Handbook of vitamin D in human health
Prevention, treatment and toxicity

edited by:
Ronald Ross Watson
Handbook of vitamin D in human health
Handbook of vitamin D in human health
Prevention, treatment and toxicity

Edited by: Ronald Ross Watson

Human Health Handbooks no. 4
ISSN 2212-375X
## Table of contents

### Overview of key vitamin D modified conditions

1. Vitamin D deficiency in the 21st century: an overview  
   S.S. Oberhelman and T.D. Thacher  
   13

2. Vitamin D in health of seniors  
   F. Lauretani, M. Maggio, C. Ruggiero, G.P. Ceda and L. Ferrucci  
   39

3. Vitamin D supplementation and public health policy  
   R. Babaria and R.R. Watson  
   53

### Vitamin D and musculoskeletal health

4. Vitamin D and fall risk  
   J. Basran, R.L. Duckham and D.B. Hogan  
   67

5. The beneficial effects of higher (adequate) vitamin D status on bone growth and muscle  
   strength in children and adolescents  
   L.H. Foo and S.J. Whiting  
   83

6. Hypovitaminosis D and muscle strength  
   T. Songpatanasilp  
   97

7. Vitamin D and growth  
   H.T. Viljakainen and E. Hypponen  
   115

8. Vitamin D and arthritis  
   U.J. Haque  
   137

9. High-dose vitamin D supplementation in the elderly  
   C.J. Bacon  
   147
Table of contents

Vitamin D and chronic disease

10. Vitamin D deficiency and premature death
   A. Zittermann and S. Prokop

11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease
    D. Hansen

12. Diseases affected by vitamin D: sun exposure
    W.B. Grant

13. Immune modulation by UV: role of vitamin D
    P.H. Hart and S. Gorman

14. Vitamin D and periodontal disease
    N. Garcia, D. Miley and D.A. Dixon

15. Vitamin D and cystic fibrosis
    T. Pincikova and M. Flodström-Tullberg

Vitamin D and infectious disease

16. Vitamin D and respiratory infections in infants and toddlers: a nutri-shine perspective
    K.V. Balan, U.S. Babu, D.E. Godar and M.S. Calvo

17. Vitamin D in HIV/AIDS: a role?
    J.-P. Viard and J.-C. Souberbielle

18. Vitamin D and tuberculosis
    K. vinh quốc Lương and L.T.H. Nguyễn

19. Vitamin D and dental health
    E. Jordan, S. Davis, P. Rakes, L.K. McCauley and J. Bashutski

20. Inflammation and vitamin D
    X. Guillot, C. Prati, N. Saidenberg-Kermanac’h, L. Semerano, G. Falgarone, M.C. Boissier and D. Wendling
Table of contents

**Vitamin D and cancer**

21. Vitamin D in the prevention and treatment of pancreatic cancer
   T.C. Chen and K.C. Chiang

22. Vitamin D and melanoma
   J.A. Newton-Bishop and J.A. Randerson-Moor

23. Vitamin D autocrine system and prostate cancer prevention and treatment
   T.C. Chen

24. Vitamin D and esophageal cancer
   H.G. Coleman, M.M. Cantwell and L.J. Murray

25. Monitoring in vivo immune modulation by vitamin D in multiple sclerosis
   A.-H. Muris, J. Damoiseaux and J. Smolders

**Vitamin D in chronic diseases**

26. Vitamin D and insulin sensitivity
   B. Larrick, S. Donkin and D. Teegarden

27. Vitamin D and diabetes mellitus: where are we?
   J. Liu

28. Vitamin D and cardiovascular risk: hype or new hope?
   J.L. Anderson and J.B. Muhlestein

29. Vitamin D and transplantation
   M. Courbebaise, J.C. Souberbielle and E. Thervet

30. Vitamin D and bone health among people with epilepsy
   R.A. Shellhaas, R.K. Singh and S.M. Joshi
### Table of contents

#### Women and children

31. Neonatal effects of enhanced vitamin D and vitamin D for premature infants  
   *H.K. Linke*  
   609

32. Lactation and vitamin D  
   *S.N. Taylor, C.L. Wagner and B.W. Hollis*  
   633

33. Vitamin D replacement in pregnant women in developing countries  
   *M.T. Sahu and M. Sahu*  
   651

34. Vitamin D in pregnancy  
   *D. Dror*  
   671

35. Design and rationale of the VITamin D and OmegA-3 Trial (VITAL)  
   *S.S. Bassuk and J.E. Manson*  
   693

#### Index

About the editor  
729
Overview of key vitamin D modified conditions
Key facts

• Historically, vitamin D deficiency was defined by the clinical disease of rickets, which was nearly eradicated through vitamin D fortification.
• Presently, defining optimal vitamin D status based on concentrations of the serum 25-hydroxyvitamin D (25(OH)D) metabolite is controversial. The functional consequences of a given 25(OH)D concentration are highly variable within the population, but up to one billion people worldwide may have less than optimal vitamin D status.
• Vitamin D is actually a hormone, not a true vitamin. It can be ingested (naturally occurring in fatty fish, fortified in some foods such as milk or in supplement form) or dermally synthesized with exposure to ultraviolet light.
• Observational studies have demonstrated relationships between vitamin D status and infectious, immune, metabolic, degenerative, and neoplastic diseases, but the beneficial effect of vitamin D in these conditions requires confirmation with randomized controlled trials.
• Identifying markers of the diverse functional effects of vitamin D will permit an individualized approach to vitamin D supplementation, based on health risks.

Summary points

• Vitamin D deficiency causes the clinical disease of nutritional rickets, which causes softening and bending of the bones.
• The incidence of vitamin D-deficiency rickets declined dramatically with fortification of milk and foods with vitamin D.
• A recent worldwide resurgence of rickets has been recognized, possibly related to reduced intake of vitamin D, breastfeeding practices, increasing prevalence of obesity, sun avoidance and sunscreen use, and air pollution.
• Vitamin D status is currently defined by the serum 25(OH)D concentration, which is a marker of storage rather than function. Laboratory assays for measurement of 25(OH)D vary widely.
• There is no universally accepted serum-based definition of vitamin D deficiency, but nutritional rickets occurs at very low 25(OH)D concentrations.
• The Institute of Medicine recommended 600 IU of vitamin D daily for most adults. However, several intermittent dosing regimens have been proposed for skeletal and non-skeletal health benefits.
• The health benefits of sufficient vitamin D may extend far beyond bone health. Observational studies suggest that vitamin D may benefit immunity, cardiovascular health, several cancers, pain, and muscle strength.
• Wide variation in individual responses to vitamin D exists. As personalized medicine becomes more of a reality, and the non-skeletal benefits of vitamin D are better defined, recommended vitamin D intake will need to be personalized.
1. Vitamin D deficiency in the 21st century: an overview

S.S. Oberhelman and T.D. Thacher
Department of Family Medicine, Mayo Clinic, 200 1st St SW, Rochester, MN 55905, USA;
thacher.thomas@mayo.edu

Abstract

Vitamin D deficiency in the past was defined by the clinical recognition of nutritional rickets, a disease nearly eradicated by vitamin D fortification. Today, the definition of vitamin D deficiency is controversial, and is based on serum concentrations of 25-hydroxyvitamin D (25(OH)D), a marker of storage rather than of function. The level of 25(OH)D that is optimal for bone health may differ from that required for non-skeletal benefits. Observational studies suggest wide-ranging effects of vitamin D status on metabolic, immunologic, infectious, cardiovascular, neoplastic, and degenerative disorders. There is likely wide individual variation in the functional implications of a given level of 25(OH)D. As randomized controlled trials confirm the benefits of vitamin D in non-skeletal diseases, the recommended intake of vitamin D and optimal 25(OH)D concentration may need to be adjusted to account for these outcomes. The future will bring an individualized approach to assessing vitamin D status and needs. Even when the factors known to influence 25(OH)D concentrations are accounted for, most of the individual variation of 25(OH)D values is difficult to explain. This variation is partially explained by genotypic variants of the vitamin D binding protein, vitamin D receptor, and the hydroxylase enzymes of the vitamin D metabolic pathway. With more widespread use of genome-wide analysis, it may be possible to personalize an individual’s vitamin D requirement, particularly in view of other disease risk factors. Measurement of the physiological effects of vitamin D that relate to bone metabolism, the immune system, or other effectors of disease could allow tailoring of the dose of vitamin D to attain maximal physiological benefit. Additional markers of the functional response to vitamin D at the individual level may be available in the future. Vitamin D has exciting potential, and a flurry of research activity is now underway to explore its potential benefits.

Keywords: rickets, hypovitaminosis D, public health, deficiency, personalized medicine
1.1 Vitamin D: yesterday

To fully appreciate the current and future implications of vitamin D insufficiency, we must explore its historical context. Historically vitamin D deficiency was defined by the clinical recognition of rickets, the childhood disease of impaired skeletal development, or osteomalacia in adults. The first descriptions of clinical manifestations of rickets appeared in ancient times. Second century Roman physician Soranus published a text in which he recommended swaddling to prevent deformities common in city dwellers (Foote, 1927; Rajakumar, 2003). Modern scientists examined a pediatric skull and radius from pre-Columbian Peru and found both consistent with rickets. In the 1920s John Foote examined fifteenth century paintings from the Netherlands and Germany and concluded that the infant subjects showed evidence of rickets (Foote, 1927).

The term ‘rickets’ first appeared in 1634 when listed as a cause of death in London’s Annual Bill of Mortality (O’Riordan, 2006). Four publications regarding rickets appeared in the mid-seventeenth century: Daniel Whistler’s doctoral defense thesis (O’Riordan, 2006; Smerdon, 1950), Arnold Boote’s chapter in his book ‘Medical Observations on Neglected Ailments’, Francis Glisson’ book which described findings seen on post mortem examinations and John Mayow’s book. For the next century and a half, little occurred in regards to rickets in the medical community (O’Riordan, 2006).

Investigators began studying the potential relationships between rickets and both cod liver oil and sunlight in the early 19th century. Unpublished rumors and subsequent case reports began spilling out of Holland, Germany and France: The Society of Science and Arts of Utrecht described the therapeutic properties of cod liver oil in 1822 (Guy, 1923); German physician Schutte published a 25 year case history of his successes in curing rickets with cod liver oil in children in 1824 (Guy, 1923); and French physician Bretonneau reported successfully treating a 15 month old suffering from rickets in 1827 (Holick, 1994; Rajakumar, 2003).

Scientists also observed a relationship with sunlight. In 1822 Sniadecki noted a higher incidence of rickets in children living in Warsaw than children living in surrounding rural communities; he blamed inferior sunlight exposure within the city as the causative factor (Holick, 1994; 2003). In 1838 Jules Guerin experimented on puppies: he weaned puppies to a dark basement and fed them raw meat while the littermates remained in a normal environment. He reported the basement
1. Vitamin D deficiency in the 21st century

puppies 'looked sad', vomited, and developed misshapen limbs and other signs of rickets while the other pups had no such signs (Mayer, 1957). Hugh Owen Thomas treated rachitic children with sunlight exposure on hospital balconies in Wales in 1878 (Chesney, 2012). While on a medical mission in Japan, Theobald Palm noted the absence of rickets. He collected prevalence data from worldwide missionaries and noted that areas of temperate latitude or heavier air pollution had more cases or rickets but that even filthy areas of the jungle did not; he concluded sunlight as the protective factor. August Hirsch and the Investigation Committee on the British Medical Association developed similar maps but blamed overcrowding and air/soil conditions (Chesney, 2012; Holick, 2003). Finally, John Bland-Sutton observed monkeys and lions in glass enclosures at the London Zoo and noted development of rickets within months of the animals' arrivals or births. However, he noted that lion cubs receiving cod liver oil and crushed bones to their usual lean-meat diet recovered from their rickets-features. He hypothesized dietary fat cured rachitic features (Findlay, 1908; Mayer, 1957; O'Riordan, 1997; Park, 1923; Rajakumar, 2003).

By the turn of the 20th century, speculations regarding the etiology of rickets varied: syphilis, scurvy, genetics, acidosis causing bony decalcification, strep infection, confinement/exercise deprivation, over-eating, or disorders of the thymus, thyroid, adrenal, parathyroid, or carotid (Cheadle, 1988; Findlay, 1908; Park, 1923). Studies of scurvy, beri-beri, and pellagra led to the discovery of 'vital substances' (later 'vitamins') which, in deficiency, led to disease. (Chesney, 2012) At this same time, rickets had become epidemic in the US and Europe: autopsy studies identified rickets in 80-96% childhood deaths (Holick, 1994; Park, 1923; Rajakumar, 2003), oral exams in London school children saw rickets in the teeth of 80% (Mellanby, 1919), and estimates of children living in industrialized cities exceeded 90% (Holick, 2003). Women with pelvises flattened by the affects of childhood rickets began requiring Cesarean births (Holick, 2003).

The early 1900s represented a turning point in rickets research. In terms of light-related work, Buchholz, Schmorl, Hess, Lundagen and Raczynski all noted seasonal variations in the incidence of rickets (Chesney, 2012; Park, 1923). German pediatrician Kurt Huldchinsky treated over 100 cases of childhood rickets with quartz mercury-vapor lamp UVB irradiation and then noted that UVB exposure to a single limb produced X-ray evidence of full skeletal healing (Chesney, 2012; Holick, 1994; 2003; Park, 1923; Rajakumar, 2003). Harriett Chick and her colleagues treated children with rickets at a Vienna hospital after World War I. They treated children with nothing, mercury-vapor lamp UVB exposure, sunlight, cod liver oil, or a combination of UVB and cod liver oil. They found several interesting results: healing rates depended on amount of UVB exposure; partial body UVB exposure produced full body healing; no new cases of rickets developed during the summer months in any group; and most rapid healing occurred in children receiving both UVB exposure and cod liver oil (Chick et al., 1922; O'Riordan, 1997). Additionally Hess studied epiphyses calcification on radiography and published that irregular sunlight exposure, in the absence of diet fortification, improved rickets (Hess and Mildred, 1925b; Holick, 1994).

From a diet-perspective, English physician Edward Mellanby varied the diets of puppies and noted the protective factor of cod liver oil, butter and whole milk. He postulated poor intake of ‘an antirachitic factor which is either fat-soluble A or has a somewhat similar distribution to the
fat soluble A' as the cause of rickets (Mellanby, 1919). Elmer McCollum, John Holand, Edward Park and Paul Shipley induced rickets in rats and then healed those manifestations with cod liver oil. They then oxidized cod liver oil to isolate the anti-xerophthalmic and anti-rachitic properties and in 1922 they deduced that the two factors were distinct (McCollum et al., 1921, 1922). They postulated that this factor was a vitamin and, as the fourth vitamin discovered, named it vitamin D (Holick, 1994; McCollum et al., 1921, 1922; Rajakumar, 2003). Two research groups (Hess and Weinstock, Steenbock and Black) noted that previously anti-rachitic-deficient foods would gain anti-rachitic activity when irradiated with UV light (Hess, 1924; Hess and Mildred, 1925a; Holick, 1994; Steenbock, 1924). University of Wisconsin's Harry Steenbock not only published his findings regarding the ability to impart anti-rachitic factor to rat feed via UV irradiation, but patented the process 'to protect the interest of the public in the possible commercial use of these findings' (Schneider, 1973; Steenbock, 1924; Steenbock and Black, 1924; Steenbock and Nelson, 1924). The University created a board of trustees to manage the patent. Quaker Oats attempted to purchase exclusive rights, but Steenbock and the board refused. They sold licenses to Quaker Oats for cereal fortification and to a pharmaceutical agency to develop Viosterol (medicinal vitamin D supplementation). They refused to sell licenses to manufacturers of chewing gum, tobacco, lipstick, beer or soft drinks, deciding that only healthy foods should receive fortification. With dairy-farmer roots, Steenbock also developed a process to fortify milk. Laws at the time prohibited any additives to milk; three manufacturers developed equipment to irradiate flowing films of milk. (Wisconsin Alumni Research Foundation)\(^1\) Initially milk was supplemented to 400 IU of vitamin D per liter, the same amount of vitamin D in one teaspoon of cod liver oil (Chesney, 2012). This process of fortification revolutionized the prevention of rickets (Wisconsin Alumni Research Foundation).

The next boom in vitamin D research started in the 1960s. Researchers at the University of Wisconsin isolated a metabolite chromatographically-distinguishable from vitamin D3, subsequently determined its structure as 25-hydroxyvitamin D (25(OH)D) and discovered it was produced in the liver (Blunt et al., 1968; Norman et al., 1964; Ponchon and DeLuca, 1969). They found a third distinct metabolite (Haussler et al., 1968; Lawson et al., 1969) and researchers in Cambridge, after determining production occurred in the kidney, diagramed our modern understanding of vitamin D synthesis and metabolism (Fraser and Kodicek, 1970). In 1971 three separate labs (University of Wisconsin, Cambridge and University of California) confirmed the structure of this metabolite as 1,25(OH)\(_2\)D (Holick et al., 1971; Lawson et al., 1971; Macintyre et al., 1977; Norman et al., 1971). Scientists re-classified vitamin D as a hormone and not a true vitamin (Holick, 1994). In 1979 Stumpf published results of \(^3\)H nuclear localization in rats that showed vitamin D receptors in the stomach, parathyroid, gonads, brain and skin. Subsequent studies found vitamin D receptors in a plethora of other body tissues (Holick, 1994; Stumpf et al., 1979). Finally, in 1980, Holick described the dermal synthesis of vitamin D (Holick et al., 1980).

---

1.2 Vitamin D: today

1.2.1 Modern understanding of vitamin D metabolism

As the 19th century scientists suspected, both diet and skin exposure to UVB provide vitamin D. It occurs naturally in few foods, namely fatty, ocean dwelling fish (salmon, sardines, mackerel, tuna, and cod), shiitake mushrooms and egg yolks. It is also fortified in some cereals (100 IU/serving), milks (100 IU/227 g), orange juice (100 IU/227 g), butters (50 IU/99 g), margarines (430 IU/99 g), cheeses (100 IU/85 g) and yogurts (100 IU/227 g) (Holick, 2007). Vitamin D exists in two forms: D2 (ergocalciferol), which is produced via irradiation of yeast, and D3 (cholecalciferol), which is the form found in fatty fish and from dermal synthesis following UV exposure (Holick, 2006). As scientists deduced in the 1960s, regardless of the source, the parent compound is metabolized to 25(OH)D (calcidiol) in the liver and then further metabolized to 1,25(OH)2D (calcitriol) in the kidney. The 1,25(OH)2D is the active hormone that acts on vitamin D receptors throughout the body.

1.2.2 Defining vitamin D deficiency... not as easy as it sounds

Today, the definition of vitamin D deficiency has expanded from the clinical diagnosis of rickets to a definition based on the serum concentration of 25(OH)D. However, this is an imperfect marker, because it represents vitamin D supply rather than vitamin D function. The 25(OH)D metabolite has no physiologic function, and its conversion to active 1,25(OH)2D is tightly regulated. Rickets or osteomalacia typically occur with serum 25(OH)D values <10 ng/ml (1.0 ng/ml = 2.5 nmol/l) (Thacher and Clarke, 2011). Optimal bone health and other non-skeletal benefits may be gained from 25(OH)D concentrations greater than those necessary to prevent rickets and osteomalacia. Currently, no universal standard definition of vitamin D deficiency exists. The most common definitions include: deficiency <20 ng/ml (<50 nmol/l), insufficiency 20-30 ng/ml (50-75 nmol/l) and sufficiency >30 ng/ml (>75 nmol/l) (Heaney and Holick, 2011; Holick, 2007), or deficiency <12 ng/ml (<30 nmol/l), insufficiency 12-20 ng/ml (30-50 nmol/l) and sufficiency >20 ng/ml (>50 nmol/l) (Institute of Medicine, 2011).

However, the clinical utility of the term ‘insufficiency’ is not at all clear. Because the functional effect of a given 25(OH)D value is widely variable, it is likely that the range defined as insufficiency represents adequate vitamin D status for some and deficiency for others. The requirement for vitamin D appears to be lower when the calcium intake is adequate. Definitions of insufficiency have been based on analysis of parathyroid suppression and/or calcium absorption. Some have argued that parathyroid suppression reaches a plateau at mean 25(OH)D concentrations of 28-40 ng/ml. However, flaws of these studies included substantial individual variation, lack of a clear inflection point, and exclusion of children (Heaney, 2004; Holick, 2007; Lee et al., 2008; Thacher and Clarke, 2011). Calcium absorption has been reported to plateau at a mean 25(OH)D concentration of 32 ng/ml. However, additional studies fail to show a relationship between serum 25(OH)D concentrations and intestinal calcium absorption, except at concentrations below 5 ng/
1.2.3 Return of rickets to a modern society

Following routine fortification of foods with vitamin D, the incidence of rickets declined dramatically, and it was considered rare by the 1960s. However, a recent worldwide resurgence of rickets has been recognized (Thacher et al., 2006). Best estimates of national prevalence come from chart reviews and case report series since rickets is not a reportable disease (Nield et al., 2006). Estimates range from 6-9 cases per million hospitalized children in the US (Centers for Disease Control and Prevention, 2001; Scanlon, 2001) or Canada (Ward et al., 2007). Additionally, meta analyses of published cases found 166 cases (in 22 studies) 1986-2003 (Weisberg et al., 2004) and 65 cases (in 11 studies) 1975-1985 (Cosgrove and Dietrich, 1985). The CDC has expressed concern regarding the prevalence of rickets, especially among darkly pigmented children (Scanlon, 2001).

1.2.4 Prevalence of hypovitaminosis D

Estimates of worldwide vitamin D deficiency exceed 1 billion individuals (Bell, 2011; Holick, 2007; Makariou et al., 2011). In the USA, NHANES reported overall national mean serum concentrations of 25(OH)D at 30 ng/ml in 1988-1994 (Ginde et al., 2009a), 24 ng/ml in 2001-2004 (Ginde et al., 2009a) and 19.9 ng/ml in 2005-2006 (Forrest and Stuhldreher, 2011). International studies show mean 25(OH)D values of 8.2 ng/ml in Iranian adults (Van Schoor and Lips, 2011); 14 ng/ml in veiled and 17.2 ng/ml in non-veiled African women in Tunisia (Van Schoor and Lips, 2011); and 11-12.8 ng/ml in Middle Eastern university students living in Riyadh (Sedrani, 1984). A British study sampled 45 year old adults from a birth cohort and found winter values below 10, 16 and 20 ng/ml in 15.5, 46.6% and 87.1% respectively (Hypponen and Power, 2007).

Several studies address postmenopausal women, given their risk of osteoporosis. The prevalence of vitamin D deficiency (25(OH)D<30 ng/ml) was 50% in Thailand and Malaysia, 75% in the USA and 90% in Japan and South Korea (Dawson-Hughes et al., 2010). In the UK, South Asian immigrants and Caucasian natives possessed mean serum concentrations of only 4.3 and 16.3 ng/ml respectively (Lowe et al., 2010). Italian investigators found values less than 12 ng/ml and 5 ng/ml in 76% and 27% of women, respectively (Isaia et al., 2003).

Children require vitamin D to establish optimal bone density during growth. Projections based on data from NHANES 2001-2004 suggested deficiency (<15 ng/ml) in 7.6 million US children and insufficiency (15-29 ng/ml) in another 50.8 million children (Kumar et al., 2009). Other studies have shown deficiency (<20 ng/ml) in 48% of healthy Maine girls ages 9-11 at some point during a three year study (Sullivan et al., 2005), 12.1% of healthy Boston infants and toddlers (Gordon et al., 2008), and 42% of Boston adolescents (with 24.1% <15 ng/ml and 4.6% <8 ng/ml) (Gordon et al., 2004). Mean serum concentrations of 11.8 ng/ml (with 55.7% below 9 ng/ml) in New Delhi children (Marwaha et al., 2005), 17 ng/ml in Lebanese youth in the spring (El-Hajj
Vitamin D deficiency also affects men. A study of community-dwelling men in several USA cities revealed a mean serum concentration of 25.1 ng/ml with 71.1% of participants below 30 ng/ml, 25.7% below 20 ng/ml and 2.9% below 10 ng/ml (Orwoll et al., 2009).

Hypovitaminosis D even extends into sunny climates. A study that recruited healthy high-sun-exposure participants from a surf shop in Honolulu Hawaii found 51% of study participants’ serum concentrations of 25(OH)D below 30 ng/ml (Binkley et al., 2007). Similarly children in Texas showed serum levels <12, 20 and 32 ng/ml in 1%, 16% and 68% of participants, respectively (Rovner and O’Brien, 2008).

Those with sub-par health commonly possess low values. Medical inpatients at Massachusetts General Hospital were found to have a mean serum concentration of 15 ng/ml with 57% below 15 ng/ml and 22% below 8 ng/ml (Thomas et al., 1998). Estimates of vitamin D deficiency in the noninstitutionalized elderly range from 40 to 100% (Holick, 2007). A study of elderly individuals >98 years found undetectable serum values in 95% (Passeri et al., 2003).

Finally, post partum women and their infants often have serum 25(OH)D concentrations shortly after birth below 15 ng/ml (Taha et al., 1984). Prevalences of deficiency have been reported at 50% (mothers <20 ng/ml) (Lee et al., 2007), 65% (Boston newborns <12 ng/ml) and 45.6% (black Pittsburgh newborns <15 ng/ml) (Rovner and O’Brien, 2008). Due to the low vitamin D content of breast milk, breastfeeding has been identified as a risk factor for vitamin D deficiency. One study found 48% of mothers and 43% of babies with 25(OH)D values <10 ng/ml (Seth et al., 2009).

1.2.5 Why has this become a problem again?

Several factors may contribute to the prevalence of hypovitaminosis D and the resurgence of rickets in our modern society.

A variety of medical conditions interfere with vitamin D absorption (i.e. celiac disease, cystic fibrosis, Crohn’s disease, or gastric bypass surgery), hepatic conversion or renal synthesis of vitamin D metabolites (Tsiaras and Weinstock, 2011). Medications, including anticonvulsants, glucocorticoids, antiretrovirals, and antirejection drugs can interfere with vitamin D metabolism (Holick, 2007).

The prevalence of obesity has increased throughout the world, and fat soluble vitamin D is sequestered in adipose tissue. A study evaluated serum levels of 25(OH)D in obese and ideal-weight-matched-controls either 24 hours after whole body UV irradiation or oral ingestion of 50,000 IU vitamin D2. Basal values between groups were similar, but the amount of increase in the nonobese subjects was 57% higher (15.3 versus 6.7 ng/ml) after UVB irradiation. Body mass
index was inversely correlated with serum concentrations after either UVB irradiation or oral ingestion (Wortsman et al., 2000).

Several barriers exist that prevent adequate sunlight exposure. Recommendations regarding skin cancer promote sun avoidance and sunscreen use. When used properly, sunscreen with SPFs of 8 or 15 reduce cutaneous vitamin D synthesis by 97.5% and 99% respectively (Holick, 2003; Holick and Chen, 2008; Scanlon, 2001). Para-aminobenzoic acid (a sunscreen) applied to human epidermis blocked production of vitamin D with UV exposure compared with marked production of vitamin D in para-aminobenzoic acid-free epidermis samples (Matsuoka et al., 1987). A study of sunscreen users and non-users with equivalent sunlight exposure revealed significantly lower serum 25(OH)D concentrations in sunscreen users (16.8 ng/ml versus 36.5 ng/ml) (Matsuoka et al., 1988). Additionally, pollution impedes cutaneous vitamin D synthesis from sunlight. Black carbon from biomass and fossil fuel combustion absorbs UVB radiation from sunlight and decreases the amount that reaches the earth’s surface by approximately 5% in typical urban environments (Highwood and Kinnersley, 2006) and up to 81% in the Brazilian rain forests (Mims, 1996).

The seasonal tilt of the earth can impact the amount of UVB available for cutaneous vitamin D synthesis. An appropriate zenith angle is required for UVB to penetrate the non-polluted ozone. Latitudes above 35°N and below 35°S produce too oblique of an angle during winter months for UVB penetration (Holick, 2003). This produces almost complete cessation of cutaneous vitamin D synthesis seasonally in some parts of the world: Rome (41.9°N) November through February (Tsiaras and Weinstock, 2011), Berlin (52.5°N) October through April (Tsiaras and Weinstock, 2011), Boston (42°N) November through February (Webb et al., 1988), Edmonton Canada (52°N) mid-October through mid-April (Holick, 1994; Webb et al., 1988).

Purdah, cultural covering, limits sunlight exposure and cutaneous vitamin D synthesis, even in the sunniest areas of the world (Holick and Chen, 2008). Window-glass blocks all UVB, eliminating any benefit for those who remain indoors (Cannell and Hollis, 2008). Melanin in the epidermis of darkly pigmented skin reduces vitamin D synthesis (Aloia, 2011). Finally, advancing age decreases cutaneous 7-dehydrocholesterol necessary for synthesis of vitamin D (Holick, 2003; Holick and Chen, 2008; Holick et al., 1989; MacLaughlin and Holick, 1985).

Fortification revolutionized the world’s approach to vitamin D deficiency. However, levels of fortification can vary significantly. For instance, fortified milk is advertised to contain 400 IU of vitamin D per quart. However, a study of randomly selected milk found that only 29% of the samples tested contained 320–480 IU/quart, 50% of samples contained <80% of advertised amount, 14% contained <5% advertised amount and 21% of the skim milk samples contained no detectable vitamin D (Holick, 1994; Holick et al., 1992; Scanlon, 2001). A four year study in New York found that only half of sampled milk complied with label declarations (Murphy et al., 2001; Yetley, 2008).
1. Vitamin D deficiency in the 21st century

Few foods naturally contain vitamin D. Ocean-dwelling salmon contains 600-1000 IU/99 g. However, canned salmon contains 300-600 IU/99 g and farmed salmon contains only 100-250 IU/99 g (Holick, 2007).

Finally, breastfeeding has played a role. HealthyPeople 2010 set a goal for 75% of infants to breastfeed for the first six months of life (US Department of Health, 2000). Between 1988 and 1999, breastfeeding among African American mothers in North Carolina increased from 5.2% to 34.7%. During this interval several cases of rickets were identified in the same region. Breast milk has low concentrations of vitamin D; therefore, the American Academy of Pediatrics recommends universal supplementation of vitamin D to exclusively breastfed infants. However, compliance with this recommendation is poor. Data regarding supplementation of lactating mothers is somewhat new and not ready for universal recommendations (Scanlon, 2001).

1.2.6 Supplementation regimens

Significant controversy exists regarding optimal supplementation dosing; partly related to the lack of universally accepted optimal serum values. Additionally, while D2 and D3 supplements both increase in 25(OH)D values, D3 has produced slightly superior results, suggesting a higher dose of D2 needed to achieve similar serum 25(OH)D concentrations (Binkley et al., 2011). Finally, the variety of dosing regimens is confusing. A review of 306 patients with vitamin D insufficiency at an Atlanta VA revealed 36 different oral vitamin D prescribing regimens. Doses ranged from 400 to 50,000 IU given twice daily to once monthly. The three most commonly utilized regimens (50,000 IU weekly ×4, then monthly ×5; 50,000 IU monthly ×6; 50,000 IU three times weekly ×6 weeks) all increased the serum 25(OH)D concentration but only achieved concentrations >30 ng/ml in 38%, 42% and 82% of subjects respectively. No toxicities were reported for any regimen (Pepper et al., 2009). Each additional 100 IU (2.5 μg) increment of daily oral vitamin D3 increases serum 25(OH)D by about 1 ng/ml (0.6-1.2 ng/ml = 1.5-3 nmol/l) (Barger-Lux et al., 1998; Dawson-Hughes et al., 2010; Heaney, 2004).

With support from the United States and Canadian governments, the Institute of Medicine updated the Dietary Reference Intakes for calcium and vitamin D in 2011. The committee concluded that neither adequate evidence regarding the cause/effect outcomes nor the dose-response relationship currently exists for vitamin D; therefore, they made recommendations solely based on bone health. The recommended RDAs strive to achieve >20 ng/ml 25(OH)D in >97.5% of the population: 600 IU daily in ages 1-70 years (including pregnant and lactating women) and 800 IU daily in elderly (>70 years). The tolerable upper intake level was set at 4,000 IU/day for individuals >9 years. The committee agreed that neither toxicity nor hypercalcemia has been reported for daily intakes <10,000 IU; however, lower values were chosen due to lack of data regarding outcomes with chronic intake. The committee stated that additional research, including better understanding of serum biomarkers, is urgently needed (Aloia, 2011; Institute of Medicine, 2011; Ross et al., 2011).
Many within the medical and research community expressed surprise at these recommendations, since several studies suggest benefit with higher doses. One study estimated a daily need of 3,440 IU for the population to achieve 30 ng/ml serum concentrations. This estimate utilized computer simulation based on data from a six month study of racially diverse adults receiving oral vitamin D supplementation with dosing adjustments performed every two months (Aloia et al., 2008). Another study compared either 1,600 IU daily or 50,000 IU once monthly in community dwelling adults >65 years. None developed toxicity but 19% failed to achieve serum concentrations >30 ng/ml after one year of supplementation (Binkley et al., 2011). Studies regarding fracture prevention have identified an optimal serum concentration of 28-40 ng/ml. Various studies have estimated 700-1000 IU daily for the average elder to achieve this goal; however, much higher doses may be necessary for the entire elder population to achieve these concentrations (Dawson-Hughes, 2004; Dawson-Hughes et al., 2010).

The intramuscular route can also be utilized: ergocalciferol 15 mg IM has been used to treat osteomalacia (Burns and Paterson, 1985).

1.2.7 Serum 25(OH)D measurement

Reported values differ when tested in different laboratories and with different methodologies. Not all labs detect both the 25(OH)D2 and 25(OH)D3 forms. One study sent twenty samples from ten healthy subjects to six laboratories. Each subject contributed one basal sample and one sample spiked with an additional 20 ng/ml 25(OH)D. The labs reported basal sample means of 25(OH)D ranging from 17.1-35.6 ng/ml. The mean increase detected in the samples spiked with 20 ng/ml of additional 25(OH)D ranged from 7.7-18 ng/ml. Individual variation for a single sample between labs ranged from 10-40 ng/ml (Binkley et al., 2004).

Two organizations are working to improve accuracy of testing: The National Institute of Standards and Technology introduced internal standards and the Vitamin D External Quality Assessment Scheme works with analysis problems in individual laboratories (Aloia, 2011).

1.3 Vitamin D: tomorrow

1.3.1 Beyond bone health

The vital role of vitamin D in bone health is universally accepted. However, a plethora of publications over the past several years shows that the role of vitamin D extends far beyond the skeleton. The future of vitamin D will be determined by establishing the non-skeletal benefits of vitamin D and individualizing vitamin D recommendations based on functional outcomes and disease risk. Vitamin D receptors are widely distributed throughout the body, as is the 1α-hydroxylase enzyme responsible for converting 25(OH)D to 1,25(OH)2D. Observational data have demonstrated relationships between vitamin D status and infectious, immune, metabolic, degenerative, and neoplastic diseases.
Both strength (see Chapter 6) and falls (see Chapter 4) improve with vitamin D sufficiency. Studies have shown a positive correlation between muscle strength and serum 25(OH)D concentrations in elderly subjects (Bischoff-Ferrari et al., 2004; Holick, 2007; Visser et al., 2003). Other studies have shown less falls with vitamin D intake >600-800 IU/day (Bischoff-Ferrari et al., 2009; Broe et al., 2007; Dawson-Hughes et al., 2010; Holick, 2007; Mason et al., 2011; Murad et al., 2011; Thacher and Clarke, 2011). Although osteomalacia, a painful bony condition, has become rare, non-specific pain is associated with hypovitaminosis D (93% patients with musculoskeletal/bone pain had 25(OH)D <20 ng/ml) (Holick, 2007; Plotnikoff and Quigley, 2003; Thacher and Clarke, 2011).

Observational studies show an inverse relationship between serum concentrations of 25(OH)D and death (Ginde et al., 2009b; Makariou et al., 2011; Thacher and Clarke, 2011; Zittermann et al., 2012). Additionally, subjects taking a vitamin D supplement have decreased mortality compared with controls (Autier and Gandini, 2007; Bjelakovic et al., 2011; Cannell and Hollis, 2008; Makariou et al., 2011; Nadir et al., 2010; Scragg et al., 2007; Thacher and Clarke, 2011).

Vitamin D may have cardiovascular benefits. Observational studies found slightly lower blood pressure in individuals with higher serum 25(OH)D concentrations (Forman et al., 2007; Lind et al., 1995; Makariou et al., 2011; Nadir et al., 2010; Reis et al., 2009; Scragg et al., 2007). Two studies have shown a 9% decrease in systolic blood pressure with initiation of supplemental vitamin D (Islam et al., 2011; Judd et al., 2010; Pfeifer et al., 2001). However, a meta-analysis of 8 studies showed a nonsignificant reduction in SBP and a small but statistically significant decrease in diastolic blood pressure (Witham et al., 2009). The WHI failed to show any blood pressure improvement, but the low compliance of 59% and the low dose of vitamin D may have impacted results (Margolis et al., 2008). Hypertensive individuals exposed to tanning beds became normotensive in 3 months while their serum 25(OH)D concentrations increased >180% (Krause et al., 1998). This relationship may be due to 1,25(OH)_{2}D regulation of the renin-angiotensin system (Li et al., 2002). Although data do not agree, several studies have shown a beneficial relationship between vitamin D adequacy and improved cholesterol values (Carbone et al., 2008; Ford et al., 2005; Hypponen et al., 2008; Lind et al., 1995; Makariou et al., 2011; Martins et al., 2007). NHANES observational data (Kendrick et al., 2009; Kim et al., 2008; Makariou et al., 2011) and a German prospective study (Makariou et al., 2011; Pilz et al., 2008) have both suggested increased risk of cardiovascular disease including stroke or death from congestive heart failure with vitamin D insufficiency. Additional observational studies have demonstrated a relationship between coronary artery disease (Grandi et al., 2010; Kim et al., 2008; Makariou et al., 2011; Temmerman, 2011) or cardiovascular death (Dobnig et al., 2008; Ginde et al., 2009b; Temmerman, 2011; Thacher and Clarke, 2011) and vitamin D insufficiency.

Vitamin D may play a role in both type 1 and type 2 diabetes. A 31-year prospective study of Finish infants given 1000 IU daily for the first year of life (Hypponen et al., 2001) and a meta-analysis of observational studies of children ever receiving vitamin D supplements (Zipitis and Akobeng, 2008) showed a lower risk of development of type 1 diabetes. Additional studies have shown potentially beneficial relationships between vitamin D and type 2 diabetes (Borissova et
The relationship of vitamin D with several different cancers has been investigated. A PubMed search revealed almost 2,000 papers published in the past decade with the medical subject headings terms 'vitamin D' and 'neoplasm'. Research includes cancers of the prostate (see Chapter 23), ovary, lung, pancreas (see Chapter 21), skin, esophagus (see Chapter 24), stomach, endometrium, kidney, and blood with the strongest literature showing a potential benefit for cancers of the breast (Chen et al., 2010; Garland et al., 2007; Gissel et al., 2008; Grant, 2010; World Health Organization International Agency for Research on Cancer, 2008) and colorectum (Fedirko et al., 2010; Gandini et al., 2011; Gorham et al., 2005; 2007; Grant, 2010; Lee et al., 2011;
1. Vitamin D deficiency in the 21st century

Ma et al., 2011; Wei et al., 2008; World Health Organization International Agency for Research on Cancer, 2008; Yin et al., 2009; 2011).

Topical 1,25(OH)₂D preparations have become firmly established as effective treatment for psoriasis. Additionally, oral 1,25(OH)₂D has shown improvement in both skin manifestations and the joint pain of psoriatic arthritis (Holick, 1994; 2007).

The health implications of vitamin D status in pregnant and lactating mothers and their infants are actively being investigated. A case-control study of pregnant women showed a dose-response relationship between serum 25(OH)D and eventual risk of pre-eclampsia during pregnancy (Bodnar et al., 2007).

1.3.2 Personalized medicine

The future of vitamin D will likely bring with it a more individualized approach to assessing vitamin D status and needs. Currently, vitamin D sufficiency is determined by a marker of storage, 25(OH)D. However, there is likely wide individual variation in the functional implications of a given level of 25(OH)D. Furthermore, a fixed amount of vitamin D intake or sun exposure produces a wide individual variation in 25(OH)D response (Aloia et al., 2008; Heaney et al., 2003). Even when all the factors known to influence 25(OH)D concentrations are accounted for, most of the individual variation of 25(OH)D values is difficult to explain. This variation is partially explained by genotypic variants of the vitamin D binding protein, vitamin D receptor, and the hydroxylase enzymes of the vitamin D metabolic pathway (Ahn et al., 2010; Wang et al., 2010). With more widespread use of genotypic analysis, it may be possible to personalize an individual’s vitamin D requirement, particularly in view of other disease risk factors. A vitamin D 25-hydroxylase genetic variant has been associated with the risk for type 1 diabetes mellitus (Ramos-Lopez et al., 2007). Genetic variants of the vitamin D receptor may modulate the individual response to vitamin D. Subjects with osteoporosis who showed a response to supplemental calcium and vitamin D expressed a difference in the vitamin D receptor polymorphisms compared with those who did not respond (Elnenaei et al., 2011).

Additional markers of the functional response to vitamin D at the individual level may be more readily available in the future and permit predicting and monitoring the effect of vitamin D supplementation. Measurement of the physiological effects of vitamin D that relate to bone metabolism, the immune system, or other effectors of disease could allow tailoring of the dose of vitamin D to attain maximal physiological benefit. As randomized controlled trials become available confirming the benefits of vitamin D in non-skeletal diseases, the recommended intake of vitamin D and optimal 25(OH)D concentration may need to be adjusted to account for these outcomes. The recommended vitamin D intake may require adjustment for calcium intake, body size, body fat, ethnicity, and usual sun exposure. As individualized medicine becomes more of a reality in all areas of medical care, the optimal role of vitamin D will certainly be better defined.
1.3.3 So much more to learn!

Although our knowledge of vitamin D has grown exponentially since ancient descriptions of rickets, many questions remain. Individuals and organizations have posed several questions still needing to be answered.

In October 2011, scientists, government agents, and academics, including representatives from the CDC and American Academy of Pediatrics, gathered for ‘A Vitamin D Expert Panel Meeting’ to examine current knowledge and future research needs. They stressed that public health recommendations should consider health implications beyond rickets and that more longitudinal studies are needed (Scanlon, 2001).

Similarly, National Institute of Health held a conference entitled ‘Vitamin D and health in the 21st century: bone and beyond’ in 2003. Along with the National Institute of Health members, participants included representatives from national institutes (National Cancer Institute, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Diabetes and Digestive and Kidney Disorders), government departments (Department of Agriculture, Office of Dietary Supplements, CDC) and industry (National Dairy Council, Coca-Cola). They identified several important areas in need of research including developing universal definitions of adequacy based on health outcomes, determination of dietary/supplement/UV requirements including better understanding of safety and toxicity in all ages and ethnic groups (Raiten and Picciano, 2004).

Vitamin D has exciting potential, and a flurry of research activity is now underway to explore its potential benefits. Enthusiasm regarding vitamin D is reminiscent of the health benefits attributed to other vitamins in the past, many of which were not eventually confirmed. Vitamin D differs from other vitamins in that it is a prohormone and not a true vitamin. The future is bound to bring clarification of the optimal role of vitamin D and new applications for vitamin D in health and disease.

References


1. Vitamin D deficiency in the 21st century


1. Vitamin D deficiency in the 21st century


Institute of Medicine, 2011. Dietary reference intakes for calcium and vitamin D. The National Academies Press, Washington, DC, USA.


1. Vitamin D deficiency in the 21st century


1. Vitamin D deficiency in the 21st century


Steenbock, H. and Black, A., 1924. The induction of growth promoting and calcifying properties in a ration by exposure to ultraviolet light. Journal of Biological Chemistry 61, 405-422.


1. Vitamin D deficiency in the 21st century


S.S. Oberhelman and T.D. Thacher


**Key facts**

- The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50%.
- Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia.
- Low vitamin D3 has been associated with significant reduction of mortality in older persons.
- A cycle for increasing risk for falling in older persons with low vitamin D has been hypothesized and reported.
- Vitamin D supplementation should be performed daily with at least 1000 UI.

**Summary points**

- The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50%. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia.
- The Institute of Medicine has recently recommended that serum 25-hydroxyvitamin D is adequate when it is higher than 50 nmol/l, which is similar to the recommendation of the Standing Committee of Europe Doctors (www.cpme.eu).
- A recent Cochrane review studying the relationship between vitamin-D and mortality reported that when the different forms of vitamin D are assessed separately, only low vitamin D3 levels significantly predict mortality, whereas vitamin D2, alfacalcidol, or calcitriol do not.
- The mechanism by which vitamin D deficiency could influence the muscle-skeletal system and can produce an increased risk for falling is described. We propose in older persons a vicious circle induced by low vitamin-D. Sarcopenia, altered cortical bone, and subsequent falls all contribute to osteoporotic fractures.
- Supplementation with cholecalciferol 1000 IU per day still remains the treatment of choice of vitamin D supplementation.
2. Vitamin D in health of seniors

F. Lauretani1, M. Maggio2, C. Ruggiero3, G.P. Ceda2 and L. Ferrucci4
1Geriatric Unit, Laboratory of Movement Analysis, Geriatric-Rehabilitation Department, University Hospital of Parma, Via Gramsci 14, 43126 Parma, Italy; 2Department of Internal Medicine and Biomedical Sciences, Section of Geriatrics, University of Parma, Via Gramsci 14, 43126 Parma, Italy; 3Institute of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, University of Perugia, S. Andrea delle Fratte, 06156 Perugia, Italy; 4Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, 2122 S. Hanover Street, Baltimore, MD 21201, USA; flauretani@ao.pr.it

Abstract

Vitamin D is a hormone with multiple biological activities that are essential for normal functioning of many biological systems including bone, skeletal muscle, brain and heart. The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50% and, because of the demographic expansion, vitamin D deficiency is becoming very important for public health. Vitamin D status has been studied in all continents and most countries over the world. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia. In this chapter, we explore potential mechanism which vitamin D could influence the muscle-skeletal system and increase risk of falling. In particular, we propose an array of mechanisms for the increased risk of falling secondary to low vitamin D. These mechanisms can be directly or indirectly related to sarcopenia (defined as reduced muscle mass with age in older persons) – and are implicated in the genesis of osteoporotic fractures. Although we mention all therapeutic options for treatment of vitamin D deficiency, we recognize that in a recent Cochrane review addressing the relationship between vitamin D and mortality, only low vitamin D3 levels were significantly associated with high risk of death whereas vitamin D2, alfacalcidol, or calcitriol were not. Therefore, the treatment of choice for vitamin D deficiency should be cholecalciferol, also known as vitamin D3. Supplementation with 1000 IU per day still remains the treatment of choice of vitamin D supplementation.

Keywords: vitamin D deficiency, mortality, sarcopenia, falls, older persons
2.1 Epidemiology of the vitamin-D deficiency

Older persons are prone to develop low vitamin D levels. This phenomenon results from reduced capacity of the skin to produce vitamin D, low sun exposure, skin pigmentation, sunscreen use, skin covering clothes and a diet low in fish and dairy products. In the elderly the reduced dermal synthesis of vitamin D is unlikely to be compensated by dietary intake of vitamin D. The estimated worldwide prevalence of vitamin D deficiency among the elderly of about 50% underlines the importance of vitamin D deficiency for public health (Lauretani et al., 2010). Vitamin D status has been studied in all continents and most countries over the world. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia (Ahmadieh and Arabi, 2011; Arabi et al., 2010; Van Schoor et al., 2011). Traditional risk groups include young children, pregnant women, and in older persons, the institutionalized and non-western immigrants.

The best determinant of vitamin D status is the serum concentration of 25(OH)D. There is no general agreement on the required serum 25(OH)D for an adequate vitamin D status. Most investigators agree that serum 25(OH)D should be higher than 50 nmol/l, but some recommends higher serum levels, e.g. higher than 75 or even 100 nmol/l (Holick, 2007). The Institute of Medicine has recently recommended that serum 25(OH)D is adequate when the serum concentration is higher than 50 nmol/l (Ross et al., 2011). This statement is similar to the recommendation of the Standing Committee of Europe Doctors (www.cpme.eu). Clinical vitamin D deficiency only occurs when serum 25(OH)D is lower than 25 nmol/l. The clinical picture includes muscle weakness, bone pain and fractures (Ahmadieh and Arabi, 2011; Van Arabi et al., 2010; Schoor et al., 2011).

In a study of community dwelling persons, representative of the older persons, performed in the Chianti area in the centre of the Italy, known for its temperate climate and sunny countryside, a high prevalence of low 25(OH)D levels was found (Figure 2.1) (Maggio et al., 2005). Serum levels of vitamin D diminish with age in both sexes, but the decline starts substantially earlier and is steeper in women starting from the perimenopausal period. In men the decline of vitamin D becomes apparent 20 years later starting from 7th decade (Maggio et al., 2005). In another study conducted in a US cohort of older men in the United States, both vitamin D deficiency and insufficiency were common. Approximately one fourth had 25(OH)D levels below the threshold
of frank deficiency (<20 ng/ml), and the majority had vitamin D insufficiency (<30 ng/ml) (Orwoll et al., 2009). Vitamin D deficiency was particularly common during the winter and spring time (especially in the northern communities) and in the oldest and more obese subjects. In fact, 86% of these subjects with multiple risk factors were vitamin D deficient (Orwoll et al., 2009).

### 2.2 Vitamin D and mortality in older persons

The relationship between vitamin D and mortality was recently reviewed in the Cochrane (Bjelakovic et al., 2011) review of fifty randomized trials conducted in 94,148 participants with data available on mortality. Most of the trials included 70 years or older women. Vitamin D was administered for a median of two years. More than one half of the trials had a low risk of bias. Overall, vitamin D decreased mortality (RR 0.97, 95% CI = 0.94 to 1.00). When the different forms of vitamin D were assessed separately, only vitamin D3 decreased mortality significantly (RR 0.94, 95% CI = 0.91 to 0.98; 74,789 participants, 32 trials) whereas vitamin D2, alfacalcidol, or calcitriol did not. Vitamin D3 combined with calcium increased the risk of nephrolithiasis (RR 1.17, 95% CI = 1.02 to 1.34). Alfacalcidol and calcitriol increased the risk of hypercalcaemia (RR 3.18, 95% CI = 1.17 to 8.68).

In accordance with these results, Heaney et al. (2011) recently published a trial comparing the supplementation of D2 or D3 on incremental value of total 25(OH)D. The trial used a single-blind, randomized design in 33 healthy adults. Calciferols were dosed at 50,000 IU/wk for 12 wk. The authors found that D3 was approximately 87% more potent in raising and maintaining serum 25(OH)D concentrations and produces 2- to 3-fold greater storage of vitamin D than does equimolar D2. Hence they concluded that given its greater potency and lower cost, D3 should be the preferred treatment option when correcting vitamin D deficiency.

Established causes of increased mortality due to vitamin D deficiency are cardiovascular events (McGreevy and Williams, 2011), such as to myocardial dysfunction (Semba et al., 2010), insulin-resistance (Liu et al., 2009), arterial hypertension (Dobnig et al., 2008) and heart failure (Pilz et
Experimental studies have also suggested that vitamin D plays a role in the regulation of several important inflammatory cytokines (such as IL-6 and TNF-alpha) (Nemerovski et al., 2009). This mechanism could explain why vitamin D deficiency has been linked to several types of cancer (prostate, breast and colon cancer) (Chung et al., 2011; Yin et al., 2009) and infection diseases (Lehouck et al., 2012; Yamshchikov et al., 2009).

Recently, the presence of vitamin D receptor and the vitamin D activating enzyme, 1-hydroxylase, in the brain has suggested a potential beneficial role of vitamin D in cognitive function (Miller, 2010). In details, the vitamin D receptor and catalytic enzymes are localized in the areas of the brain involved in complex planning, processing, and the formation of new memories. These findings potentially implicate the role of vitamin D in cognitive impairment (Llewellyn et al., 2010), depression (Milaneschi et al., 2010) and also multiple sclerosis (Soilu-Hänninen et al., 2008).

2.3 Vitamin D and falls in older persons

Falls in older persons are a public-health problem because of the high prevalence (about 30% among subjects aged 65 or older) and because they often result in adverse outcomes (Annweiler et al., 2010). In particular, fall-related fractures are associated with higher morbidity and mortality, and substantial financial cost (Tinetti et al., 2008). In order to delay the occurrence of falls and to reduce its individual and public health impact, effective preventive interventions and strategies need be identified. Data accumulated since the original publication by Chapuy et al. (1992) on the effects of vitamin D supplementation showed, despite several negative results (Grant et al., 2005; Porthouse et al., 2005), a reduction of fall and bone fracture rates (Chung et al., 2011; Gillespie et al., 2009). As a consequence, a vitamin D-calcium supplementation appears to be a low-cost and effective strategy for the reduction of fall and fracture (Bischoff-Ferrari et al., 2003, 2004a, 2009; Chung et al., 2011).

The mechanism by which low vitamin D could negatively influence the muscular-skeletal system producing an increased risk for falling is shown in Figure 2.2. We proposed in older persons a vicious circle for the risk of falling induced by vitamin D, sarcopenia (defined as reduced muscle mass with age in older persons) and altered cortical bone, as the main mediators of the final event represented by osteoporotic fractures (Figure 2.3).

Several studies clearly reported that an impairment of the cortical bone is the main factor responsible of the development of osteoporotic fractures (Dempster et al., 1994; Mayhew et al., 2005; Seeman and Delmas, 2006; Zebaze et al., 2010). There is evidence that most of bone loss occurs after the age of 60 and is cortical, rather than trabecular, at peripheral sites (Seeman and Delmas, 2006). About 50% of cortical bone loss at peripheral sites is the result of remodeling within the cortex adjacent to the marrow (Zebaze et al., 2010).
2. Vitamin D in health of seniors

We previously showed, using data from the InCHIANTI, a representative study of the older persons living in the community, that calcitropic hormones were strong independent correlates of cortical bone characteristics in women, being 25(OH)D positively associated with cortical bone area and volumetric cortical bone density (Figure 2.4) (Lauretani et al., 2006). We found a clear dose-effect in the relationship between cortical bone density and serum level of vitamin D in older women, which is maximal below 30 nmol/l.

Both trabecular and cortical bone density were lower in old age in both sexes; while changes in bone geometry were substantially different in two sexes (Russo et al., 2006). In men, cortical density but not cortical area was lower in old age, with a significant increase in bone size. In women, both cortical bone area and density were lower in old age, but the increase in total bone area was less pronounced than in men (Lauretani et al., 2008). Factors known to be associated with increased bone resorption (such as 25(OH)D) mainly affected volumetric BMD, while others

Figure 2.2. Relationship between vitamin D and muscle mass in older persons.

Figure 2.3. Spirality of falls vitamin D: deficiency induced in older persons considering musculo-skeletal system.
associated with delivering mechanical forces on bone such as muscle mass primarily modulated bone geometry, producing bone fragility and subsequent falls from osteoporotic fractures in non-vertebral sites (Lauretani et al., 2006). Since these physiological factors are potentially modulated by different preventive or pharmacological treatment of osteoporosis, these data may help to predict which bone site can be affected by a particular intervention. For instance, based on our data, we may hypothesize that an increase in muscle mass obtained by means of progressive resistance training will have a greater effect on bone geometry parameters rather than on volumetric BMD. Similarly, vitamin D compounds or teriparatide will be more effective in modulating directly vBMD rather than geometric parameters.

2.3.1 Examining effects of vitamin D on bone

Vitamin D deficiency has both direct and indirect consequences on bone cell function (Holick, 2007). Direct effects mainly concern reduced recruitment and differentiation of osteoclastic progenitors into mature osteoclasts, mostly mediated by osteoblast secretion of activating factors. There is also a diminished synthesis of specific collagenous and noncollagenous proteins in the osteoblasts. The main indirect consequences of vitamin D deficiency on bone cell function are defective mineralization of osteoid seams, due to inadequate intestinal absorption of calcium and phosphate, and an age-related form of compensatory hyperparathyroidism, which drives an accelerated bone loss. We suggested that the minimal 25(OH)D serum concentrations needed to avoid compensatory hyperparathyroidism are significantly higher at older ages (Maggio et al., 2005). In a prospective, observational study designed to analyze risk factors for fracture in an ambulatory population aged >55 years, 73 (88%) had evidence of osteopenia or osteoporosis (T-score <-1.5) and/or low 25(OH)D (Seton et al., 2005). Similar results were observed in patients

Figure 2.4. Relationship between vitamin D and cortical bone density.
enrolled in two Finnish hospitals for fracture during approximately 13 months with hip fracture, fresh of previous events (Nurmi et al., 2005).

Recent data from the Osteoporotic Fractures in Men (MrOS) study performed in healthy older men and focusing on osteoporosis showed that 25(OH)D level <20 ng/ml is associated with greater rates of hip bone loss, while rates of bone loss were similar among men with higher levels of total 25(OH)D (Ensrud et al., 2009). The association between 25(OH)D levels and bone loss was stronger among men 75 years and older (Ensrud et al., 2009). These findings suggest that low 25(OH)D levels are detrimental to BMD in older men.

2.3.2 Examining effects of vitamin D on skeletal muscle

Muscle weakness has long been associated with vitamin D deficiency. A vitamin D receptor is present in skeletal muscle (Simpson et al., 1985), and vitamin D deficiency has been associated with proximal muscle weakness, increase in body sway, and an increased risk of falling (Dam et al., 2009).

Vitamin D deficiency in adults can also cause a skeletal mineralization defect. The non-mineralized osteoid provides little structural support for the periosteal covering. As a result, patients with osteomalacia often complain of isolated or global bone discomfort along with aches and pains in their joints and muscles (Hicks et al., 2008). These patients may be misdiagnosed with fibromyalgia, dysthymia, degenerative joint disease, arthritis, chronic fatigue syndrome, and other diseases (Plotnikoff and Quigley, 2003).

Speed performance and proximal muscle strength were markedly improved when 25(OH)D levels increased from 4 to 16 ng/ml (10 to 40 nmol/l) and continued to improve as the levels increased to more than 40 ng/ml (100 nmol/l) (Bischoff-Ferrari et al., 2004b). Interestingly, persons with low (<25 nmol/l) baseline 25(OH)D levels were 2.57 (95% confidence interval 1.40-4.70, based on grip strength) and 2.14 (0.73-6.33, based on muscle mass) times more likely to experience sarcopenia, compared with those with high (>50 nmol/l) levels (Visser et al., 2003). These results are in accordance with data reported in Figure 2.3. Using data from the InChianti study, we found a direct correlation between the cross-sectional muscle area – which is a proxy of the muscle mass – of the lower leg and the serum levels of vitamin D from 0 to 100 nmol/l. Thereafter, this correlation was lost, probably because of achievement of the plateau effect on the vitamin D on muscle mass.

In a previous cross-sectional analysis conducted at baseline in the InCHIANTI study (Houston et al., 2007; Shardell et al., 2009) low serum 25(OH)D levels was associated with impaired physical performance. Low 25(OH)D may affect physical performance and frailty, defined as ‘a biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems and causing vulnerability to adverse outcomes’, via effects on muscle strength. VDRs are located in skeletal muscle cells, and low 25(OH)D may result in decreased muscle strength from both decreased muscle synthesis and altered contractile
properties of muscle. Muscle protein synthesis is initiated by binding 1,25(OH)_2D to its nuclear receptor. The influence of 1,25(OH)_2D on calcium homeostasis is believed to influence contractile properties of muscle cells via both a VDR-mediated genomic pathway and a non genomic rapid mechanism. Thus, the association between low 25(OH)D and frailty may be explained by associations of insufficient 25(OH)D with sarcopenia and muscle weakness because both exert a central role in the development of the frailty syndrome. In a cross-sectional study, 6 minute walk distance was correlated with higher 25(OH)D level even in patients affected by chronic heart disease (Boxer et al., 2008). In the Longitudinal Aging Study Amsterdam, an association between vitamin D and physical function has been described. Compared with individuals with serum 25(OH)D levels above 30 ng/ml, physical performance was poorer in participants with serum 25(OH)D less than 10 ng/ml [regression coefficient (B) = -1.69; 95% CI = -2.28 to -1.10], and with serum 25(OH)D of 10-20 ng/ml (B = -0.46; 95% CI = -0.90 to -0.03). After adjustment for confounding variables, participants with 25(OH)D less than 10 ng/ml and 25(OH)D between 10 and 20 ng/ml had significantly higher OR for 3-year decline in physical performance (OR=2.21; 95% CI=1.00-4.87; and OR=2.01; 95% CI=1.06-3.81), compared with participants with 25(OH)D of at least 30 ng/ml (Wicherts et al., 2007).

In a meta-analysis recently published (Annweiler et al., 2009), this relationship was further investigated. Of the 102 selected studies, 16 met the selection criteria and were included in the final analysis. There were 8 observational studies and 8 intervention studies. The number of participants ranged from 24 to 33,067. A majority of studies examined community-dwelling older women. Five observational studies showed a significant positive association, whereas three studies did not. Four of the 5 studies and two of the 3 studies which tested the vitamin D supplementation effect, respectively on balance and gait, showed no significant effect. Four studies showed a significant effect on muscle strength, while this effect was not observed in three other studies. In addition, there was no significant association between vitamin D supplementation and an improvement of the sit-to-stand test in 50% of the studies. Authors concluded that, the association between vitamin D and physical performance remains controversial. Observational studies and clinical trials yielded divergent results, which highlights the complex and to date still poorly understood association between serum vitamin D concentration or vitamin D supplementation and physical performance (Annweiler et al., 2009).

### 2.4 Options for vitamin D therapy in older persons

The treatment of choice for vitamin D deficiency is vitamin D, cholecalciferol, also known as vitamin D3. Cholecalciferol is available in 400, 1000, 2,000, 5,000, 10,000, 50,000 and even 300,000 UI capsules. Supplementation with 1000 IU per day will usually result in about a 10 ng/ml elevation of serum 25(OH)D when given over 3-4 months. Formulation of vitamin D3 of 300,000 IU is also present as intramuscular options, and given 300,000 IU annually corresponds to about 800 IU daily. A recent double-blind, placebo-controlled trial of 2,256 community-dwelling women, aged 70 years or older, considered to be at high risk of fracture were randomly assigned to receive cholecalciferol (a single annual dose of 500,000 IU of cholecalciferol administered orally)
or placebo each autumn to winter for 3 to 5 years. Contrary to their hypothesis, they found that participants receiving annual high-dose oral cholecalciferol experienced 15% more falls and 26% more fractures than the placebo group (Sanders et al., 2010). Therefore, supplementation with 1,500-2,000 (Pramyothin and Holick, 2012; Wimalawansa, 2012) IU per day still remains the treatment of choice for vitamin D supplementation. Specific conditions are:

- Primary and secondary prevention for bone loss – cholecalciferol 1000 IU/day plus calcium 1 g/day.
- Inflammatory Bowel Diseases (IBD) – cholecalciferol 1000 IU/day plus calcium 1 g/day.
- Liver diseases – calcifediol (25(OH)D3) or 1-alpha (OH) calcidiol.
- Kidney diseases – calcitriol 0.5 μg/day plus calcium 1 g/day.

In conclusion, vitamin D is a hormone with several actions, and is fundamental for many biological systems including cortical bone and skeletal muscle.

Recent studies suggest that assessment of Vitamin D status should be recommended not only for prevention and treatment of osteoporosis but also included in the global evaluation of risk of falling, sarcopenia, neurological diseases, cardiovascular disease and cancer especially in older populations.

We should always consider that low vitamin D observed during chronic disease (such as diabetes and heart failure) could be a surrogate marker rather than be a causal mechanism. In addition because it is a hormone, vitamin D replacement in older people should be carefully monitored for avoiding side-effects of the supplementation such as nephrolithiasis.

References


2. Vitamin D in health of seniors


2. Vitamin D in health of seniors


Key facts

- A scientist received the Nobel Prize for isolating vitamin D and used cod liver oil to cure dogs with rickets.
- Vitamin D deficiency is a common condition but frequently seen in northern latitude countries.
- Sun exposure is the best way to increase vitamin D levels in the body.
- Vitamin D supplementation can improve vitamin D status drastically.
- A healthy vitamin D status can provide protection against many serious diseases such as rickets, osteomalacia, cancer and osteoporosis.

Summary points

- Vitamin D is an important micronutrient to healthy outcomes.
- Supplementation is necessary among vulnerable populations that may lack exposure to sunlight or possess risk factors.
- Dietary intake may be inadequate to maintain optimal vitamin D concentrations in the body.
- Several studies demonstrate increased supplementation leads to a healthy vitamin D status.
- Policies should incorporate a multi-strategic approach to supplementation at local and national levels.
3. Vitamin D supplementation and public health policy

R. Babaria and R.R. Watson
Mel and Enid Zuckerman College of Public Health, P.O. Box 245155, 1295 N. Martin, Tucson, AZ 85724-5155, USA; radhikababaria@gmail.com

Abstract

Vitamin D deficiency is a common condition among people throughout the world. It affects any age, gender, and ethnicity. However, certain individuals are at a greater risk due to a genetic predisposition or lifestyle. Sun exposure is the greatest source of vitamin D synthesis in the body. Often times, individuals may lack adequate exposure due to internal and external factors. Dietary food intake often is not sufficient to meet an adequate vitamin D status. Public health policies should target supplementation among vulnerable populations in order to decrease the prevalence of vitamin D deficiency. Successful public health strategies collaborate and network with various organizations dedicated to improving vitamin D status and incorporate a multi-strategic approach at both local and national levels.

Keywords: fortification, strategies, vulnerable populations, sunlight exposure
3.1 Introduction

The significance of vitamin D in human health is evident since the early 20th century. Scientific researcher Elmer McCollum was the first to isolate vitamin D. McCollum received a Noble Prize for an experiment that used modified cod liver oil to cure dogs with rickets (Mohr, 2009). Scientists later discovered that the human body could synthesize vitamin D through UVB light exposure, giving the micronutrient the nickname, the ‘sunshine vitamin.’ In the 1930s, rickets was a major public health concern in the United States (ODS, 2011). A milk fortification program was a key method implemented to combat rickets and prevent a low vitamin D status.

According to the Center for Disease Control and Prevention, vitamin D deficiency is defined as a condition that causes ‘inadequate mineralization or demineralization of the skeleton (CDC, 2008).’ It is associated with rickets and osteomalacia in children and adults, respectively (Misra et al., 2008). Vitamin D acts as a biological hormone within the body. By maintaining sufficient calcium and phosphate levels, adequate vitamin D concentrations promote bone and muscle health (ODS, 2011). Several studies associate vitamin D insufficiency to poor immune, respiratory, cardiovascular, cell proliferation, and metabolic functions; however, further research is necessary (Schwalfenberg and Genuis, 2010).

Determining a standard method to define and measure vitamin D status can improve the variability in data seen across countries. The best indicator to determine vitamin D status is serum 25(OH)D concentration (ODS, 2010). Although vitamin D deficiency is well-documented worldwide and numerous reports indicate the benefits from supplementation, there is a lack of supplementation policies and programs preventing and treating the health consequences due to this insufficiency.

3.2 Epidemiology

Vitamin D deficiency is common in Europe but varies greatly among European countries (Lips, 2007). Other regions with a high prevalence include the United States, Middle East, India, China and Japan (Holick and Chen, 2008). Vitamin D deficiency has no boundaries, affecting any age, gender, and ethnicity; however, certain populations are affected disproportionally. Children, elderly, and pregnant women are vulnerable subgroups for vitamin D deficiency. The prevalence
3. Vitamin D supplementation and public health policy

of vitamin D deficiency in the USA is especially common among minority groups. Data from the National Health and Nutrition Examination Survey conducted between 2005 and 2006 defined vitamin D deficiency as a serum 25(OH)D concentrations less than or equal to 20 ng/ml (50 nmol/l) (Forrest and Stuhldreher, 2011). The study found an overall 41.6% prevalence of vitamin D deficiency among the study participants (Forrest and Stuhldreher, 2011). Among the participants, 82.1% of the African Americans and 69.2% of the Hispanic were deficient (Forrest and Stuhldreher, 2011). Other risk factors for a poor vitamin D status include a lack of a college education, obesity, poor health status, and no daily milk consumption (Forrest and Stuhldreher, 2011). Skin pigmentation, gender, nutrition, lack of supplementation, and clothing style are also genetic and lifestyle risk factors (Lips, 2007).

3.3 Sources of vitamin D

Several sources of vitamin D include sun exposure, natural diet foods, fortified foods, and supplements (WHO, 2012). The best way to obtain vitamin D is sun exposure. Sun exposure enables UVB rays to reach exposed skin to produce vitamin D3. Factors that affect synthesis include the season, time of day, duration of exposure, pollution, skin pigmentation, and sunscreen use. Researchers recommend sun exposure of at least 15 to 30 minutes, twice a week (ODS, 2011). Example actions include increasing the amount of time exposed to sunlight or the type of clothing worn. Often times in countries that have a predominant religious association such as the Middle East, women are unable to alter clothing form. It becomes more crucial for these vulnerable populations to seek vitamin D through diet and supplementation. Individuals who have limited activity in sunlight should rely on diet and supplementation to benefit from optimal vitamin D effects.

Vitamin D, a fat-soluble micronutrient, is obtained from a limited number of naturally occurring foods. It is primarily found abundantly in fatty fish and eggs. Children are able to obtain vitamin D from fortified sources such as cereals, cheese, and milk. However, a vulnerable population includes darker skinned individuals lacking sufficient UVB exposure. Among this population, an emphasis on supplementation to meet adequate levels is crucial. Fortified foods are often inadequate to meet vitamin D requirements. In adults, diet alone constitutes only 10-20% of vitamin D concentrations (WHO, 2011). However, in the USA, fortified foods provide the most diet obtained vitamin D. In Canada, it is a law to fortify milk and margarine with vitamin D at 35 to 40 IU/100 ml and at or greater than 530 IU/100 g, respectively (ODS, 2011). It is also a law to fortify infant formula in the USA and Canada. The USA requires 40 to 100 IU/100 kcal vitamin D while Canada requires 40-80 IU/100 kcal (ODS, 2011). Taking into consideration a risk of inadequate sun exposure, children and adults may need at least 800-1000 IU vitamin D to maximize health benefits (Holick and Chen, 2008).
3.4 Public health strategies to maintain an adequate vitamin D status

Public health policies and strategies should target populations at risk for a compromised vitamin D status. Planned regular sun exposure is one of the best ways to synthesize vitamin D in the body but for public health policy purposes, the focus is on natural food sources, fortification, and supplementation.

3.4.1 Natural food sources

Very few naturally occurring foods are rich in vitamin D. Typically, animal foods contain cholecalciferol, while plant foods contain ergocalciferol. One study demonstrates that consuming fatty fish frequently is effective in maintaining serum 25(OH)D levels in elderly Japanese women (Nakamura et al., 2000). Study participants who consumed fish more than four times a week had greater serum 25(OH)D levels than those women who ate fish less frequently or not at all. In addition, adequate serum 25(OH)D levels were maintained in the women who consumed fish more frequently during the winter. Liver meat is another source of abundant vitamin D. For vegetarians or vegans, all edible mushrooms have some content of vitamin D2 and ergosterol, which becomes activated with UVB exposure. Shitake mushrooms can have up to 25 μg/g vitamin D2 content (Nakamura et al., 2000). Other mushroom types vary in vitamin D levels and optimal benefit is also contingent upon sunlight exposure.

One of the barriers documented in some studies is whether or not obtaining vitamin D through natural food sources is sufficient for an adequate vitamin D status. Recommendations for vitamin D vary between countries if provided at all. Estimating the amount of vitamin D intake is difficult because vitamin D content is not always accessible on food labels (Lamberg-Allardt, 2006). In Canada, nutritional content for vitamin D is generally available. Reporting vitamin D content on food products is one public health strategy that can facilitate public awareness towards conscious vitamin D intake.

3.4.2 Fortification

Fortification is one of the best methods to reach the general populations for vitamin D intake. It is a cost effective and feasible option for many foods in comparison to the treatment of problems that arise from vitamin D deficiency (Calvo and Whiting, 2006). One challenge in fortifying foods with vitamin D is that it may not be effective in meeting recommended levels for vitamin D intake. In the USA and Canada, fortifying milk levels are too low to increase or maintain serum 25(OH)D levels. A higher fortification as seen in powdered milk proved to be more effective in bone mineralization in women (Calvo and Whiting, 2006). Another limitation in fortification is that policies fortify select staple foods that may not reach all vulnerable risk groups or exclude individuals with specific dietary restrictions. A possible strategy to overcome this barrier is to increase the number and type of foods fortified, taking into consideration cultural preferences and accessibility.
3. Vitamin D supplementation and public health policy

3.4.3 Supplementation

When dietary intake of vitamin D is restricted or otherwise unavailable, supplementation is another option to help improve vitamin D status. Supplementation for vulnerable populations as a preventative strategy is more cost effective than screening for vitamin D status among all individuals. A challenge in supplementation strategies is compliance. In a European study assessing the vitamin D intakes in Denmark, Finland, Ireland, and Poland, use of supplements varied by country and age. Generally, only 38% of elderly women used supplements (Lamberg-Allardt, 2006). Of this, Denmark had the highest use while Poland had the lowest. In comparison, only 19% of young girls used supplements with Denmark again showing the highest use and Poland the least (Lamberg-Allardt, 2006). Similar variations are seen among racial and ethnic groups in the USA and Canada according to the National Health and Nutrition Examination Survey.

When comparing African American and Caucasian women taking similar intake guidelines for vitamin D supplements, African Americans illustrated a greater prevalence of vitamin D deficiency. Markedly different vitamin D statuses among racial groups suggest a need to reflect racial and ethnic backgrounds in dietary guidelines. Another limitation on an individual basis is financial barriers in obtaining supplements. The best strategy would be to create evidence based supplementation programs for vulnerable populations in a community.

Data for vitamin D recommendations for infants, pregnant women, and adolescents is difficult to assess due to the lack of sufficient information (Cashman and Kiely, 2011). In Southeast China, a study conducted in the Hangzhou Zhejiang province demonstrated a very high prevalence of vitamin D deficiency among 1 month to 16 year olds (Zhu et al., 2012). The study results recommend supplementing children through adolescence. The physiological mechanism of vitamin D during pregnancy and early infant development is not well known. Controversial findings of fetal skeletal development and adverse health outcomes such as low birth weight in infants and pre-eclampsia in mothers signify the need for further research in maternal vitamin D during pregnancy (Cashman and Kiely, 2011). In a systematic review, six randomized control trials examined vitamin D effects during pregnancy. Five trials tested vitamin D supplementation versus no supplementation or a placebo. Another trial compared vitamin D supplementation with calcium versus no supplementation. All trials demonstrated that supplementation increased serum 25(OH)D levels during pregnancy (De-Regil et al., 2012). However, since there is no evidence of clinical benefits in preventing pregnancy or infant outcomes, WHO does not recommend vitamin D supplementation to prevent pre-eclampsia during pregnancy (WHO, 2011). Varying dosages, undetermined effects, and unknown safety of supplementation during pregnancy are a few examples that illustrate the need for guidance. With future research findings, evidence based public health strategies can be potentially made to establish recommended vitamin D supplementation levels for pregnant women (Cashman and Kiely, 2011).
3.5 Recommended supplementation

There are two physiologically important forms of vitamin D for supplementation: D2 and D3. These forms differ in the chemical side chain structures and both are used as nonprescription supplementation sources. In the USA, vitamin D2 is available through prescription (CDC, 2008). Vitamin D2, also known as ergocalciferol, is engineered by UV irradiation of ergosterols usually found in molds, yeasts, and higher order plants (CDC, 2008). Vitamin D3, also known as cholecalciferol, is synthesized by the UVB radiation of 7-dehydrocholesterol on the human skin (ODS, 2011). The National Institutes of Health compares the two forms of vitamin D supplementation and although firm conclusions about the different effects cannot be drawn, both forms increase serum 25(OH)D concentration (ODS, 2011). Ergocalciferol and cholecalciferol are biologically inert forms and both have similar mechanisms for metabolism in the human body (CDC, 2008). Vitamin D supplementation is often times mixed with calcium because vitamin D helps absorb calcium in the gut and maintain serum calcium and phosphate levels for bone mineralization (ODS, 2011).

In the USA, the IOM recommends dietary allowance of 600 IU per day for 1 to 70 years of age and over 20 IU per day for individuals older than 71 years of age (IOM, 2010). Pregnant and lactating women are recommended to take 600 IU of vitamin D per day (IOM, 2010). Adequate intake for infants is 400 IU per day (IOM, 2010). The tolerable upper intake level refers to the highest safe amount of vitamin D an individual can consume. The upper level intake for infants less than 6 months is 1000 IU per day and 1,500 IU per day for infants 6 months to 12 months of age (IOM, 2010). Children 1 to 3 years of age have an upper intake limit of 2,500 IU per day and children 4 to 8 years of age can have 3,000 IU per day (IOM, 2010). Individuals 9 to older than 71 years of age have an upper intake level of 4,000 IU per day along with pregnant and lactating women (IOM, 2010). Recommended dietary allowances for Canada are similar to that of the USA.

Based upon literature reviews, each institute in various countries has different recommendations for vitamin D supplementation and different definitions for vitamin D inadequacy. It is important to note that dietary intake of vitamin D can also vary among countries and individuals. For instance, Lamberg-Allardt (2006) discusses various studies to demonstrate that current dietary intake recommendations are too low to reach and maintain optimal serum 25(OH)D concentrations when sunlight exposure is absent. Supplementation results in increased serum 25(OH)D concentrations as exemplified in a study that involved two adolescent groups. One group was given a daily dose of 5 mg of vitamin D3 while the other group was given 10 mg for a total duration of 1 year (Lips, 2007). Comparing the two groups, the adolescents receiving 10 mg of vitamin D3 had greater vitamin D concentrations and maintained adequate levels from autumn to spring (Lips, 2007).

In Europe, the European Food Safety Authority adopted the upper intake values for vitamin D as recommended by the IOM. Representative data on vitamin D intake exists only for a limited number of European countries; however, the method for data collection using surveys and instruments is inconsistent and unreliable to make comparisons (Cashman and Kiely, 2011).
3. Vitamin D supplementation and public health policy

One of the challenges the DRI committee encountered in the 2010 review was assessing the dose-response relationship for vitamin D. It was a challenge to accurately determine sunlight exposure, and there was limited information on vitamin D status. Therefore, a key step in addressing vitamin D deficiency is quantifying the data to make comparisons among DRIs and vitamin D status in the population and addressing the gaps.

3.6 Supplemental toxicity

Vitamin D supplementation discussions usually bring a concern for toxicity. The upper intake limit for serum 25(OH)D concentrations is not established for various groups (Veith, 2007). Symptoms of toxicity include those of hypercalcemia, lethargy, dehydration, and nausea. One study states that sunlight can provide up to 10,000 IU per day if converted to the oral consumption equivalent (Veith, 2007). In comparison to the DRIs in the United States, this is a very large dose. Clinical trials conducted illustrate that current DRIs are very conservative and a prolonged intake of a higher dose such as 10,000 IU per day poses no adverse effect on health (Veith, 2007). Similar results are demonstrated in a study done by Hathcock et al. (2007). Although these intake levels seem alarming, the main purpose of presenting the finding is to exemplify the need for an established and uniform upper intake level to support supplementation. Overall, there is a low toxicity risk for supplemental use based on the current DRIs and fortification (Calvo and Whiting, 2006).

3.7 Global supplementation studies

3.7.1 North America

One possible method to target a vulnerable subgroup, such as the elderly, is to focus on controlled environments. An example of such an environment is institutional infrastructures. Now more than before, an increased number of seniors are utilizing nursing home facilities. A nursing home population is one of the groups susceptible to vitamin D insufficiency. Factors that lead this high risk of vitamin D deficiency include limited access to sunlight due to mobility issues, decreased vitamin D production with age. Individuals living at higher latitudes also have difficulty producing vitamin D skin synthesis due to weak UVB intensity. In a geriatric study, 68 nursing home patients were given 2,000 IU of daily vitamin D3 supplementation (Schwalfenberg and Genuis, 2010). Each patient involved in the retrospective chart review continued supplementation for at least five months. Approximately 94.1% of the patients improved their vitamin 25(OH)D status and none of the study residents reached toxicity (Schwalfenberg and Genuis, 2010). The study makes a strong recommendation to routinely supplement all nursing home residents daily unless otherwise noted for other health concerns. With this effort, vitamin D status and quality of life can be improved in nursing home patients and morbidity and mortality can be prevented (Schwalfenberg and Genuis, 2010).
The vitamin D initiative in the United States became more significant due to recent advances in vitamin D research over the past decade. The Office of Dietary Supplements at the National Institutes of Health coordinates the initiative. Key stakeholders and partners of the initiative include the US Department of Health and Human Services such as the Center for Disease Control and Prevention, National Institutes of Health, Food and Drug Administration, Agency for Healthcare Research and Quality, National Institute of Standards and Technology, US Department of Defense, US Department of Agriculture, and Health Canada (ODS, 2010). The Vitamin D Standardization Program began in 2010 as part of this initiative. The Office of Dietary Supplements at the National Institutes of Health aims to provide a standardized method of measuring vitamin D in foods and supplements and vitamin D status in the USA population. With standardized measurements, international efforts can expand to public health activities worldwide. The program includes national health survey participation from Australia, Canada, Germany, Ireland, Mexico, South Korea, the United Kingdom, and the USA. The representative method of data collection can provide a consistent manner for evaluation and comparison to inform public policy, research efforts, and guidelines (ODS, 2010).

3.7.2 Europe

In the European region, PA International is a key agency that focuses on health promotion and protection. More specifically, board members address vitamin D deficiency by collaborating with other organizations with missions that promote vitamin D health among populations (PA International, 2012). The stakeholders include Shine on Scotland, the Irish Osteoporosis Society, the Dutch Fall Prevention Network, and the German Osteoporosis Patient Society, and the Health Research Forum. According to PA International, one of the initial steps in addressing this issue is to collaborate between doctors, scientists, patients and representatives of politics, industry and the media. Further steps include developing policy briefs and increasing awareness of vitamin D deficiency as a public health concern. In 2010, WHO/Europe and PA International convened to address the vitamin D deficiency in Europe. WHO/Europe planned on developing an integrated strategy based on the Action Plan for Food and Nutrition Policy while PA International focused on fundraising opportunities. Through international collaboration and coordination of local stakeholders, vitamin D deficiency can be addressed more effectively (PA International, 2012).

A Finland study exemplifies how public policy to fortify specific foods with vitamin D can increase the levels of vitamin D status (Laaksi et al., 2006). During the wintertime, vitamin D deficiency increases substantially among people in northern countries in response to decreased sun exposure; therefore, along with supplementation, fortification of vitamin D is another method to help increase vitamin D concentrations among populations. Finland exemplifies a successful fortification national public health policy based on the Ministry of Social Affairs and Health's recommendation. The policy targets fortifying liquid milk products, margarines, and butter with vitamin D across the country. Vitamin D status is determined using serum 25(OH)D as a marker in Finnish men aged 18 to 28 years (Laaksi et al., 2006). Data on the vitamin D status of these men was collected in January 2003 and after the implementation of the policy in January 2004 (Laaksi et al., 2006). Mean serum 25(OH)D concentrations increased by 50% after implementation.
3. Vitamin D supplementation and public health policy

Ireland demonstrates how public health policy on vitamin D supplementation can target infants. The Food Safety Authority of Ireland recommends a universal vitamin D supplementation for all infants from birth to 1 year of age. The Department of Health and Children validated this evidence-based recommendation and has fulfilled responsibility to the Health Service Executive to implement the policy. More specifically, the policy states, ‘…all infants, from birth to 12 months, whether breastfed or formula fed, be given a daily supplement of 5 μg (200 IU) vitamin D. This should be provided by a supplement containing vitamin D exclusively (HSE, 2010).’ According to the policy brief, 200 IU of vitamin D daily is sufficient to maintain serum 25(OH)D levels greater than 27.5 nmol/l (HSE, 2010). It is the responsibility of health professionals, community and hospital dietitians, practitioners, pharmacists, and other health care staff to inform parents about daily supplementation. It is important to note that the public health policy generalizes the population and parents of infants with specific medical conditions should seek medical advice from a physician. Between October to March, sunlight exposure is inadequate in the northern latitude of Ireland (HSE, 2010). Infants and young children are not recommended to seek direct UVB exposure and the infant diet is limited in vitamin D (HSE, 2010). These factors along with incidents of rickets in Ireland helped endorse the universal supplementation policy. The universal supplementation of 200 IU does not pose an issue for toxicity even if an infant is fed formula fortified with vitamin D (HSE, 2010). The public health implications of this policy include suggesting a cost-effective strategy to prevent vitamin D deficiency in infants by promoting collaboration at local, regional and national levels in Ireland.

3.7.3 Asia

Vitamin D deficiency is highly prevalent among the people residing in India even though sunlight exposure is available all year long. The urbanization of India, the darker pigmented skin, low use of supplements, and a lack of a national fortification of staple foods are several factors that lead to this insufficiency. An increased number of infants and children have bone deformities and rickets; however, the country has not prioritized vitamin D supplementation or fortification. Many inhabitants of India are vegetarian, limiting the dietary intake of vitamin D found in fish and meats. Due to a low use of supplements and a cultural diet that limits vitamin D intake, it is important to target food fortification with vitamin D as an alternative method.

One very common staple food among many Indians, vegetarians and non-vegetarians, is the whole-wheat ‘chapatti’ or bread. Fortifying the flour used to make this bread can help increase daily intake of vitamin D. Although in the United States and Canada milk is required to be vitamin D fortified, in India, many families, especially rural residents do not have access to fortified milk or facilities to store pasteurized milk (Babu and Calvo, 2010). Jordan is another country that was in a similar situation as India. Both countries receive sunlight all throughout the year. However,
in 2009, 87% Jordanians aged 18 to 70 had serum 25(OH)D levels less than 50 nmol/l (Babu and Calvo, 2010). Fortunately, the government of Jordan implemented a fortification policy for bread to help improve the vitamin D status. Urban populations had significantly lower serum 25(OH)D levels in comparison to rural populations (Babu and Calvo, 2010). It is important to note that there is no official recommendation for sufficient dietary intake for any age, gender, or factors such as pregnancy (Babu and Calvo, 2010). In order to provide national policies on supplementation, India first needs to implement a nationwide program that enables standard measurements of serum 25(OH)D. With these guidelines, pregnant women and infants can be targeted groups to prevent and treat vitamin D deficiency.

3.8 Barriers to supplementation

There are several challenges to incorporating vitamin D supplementation into public health policies. Each individual has a unique requirement for adequate vitamin D intake due to various exposure, genetics, and diet. The controversy of the recommended DRI is largely due to the gap in vitamin D research (Cashman and Kiely, 2011). However, recent advances in vitamin D knowledge enabled the IOM to propose estimated average requirements. More specifically, the evidence based proposal suggests a ‘40 nmol/l median value of a serum 25(OH)D level in which about 50% of the population might meet this requirement and a value of 50 nmol/l of a serum 25(OH)D level in which nearly all of the normal healthy population can meet this requirement (Cashman and Kiely, 2011).’

The IOM establishes the DRI values and the most recent review of vitamin D in 2010 utilized a risk assessment strategy. These steps include hazard identification, hazard characterization, intake assessment, and risk characterization. Once a report is completed, agencies can implement public health policies and educational programs to address the gaps identified in the risk assessment. One of the concerns for establishing guidelines for supplementation is the risk of consuming too little vitamin D levels resulting in no health benefits or consuming an increased level that would be toxic and result in adverse effects. Cashman and Kiely (2011) presents that utilizing the risk assessment approach as exemplified by the IOM has the potential to promote a national policy for vitamin D and improving international collaboration.

3.9 Conclusion

Vitamin D deficiency is a public health issue that has become pandemic to many regions of the world. As research advances on vitamin D and health, gaps are continually addressed and further research will help guide public health aims to eliminate the prevalence of vitamin D deficiency. A multi-strategy approach involving education, fortification, and supplementation is best to address the vitamin D problem. Education is key to informing the public of how to prevent vitamin D deficiency.
3. Vitamin D supplementation and public health policy

From a public health standpoint, fortifying foods common to a region or a whole population is a good way to increase vitamin D sources. Naturally occurring vitamin D in foods may not be consumed every day or there may be dietary cultural restrictions. Infants who exclusively breastfeed may need additional supplementation but most formulas are vitamin D fortified in the USA.

At an international level, once data on vitamin D deficiency prevalence in a community is quantified, health agencies or public health officials should recruit stakeholders to help obtain funding and disseminating knowledge about vitamin D insufficiency. A community-based approach in addressing this problem can be more sustainable in comparison to individual efforts. Economical and financial barriers can limit obtaining fortified or supplements to improve vitamin D deficiency in low and middle-income countries. With additional public health activities supported through funding, a larger population can be reached.

Public health programs that focus on supplementation can be either community based or clinical programs. Both need to incorporate cultural sensitivity, feasibility, and affordability. Individual compliance varies in attending clinics or taking supplements and therefore an educational component can increase support for supplementation through awareness. Overall, sustainability increases when people know the benefits of supplementation. Due to the toxicity concerns with vitamin D, it may be beneficial to channel supplementation programs through local clinics where supplementation can be based on blood tests. Recommended dosage levels and clinical advice can be personalized to an individual based upon medical history as well. In conclusion, supplementation requirements vary on an individual basis and therefore, individuals should seek the advice of a medical professional.

References


Vitamin D and musculoskeletal health
**Key facts**

- Falls are the most common cause of accidental mortality and morbidity in older adults.
- Vitamin D insufficiency is common in older adults – among other adverse consequences this insufficiency can be associated with weakness and an increased risk of falls.

**Summary points**

- Among those at a high risk of falling (e.g. prior fall within the last year), a dose of 800-2,000 IU of vitamin D per day appears to be a safe economical intervention that can reduce their risk of future falls by up to 34%, particularly if they are deficient prior to treatment.
- Although not well understood, vitamin D most likely mediates its effect on falls through multiple pathways, including those involving skeletal muscle cells and neurotransmitter activity.
- The estimated effective serum 25-hydroxyvitamin D threshold to prevent falls is 60 nmol/l, to improve muscle strength and balance is 75 nmol/l, and possibly even higher to prevent fractures.
4. Vitamin D and fall risk

J. Basran¹, R.L. Duckham² and D.B. Hogan³
¹Division of Geriatric Medicine, Department of Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B2, Canada; ²College of Kinesiology, University of Saskatchewan, 87 Campus Drive, Saskatoon, SK S7N 5B2, Canada; ³Division of Geriatric Medicine, Department of Medicine, Faculty of Medicine, University of Calgary, HSC-3330 Hospital Dr. NW, Calgary, Alberta T2N 4N1, Canada; jenny.basran@saskatoonhealthregion.ca

Abstract

Falls are a major health care concern, particularly in the growing older adult population. Falls not only contribute to escalating health care costs, but more importantly are associated with detriments to an individual’s quality of life, as well as morbidity and mortality. The association between vitamin D deficiency and muscle weakness and/or worsening balance likely underlies the link between low serum 25-hydroxyvitamin D (25(OH)D) levels and increased rates of falls. Although the mechanisms by which vitamin D affects the likelihood of falling are not fully understood, adequate levels of this micronutrient enhance skeletal muscle strength and improve postural stability. Vitamin D3 at daily doses between 800-2,000 IU will elevate serum 25(OH)D levels to at least 60 nmol/l, a level (where a fall reduction effect has been demonstrated) in most older persons. Although still debated, we feel there is convincing evidence that vitamin D can reduce falls by between 13-34%. Vitamin D3 is a safe, economical, easily administered and well tolerated intervention that should be added to the customized fall prevention plan of high risk fallers.

Keywords: 25-hydroxyvitamin D, cholecalciferol, ergocalciferol, fall related injury, vitamin D receptors
4.1 Introduction

Falls are the most common cause of accidental injury and death among older adults (Kalyani et al., 2010). While the definition of a fall seems self-evident, for clinical and research purposes a standardized definition is required to ensure accuracy and consistency. The Prevention of Falls Network, an international working group on falls, defines a fall as ‘an unexpected event in which the participant comes to rest on the ground, floor or lower level’ (Lamb et al., 2005). Despite this definition, there remains uncertainty about whether to include events arising from a loss of consciousness or unusual external forces (e.g. strong push or shove) when counting falls.

As society ages, the number of fall-related hospitalizations is rapidly rising within the more economically developed nations of the world. In the United States, fall-related hospitalizations have increased from 373,128 to 559,355 between 2001 and 2008 (Hartholt et al., 2011) while in the Netherlands, fall-related hospitalizations rose 137% between 1981 and 2008 among those 65 years of age and older (Hartholt et al., 2010). Approximately one in three community dwelling adults aged sixty-five years and older (65+) experience at least one fall every year, a rate that increases to one in two adults over the age of 80 (Tinetti et al., 1988). Between a quarter and a half of these older fallers will have multiple falls within that year (Souberbielle et al., 2010), with up to 30% seeking medical attention as a result of a fall (Bischoff-Ferrari et al., 2004; Peel, 2011).

In 2000, the estimated direct medical costs of falls among older adults in the US was over $19 billion dollars (or $25.6 billion dollars in 2012) (Stevens et al., 2006). The most costly component (approximately $12 billion dollars) was for hospitalization costs, with expenditures for women 2-3 times higher than for men. Falls are the presenting complaint for between 18-40% of all emergency visits by older adults and account for over 80% of all injury related hospitalizations in this age group (Peel, 2011; Scott et al., 2010). Fractures are both the most frequent and most expensive type of non-fatal injury, although head injuries are also a concern. Although thoracic spine and radius fractures frequently occur, falls account for 90% of all hip fractures (Zuckerman, 1996). The latter fracture is particularly concerning, given the high occurrence of
4. Vitamin D and fall risk

permanent disability and the one-year 20% mortality rate associated with falls resulting in hip fractures (Zuckerman, 1996).

A recent Canadian report found that once hospitalized for a fall-related injury, an individual had an average acute care length of stay 70% longer than compared to the average length of stay for all other hospitalizations (Scott et al., 2010). In addition, older adults in acute care facilities also have high rates of falls – up to 20 falls per 1000 bed-days (Haines and Waldron, 2011; Oliver et al., 2004). Accidental inpatient falls are the most commonly reported safety incident (approximately 30%) with injuries occurring in almost of third (Oliver et al., 2008; Peel, 2011).

The occurrence of falls is associated with an increased risk of institutionalization. Compared with similarly aged persons who have not fallen, those with a single non-injurious fall will have nearly a five-fold higher risk of admission to a long term care facility within three years. Among older persons with a fall causing serious injury the relative risk of admission is approximately twenty times higher (Tinetti and Williams, 1997). In an American study, the population attributable risk of a permanent admission to a long-term facility due to falls related causes was approximately 26% (Tinetti et al., 1988). In other words, about a quarter of all permanent long term care admissions could be attributed to a fall. Not surprisingly falls are also frequent among the generally disabled group of seniors found in these facilities, where the incidence of falls is 1.5 falls per bed per year (Peel, 2011; Rubenstein et al., 1994).

In addition to an injury, a fall may negatively impact an individual’s quality of life by causing a fear of falling. This often triggers a vicious cycle where the fear initiates a restriction in activity leading to decreased mobility and social isolation causing a further increase in their risk of falling. The mortality, morbidity, disability, pain, other detrimental effects on health-related quality of life, and the growing economic burden of falls and related injuries underscore the need for effective interventions for fall prevention (Rosen, 2011a).

It has long been recognized that the more fall risk factors present, the greater the likelihood of falling (Tinetti et al., 1988) and that most falls in older adults result from an inter-play of predisposing and precipitating factors (Berry and Miller, 2008). Predisposing factors include increasing age, female sex, age-associated changes in strength and balance, sensory impairments (e.g. poor vision, polyneuropathies, vestibulopathies), and chronic diseases (e.g. lower extremity arthritis and various neurological conditions like dementia, cerebrovascular disease, and extrapyramidal disorders). Falls are then precipitated in at risk individuals by such factors as: acute illnesses (e.g. delirium, sleep disturbances with fatigue, dehydration with orthostatic hypotension, or weakness), medications (e.g. psychotropics), lower urinary tract symptoms, orthostatic hypotension and weakness. This risk is further exacerbated by inappropriate footwear and environmental factors such as a wet floor and poor lighting, or being placed in a new and confusing environment (e.g. a hospital or a long term care facility). The current recommended approach to fall prevention focuses on searching for modifiable risk factors coupled with tailored interventions to deal with those factors identified. These interventions can include one or usually a combination of: patient education, adaptation or modification of home environment, withdrawal...
or minimization of psychoactive and other medications, management of postural hypotension, management of foot problems and footwear, and exercise – particularly balance, strength, and gait training (Panel on Prevention of Falls in Older Persons and British Geriatrics, 2011).

Increasingly vitamin D supplementation has become recognized as an addition to the list of fall reduction interventions. It is particularly attractive due to its low cost and ease of implementation. In the rest of this chapter we will review the data linking vitamin D deficiency with fall risk and examine the evidence of benefit with supplementation.

4.2 Vitamin D and falls

The serum concentration of 25(OH)D is felt to be the ‘best’ single indicator of nutritional and functional status of vitamin D (Rosen, 2011b). Low levels of 25(OH)D encompasses both ‘deficiency’ (serum 25(OH)D levels <25 nmol/l) and ‘insufficiency’ (serum 25(OH)D levels 25-75 nmol/l) states (Hanley et al., 2010). Risk factors for insufficiency or deficiency states include female sex, dark skin pigmentation, avoidance of ultraviolet radiation, diet low in food sources of vitamin D or malabsorption (though most circulating vitamin D is derived from sunlight exposure), obesity, and impaired renal function. Older adults are particularly prone to low levels of vitamin D and have diminished responsiveness of tissue targets of vitamin D due to reduced receptor numbers. Between the ages of 20 and 70 the ability to synthesize vitamin D in our skin declines by 75% (Holick, 2011). This is compounded by reduced exposure to sunlight in many older adults, particularly those in institutional care (Kalyani et al., 2010; Menant et al., 2012). With age, gastrointestinal absorption of vitamin D is also reduced, along with a decline in the metabolism to active forms of vitamin D by the kidneys and peripheral tissues (Muir and Montero-Odasso, 2011).

In the northern hemisphere, people who live above the 35° N latitude will have little or no vitamin D synthesis in their skin from November to February (Holick, 2004). Low levels of vitamin D are found in a third or more of adults in Canada and among those living in the northern half of the USA (Hanley and Davison, 2005; Liu et al., 1997; Rucker et al., 2002). Interestingly, low vitamin D levels are also common among those who fall and have been found to be an independent predictor of incident falls (Flicker et al., 2003; Stein et al., 1999). Nearly three-quarters of patients attending a falls clinic in the United Kingdom had a vitamin D deficiency (Dhesi et al., 2002).

In addition to its effects on bone health, vitamin D has a variety of non-skeletal functions, including enhancing muscle health (Bischoff-Ferrari, 2012; Thacher and Clarke, 2011). Clinically, severe levels of vitamin D deficiency are associated with proximal muscle weakness, wasting, pain and changes in walking such as waddling or myopathic gait (the ‘waddling’ is due to the weakness of the proximal muscles of the pelvic girdle) (Glerup et al., 2000; Stockton et al., 2011). Benefits might arise from activating vitamin D receptors in muscle that in turn will promote protein synthesis. Observational studies have shown direct correlations between 25(OH)D levels and muscle strength as well as lower extremity function. Low levels of vitamin D are also specifically
4. Vitamin D and fall risk

associated with postural instability (Boersma et al., 2012). Finally, numerous cross-sectional studies in community dwelling older adults have shown a direct relationship between vitamin D status and physical performance measures, especially when levels are low (Houston et al., 2011; Muir and Montero-Odasso, 2011).

The foregoing all suggests that vitamin D supplementation might be an effective and feasible approach to fall prevention in older people (Annweiler et al., 2010). Intervention studies have shown that supplemental vitamin D at daily doses of 800 to 1000 IU consistently lead to small but clinically significant improvements in surrogate markers for fall risk including improved lower extremity muscle strength, decreased postural sway, and lower ‘Timed Up and Go’ times but not for gait variables (Muir and Montero-Odasso, 2011; Zhu et al., 2010). Most importantly, vitamin D supplementation has been associated with a 14 to 34% reduction in actual falls. The effect on rates varies depending on the dose of vitamin D used, how it was administered and the proportion with deficiency prior to the initiation of therapy (Annweiler et al., 2010; Bischoff-Ferrari, 2012).

With regards to the latter point, the effect appears to be more prominent in patients who are vitamin D deficient prior to the initiation of supplementation (Murad et al., 2011).

4.3 Potential mechanisms

How vitamin D specifically reduces the likelihood of falls at a cellular level is still being elucidated but several theories exist. Activation of fast twitch (type II) muscle fibers occurs when an individual requires a fast reaction, such as would be needed in regaining balance to prevent a fall (Flicker et al., 2003). Aging itself is associated with greater atrophy of these fast twitch (type II) muscle fibers relative to slow twitch fibers (Lips and Van Schoor, 2011). It is possible that the age related decline in fast twitch muscle fibers may, in part, be explained by vitamin D deficiency. Their loss could lead to an increased risk for falling (Bischoff-Ferrari et al., 2004; Flicker et al., 2003; Hamilton, 2010).

A number of studies (Bischoff-Ferrari et al., 2004; Bischoff et al., 2001; Costa et al., 1986) suggested that the effect of vitamin D on muscle was likely direct and via genomic transcription (Bischoff-Ferrari, 2012). Earlier research showed VDRs to be present in all skeletal muscle cells. When VDRs are bound by active vitamin D metabolites (i.e. 1,25(OH)_{2}D), protein synthesis is increased leading to muscle cell growth (Bischoff et al., 2001; Freedman, 1999; Simpson et al., 1985; Sorensen et al., 1979). It has been argued that reduced VDR binding and expression eventually results in the sarcopenia seen at an advanced age (Bischoff-Ferrari et al., 2004; Bischoff et al., 2001; Simpson et al., 1985). More recently, however, Wang and De Luca (2011) have rebutted the notion that VDRs are found in muscle. They suggest that earlier studies using chick monoclonal VDR antibody 9A7 identified non-VDR proteins, which stain with this antibody (Wang and DeLuca, 2011). They postulate that the effect of 1,25(OH)_{2}D on muscle function is likely indirect (Pfeifer et al., 2001; Wang and DeLuca, 2011), possibly through the nervous and/or endocrine-metabolic systems (Binkley, 2012).
Vitamin D deficiency may alter neurotransmitter activity, thereby adversely affecting neurological function. VDR have been located at a cellular level in neuronal and glial cells (Binkley, 2012; Muir and Montero-Odasso, 2011; Vandervoort, 2002). With aging, VDR expression and activation will decline resulting in reductions in neural functions such as nerve conduction (Annweiler et al., 2010; Dretakis et al., 2010). This could result in poor balance and reduced reaction time (Ceglia, 2009; Gallagher et al., 2007; Muir and Montero-Odasso, 2011). Moreover, impaired cognition from reduced VDR expression could lead to slower processing speeds in older adults (Kalueff and Tuohimaa, 2007; Menant et al., 2012; Michael et al., 2010).

An alternative mechanism that has been proposed is via changes in sex steroid levels. Consistent with the seasonal variation seen in vitamin D levels, there is distinct seasonal variability with testosterone. Highest levels are observed during the summer (Andersson et al., 2003). As testosterone has anabolic effects on bone and muscle, it is possible that vitamin D supplementation in vitamin D deficient individuals might indirectly result from increases in circulating testosterone (Binkley, 2012). Further work to confirm or refute this suggested mechanism is required.

Which of these (or some other) mechanisms best explains the relationship between falls and vitamin D deficiency in older adults remains unknown (Janssen et al., 2002). Given the breadth of literature, it is likely that vitamin D mediates its effect through multiple pathways.

4.4 Extent of fall risk reduction with vitamin D

In spite of the number of systematic reviews and meta-analyses, there continues to be controversy over the role of vitamin D for the specific indication of preventing falls. Over the last several years, a number of organizations have produced guidelines and/or position papers commenting on its utility in older individuals for this purpose (Table 4.1). While most other groups have endorsed the use of vitamin D for fall prevention in at least some circumstances, the IOM drew criticism and controversy by concluding that the evidence was too inconsistent to allow them to make a favorable recommendation (Ross et al., 2011).

Recent systematic reviews and meta-analyses have indicated that vitamin D supplementation can lead to anywhere between a 13% to 34% reduction in falls, although there was moderate heterogeneity in the studies and potential publication bias (Table 4.2). The evidence for falls prevention was predominantly in patients who were vitamin D deficient and then received a dose greater than 700-800 IU once a day. Co-administering calcium was beneficial in subgroup analyses of some of the systematic reviews (Kalyani et al., 2010; Murad et al., 2011). While no difference in effect was seen when comparing those dwelling in the community with those institutionalized (Murad et al., 2011) many of the community based studies recruited individuals at high risk for falls and/or deficient in vitamin D. Those enrolled may have had a closer resemblance to institutionalized adults than a more diverse community sample. Although fall rates are high in acute care, there is limited evidence for this setting. Despite a negative result, one acute care randomized controlled trial (RR of 800 IU of vitamin D on risk of falling = 0.82, 95% CI = 0.59-
4. Vitamin D and fall risk

Table 4.1. Guideline/position paper summary.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine Society 2011 (Holick et al., 2011)</td>
<td>Recommends prescribing vitamin D supplementation for fall prevention.</td>
</tr>
<tr>
<td>IOF Position Paper 2010 (Dawson-Hughes et al., 2010)</td>
<td>From a meta-analysis of available data, they concluded that a mean serum 25(OH)D level of at least 60 nmol/l (24 ng/ml) is needed for optimal fall risk reduction. Supplementation in amounts of 17.5 to 25 μg/day (700-1000 IU/day), lowered their risk of falling by 20% in older individuals, independent of their calcium intake level.</td>
</tr>
<tr>
<td>Osteoporosis Canada 2010 (Hanley et al., 2010)</td>
<td>Evidence that supplementation with 20 μg (800 IU) vitamin D3 daily reduces the risk of falls, particularly from trials with adequate ascertainment of falls (level 2 evidence).</td>
</tr>
<tr>
<td>AGS/BGS Clinical practice guidelines - prevention of falls in older persons 2010 (Panel on prevention of falls in older persons and British geriatrics, 2011)</td>
<td>Vitamin D supplements of at least 800 IU per day should be considered for people with suspected vitamin D deficiency or who are otherwise at increased risk for falls [Strength of recommendation – B].</td>
</tr>
<tr>
<td>CADTH – Vitamin D supplementation in long term care residents: a review of the clinical effectiveness and guidelines (CADTH, 2010)</td>
<td>Overall, felt that the identified evidence supports vitamin D supplementation at a dose of at least 800 IU daily in residents of long-term care facilities to reduce the rate of falls.</td>
</tr>
<tr>
<td>ACSQHC – Prevention of falls and harm from falls in older adults (ACSQHC, 2009)</td>
<td>Vitamin D and calcium supplementation recommended as an intervention strategy to prevent falls in residents of residential aged care facilities (Level I). Although future research may elucidate clear benefits and possibly even different requirement levels for vitamin D in relation to these non-skeletal outcomes (including falls), existing data cannot support such conclusions.</td>
</tr>
<tr>
<td>The 2011 Report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine (Ross et al., 2011)</td>
<td></td>
</tr>
</tbody>
</table>

1 ACSQHC: Australian Commission on Safety and Quality in Health; CADTH: Canadian Agency for Drugs and Technology in Health; IOF: International Osteoporosis Foundation.

1.16) (Burleigh et al., 2007), did suggest a benefit trend and interest in the role of vitamin D supplementation for fall prevention in hospitalized seniors persists.

4.5 Type, dose and toxicity of vitamin D

The two main forms of vitamin D are D2 and D3. Vitamin D3 (cholecalciferol), is produced in the skin in response to UVB radiation. Lanolin can be used to produce vitamin D3 supplements and in some countries is used to fortify food (Holick, 2011). Vitamin D2 (ergocalciferol) is
made by yeast and mushrooms exposed to UVB radiation (Holick, 2011). It is often given as high dose boluses when used for treatment. Both forms of vitamin D can be metabolized to 1,25-dihydroxyvitamin D, the active form of the vitamin. The two forms of vitamin D appear to be effective in falls reduction, although vitamin D3 has been more commonly used in studies as there is some evidence that D2 may not be used as efficiently in \textit{in vivo} studies (Armas \textit{et al.}, 2004; Kalyani \textit{et al.}, 2010; Murad \textit{et al.}, 2011). There is less evidence available on other forms of vitamin D as it relates to fall prevention. No significant reduction in falls was found with alfacalcidol (RR = 0.84, 95% CI = 0.58-1.22) (Kalyani \textit{et al.}, 2010). A few trials have been performed using the 25(OH)D3 metabolite itself. It is more hydrophilic than the parent compound, has a short half life (8 to 11 hours), and causes a rapid increase in serum 25(OH)D levels. Although, trials have shown improvements in bone density, the mechanical strength of bone, and lower extremity function, an effect on fall rates has not been reported (Bischoff-Ferrari \textit{et al.}, 2012).
Adherence is not a major issue with oral vitamin D supplement. Missing a dose now and then when taken on a daily basis would not be expected to lead to significant issues. However there remains interest in looking at different dosing approaches to quickly and reliably reach desired levels of 25(OH)D. The estimated effective threshold of 25(OH)D is 60 nmol/l to prevent falls, 75 nmol/l to improve muscle strength and balance, and possibly even higher for fracture prevention (Dawson-Hughes, 2012; Glendenning and Inderjeeth, 2012; Muir and Montero-Odasso, 2011). Relatively long periods of time, at least three months, are required with regular oral supplementation with vitamin D3 to achieve these levels (Bischoff-Ferrari et al., 2012; Hanley et al., 2010). Hanley provides a clinically useful estimate, whereby 1 μg (40 IU) of vitamin D3 will increase serum 25(OH)D by 0.7-2.0 nmol/l (Hanley et al., 2010). For most adults, a supplement of 20-25 μg (800-1000 IU) daily will raise serum 25(OH)D levels by approximately 15-30 nmol/l in about 3 months. It is unclear, though, whether getting to these desired levels any quicker is important. 25(OH)D3 metabolite at a dose of 20 μg, reached a 25(OH)D threshold greater than 75 nmol/l in about a month, but has not yet been shown to be effective in fall prevention (Bischoff-Ferrari et al., 2012).

Three trials have raised concerns that high-dose bolus therapy may have overall detrimental effects on health. Smith et al. (2007) administered 300,000 IU ergocalciferol (vitamin D2) intramuscularly annually for three years. There was no effect on falls (HR = 0.98 (0.93-1.04), but there was a significant increase in the risk of hip, femur and wrist fractures among women in the treatment group (HR = 1.59; 95% CI = 1.17-2.16, P=0.003) (Smith et al., 2007). Sanders et al. (2010), administered 500,000 IU of oral cholecalciferol annually for 3 years and found a 26% increased risk of fractures (RR = 1.26; 95% CI = 1.00-1.59, P=0.047) and a 15% increased risk of falls (RR = 1.15; 95% CI = 1.02-1.30, P=0.03) with treatment compared to the control arm (Sanders et al., 2010). Recently, Glendenning et al. (2012) administered 150,000 IU oral cholecalciferol every 3 months for a total of 9 months. Although there were no statistically significant differences in either the number of falls or the measures of mobility or hand strength compared to placebo, there was a trend suggesting the treatment group was more likely to experience multiple falls as compared to the control group (OR = 1.58; CI = 0.83-2.99) (Glendenning et al., 2012).

Why would bolus therapy be detrimental? Rosen (2011a) suggested the following explanations: (1) high doses generate higher concentrations of dangerous metabolites; (2) vitamin D, a prohormone, acts in a catabolic manner at high doses (like parathyroid hormone); or (3) that high serum levels are dangerous, a reverse J-curve, as suggested by the IOM report (while levels between 50-80 nmol/l are good, both low levels and levels >80 nmol/l are associated with health issues) (Rosen, 2011a).

Though serum 25(OH)D levels above 80-125 nmol/l are potentially harmful, toxicity is rare and occurs when vitamin D doses exceed 10,000 IU per day, with serum 25(OH)levels >375 nmol/l (Jones, 2008). Toxicity, if it occurs, typically presents as hypercalcemia, possibly nephrolithiasis, and/or renal damage. Caution should be used in conditions that can be associated with hypercalcemia and/or hypercalciuria such as the chronic granulomatous disorder sarcoidosis. With a half life of 15-20 days (Hanley et al., 2010), vitamin D can accumulate in the body and take
a long time to clear so any toxicity may have long lasting effects. In Canada, daily intakes of up to 2,000 IU are considered safe without the need for any monitoring of vitamin D levels (Hanley et al., 2010). The IOM reported the risk for harm begins to increase (upper tolerable level) at dosages beyond 4,000 IU/d and serum 25(OH)D levels >125 nmol/l (Ross et al., 2011).

There is no need to obtain 25(OH)D levels either before or during therapy (Hanley et al., 2010). Monitoring should be reserved for those with recurrent osteoporotic fractures, continued bone loss in spite of treatment, or co-morbid conditions that might affect vitamin D absorption or action (e.g. malabsorptive states, anticonvulsant therapy). The increase in the volume of questionably useful vitamin D testing has been staggering (Sattar et al., 2012). In the Canadian province of Ontario the number of tests increased over 20-fold in only five years from 29,000 (2004) to over 700,000 (2009) (CBC News: http://www.cbc.ca/news/canada/calgary/story/2011/01/28/calgary-vitamin-d-blood-testing.html). Aside from issues of cost (the cost per test in Canada is $50 to $100 CDN) and lack of demonstrated benefit from the expenditure, there is significant inter-assay and inter-laboratory variability in the 25(OH)D assay (Lai et al., 2010).

Researchers and clinicians alike have shown increasing interest in vitamin D as demonstrated by the sharp rise in the number of vitamin D publications. The lack of agreement on the benefit of vitamin D supplementation in reducing fall risk could be partially explained by the noted heterogeneity in the published studies and methodological shortcomings. Most community-based studies selectively recruited patients at a high risk of falling. By potentially exaggerating the risk reduction seen, generalizability of the results obtained is limited. Research in the area of fall prevention with vitamin D would benefit from more inclusive recruitment of subjects, careful assessment of potential confounders and explanatory variables (e.g. 25(OH)D levels), use of an array of outcome measures (including carefully ascertained falls and objective measures of strength, balance, and gait) and studies of a longer duration.

4.6 Conclusions

We feel there is compelling evidence that low vitamin D levels are associated with muscle weakness and impaired balance. This likely underlies the documented higher fall rates found in vitamin D deficient individuals. Although there are inconsistencies in the evidence base, the dominant theme is that vitamin D supplementation among those with low pre-treatment levels can reduce of further falls by as much as a third. To achieve benefits in fall prevention a 25(OH)D level of at least 60-75 nmol/l is required.

Pending the results of further research, what can be suggested now? Current recommendations for vitamin D intake in older adults have increased to 800 IU or more per day. Among those at a high risk of falling (e.g. prior fall within the last year), we would routinely suggest a dose of 800-2,000 IU per day. This dose range appears to be safe. It can be given with or without calcium (we normally recommend calcium supplementation). This dose range of vitamin D should guarantee vitamin D sufficiency unless there are mitigating circumstances. In addition, we encourage
exposure to natural sunlight in moderation (e.g. 5-15 minutes, 3 times a week between 10:00 and 15:00 during the summer) (Holick, 2011). At this time, intermittent high-dose therapy with vitamin D is not recommended.

Moderate vitamin D supplementation is an easy and inexpensive, yet effective, addition to any care plan for the reduction of falls and fall related injuries in older adults.

References


Gallagher, J.C., Rapuri, P.B. and Smith, L.M., 2007. An age-related decrease in creatinine clearance is associated with an increase in number of falls in untreated women but not in women receiving calcitriol treatment. Journal of Clinical Endocrinology & Metabolism 92, 51-58.


4. Vitamin D and fall risk


4. Vitamin D and fall risk


Key facts

- The primary function of vitamin D is the maintenance of calcium and phosphate homeostasis and skeletal integrity throughout life.
- Vitamin D deficiency and/or insufficiency are global problems for growing children and adolescents.
- Vitamin D deficiency might arise from an inadequate supply, impaired utilization or enhanced excretion of vitamin D.
- An adequate vitamin D status is associated with higher muscle strength and muscle functions in children and adolescents.

Summary points

- High proportions of vitamin D deficiency and insufficiency have been reported in apparently healthy children and adolescents worldwide.
- A persistent severe vitamin D deficiency results in the bone disorder disease of rickets in children and osteomalacia in adults.
- Vitamin D deficiency is associated with an increase in parathyroid hormone concentration in blood, increase rate of bone turnover and a decreased rate of bone mass accumulation in growing children and adolescents.
- An inadequate vitamin D status exerts detrimental effect on musculoskeletal health in growing populations.
- Preventing vitamin D deficiency by ensuring an adequate vitamin D supply throughout childhood and adolescence might enhance physical growth and bone mass accretion, which ultimately could reduce the risk of osteoporotic fracture later in life.
5. The beneficial effects of higher (adequate) vitamin D status on bone growth and muscle strength in children and adolescents

L.H. Foo\textsuperscript{1} and S.J. Whiting\textsuperscript{2}

\textsuperscript{1}Program of Nutrition, School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kelantan, Malaysia; \textsuperscript{2}Division of Nutrition and Dietetics, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatchewan, SK S7N 5C9, Canada; lhfoo@kb.usm.my

Abstract

Vitamin D is chemically a seco-steroid, which acts biologically as a prohormone and requires two hydroxylation processes, first in liver and the other in kidney (for those functions related to bone) before becoming active. The primary function of vitamin D is thought to be the maintenance of calcium and phosphate homeostasis and skeletal integrity throughout life. Numerous epidemiological studies suggest that vitamin D deficiency and/or insufficiency are global problems for growing children and adolescents. Although it is well established that persistent severe vitamin D deficiency results in the development of rickets in children and osteomalacia in adults, it is now suggested that a low 25-hydroxyvitamin D concentration is not only a biochemical abnormality, but also associated with physiological, pathological and clinical signs of vitamin D deficiency, along with secondary hyperparathyroidism, and increased bone remodelling across the lifespan. A growing body of evidence indicates that higher and adequate vitamin D status in the body plays an important role in maintaining musculoskeletal integrity at any age. However, the adverse effects of vitamin D deficiency on bone mass has been studied extensively in the postmenopausal women and in the elderly with only a few studies in children and adolescents. Therefore, the review will discuss the current evidence pertaining to the relationships between vitamin D status and musculoskeletal health in children and adolescents.

Keywords: vitamin D deficiency, musculoskeletal health, bone growth, children, adolescents
5.1 Introduction

Osteoporosis is defined as a systemic skeletal disorder characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture risk (World Health Organization, 1994). Osteoporotic fractures result in substantial mortality, morbidity and are destructive in terms of productivity and quality of life. The economic and social burden of such fractures will increase dramatically worldwide as the population ages. So serious are the long-term consequences of osteoporotic fracture, in terms of economic and human cost that osteoporosis has been recognized as a major public health concern and a major contributor to medical care costs in many regions of the world (Cummings and Melton, 2002; Johnell and Kanis, 2004). A growing body of evidence suggested that osteoporosis may have its origins at an earlier stage in life by failure to achieve optimal peak bone mass during childhood and adolescence. Peak bone mass is defined as the amount of bony tissue present at the end of skeletal maturation (Heaney et al., 2000). The quantity of bone when peak bone mass is reached is regarded as a major determinant of the risk of osteoporotic fractures later in life. Indeed, the adolescent years determine lifelong skeletal health because this period represents the time of greatest skeletal growth, during which bone mass is largely accrued. For instance, almost 40% of the bone mass accrual in girls occurs during this period (Bailey et al., 1999; Matkovic et al., 1994). Hence, osteoporotic fractures related to bone loss in later life may perhaps be prevented by strategies to promote and maximize bone mineralization during growing years. Therefore, better understanding the factors associated with greater bone mass accretion in growing children and adolescents is ultimately crucial, in order to prevent excessive age-related bone loss and osteoporotic fractures later in life. Of all these factors, vitamin D nutrition has been emerging as an important factor in relation to musculoskeletal health across the lifespan.

It is generally agreed that persistent severe vitamin D deficiency results in the development of rickets in children and osteomalacia in adults. On the other hand, mild vitamin D deficiency as known as hypovitaminosis D is regarded as an emerging global health problem for growing children and adolescents, with the prevalence of vitamin D deficiency increasingly being

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>aBMD</td>
<td>Areal bone mineral density</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary reference intake</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-photon energy X-ray absorptiometry</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended dietary allowance</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>Ergocalciferol</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Cholecalciferol</td>
</tr>
</tbody>
</table>

L.H. Foo and S.J. Whiting

Handbook of vitamin D in human health
5. Effects of vitamin D on bone growth and muscle strength

reported in apparently healthy children and adolescents worldwide, in both the developed and the developing countries (Arabi et al., 2010; Docio et al., 1998; Foo et al., 2009a; Fraser, 2004; Gordon et al., 2004; Guillemant et al., 1999; Lehtonen-Veromaa et al., 2002a; Looker et al., 2002). Surprisingly, hypovitaminosis D has been found in sunny countries at tropical latitudes where cutaneous production of vitamin D can occur throughout the year (Arabi et al., 2010; El-Hajj Fuleihan et al., 2001; Gannage-Yared et al., 2000; Hatun et al., 2005). Long term vitamin D deficiency is associated with increased parathyroid hormone levels in blood. Persistent elevation of PTH results in a net loss of bone, and in children, the development of rickets. In contrast, little is known about the effects on musculoskeletal health in children and adolescents of low vitamin D status without clinical signs of rickets. Nevertheless, several studies have found that vitamin D deficiency and insufficiency in children and adolescents resulted in secondary increases in serum PTH (El-Hajj Fuleihan et al., 2001; Foo et al., 2009a; Gordon et al., 2004; Guillemant et al., 1999; Outila et al., 2001), increased bone turnover (Cheng et al., 2003; Foo et al., 2009b; Lehtonen-Veromaa et al., 2002b) and a decrease in the rate of bone mass accumulation (Foo et al., 2009b; Jones and Dwyer, 1998; Lehtonen-Veromaa et al., 2002b).

5.2 Effects of vitamin D status on bone growth in children and adolescents

A growing body of evidence which suggest that mild vitamin D deficiency also called ‘insufficiency’ in children and adolescents may also be deleterious to bone health in children and adolescents. Adequate vitamin D status may be important for skeletal health because of its primary function in maintaining calcium and phosphate homeostasis (Fraser, 1995). Numerous studies performed in growing children and adolescents have found that a persistently low vitamin D status with prolonged elevation of PTH and a subsequent increase in bone turnover, which consequently leads to sub-optimal bone mass accretion. However, the positive effects of vitamin D status on bone mass accretion in healthy growing children and adolescents have produced inconsistent results, especially among adolescents at the time of pubertal growth development. In Caucasian adolescent girls, a positive association has been found in some studies between vitamin D status and bone mass measurements (Cheng et al., 2003; Lehtonen-Veromaa et al., 2002b; Outila et al., 2001). However, this association was not found in other studies (Kristinsson et al., 1998; Willett, 2005). In a cross sectional survey of adolescent girls aged 14 to 16 years, a positive association was found between serum 25(OH)D concentrations and forearm aBMD, but only in adolescents who had a 25(OH)D levels of <40 nmol/l (Outila et al., 2001). Similarly, in a 3-year longitudinal investigation of bone development in Finnish adolescent girls aged 9 to 15 years, Lehtonen-Veromaa et al., (2002b), found a positive association between changes of serum 25(OH)D levels from baseline as well as the baseline 25(OH)D levels themselves on lumbar spine aBMD. Both studies used aBMD as their bone outcome variable. However, the use of aBMD in growing children and adolescents has been generally accepted as an inaccurate measure of bone mass accretion because it depends on bone size and body growth factors (Prentice et al., 1994). The increase in aBMD reported in these studies may have been related to differences in bone size between individuals rather than to vitamin D status. Hence, there is still uncertainty about
the importance of vitamin D deficiency during childhood and adolescence on final bone mass at maturity.

There is limited data about the influence of vitamin D status on bone growth in children and adolescents of non-Caucasian origin. In a population-based study of Chinese adolescent girls aged 15 years, adequate vitamin D status of at least 50 nmol/l was found to be associated with a significant increase in size-adjusted BMC of the total body, and distal and proximal forearm compared to those with poor vitamin D status (Foo et al., 2009b). These relationships persisted after adjusting for stage of pubertal development, dietary intake of calcium and vitamin D, and total time spent in physical activity thus demonstrating that these variables did not influence the changes in bone measurements in relation to vitamin D status. In contrast, there were no significant differences in bone area as an indicator of bone size at the skeletal sites measured between the three groups of vitamin D status. These findings were no different from those of Kristinsson et al. (1998) where adolescent girls aged 16 to 20 years, showed a significant positive association between 25(OH)D concentration and total forearm BMD and BMC when it was analyzed according to individual age.

Two recent intervention studies of vitamin D supplementation on bone growth in growing adolescents (El-Hajj Fuleihan et al., 2006; Viljakainen et al., 2006) corroborate the prospective cohort studies. In the study of 212 Finnish pre-pubertal adolescent girls aged between 11 and 12 years who were randomly given different doses of vitamin D supplements of either 200 IU or 400 IU daily for a year, it was found that bone mineral augmentation of the proximal femur was 14% and 17% significantly greater in participants who received 200 IU and 400 IU of vitamin D per day, respectively, compared to control girls (Viljakainen et al., 2006). The second investigation was in Lebanese premenarcheal adolescent girls aged 10 to 17 years, supplemented with either 1,400 IU/wk or 14,000 IU/wk vitamin D3 for one-year. They had significantly increased in bone mineral content and bone area of the total hip as compared to the controls (El-Hajj Fuleihan et al., 2006). These findings are further supported by a recent meta-analysis of six randomized-controlled trials of vitamin D supplementation in 541 apparently healthy children and adolescents aged 1 month to <20 years, whose were supplemented for vitamin D for at least three months, showing a significant effects on BMC of the total body and BMD of the lumbar spine, especially among those children and adolescents whose had low vitamin D status of blood (25(OH)D of <35 nmol/l) (Winzenberg et al., 2011).

Most of the published reports investigating the influence of vitamin D status on bone mass in children and adolescents focused on BMC and/or aBMD, assessed by DXA as the endpoint bone variable. This assessment method does not measure bone geometry and structural properties of the skeleton and only provides a two-dimensional rather than a more complex three-dimensional analysis of the bones measured. There are two reports of investigations into the association between vitamin D status and bone geometry. One of these was with Caucasian pre-pubertal girls aged 10 to 12 years (Cheng et al., 2003) and the other was with Hispanic and Caucasian post-pubertal females aged 16 to 22 years (Kremer et al., 2009). The study by Cheng et al. (2003) using peripheral quantitative computed tomography, found that girls with vitamin D deficiency, defined
5. Effects of vitamin D on bone growth and muscle strength

as 25(OH)D levels ≤25 nmol/l, had significantly lower cortical volumetric BMD in the distal radius compared with subjects with higher vitamin D status. By contrast, however, no significant differences were found between vitamin D status and any BMC or BMD measurements for the total body, femur and lumbar spine, measured by DXA device (Cheng et al., 2003). The authors concluded that when only DXA was used to determine the influence of vitamin D status on bone mass, there could be a misinterpretation of the role of vitamin D on bone mass accretion. When peripheral quantitative computed tomography was used to measure volumetric bone density, vitamin D status was found to influence the results (Cheng et al., 2003). On the other hand, no significant association was found between the adequacy of vitamin D status and bone growth as measured by either computed tomography or DXA methods (Kremer et al., 2009).

Although it is well established that low vitamin D status in postmenopausal and elderly women is considered as a major risk factor associated with increased risk of rapid bone loss and osteoporotic fractures (Dawson-Hughes et al., 2005; Lips, 2001), on the contrary, the precise beneficial effects of vitamin D status on bone mass growth and development in growing children and adolescents is still inconclusive. Interpretation of the effects of vitamin D status and bone mass accretion in growing children and adolescents is complicated and confounded by various biological growth factors such as sexual maturation and changes in the rate of linear growth. The interaction between these variables is complex during this rapid growth period (Heaney et al., 2000; Saggese et al., 2002). For instance, there is a strong confounding effect of puberty on skeletal modeling and remodeling, where plasma calcium and phosphate concentrations may be affected by factors other than vitamin D status (Kristinsson et al., 1998; Saggese et al., 2002). This suggests that these biological variations may confound the independent influence of vitamin D on bone mass accretion. In the study of Chinese girls, the significant association between vitamin D status and bone mass remained unchanged, even after adjusting for pubertal stage (Foo et al., 2009b). This is not surprising since most of the subjects were at a post-pubertal stage of development, where changes in sex hormones are less pronounced compared to those in subjects at an earlier stage of puberty (Saggese et al., 2002).

The mechanism by which adequate vitamin D status leads to higher bone mass in healthy growing children and adolescents is not clearly elucidated. However, based on the current published evidence available, it is suggested that the increased bone mass with adequate vitamin D status may be in part a consequence of lower secretion of PTH, followed by a decrease in the rates of bone remodeling, as indicated by the biochemical markers for bone formation and resorption (Foo et al., 2009b; Hill et al., 2010; Outila et al., 2001).

5.3 Effects of adequate vitamin D levels on muscular strength in children and adolescents

It is well documented that long term severe vitamin D deficiency can cause rickets. Rickets is characterized as a failure of mineralization of the organic matrix of bone particularly at the epiphyseal growth plate with deformities of the skeleton, enlargement of the joints of the long
bones and rib cage, curvature of the spine and thighs. Rickets also includes muscle spasms, weakness and atrophy in children, however, there is limited evidence of the effects of vitamin D deficiency and/or insufficiency on muscle functions in growing children and adolescents. A significant positive influence of adequate vitamin D status on muscle strength has been found in a representative population-based study of Chinese post-pubertal adolescent girls aged 15 years, in which adolescent girls with adequate 25(OH)D levels of more than 50 nmol/l showing significantly greater handgrip muscle strength compared with those of lower vitamin D status. This relationship was independent of pubertal growth parameter, body size, level of physical activity and dietary calcium and vitamin D intakes (Foo et al., 2009b). This finding is further supported by another study of 99 post-menarcheal girls aged between 12 and 14 years, also showing a significant positive association between serum 25(OH)D concentration and muscle power and force of the lower limb, assessed by the jumping velocity, height and power, the Esslinger Fitness Index and maximum voluntary force (Ward et al., 2009). Adolescent girls with low 25(OH)D concentrations generated less muscle power, and so jump height and velocity were lower compared to those with higher vitamin D status (Ward et al., 2009), suggesting that muscle contractility is affected by vitamin D status.

The observations from cross-sectional studies are further supported by a recent randomized-controlled trial of vitamin D supplementation on musculoskeletal health assessment in postmenarchal girls aged 12 to 14 years-old with low baseline serum 25(OH)D concentrations of 18 nmol/l, which also found that a significant increase in muscle function, as measured by an efficiency of movement of the lower limb in adolescent girls whose were supplemented with 4 doses of 150,000 IU vitamin D2 in a year compared to the untreated participants (Ward et al., 2010). In addition, there was an significant interaction between baseline serum 25(OH)D concentration and response to vitamin D supplementation for muscle jump velocity. On the contrary, not such significant positive effect of vitamin D supplements on bone mass was found (Ward et al., 2010). These findings in adolescents are comparable to those in postmenopausal women and elderly populations where higher vitamin D status, as assessed by a blood 25(OH)D levels was significantly associated with increased muscle strength and improved lower extremity function (Bischoff-Ferrari, 2012; Bischoff et al., 2003; Dawson-Hughes et al., 1995). In the elderly this improved muscular function and strength may lower the risk of falls and consequent osteoporotic fractures. However, more vitamin D is needed compared to protection against bone loss (Bischoff-Ferrari, 2012).

Until recently, the mechanism of action of higher vitamin D status on greater muscle strength levels and muscle function is not well elucidated in children and adolescents. However, there are several plausible explanations on such positive effect of adequate vitamin D status on muscle. One possible mechanism may involve a direct role of vitamin D (either as 1,25(OH)2D or 25(OH)D) in muscle action. Although such an effect has not been shown, there has been much research on the molecular action of vitamin D in muscle through different pathways involved in genomic and non-genomic actions (Hazell et al., 2012). It has been demonstrated that a direct physiological role of vitamin D receptor in muscle development, in which 1,25(OH)2D stimulates the growth and proliferation of muscle cells and regulates intracellular calcium in muscle cells (Boland, 1986;
5. Effects of vitamin D on bone growth and muscle strength

Boland et al., 1995) and it is also suggested that the absence of vitamin D receptor or unbound vitamin D receptor in low vitamin D status may produce abnormal muscle growth (Hazell et al., 2012). It may be that vitamin D has a role in modifying the transport of calcium in muscle cells through separate non-genomic mechanisms or it may have a role quite independent of that. For instance, low vitamin D status may result in fatigue and diminished muscle oxygenation, and consequently may affect muscle strength and function, and give rise to muscle weakness (Boland, 1986). On the other hand, a separate observation that 25(OH)D is apparently stored in muscle in newborn rats may indicate a special role of muscle in vitamin D homeostasis, which is quite independent of any role of vitamin D in muscle function (Clements and Fraser, 1988). Therefore, more studies are required to identify the mechanisms for these interactions. Such information is much needed because the development of optimum muscle strength has an important influence on bone mass accrual and also for the maintenance of bone mass integrity during this critical period of growth. For instance, several studies in children and adolescents found that increases in muscular strength were significantly associated with both changes in bone mass (Foo et al., 2007) and also changes in bone geometry, and hence the structural properties of bone, independent of the bone mass properties (Haapasalo et al., 1998). This is compatible with the functional model of bone development, described as the mechanostat hypothesis, in which muscle and bone together form a functional unit, so that greater muscle strength leads to increased bone strength and mass (Frost and Schonau, 2000).

5.4 Recommended daily intake of vitamin D for children and adolescents

The DRI panel for calcium, phosphorus, magnesium, vitamin D and fluoride was first established in 1997 to provide intake recommendations for Americans and Canadians (Institute of Medicine, 1997). At that time, an adequate intake was set for vitamin D as there was not enough data to set an RDA (Weaver and Fleet, 2004). At that time, vitamin D was thought to be mainly acquired by solar irradiation of skin. In 1997, adequate intake for vitamin D was set at 200 IU for infants, children and adolescents, regardless of sunlight exposure, age, degree of skin pigmentation, geographic region and gender. The upper levels were set at 1000 IU for infants and 2,000 IU per day (50 μg/day) for all other age groups. The upper level was set to prevent hypervitaminosis D when serum 25(OH)D concentrations are in excess of 400 nmol/l. Since 1997, there has been debate about whether the adequate intake level for vitamin D is really ‘adequate’ and ideal for optimal bone health (Dawson Hughes et al., 2005; Vieth, 1999). Since then, progress was made toward establishing the vitamin D intake needed to exert significant effects on bone health. The revised DRI values included an RDA of 600 IU for vitamin D in 2011 (Institute of Medicine, 2011) (Table 5.1). This RDA for vitamin D for growing children and adolescents is three-fold higher than the first recommendation set in 1997. The difference is in the intended level of 25(OH)D thought necessary in order to promote appropriate bone growth; in 1997 the level of 25(OH)D was 25 nmol/l while in 2011 it is 50 nmol/l. DRI values pertain to healthy individuals, while unhealthy children and adolescents may need more vitamin D, for whatever reason may undermine health, from overweight/obesity to malabsorption disorders (Holick et al., 2011). The Endocrine Society has made vitamin D recommendations for ‘unhealthy’ children and adolescents, as shown in
Table 5.1. In contrast to the DRI values for RDAs, the Endocrine Society has indicated a need to achieve a level of 25(OH)D at or above 75 nmol/l.

### 5.5 Dietary sources of vitamin D

There are two forms of dietary vitamin D. Vitamin D2 originates from a plant-origin. It is produced by exposure of the ergosterol present in fungi and yeasts to ultraviolet-B radiation. Vitamin D3 is the natural form produced by the cutaneous synthesis by exposure to sunlight in the body in most animals. In general, food sources of vitamin D are extremely limited. The only food sources relatively rich in vitamin D are some oily fish, fish liver oils, and egg yolk. In some developed countries such as Canada and the USA, foods such as breakfast cereals, fruit juices, margarine, and some dairy products are fortified with vitamin D (Calvo et al., 2004). Dietary supplements containing vitamin D in amounts that will increase vitamin D status may also be consumed in these countries (Whiting et al., 2011).

#### Table 5.1. Dietary reference recommended intake for vitamin D for children and adolescents of healthy populations from the US Institute of Medicine (IOM)¹ and populations at risk of vitamin D deficiency from the endocrine practice guidelines committee.²

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 years</td>
<td>600 IU</td>
<td>600-1000 IU</td>
</tr>
<tr>
<td>4-8 years</td>
<td>600 IU</td>
<td>600-1000 IU</td>
</tr>
<tr>
<td>Males and females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-18 years</td>
<td>600 IU</td>
<td>600-1000 IU</td>
</tr>
<tr>
<td>19-30 years</td>
<td>600 IU</td>
<td>1,500-2,000 IU</td>
</tr>
</tbody>
</table>

¹ New revised recommended vitamin D intakes from the IOM (2011).
² Recommended vitamin D intake for children and adolescents at risk for vitamin D deficiency from the endocrine practice guidelines committee (Holick et al., 2011).
³ Conversion factor: 1 μg vitamin D₃ = 40 IU.
5. Effects of vitamin D on bone growth and muscle strength

5.6 Conclusions

Low vitamin D status, is not only a biochemical abnormality, but is also associated with physiological, pathological and clinical signs of vitamin D deficiency, along with secondary hyperparathyroidism, and increased bone remodeling, which could have long-term detrimental effects on skeletal development and growth. Moreover, low vitamin D levels also associated with impairment of optimal muscle function in children and adolescents, in which these conditions would therefore compromise bone mass accretion and muscular strength and function during the growing years. It is therefore, an adequate nutritional vitamin D status during growth is important for the maintenance of musculoskeletal health, which significantly could contribute to attaining optimal peak bone mass in growing children and adolescents. It is strongly recommended that children and adolescent should participate in intervention programs to combat vitamin D deficiency. Several preventive strategies to maintain optimum nutritional vitamin D status, such as promoting the production of cutaneous vitamin D through sunlight exposure and intake of vitamin D rich foods such as milk or foods fortified with vitamin D. On the other hand, longitudinal studies with large growing populations warrant further investigation in order to determine what the optimal serum 25(OH)D concentration might be for maximal bone accretion during the time of rapid growth and also to identify the optimal levels of vitamin D intake in relation to greater bone mineralization and physical growth in children and adolescents.

References


5. Effects of vitamin D on bone growth and muscle strength


Key facts

- Our body can synthesize vitamin D from beneath the skin when exposed to ultraviolet light.
- 25-hydroxyvitamin D (25(OH)D) is the main reservoir of vitamin D in our body.
- 1,25-dihydroxyvitamin D is the active form of vitamin D and also a hormone.
- Hypovitaminosis D is defined as the level of 25(OH)D less than 30 ng/ml.
- Prevalence of hypovitaminosis D range from 40 to 70% in postmenopausal women and increases with age.
- The elderly above 70 years old are capable of synthesizing less than 30% vitamin D compared to the young when equally exposed to the sun.

Summary points

- Vitamin D plays a major role in calcium and phosphorus homeostasis.
- Vitamin D also plays a major role in muscle functions.
- Hypovitaminosis D is more prevalent in postmenopausal women and increases with age.
- Usually, hypovitaminosis D presents with no symptoms accept for some muscle fatigue and low bone mineral density.
- Replacement of vitamin D can improve muscle function and reduce the risk of falls in subjects that have hypovitaminosis D.
6. Hypovitaminosis D and muscle strength

T. Songpatanasilp

Division of Orthopaedic Oncology and Bone Metabolism, Department of Orthopaedics, Phramongkutklao College of Medicine, 315 Rajavithi Rd., Rajathavee, Bangkok 10400, Thailand; thaweesps@yahoo.com

Abstract

Vitamin D plays a major role in calcium and phosphorus homeostasis. The natural forms of vitamin D are ‘ergocalciferol’ from vegetable food, and ‘cholecalciferol’ from the synthesis beneath our skin when exposed to ultraviolet light. These two forms of vitamin D have to change into 25-hydroxyvitamin D (25(OH)D), the main reservoir of vitamin D in our body, by hydroxylation in the liver. Then 25(OH)D undergoes further hydroxylation in the kidney to 1α,25(OH)₂D, the active functioning hormone in our body. 1α,25(OH)₂D will interact with vitamin D receptor (VDR) at the cellular level and result in the transcription and translation of many vitamin D dependent proteins. Nowadays we can detect VDR in various tissues which confirms the many biological effects that 1α,25(OH)₂D might have, apart from calcium and phosphorus homeostasis. Those unexpected tissues include pancreas, placenta, pituitary, ovary, testis, mammary gland, muscle and heart. Our understanding of vitamin D deficiency syndrome has changed over the past 10 years. Hypovitaminosis D or vitamin D insufficiency refers to the low level of serum 25(OH)D which induces the depletion of tissue levels and the elevation of parathyroid hormone levels. Hypovitaminosis D can be defined as the serum level of 25(OH)D below 30 ng/ml (75 nmol/l). Hypovitaminosis D is more prevalent among postmenopausal women, ranging from 40 to 70% of this population and increasing with age. This high prevalence of hypovitaminosis D causes depletion of muscle function as well as an increased risk of falls in this elderly group. Many studies show that replacement of vitamin D can improve muscle function and reduce the risk of falls in subjects that have hypovitaminosis D.

Keywords: vitamin D insufficiency, vitamin D inadequacy, osteoporotic fractures, myopathy, falls
Abbreviations

1α,25(OH)₂D  1α,25-dihydroxyvitamin D  
25(OH)D  25-hydroxyvitamin D  
25(OH)D₃  25-hydroxycholecalciferol  
BMD  Bone mineral density  
DBP  Vitamin D binding protein  
HDM  Hypovitaminosis D myopathy  
PTH  Parathyroid hormone  
RXR  Retinoid-X receptor  
VDR⁻/⁻ mice  VDR knockout mice  
VDR  Vitamin D receptor  
Vitamin D2  Ergocalciferol  
Vitamin D3  Cholecalciferol

6.1 Introduction

Vitamin D is very well known for its vital role in controlling the metabolism of calcium and phosphorus in all kinds of vertebrates by working through 3 major organ systems, namely the intestinal, renal and skeletal system. 1α,25(OH)₂D, or the active form of vitamin D, functions as a major gear wheel within these systems which might be regarded as one of our body’s hormones and is sometimes called the ‘D-hormone’. Currently, it is known that 1α,25(OH)₂D has various target tissues and organs. We can classify these target tissues into 2 main categories (Demay, 2003): (1) target tissues related to mineral homeostasis including intestine, renal, bone and parathyroid gland are called the ‘traditional target tissues’; (2) target tissues unrelated to mineral homeostasis are called the ‘non-traditional target tissues’. Advancements in research involving VDR and VDR knockout mouse have vastly broadened our knowledge and understanding of the non-traditional target tissues (Li et al., 1998). For the past 10 years, VDR has been detected within various cells and tissues beyond the bone as well as the tissues related to the mineral metabolism. This suggests 1α,25(OH)₂D has a large number of non-traditional functions which have not been clearly investigated, involved with the function of tissues within various systems beyond the skeletal system. There are several lines of evidence supporting the theory that 1α,25(OH)₂D might play a role in controlling the proliferation and differentiation of cells and other tissues besides an osteoblast. Moreover, there is activity in immunoregulation, hair follicle cycling, blood pressure regulation, mammary gland development and also the ability to modulate the secretion of insulin and prolactin (Walters, 1992). Several lines of evidence report that muscle cells are one of the important target tissues of vitamin D. It has also been found that muscle cells can express classical VDR (Boland, 2005; Boland et al., 1985; Demay, 2003) as well as demonstrate the non-genomic actions (De Boland et al., 1994; Morelli et al., 1993) of vitamin D within muscle cells which will be described in detail later.
6. Hypovitaminosis D and muscle strength

6.2 The vitamin D endocrine system

The vitamin D endocrine system is very important to mineral homeostasis and skeletal integrity as well as to the development and physiology of other tissue systems. The question, therefore, is this: how is 1α,25(OH)2D which is a key functional molecule of this system, created? The source of 1α,25(OH)2D comes from a substance within a group of secosteroids called vitamin D2 which occurs naturally, and also comes from self-synthesis in the skin called vitamin D3. Vitamin D2 is a natural vitamin found in various types of plants, while vitamin D3, which is a precursor of 1α,25(OH)2D3, can be obtained from 2 sources, namely food and most importantly from the synthesis in the skin when exposed to ultraviolet light. Since our body can synthesize vitamin D3 by itself, cholecalciferol does not seem to fit well with the definition of a vitamin (theoretically, a vitamin should be categorized as a vital substance that our body needs but is unable to produce itself). The precursor of vitamin D3 is ‘7-dehydrocholesterol’ in skin, which requires ultraviolet light with frequency of 290-315 nm in sunlight (Holick, 2008) to turn it into vitamin D3. Then vitamin D3 will be bound by DBP and carried to the liver. Vitamin D3 will then be turned into 25(OH)D3 by the enzyme vitamin D-25-hydroxylase. 25(OH)D3, which is a major circulating form of vitamin D within mammals, is transported to the kidney by DBP. 25(OH)D3-DBP will be filtered by the glomerulus and reabsorbed via the active endocytotic process by the receptor called ‘Megalin’ (Holick, 2008) in order to preserve 25(OH)D3 and be conveyed to the renal tubular cells. 25(OH)D3 is turned into 1α,25(OH)2D3 by cytochrome P450 enzymes called ‘renal-25(OH)D3-1α-hydroxylase’. 1α,25(OH)2D3, or the active form of vitamin D, is held to be one of our body’s hormones for which the kidney is a key endocrine gland. The renal proximal tubular cells will create and adjust this hormone according to the physiological stimulation of the body, thus this whole system is known as the ‘vitamin D endocrine system’ (Figure 6.1). 1α-hydroxylase will be stimulated from the conditions of low serum calcium, low serum phosphate and high serum PTH. In contrast, 1α-hydroxylase is negatively regulated by the increased level of 1α,25(OH)2D3 itself (Holick, 2008). The inactivation and catabolism of 1α,25(OH)2D3 derives from the enzyme 25-hydroxyvitamin D3-24-hydroxylase which will hydroxylase 25(OH)D3 and 1α,25(OH)2D3 and turn it into 24,25(OH)2D3 and 1,24,25(OH)3D3 respectively (Holick, 2008), before being degraded and excreted from the body. This 24OHase system is regarded as the system which helps to keep the levels of 1α,25(OH)2D3 stable.

1α,25(OH)2D3 plays an important role in controlling various bodily systems by having the genomic transcription mechanism bound mainly to one of the nuclear receptors called VDR. VDR is one of the members of the classical endocrine nuclear receptor super-family as well as thyroid hormone receptors, receptors of sex hormone (estrogen receptor, progesterone receptor, etc.) and receptors of corticosteroid hormone. Upon binding to 1α,25(OH)2D3 the VDR will combine with RXR. This complex acts as a transcription factor regulating the transcription of various important genes within our body. Especially in the skeleton system, there are calcium binding proteins that regulate genes encoding proteins associated with calcium metabolism, genes encoding osteocalcin and osteopontin, as well as the genes that encode various proteins related with osteoblast proliferation and differentiation such as Runx2, COL1A1, RANKL, etc. (Atkins et al., 2005). This mechanism is commonly known as the ‘genomic pathway’. Moreover,
not only can 1α,25(OH)₂D₃ function without the binding process to the nuclear receptor; it is also capable of stimulation at the plasma membrane through the transmembrane receptor and forwarding signals internally via the signal transduction pathway. This process is similar to growth factors and other peptide hormones, and is called the ‘non-genomic pathway’ (De Boland and Nemere, 1992; Nemere and Farach-Carson, 1998).

6.3 The vitamin D receptor

The idea that vitamin D may act as a type of steroid hormone appeared in 1968 (Norman, 1968). Later 1α,25(OH)₂D was discovered to be an active hormonal form of vitamin D (Holick et al., 1971; Lawson et al., 1971; Norman et al., 1971). The fact that 1α,25(OH)₂D₃ is a lipophilic molecule and localized in the nucleus of target tissue, as well as the fact that vitamin D response is often inhibited by transcriptional inhibitors, have resulted in theories that there might be a protein which acts as a vitamin D nuclear receptor similar to other steroid hormones. The VDR itself was successfully cloned for the first time in poultry intestinal tissues (McDonnell et al., 1987). Current-day technologies allow us to detect VDR in various tissues which confirms
VDR belongs to a member of ligand-activated transcription factors in the nuclear receptor superfamily. $1\alpha,25(\text{OH})_2D$ upon binding to VDR will initiate several continuous reactions, beginning by forming itself into a heterodimer with RXR. Ligand VDR-RXR heterodimer then functions as an active transcription factor binding with high affinity to vitamin D response elements of the target genes which then induces the transcription (Sutton and McDonald, 2003). The process of ligand activated transcription is actually far more complicated and will be discussed in detail elsewhere in this book. The facts show that all the responses of each cell type to $1\alpha,25(\text{OH})_2D$ are different. In addition to the previously mentioned level of VDR as a determinant, more important factors are the quantities and types of co-modulators which vary within each cell type. There are 2 major co-modulators comprising the co-activators which promote the transcription upon binding to ligand VDR-RXR heterodimer complex, as well as the co-repressors which conversely inhibit the transcription when binding to the ligand VDR-RXR heterodimer complex (Sutton and McDonald, 2003). These co-modulator molecules are the main factors for determining the specific cellular response of each individual’s tissue type to the steroid hormone or what we call the ‘selective receptor modulator concept’.

### 6.4 Vitamin D deficiency and hypovitaminosis D

Age, location, time of day, season and skin color are all factors which affect the synthesis of vitamin D via skin. It has been shown that the elderly are more at risk of vitamin D deficiency syndrome for many reasons. When compared to the general youth, the over-70s are capable of synthesizing less than 30% vitamin D when equally exposed to the sun (Holick et al., 1989). Furthermore, the elderly might consume food with low levels of vitamin D or they might have worse intestinal absorption when compared to younger people. The other important reason could be impaired hydroxylation in the liver and the kidney; functional problems of these 2 organs are often found among the elderly. The level of $25(\text{OH})D$ in serum, which is the precursor of $1\alpha,25(\text{OH})_2D$, will be 1000 times higher than $1\alpha,25(\text{OH})_2D$ level and $25(\text{OH})D$ acts as the storage source of $1\alpha,25(\text{OH})_2D$. Therefore, it is preferable to measure the serum $25(\text{OH})D$ level rather than the vitamin D level in our body.

Our understanding of vitamin D deficiency syndrome has changed over the past 10 years. Vitamin D deficiency syndrome is determined by low levels of serum $25(\text{OH})D$, until the patient shows signs of a lack of vitamin D such as hypocalcemia, secondary hyperparathyroidism, muscle pain and weakness, sarcopenia, bone pain, insufficiency fractures, osteomalacia, etc. At this stage the serum $25(\text{OH})D$ level often remains very low. Most laboratories use the minimum value of the normal serum $25(\text{OH})D$ level of 6-20 ng/ml (15-50 nmol/l) as a criterion (Binkley et al.,
2004). Nonetheless, there are some discrepancies in differentiating ‘vitamin D insufficiency’ from ‘vitamin D deficiency’ as some researchers believe both syndromes have different effects as regards calcium homeostasis and bone metabolism. The term ‘vitamin D sufficiency’ is defined as the serum 25(OH)D3 level which has absolutely no effect on calcium homeostasis and signifies the normal condition. Whereas ‘vitamin D insufficiency’, which is sometimes called ‘hypovitaminosis D’ (Zittermann, 2003) or ‘vitamin D inadequacy’ or ‘mild vitamin D deficiency’, refers to the low level of serum 25(OH)D which induces the depletion of tissue levels and the elevation of PTH level. Hypovitaminosis D usually has no distinct clinical signs other than possibly some fatigue or aches and pains, and the serum calcium level still remains within the normal range while the serum 25(OH)D level which enhances the serum PTH level is different among each population group. The study by Malabanan (1998) designated the serum 25(OH)D level at 20 ng/ml (50 nmol/l) as a threshold. Also, Chapuy et al. (1997) found that the serum PTH level started to increase when the serum 25(OH)D3 level was lower than 31 ng/ml (78 nmol/l). Furthermore, Heaney (2003a,b) found that a serum 25(OH)D3 level of 32 ng/ml (80 nmol/l) did not increase of serum PTH level. Contrary, in a study in North Eastern Thailand (Soontrapa et al., 2001) conducted in elderly women (average age 69 years old) found that the serum PTH level started to increase when the serum 25(OH)D level was lower than 35 ng/ml (87.5 nmol/l), and significantly increased when the serum 25(OH)D level was lower than 30 ng/ml (75 nmol/l). It is still somewhat controversial to use the serum 25(OH)D value as an indicator of a patient’s conditions, especially when the clinical criteria are used to judge whether the symptoms will occur or not, which are often variable in each person. The point at which the serum PTH level starts to increase is still unclear due to the difference in threshold within each population. Moreover, the value of serum 25(OH)D which is regarded as a normal value for each laboratory is still controversial. On average, the prevalent use of normal serum 25(OH)D values is much lower than it should be due to the overestimation of vitamin D toxicity state (Vieth, 1999, Holick, 2003).

Figure 6.2. shows the ranges of the serum 25(OH)D values for the classification of the vitamin D condition. The criteria for assigning the serum 25(OH)D values to different classes are as follows:

- **Vitamin D deficiency: less than 20 ng/ml (50 nmol/l).** The serum 25(OH)D levels below 20 ng/ml (50 nmol/l) significantly determine the condition of vitamin D deficiency. However, some authorities believe that this value is too low. Heaney (2003c) and Holick (2003) still affirm that the serum 25(OH)D level should be higher than 30 ng/ml (75 nmol/l) in order to have no symptoms.

- **Vitamin D insufficiency: less than 30 ng/ml (75 nmol/l).** According to Zittermann (2003), the condition of hypovitaminosis D which started to cause a decrease in vitamin D tissue levels and an increase in serum PTH levels would correlate with the serum 25(OH)D at approximately 30-40 ng/ml (75-100 nmol/l). Dawson-Hughes et al. (1997a) demonstrated that the PTH level increased when the serum 25(OH)D level was lower than 45 ng/ml (112 nmol/l) among elderly men and women. Kinyamu et al. (1998) also conducted the study specifically in elderly women and found that serum PTH level started to increase when the serum 25(OH)D level was lower than 49 ng/ml (122 nmol/l). However, as mentioned before, the studies by Chapuy et al. (1997), and Heaney et al. (2003a,b), have concluded that the optimal cutoff level of 25(OH)D from which the serum PTH level started to increase should
be 31-32 ng/ml (77.5-80 nmol/l). We conclude from the above-mentioned study that the serum 25(OH)D level which is classified as vitamin D insufficiency should be around 20-40 ng/ml (50-100 nmol/l). Recent recommendations by several organizations suggest that the normal 25(OH)D level should begin at 30 ng/ml (75 nmol/l) and above (Dawson-Hughes et al., 2005; Hanley et al., 2010; Mithal et al., 2009; National Osteoporosis Foundation, 2008). There are two reasons for setting the low end of the normal range for 25(OH)D at 30 ng/ml (75 nmol/l): (1) studies published in the last years, mostly suggests that levels of PTH rise when levels of 25-hydroxyvitamin D fall below 30 ng/ml (75 nmol/l) (Heaney, 2005; Holick, 2007; Steingrimsdottir et al., 2005); (2) earlier studies suggests that active calcium absorption is optimal when the level of 25(OH)D is 30 ng/ml (75 nmol/l) (Chapuy et al., 1997).

- **Optimal vitamin D status: 30-65 ng/ml (75-160 nmol/l).** Zittermann (2003) stated that the sufficient and normal value of serum 25(OH)D should fall within the range of 40-80 ng/ml (100-200 nmol/l). After having reviewed many of the above-mentioned studies, the lower limit of normal range has been accepted as 30 ng/ml (75 nmol/l) (the normal vitamin D level should not be less than this) whereas the upper limit value is still unclear. But it is clear that the value must be safe from vitamin D excess and vitamin D toxicity. Zittermann (2003) concluded that the serum 25(OH)D level which was safe from any toxicity could rise up to 100 ng/ml. However, according to the study of the safe upper limit, it was found that respondents who only exposed themselves to sunlight could raise the serum 25(OH)D level higher than 80 ng/ml (200 nmol/l). And those who consumed vitamin D via oral supplements up to 10,000 IU daily would raise the serum 25(OH)D level higher than 80 ng/ml (200 nmol/l) with no toxicity found for both cases (Heaney, 2003a). Thus, the use of 65 ng/ml (160 nmol/l) as a criterion would be the most appropriate value to ensure higher safety levels.

- **Vitamin D excess: greater than 80 ng/ml (200 nmol/l) with accompanying hypercalcemia.** As mentioned above, a serum 25(OH)D level higher than 80 ng/ml (200 nmol/l) does not specify an occurrence of toxicity except if the clinical signs of accompanying hypercalcemia appear. Vieth (1999) stated that for hypercalcemia from hypervitaminosis D the serum 25(OH)D level must always be higher than 88 ng/ml (220 nmol/l). Holick (2000) also reported that vitamin D intoxication would not occur until the level was higher than 125 ng/ml (312 nmol/l). Therefore, the criterion expressing the vitamin D excess level should be higher than 80 ng/ml (200 nmol/l), and the vitamin D toxicity level is indicated when are also clinical signs of hypercalcemia.

![Figure 6.2 The classification of deficient-excessive conditions of vitamin D by the serum 25(OH)D3 levels, based on the current research. (Vasquez, 2004)]
There are not many studies regarding vitamin D deficiency and hypovitaminosis D in Thailand as it is often believed that a tropical country should have no problem of vitamin D deficiency owing to the abundance of sunlight. The study by Chailurkit et al. (1996) in 158 men and women aged between 20-80 years found that the average value was 42 ng/ml (106 nmol/l) and concluded that there was no vitamin D deficiency in Thailand. However, there were only 39 postmenopausal women among volunteers within this study. The study by Soontrapa et al. (2001) among 106 Thai elderly women (average age at 69 years) in Khon Kaen province found that the serum PTH level would increase when the serum 25(OH)D level started to decrease by 35 ng/ml (87.5 nmol/l), and it would increase significantly when the serum 25(OH)D level was lower than 30 ng/ml (75 nmol/l). It was shown from this study that at a threshold level of 30 ng/ml (75 nmol/l), 35% would have hypovitaminosis D, but when a threshold level of 35 ng/ml (87.5 nmol/l) was used, 65% would have hypovitaminosis D.

A study by Songpatanasilp et al. (2009) measuring the serum 25(OH)D level among 72 elderly women aged above 65 years old (average age 71 years) who all attended the Orthopaedics clinic at Phramongkutklao hospital with various diseases, found that hypovitaminosis D [defined as the level below 30 ng/ml (75 nmol/l)] was detected up to 64%, the same very high level as found by Soontrapa et al. (2001). It might be possible that even though vitamin D deficiency is rarely found among the general population, it can be commonly found in some subpopulations, such as very old postmenopausal women (>65 years) who attended the hospital with various kinds of musculoskeletal pain. All the above-mentioned studies in Thailand correspond to other studies carried out in neighboring tropical countries (Gannage-Yared et al., 2000; Ghannam et al., 1999; Goswami et al., 2000). Thus it is possible to conclude that a problem of hypovitaminosis D and vitamin D deficiency exists, and that it is more prevalent than expected in tropical countries.

6.5 Osteomalacic myopathy and hypovitaminosis D myopathy

The symptom of ‘osteomalacic myopathy’ can often be found in patients with osteomalacic bone disease, and both conditions can be considered major symptoms of vitamin D deficiency muscle weakness. Hypotonia is also found a major symptom of these conditions, sometimes referred to as ‘vitamin D deficiency-related myopathy’. The classical condition will be the proximal muscle weakness especially in the areas of extensor, flexor and abductor muscles of hip, and also the flexor, extensor muscles of knee, as well as the accompanying diffuse skeletal pain. Skaria et al. (1975) reported the 25 osteomalacia patients who expressed abnormality in a muscle electromyogram which showed the characteristics of both the myopathy and also the decrease in nerve conduction velocity. However, whether the peripheral nerve is involved or not remains controversial. Yoshikawa et al. (1979) conducted the muscle biopsy in osteomalacia patients and found atrophy of type-II muscle fibers as well as dilation of interfibrillar spaces which were infiltrated with fat, fibrosis and glycogen granules. These characteristics were different from the neuropathic muscle in which the atrophy was found in both type-I and type-II muscles, and they were also different from immobilization muscle atrophy in which the atrophy was found only in type-I muscle. In circumstances where a sudden movement is required, the type-II muscle...
6. Hypovitaminosis D and muscle strength

fibers which can contract stronger and faster will function first to prevent falling (McComas, 1996). This helps to explain why patients with vitamin D deficiency, mainly affecting type-II fibers, will have a higher tendency to fall. We have discovered that the myopathy state can be detected before the biochemical signs of osteomalacia are present (Glerup et al., 2000) or even found in falling patients who have a hip fracture even before secondary hyperparathyroidism has occurred (Bischoff-Ferrari et al., 2003). We refer to the myopathy state which occurs without other symptoms of vitamin D deficiency as HDM (Glerup and Eriksen, 2005). Such a myopathy state has long been overlooked and misdiagnosed for 2 reasons: (1) the decrease in muscle strength is a phenomenon which gradually and continually occurs whereas the loss of functional ability is not a continual phenomenon; in addition the muscle strength of patients might slowly be wearing down anyway, and patients will only complain if they really feel tired or unable to do what they could do before. (2) the symptoms of HDM are quite unspecific and there are many symptoms of other diseases which are similar to the group of non-specific rheumatic diseases such as polymyalgia, fibromyalgia and psychoneurotic disorders, etc. Recent studies have found that the most distinct symptom of HDM (Glerup and Eriksen, 2005) is a diffuse muscle pain especially in the proximal muscles of lower limbs, arms and muscle around the shoulder which could later develop into deep bone pain, fatigue, inability to walk upstairs, inability to stand up from a chair or lift things (even light objects), and probably peripheral paresthesia, muscle cramps as well as joint pain. All the above-mentioned symptoms will correlate significantly with the serum 25(OH)D but not quite correlate with the serum 1α,25(OH)₂D (Glerup, 1999).

A study conducted in elderly patients (65-95 years old) whose 25(OH)D serum level was <12 ng/ml (30 nmol/l) (Bischoff-Ferrari et al., 1999) found a significant correlation between the serum 25(OH)D and the leg extension power. This was in line with a study by Mowe et al. (1999) in 349 elderly people (>70 years old) whose serum 25(OH)D level was significantly lower in the group that had less handgrip strength, that were unable to walk upstairs, that undertook no outdoor activities and that had fallen at least once within the past month. Moreover, Geusens et al. (1997) conducted an interesting study to explore the relationship between the VDR polymorphism and the muscle function in nonobese women over 70 years old and found the difference between quadriceps muscle strength statistically significant (P<0.01) between the group that has bb genotype and BB genotype. A study by Songpataesilp et al. (2009) in Thai postmenopausal women [average age of 71 years old] also demonstrated that the serum 25(OH)D level of respondents was below 30 ng/ml (75 nmol/l). The respondents were randomized and allocated to two groups and the focus group received alfacalcidol (1α-hydroxyvitamin D) combined with calcium. It was shown that the quadriceps muscle strength measured using an isokinetic dynamometer could be improved significantly compared to the other group which received calcium only.

Until now, only two studies have examined whether vitamin D supplementation may have an impact on muscle fiber composition. In a small uncontrolled study, Sorensen et al. (1979) obtained muscle biopsies from elderly women after treatment with 1α-hydroxyvitamin D and calcium for 3-6 months. Results showed an increase in relative fiber composition and in fiber area of type II muscle fibers. More recently, a randomized controlled study found that treatment
of elderly stroke survivors with 1000 IU of vitamin D2 daily significantly increased mean type II muscle fiber diameter and percentage of type II fibers over a 2 year period (Sato et al., 2005). There was also a correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after two years of follow-up (Sato et al., 2005). It remains unclear, however, if the increase in type II muscle fiber number is caused by new formation of type II fibers or a transition of already existing fibers from type I to type II. Nevertheless, the effect on muscle caused by vitamin D appeared to be the direct result of vitamin D itself rather than the indirect result of the increase in calcium.

6.6 Genomic and non-genomic effects of vitamin D on muscle

We now recognize that striated muscle cells can express VDR and also find that vitamin D can stimulate the synthesis of various important muscle proteins such as troponin-C and actin in the sarcoplasmic reticulum and the inner membrane of mitochondria by genomic transcription process (Glerup and Eriksen, 2005). Endo et al. (2003) conducted study in VDR⁻/⁻ mice which had distinct clinical signs of vitamin D resistant rickets (i.e. hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and osteopathy). When VDR⁻/⁻ mice received a high calcium diet [rescue diet] from birth, those mice remained normal until this diet was no longer provided. However, the muscle abnormality was detected in the diseased VDR⁻/⁻ mice and the VDR⁻/⁻ mice that were on the rescue diet, showing that such muscle abnormality was independent of the secondary effect from metabolism change. The study in cultured myoblast found that the VDR was necessary in the normal development and gene expression of muscle cells. Endo et al. (2003) also found that the abnormality of muscle which occurred as a result of the ligand-dependent action of VDR in the protein transcription was related to muscle cell proliferation and differentiation. In addition, the genomic effects of vitamin D in muscle cells are also related to the synthesis of proteins used in the regulation of muscle cell proliferation and differentiation (Drittanti et al., 1989b), calcium transport (Drittanti et al., 1989a) and phospholipid metabolism (Drittanti et al., 1988). Calcium binding proteins, such as calbindin-D9K, are examples of newly synthesized myoblast proteins expressed by activation of the nuclear VDR (Zanello et al., 1995). Prolonged treatment with 1α,25(OH)₂D has been shown to affect the synthesis of certain muscle cytoskeletal proteins which are important in controlling muscle cell functions (Boland et al., 1995). Other 1α,25(OH)₂D-dependent proteins have been described as calmodulin-binding components of the myoblast cytoskeleton (Brunner and de Boland, 1990). Calmodulin is a calcium-binding protein that regulates several cellular processes including muscle contraction. Experiments in mitotic myoblasts treated with 1α,25(OH)₂D revealed increased synthesis of calmodulin (Drittanti et al., 1990). In vitro and in vivo experiments in chick skeletal muscle have shown that 1α,25(OH)₂D regulates muscle calcium uptake by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma. It also regulates the calcium influx via voltage-sensitive calcium channels thereby altering intracellular calcium (Boland, 1986). Modifications in intracellular calcium levels control muscle contraction and relaxation, thus impacting muscle function (Boland et al., 1995, Ebashi and Endo, 1968). 1α,25(OH)₂D also appears to play a significant role in the regulation of phosphate metabolism of myoblastic cells (Boland et al., 1995). In skeletal
muscle cells phosphate in the form of ATP or inorganic phosphate is necessary for the structural and metabolic needs of the cell. Exposure to 1α,25(OH)2D stimulates accelerated phosphate uptake and accumulation in these cells.

Apart from the classical genomic effects of vitamin D, also the various non-genomic effects of vitamin D were discovered (Figure 6.3). The definition of non-genomic pathway means that 1α,25(OH)2D could act by binding to the membrane receptor, causing the stimulation of ‘second messenger systems’ which will transmit the signal to the cytoplasm; this effect is sometimes called the ‘fast effect’ (Boland, 2005). Such stimulation results in: (1) the activation of Ca2+ flows into cells via G-protein-mediated voltage-dependent Ca2+ channels by means of the adenylyl cyclase/cAMP/protein kinase A pathway (De Boland et al., 1995) and the PLC/DAG+IP3 pathway (Morelli et al., 1996). Moreover, the Ca2+ release can also be stimulated from the intracellular stores – the store-operated calcium channels; (2) the proliferation and growth of muscle cells by the stimulation of tyrosine phosphorylation of the MAP kinase pathway (Boland, 2005); and (3) induction of rapid translocation of VDR towards the cell membrane via the tyrosine kinase pathway (Boland, 2005).

It has been shown that 1α,25(OH)2D has a significant and direct effect on muscle calcium action as well as the growth and differentiation of muscle cells, whether it is a genomic or non-genomic effect. 1α,25(OH)2D has a similar effect on the muscular system to one of the hormones in this system which performs the control work extremely well. Previously there has not been much interest in this subject, however, recently, we have begun to understand it better.

Figure 6.3. Non-genomic action of 1,25(OH)2D3 in the stimulation of membrane vitamin D receptor (mVDR) towards second messenger of protein kinase A (PKA) and C (PKC) pathways (Feldman et al., 2005).
6.7 Vitamin D and falls

Chapuy et al. (1992) inspired many researchers on the relationship between muscular effects and vitamin D. The authors conducted the study on 3,270 elderly people who lived in a nursing home showing that the hip fracture incidence could be lowered by 43% in the group that received the 800 IU of vitamin D3 and 1,200 mg of calcium per day compared to the placebo group over a period of 18 months. The study caused great astonishment since there was almost no change in BMD (~2% increase from baseline), and the hip fracture reduction still remained after 3 years. Moreover, the study of Dawson-Hughes et al. (1997b) found 58% reduction in non-vertebral fracture in 399 elderly people (>65 years) who received 700 IU of vitamin D supplement and 500 mg of calcium. The idea that vitamin D + calcium could lower the incidence of fractures without increasing BMD might derive from the ability to improve the muscle strength and reduce the risk of falls by vitamin D.

On average, 33% of elderly people fall at least once a year and approximately 6-7% of them experience a hip fracture as a result (Graafmans et al., 1996; Tinetti et al., 1988). As mentioned, vitamin D deficiency and hypovitaminosis D often cause muscle weakness especially in the weight-bearing muscles of lower limbs which are very important to postural balance and walking. More muscle weakness will affect the postural balance in standing, walking and the changing of posture. Body sway and poor functioning of weight-bearing muscles also contribute to falling more easily. Moreover, a reflex to prevent falling as well as a change of posture lower than expected will normally cause elderly people to fall on their back, having the hip, the back and the head hit by the floor, rather than falling forward using their hands to stop the fall.

The study by Mowe et al. (1999), as well as the study by Stein et al. (1999) on elderly people found a significant correlation between serum 25(OH)D3 and occurrence of falls. A study in a ‘falls clinic’ in London (Dhesi et al., 2002), with patients that have fallen at least once within 8 weeks attending the clinic, found that up to 32% of patients had vitamin D deficiency (25(OH)D <12 ng/ml), while up to 73% patients had mild vitamin D deficiency (25(OH)D <20 ng/ml). The patient group with serum 25(OH)D3 level less than 12 ng/ml (30 nmol/l) was found to have significantly more accompanying impaired psychomotor function, increased body sway, impaired reaction time and decreased isometric quadriceps muscle strength.

6.8 Therapeutic implications

Chapuy et al. (1992) and Dawson-Hughes et al. (1997b) demonstrates the significant antifracture efficacy of vitamin D in combination with calcium while being independent of BMD. The fracture reduction is very distinct, especially for hip and non-vertebral fractures. It seems that the anti-fracture efficacy of vitamin D + calcium is not the result of increasing BMD, but possibly the fact that vitamin D + calcium can lower secondary hyperparathyroidism. Alternatively, it can result from the preservation of bone strength, and the decrease in bone turnover, both of which preserve bone quality. However, a very interesting and plausible issue is the effect of vitamin
D in combination with calcium in decreasing the risk of falls. Bischoff-Ferrari et al. (2004) conducted another meta-analysis study among 1,237 subjects as a collection of 5 randomized controlled trials. They found that the group that received vitamin D in combination with calcium decreased the risk of falling by 22% [corrected OR, 0.78, 95% CI 0.64-0.92] compared to the group that received only calcium or the placebo. This study showed the number needed to treat to be 15 [95% CI, 8-53] which is encouraging in terms of health economy. To conclude, vitamin D supplementation with the administration of calcium could effectively decrease the risk of falls in elderly people by more than 20% (Bischoff-Ferrari et al., 2004).

However, this study did not answer questions about the appropriate dose of vitamin D and calcium, the appropriate frequency and the method of treatment as well as the appropriate length of treatment. However, Bischoff-Ferrari et al. (2004) recommended the dose of vitamin D at 700-800 IU/day and recommended that the accompanying calcium supplement should always be administered as well. Generally, we can see that the treatment of osteoporosis will focus on the baseline of patients, i.e. that they should receive adequate treatment with calcium and vitamin D. As mentioned before, even elderly people living in hot areas might still receive less than adequate amounts of vitamin D. Also, the general diet in Thailand mostly contains low levels of calcium, hence the treatment of osteoporosis in Thai elderly people should always put great emphasis on treatment with vitamin D and calcium supplement. In addition to antiresorptive treatments for the general indication, vitamin D and calcium supplements can at least help decrease the risk of falls which is an alternative mechanism for decreasing the risk of fracture, as well as enhancing the better anti-fracture efficacy of other medicines.

Reference


T. Songpatanasilp


6. Hypovitaminosis D and muscle strength


6. Hypovitaminosis D and muscle strength


Songpatanasilp, T., Chailurkit, L., Nichachotsalid, A. and Chantarasorn, M., 2009. Combination of alfacalcidol with calcium can improve quadriceps muscle strength in elderly ambulatory Thai women who have hypovitaminosis D: a randomized controlled trial. Journal of the Medical Association of Thailand 92(Suppl5), S30-41.


Key facts

- Vitamin D deficiency is highly prevalent among children and adolescents due to limited sunlight exposure and inadequate dietary intake.
- Vitamin D is required for a normal function of the body.
- Altogether 37 tissues present vitamin D receptor (VDR).
- Deficiency affects growth and development, but if corrected while growing most of the consequences are overcome.
- VDR polymorphism may modify the response of vitamin D supplementation.

Summary points

- Vitamin D deficiency causes rickets affecting the well-being of an individual in multiple ways, i.e. skeletal mineralization, axial growth, immune responses and muscle function.
- Maternal vitamin D status is associated with bone size and bone mass in newborn in several studies, and this association may remain throughout childhood.
- In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth.
- High doses are needed to overcome rickets and its skeletal consequences.
- Vitamin D supplementation benefits bone mineral accrual in apparently healthy prepubertal girls. Most pronounced effects are seen on cortical rich sites.
- Although VDR polymorphism may explain differences observed in growth pattern, stature or bone mineral density, it is more likely to modify the response to vitamin D supplementation.
7. Vitamin D and growth

H.T. Viljakainen¹² and E. Hyppönen³
¹Children’s Hospital, University of Helsinki, P.O. Box 705, 00029 HUS, Finland; ²Musculoskeletal Research Unit, School of Clinical Sciences, University of Bristol, Southmead Hospital, BS10 5NB Bristol, United Kingdom; ³Centre for Paediatric Epidemiology and Biostatistics and Medical Research Council Centre for the Epidemiology of Child Health, University College London Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom; heli.viljakainen@helsinki.fi

Abstract

Linear growth requires health and adequate nutrition. Stunted growth is a growth disorder with multiple symptoms. In Asia 35-65% of under 5-year olds have stunted growth. Rickets is a consequence of severe vitamin D deficiency and can be seen as a special case for stunted growth. Nutritional vitamin D status is commonly measured with serum 25-hydroxyvitamin D (25(OH)D). Clinical symptoms of rickets are typically associated to 25(OH)D level below 12.5 nmol/l, but the normal function of body may require concentrations above 50 nmol/l. Rickets may relate to delayed or difficulties with crawling and walking and it commonly presents with comorbidities such as continuous or recurrent respiratory infections. We reviewed studies reporting both bone mass accrual and linear growth as an outcome and vitamin D as an exposure. ‘Vitamin D’ includes here intake of vitamin D, it’s nutritional status, and closely related genetic variations. We further evaluate studies using vitamin D to prevent stunting of growth, and also characterize the effect of vitamin D deficiency induced rickets on growth. Vitamin D deficiency causes rickets affecting the well-being of an individual in multiple ways, i.e. skeletal mineralization, axial growth, immune responses and muscle function. Maternal vitamin D status is associated with bone size and bone mass in newborn in several studies, and this association may remain throughout childhood. In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth, but to overcome rickets and its skeletal consequences high doses are needed. Vitamin D intervention benefits the bone mineral accrual in apparently healthy prepubertal girls. Most pronounced effects are seen on cortical rich sites and to lesser extent in trabecular bone. Although vitamin D receptor polymorphism may explain differences observed in growth pattern, stature or BMD, it is more likely to modify the response to vitamin D supplementation.

Keywords: growth, skeletal outcomes, children, adolescent, long-term consequences, VDR polymorphism, supplementation
7.1 Introduction

Linear growth requires health and adequate nutrition. Careful monitoring of growth helps to reveal underlying disease in an individual. At the population level, growth patterns reflect general well-being and changes in living standards over decades. A pragmatic way to describe growth is to measure increases in height, weight or circumference (head or mid-upper arm) over time. During childhood, growth and development are tightly linked, and it is often difficult distinguish influences on linear growth from those affecting the process of maturation.

Stunted growth is a growth disorder with multiple symptoms. It is very common in low income countries and is thought to be a complex implication of nutritional, environmental and social factors. According to a Unicef report (Unicef, 2012) as many as 35-65% of under 5-year olds have stunted growth in the region of India and China. This is illustrated by lower than -2 SD (or less than 10th percentile) average birth weight in these populations, ceased growth rate and eventually small size in adulthood. Improved standards of living associate with lower rate of stunted infant growth. Because of its complexity interventions to prevent stunted growth are hard to establish. Rickets is a well-known consequence of severe vitamin D deficiency and can be seen as a special case for stunted growth. Nutritional vitamin D status is commonly measured by circulating concentrations of 25(OH)D. Clinical symptoms of rickets are typically associated to 25(OH)D level below 12.5 nmol/l (always below 25 nmol/l) (Bishop, 1999). While there is no general consensus for adequate vitamin D concentrations, recent review by Institute of Medicine indicated that concentrations over 50 nmol/l are required for normal function of body (Institute of Medicine, 2011). In this chapter, we will review studies reporting influences both on bone mass accrual and linear growth with the exposure ‘vitamin D’ including the intake of vitamin D, it’s nutritional status, and closely related genetic variations. We will further evaluate studies using vitamin D to prevent stunting of growth, and also characterize the effect of vitamin D deficiency induced rickets on growth.
7.2 General about growth and bone mass accrual

During fetal life increase in bone mass occurs mainly through intramembranous ossification. Highest rate of calcium accretion is noted during the third trimester (Prentice et al., 2006) together with highest growth velocity (Gardosi, 1997). Growth velocity continues to be high immediately after birth, slows down after 2 years and accelerates again later in childhood, finally ceasing after puberty. Typically longitudinal bone growth in infancy affects more appendicular than axial skeleton, which matures in puberty (Boot et al., 1997). Growth in the length of the long bones occurs in the growth plates through endochondral ossification; a cartilage rich form of bone matures and calcifies into bone (Heaney et al., 2000). Longitudinal bone growth ceases after puberty as the growth plates fuse, but modeling in term of mineral apposition continues throughout the life (Heaney et al., 2000). Periosteal apposition increases the outer diameter of bone: mineral is placed far away from central axis to increase mechanical strength. Androgens and weigh bearing exercise stimulate periosteal apposition, which explains why men and athletes have bigger bones. Opposite to this is modifications of endosteal surface, thus a phenomenon responsible for wall thickness. In females extra mineral is placed under endosteal envelope, where it can be utilized for increased requirements later in life (Prentice et al., 2006).

Growth trajectory is suggested to be set during the fetal period; metabolic and endocrine systems are programmed to adapt the fetus to the outside-utero environment (Jansson and Powell, 2007). This may explain seasonal variation of birth outcomes, e.g. birth weight, overall size, limb length, head size and skin-fold thickness observed in several studies (McGrath et al., 2005; Murray et al., 2000). Influences on fetal growth may be mediated through growth hormone – IGF-I axis, whereas bone quality may be determined by factors related to differentiation of mesenchymal stem cells (Lanham et al., 2008; Zhou et al., 2010). The intrauterine environment is suggested to have long-term consequences on growth up until puberty: intrauterine nutritional deficits have permanent consequences and that a newborn’s metabolism may not adapt to improved nutritional status (Gluckman and Hanson, 2004). To support this proposition, several studies have reported a relationship between growth pattern (during the fetal period, infancy and early childhood) and lower adult BMD or increased fracture risk later in life (Cooper et al., 1997, 2001; Yarbrough et al., 2000).

7.3 Rickets and growth

Vitamin D deficiency in childhood is the most common cause of rickets, which is characterized by disturbed bone formation: bone formation rate is accelerated but mineralization of growth plates is stunted (Parfitt, 2003). This affects the shaping and the strength of bones, leading to low BMD and bowing of weight-bearing bones (Bishop, 1999). Metaphyseal bone sites are typically widened in patients with rickets. Rickets may relate to delayed or difficulties with crawling and walking (Agarwal et al., 2009; Thacher et al., 2000), and it commonly presents with comorbidities such as continuous or recurrent respiratory infections (Karatekin et al., 2009; Laaksi et al., 2007; Wayse et al., 2004) and muscle weakness (Pettifor and Prentice, 2011). Osteomalasia is the adult form of rickets, and results from a chronic vitamin D deficiency affecting both bone...
and muscle. It is more difficult to diagnose because of a mixture of related symptoms which include muscle or bone pain, increased risk of fractures, and progressive deformities (Narchi et al., 2001). Neonatal consequences of vitamin D deficiency include hypocalcemia, big fontanelles, and bone deformities. Severe cases can present with seizures with extreme complications such as cardiac failure reported in some studies (Maiya et al., 2008; Uysal et al., 1999). Asymptomatic, mild rickets may remain unrecognized during growth, but related consequences can sometimes present later in life with symptoms such as increases in the risk of fractures or complications with labor due to related bone malformations in the pelvis.

Bone modeling and mineralization occurs in metaphysis of long bones whereas epiphysis is responsible for longitudinal growth (Rauch et al., 2001). In rickets newly formed bone is hypomineralized, but the rate of longitudinal growth is normal or even accelerated (Parfitt, 2003). This means that in rickets the collagenous format of the bone remains longer and is readily deformed to bowlegs, asymmetrical skull or bulges in the rib cage (Petitfor and Prentice, 2011). Bone growth is illustrated by bone turnover markers: during growth both formation and resorption markers are increased. Growth is an anabolic situation and formation overrules bone turnover. Several studies have demonstrated that in rickets especially bone specific alkaline phosphatase and pro-collagen type I aminoterminal peptide are elevated because of activated osteoblasts but not osteocalcin (Brooke et al., 1980; Soliman et al., 2010). However, after correction of vitamin D deficiency, bone turnover typically decelerates and markers of mineralized matrix such as osteocalcin are normalized.

7.4 Maternal vitamin D status, fetal bone mineralization and growth

The effect of maternal vitamin D status on fetal bone mass has been a focus in several prospective studies (Akcakus et al., 2006; Viljakainen et al., 2010; Weiler et al., 2005), but as reviewed below, findings have been somewhat inconsistent. In a Finnish study maternal vitamin D status was measured twice during pregnancy at 13th week and 2 days postpartum, the mean of these two was most strongly associated with newborn bone characteristics (Viljakainen et al., 2010). The median S-25(OH)D in the group of 100 women was less than 50 nmol/l, with nearly 70% of mothers having concentration below 50 nmol/l. Children born to mothers with inferior vitamin D status had 16% smaller bone CSA and 14% lower mineral content in distal tibia compared to children born to mothers with superior vitamin D status (Viljakainen et al., 2010). The bone measures were performed from the diaphysis of tibia with peripheral quantitative computed tomography. Larger bone size accompanied with higher mineral content accounts for higher bone strength at birth. In contrast, Akcakus et al. (2006) found no relationship between maternal vitamin D status and infant bone indices at birth. This could be due to extremely poor vitamin D status in the whole cohort, since nearly 90% of mother-child pairs had 25(OH)D below 25 nmol/l. In another study, lower maternal 25(OH)D was related to greater fetal femoral metaphyseal CSA, but not to femoral length at 19 and 34 weeks’ gestation (Mahon et al., 2010). Femoral characteristics were measured with high-resolution 3D ultrasound from over 400 mothers in Southampton in UK. Authors calculated spaying index (a ratio of CSA to length) which is used to characterize rickets and this was also associated to lower maternal 25(OH)D concentration.
7. Vitamin D and growth

Findings were somewhat controversial from a study on full-term and normal birth weight infants, where vitamin D deficiency was associated with greater weight and length but lower bone mass relative to body weight (Weiler et al., 2005). This finding could be a consequence of faster growth in utero which intervenes maternal vitamin D status since corresponding has been reported (Viljakainen et al., 2010). These findings could also suggest that mothers with larger babies had lower vitamin D status at the end of the gestation than mothers with smaller babies. Similarly, it is proposed that maternal parathyroid hormone concentrations increase due to greater mineral demands of bigger babies (Brooke et al., 1980). Vitamin D status of pregnant women is typically lower compared to non-pregnant women (Holmes et al., 2009), with concentrations declining as the pregnancy proceeds (Holmes et al., 2009). Declining concentrations may be caused by volumetric dilution, or possibly, because of increased vitamin D requirement induced by the growing fetus.

There are only a few studies investigating the association between maternal vitamin D status and birth length, while more studies have focused on birth weight. In an Australian study, newborns to vitamin D deficient mothers had 4.5 mm shorter knee-heel length; thought to be most reliable measure of intrauterine long bone growth (Morley et al., 2006). Newborns to vitamin D deficient mothers also had smaller mid-upper arm and calf circumference than newborns to mothers with superior vitamin D status. In this study, vitamin D deficiency was also associated with shorter duration of gestation by 0.7 week, which appeared to explain differences in knee-heel length at least partly, and no differences in circumferences were noted after adjusting for gestation length.

There are conflicting reports on maternal intake of vitamin D and offspring birth weight (Scholl and Chen, 2009). In a study from Teheran correlations were found between adequate maternal calcium and vitamin D intake with both appropriate birth weight and weight gain of mothers during pregnancy (Sabour et al., 2006). A large-scale prospective study in Netherlands searched for an association between maternal vitamin D status with fetal and neonatal growth in a multi-ethnic cohort (n=3,730) (Leffelaar et al., 2010). They compared newborn size and growth in three groups which were defined by maternal vitamin D status (≤29.9, 30-49.9, ≥50 nmol/l) at 12th week of gestation. Mothers and their newborns were followed 12 months after the birth. Altogether 2,355 children completed the study with detailed growth records. Compared to group with adequate vitamin D status (≥50 nmol/l) children born to mothers with inferior 25(OH)D had lower birth weight (3,418, 3,506 and 3,560 g, in above mentioned groups, respectively) and higher proportion of them were considered as SGA (14.8, 9.4 and 6.8%, in groups, respectively). However, ethnicity was not equally distributed across groups, which complicates the interpretation of results. Crude values showed that difference in birth weight between deficient and adequate group was 64 (29-107.5) grams in favor of adequate vitamin D status and similarly the risk for SGA was 1.9 times higher (1.4-2.7) after adjusting for possible confounders including ethnicity (Leffelaar et al., 2010).

Experimental evidence for an association with maternal vitamin D intake and infant growth from supplementation trials with vitamin D is scarce, but results are relatively consistent. One of the earliest randomized controlled trials studied Asian originated immigrants in United
Participants in the study, although a part of an affluent society in south London, were severely vitamin D deficient (mean 20 nmol/l) at enrolment which was between 28 and 32 weeks’ gestation (Brooke et al., 1980). Daily supplementation with 1000 IU improved maternal and cord 25(OH)D concentration and maintained normalized serum calcium and total alkaline phosphate activity in cord blood when compared to placebo group. However, no differences in mean weight, length, or other anthropometrics were observed at birth. On the other hand, 50% lower proportion of newborns in the supplemented group were SGA when compared to placebo (Brooke et al., 1980). These findings were supported with a similarly study design (Maxwell et al., 1981). In addition, they observed that maternal weight gain was greater in supplemented group. Brooke’s group extended the follow up of the mother and child pairs (Brooke et al., 1981). After 1 year of follow up they reported that children whose mothers received supplementation during pregnancy continued to grow more efficiently and achieved higher weight and length at the age of 12 months (Brooke et al., 1981). Recently, Hollis and Wagner (2013) performed randomized trial on pregnant women with daily dose of 400 IU, 2,000 IU or 4,000 IU throughout the pregnancy. They showed that delivery mode and co-morbidities such as preeclampsia differed between groups in favor of higher supplementation. A tendency for longer gestational age (P=0.1) with increasing supplementation was also noted, but no difference in mean birth weight. Shorter gestational length induced by poor maternal vitamin D status could potentially explain some of the differences in birth size as discussed above.

7.5 Maternal vitamin D status, bone density and growth in childhood

There are some studies to suggest that maternal vitamin D status has long-term consequences on bone growth and mineralization in the offspring. A prospective study of Javaid et al. showed in 198 subjects that maternal vitamin D status during late pregnancy predicts total body and lumbar spine BMC and BMD at age 9 (Javaid et al., 2006). In the same cohort, higher maternal vitamin D status was associated also with greater head circumference, but there were no consistent associations with growth, intelligence, or psychological health (Gale et al., 2008). However, some caution is required when interpreting these data, as only 38% of the original cohort attended the 9-year visit.

Based on correlations with estimated maternal UVB exposure and bone outcomes of offspring, Sayers and Tobias suggested maternal vitamin D status to affect skeletal development in child directly through influences on periosteal bone formation (Sayers and Tobias, 2009). Their study was done in Avon Longitudinal Study of Parents and Children birth cohort consisting of nearly 7,000 subjects (Sayers and Tobias, 2009), and they reported associations between estimated maternal UVB exposure in late pregnancy with offspring bone mass and size, height, and lean mass at 10 years. At the time of the study, information on maternal 25(OH)D concentrations was available only for a sub-sample of 55 mothers (mean 53 (SD=32) nmol/l), nevertheless, a weak positive correlation with estimated UVB dose was reported. Although the maternal vitamin D status appears crucial for bone growth, the prospective study from Finland (Viljakainen et al.,
suggests that catch-up growth occurs in bone mineral accrual, but not in bone size, when vitamin D status is corrected in initially deficient infants.

7.6 Infant vitamin D supplementation, bone density and growth in childhood

A retrospective study of Zamora et al. (1999) tracked the effect of infant vitamin D supplementation (dosage 400 IU) on bone health in pre-pubertal Swiss girls. Children who had taken vitamin D supplements in infancy had higher areal BMD in radial metaphysis, femoral neck, and trochanter, but not in trabecular rich bone sites, e.g. lumbar spine. Unfortunately, authors did not analyze supplementation in relation to changes in childhood height or weight gain. However, this study design might be biased due to low number of subjects in the control group (n=15) and no data on current use of vitamin D supplementation.

The effect of vitamin D supplementation on infant growth has been a neglected topic, but a high quality study was recently published in British Medical Journal. In this RCT the effect of weekly supplementation of vitamin D on mortality and morbidity in Indian low birth weight, full term infants was investigated (Kumar et al., 2011). Secondary outcome in this trial was growth at the age of 6 months. Stunted infant growth is typical in India and it was one of the outcomes in the study. Despite adequate power calculations, mortality or morbidity were not affected by vitamin D supplementation, but increases in length, weight, and arm circumference were significantly higher in supplemented group compared to placebo at 6-months. Authors emphasized that impact on anthropometrics were relatively small as difference between groups were about 0.11 Z-scores in favor of supplementation (Kumar et al., 2011). Vitamin D dosage which was used in the study was relatively modest (1,400 IU/35 μg per week) and although it improved the mean 25(OH)D concentration by 19 nmol/l, 43% of children remained vitamin D deficient (25(OH)D <25 nmol/l) in the supplemented group. Nevertheless, these results are promising by suggesting clear benefits with correction of extreme vitamin D deficiency, but studies with a higher dose and longer follow-up time will help to clarify the results for anthropometric outcomes. In addition to vitamin D deficiency Indian infants in the study of Kumar et al. might suffer from multiple nutritional deficiencies (Kumar et al., 2011). This is supported by a study of Ekbote and colleagues (2011) in which 47% were severely vitamin D deficient, 70% had anemia, roughly half presented stunted growth, and possibly also protein malnutrition at baseline. Laddoon, a traditional Indian sweet was tested as a vehicle for calcium supplementation to 60 Indian toddlers aged between 2 and 3 years. In addition to calcium fortified laddoon which was provided five days a week both groups received once a month an oral dose of vitamin D 30,000 IU. The intervention did not affect growth per se, but children in a group receiving higher calcium fortification gained more mineral to their skeleton and presented higher BMD at the end of the study compared to the control group.

In contrast to the proposed benefits for vitamin D supplementation on growth outlined above, a high dose vitamin D supplementation has also been suggested to be harmful for linear growth. This suggestion has been largely based on relatively old and small case studies on children, in
particular one study from 1937 which reported data for 9 cases suggestive of potential reductions in growth (Jeans and Stearns, 1938). However, using data from a large prospective population-based birth cohort of nearly 10,000 children, Hyppönen and colleagues did not find any evidence for stunted growth for infants who had received high dose vitamin D supplementation (typically 2,000 IU/day) during the first year of life (Hypponen et al., 2011); indeed before confounder adjustment supplemented children were significantly taller than others. However, it was not possible to exclude a possible adverse association between high-dose vitamin D supplementation and growth in height in infants who are born small at birth based on this study, and a possibility also remains that a co-supplementation with vitamin A may have reduced any possible adverse effects by high dose vitamin D supplementation (Hypponen et al., 2011). Interestingly, rickets at 1 year of age was not associated with length of the child in this study. However, infants who had been affected by rickets during the first year of life tended to be relatively tall in adulthood, and once the authors accounted for rickets treatment and adult height (as a proxy for growth potential) rickets became expectedly associated with shorter length. This could be due to children who became tall adults being more susceptible to rickets possible due to higher requirements for growth (Hypponen et al., 2011).

### 7.7 Vitamin D supplementation and childhood growth: intervention studies

A sizeable amount of peak bone mass accrues during puberty and many researchers have focused on nutrition and bone mass accrual during this critical period. After birth, vitamin D has a role in bone development, particularly with bone mineralization (El-Hajj Fuleihan et al., 2006; Lehtonen-Veromaa et al., 2002b; Viljakainen et al., 2006). It has also been suggested that effect of vitamin D on bone may act through muscle (Bartoszewska et al., 2010), but the current evidence on the association of muscle and vitamin D is conflicting (El-Hajj Fuleihan et al., 2006; Ward et al., 2010) and a direct effect of vitamin D on bone cells via regulation of the calcium balance is more commonly accepted (Norman, 2008).

There are several studies investigating the effect of varying dosages and types of vitamin D supplementation on bone mineralization and growth, with largely inconsistent findings. In an early study by Ala-Houhala et al. (1988), 54 girls aged between 8 and 10 years were randomized to receive either vitamin D (400 IU/day) or placebo for 12 months (Ala-Houhala et al., 1988). The study was underpowered to detect differences between groups either in height increment or bone mineral accretion in distal radius. More recently, another Finnish group investigated the effect of wintertime vitamin D2 supplementation on bone mineral accrual in initially 9-15 year old girls during a 3-year trial (Lehtonen-Veromaa et al., 2002b). They found that the baseline S-25(OH)D predicted the 3-year change in lumbar BMD, however, they failed to show any effects on bone mineral accrual by supplementation with vitamin D (10 or 20 μg/day). The higher dose given during wintertime raised S-25(OH)D concentration to 44 nmol/l, which was significantly higher than in the placebo group (32 nmol/l) (Lehtonen-Veromaa et al., 2002a).
Also in the 24-month trial of Cheng et al. (2005) vitamin D supplementation with calcium to girls with habitually low calcium diet did not improve bone mineral accrual in whole body or hip and did not increase tibian strength compared to placebo. However, in their study calcium supplementation in terms of high consumption of cheese appeared to increase cortical thickness tibia more when compared to other groups.

In contrast to earlier studies with smaller samples, we identified four RCTs with positive effects. A dose-response effect of vitamin D3 on bone mineral accrual both in total hip and lumbar spine area was shown in prepubertal Finnish girls (n=228) with high habitual calcium intake (Viljakainen et al., 2006). In this study, the participants were first stratified by their pubertal status and then randomized into three study groups. The intervention overlapped with a period of high growth rate. Interestingly, girls in the vitamin D groups gained more weight as well as mineral to their bones than in the placebo (Viljakainen et al., 2006). After adjusting for changes in weight, bone size and pubertal status; BMC accretion in the hip was 14% and 17% higher in the groups receiving 5 μg (200 IU) and 10 μg (400 IU) of vitamin D, respectively, compared with the placebo group. Only the higher dose increased lumbar spine BMC significantly. Also a Canadian study with vitamin D 10 μg and calcium a day showed an association with BMC and BMD, while the intervention did not affect tibian length. In this study the subjects were Caucasian girls (n=77), aged 12 years, and they had an early pubertal status (Moyer-Mileur et al., 2003). Furthermore, in the recent study by Mølgaard et al. (2010) the effect of vitamin D supplementation on bone mineral accrual was modified by VDR genotype. This study on apparently healthy 11-year old Danish girls, did not find any overall differences in whole-body or lumbar spine size-adjusted BMC after a 12 month vitamin D supplementation (5-10 μg day) when compared to placebo. However, the group reported an interaction with supplementation and FokI VDR gene polymorphisms in whole-body BMD: vitamin D supplementation improved BMC over placebo in a sub-group of girls who carried the FF VDR phenotype (Mølgaard et al., 2010). Further studies are required to explore gene-environment interaction in affecting response to vitamin D supplementation, as this may explain why some individuals are more responsive to vitamin D supplementation than others.

A Lebanese study reported increments in lean mass, bone size, and BMC in hip, but not in lumbar spine, or total body (El-Hajj Fuleihan et al., 2006). Altogether 179 girls were randomly assigned to receive either weekly dose of 14,000 IU, 1,400 IU, or placebo for 12 months. The increments in bone mass appeared largest at the highest dose and especially in the subgroup of premenarcheal girls. Interestingly, this study also reported a weak tendency for enhanced height increment across vitamin D supplemented groups (5.6%, 5.0%, 3.8%, in groups 14,000 IU, 1,400 IU, or placebo, respectively (P=0.07)).

Milk interventions may provide wider understanding on impact of nutrients on growth and bone development. Depending on the study design milk may be fortified with extra calcium and vitamin D. However, in different study populations the effects of the trials vary (Cheng et al., 2005; Zhu et al., 2006). Differences in response may be explained with differences in general nutritional status, growth pattern, and activity levels. A 2-year school milk intervention in China...
consisted of three arms (Zhu et al., 2006): no milk, 330 ml milk fortified with Ca, and 330 ml milk fortified with both Ca and vitamin D. Two of the latter ones provided extra calcium and vitamin D 560 mg, 0 μg and 560 mg, 5-8 μg, respectively. After the 2-year intervention both fortified groups showed greater increase in height (0.7%), sitting height (1%) and BMC (1.5%) and BMD (4.3%) compared to placebo group (Zhu et al., 2006). In the beginning of the study Chinese girls aged 10 years and were mostly prepubertal.

Soliman et al. (2010) observed that Nigerian children suffering from severe vitamin D deficiency were short for age, had lower BMI and their growth velocity was decreased compared to apparently healthy controls. The children were treated with mega doses of vitamin D (10,000 IU/kg) intramuscularly. After 6 months children were still shorter, but their other anthropometric measurements had improved to comparable level with controls with a significant increase in growth velocity. One of the most intriguing observations the group made was that S-25(OH)D concentration was associated with circulating IGF-1 level (Soliman et al., 2010), a finding that has also been seen in adults (Hypponen et al., 2008). It has been proposed that regain in growth velocity after the correction of vitamin D deficiency is mediated through growth hormone/IGF-I axis (Soliman et al., 2010).

The short-term benefit of vitamin D supplementation on bone mass accrual is at least promising, but in a long follow up the effects tend to attenuate. On the other hand, calcium supplementation is suggested to extend growth period (Chevalley et al., 2005b; Rozen et al., 2003) which implies the plasticity of growing phenotype is maintained in optimal environment. It is speculated that intervention with vitamin D supplementation during puberty might have persistent effects (Chevalley et al., 2005a), but this is not supported by all studies (Zhu et al., 2006).

7.8 Vitamin D and growth: animal models

There is some evidence for the importance of vitamin D on growth from animal studies and in particular, features of vitamin D dependent rickets in domestic animals appear to be very similar to those observed in humans (Dittmer and Thompson, 2011). Most frequently rickets was observed in sheep or pigs with scarce or no UVB exposure accompanied with poor, unbalanced diet. The musculoskeletal symptoms in pigs were similar to humans: difficulty in rising, bowed forelegs, joint swellings, and pain on moving. Although sheep in many countries graze outside throughout the year, the covering fleece reduces exposure to ultraviolet light. Similarly to observations in humans, vitamin D deficiency in sheep is exacerbated during pregnancy and S-25(OH)D concentration of lambs and dam are closely related. Rickets was observed most commonly in rapidly growing lambs.

In contrast, rodents do not typically become totally vitamin D deficient as discussed (Hollis and Wagner, 2013) and there is evidence to suggest that vitamin D may not be required for fetal growth or development because the influences of calcitriol on calcium absorption are compensated by other hormones (Fudge and Kovacs, 2010). It is possible, that in rodents during
weaning these compensatory mechanisms may maintain normal skeletal mineralization in the offspring (Fudge and Kovacs, 2010), however, it is unclear if such influences may be applicable to humans. Differences in the length of pregnancy and placental structure may also contribute to the extent by which studies on maternal vitamin D status and fetal development done in rodents will apply to humans.

Despite differences between the mice and men, the phenotype of genetically modified animals such VDR- or 1-α-hydroxylase null mice can provide valuable information on the possible role of vitamin D for well-being. A recent review discussed these in detail in comparison with findings in man (Bouillon et al., 2008). At birth fetuses of VDR null mice were slightly growth-retarded and their skeleton undermineralized: total Ca content per weight and the mineralized bone in the CSA of tibia were reduced (Rummens et al., 2003). On the contrary, growth plate was normal. A rescue diet with high calcium, phosphorus and lactose recovered the metabolic and skeletal abnormalities of VDR null mice, but in the fetuses, the rescue diet reversed only the bone phenotype but not the growth retardation. Interestingly, VDR null fetuses on the rescue diet gained less weight than on the standard diet, hence, the VDR genotype seems to determine fetal growth in mice as well as in humans as reviewed below. However, VDR null mice appear to grow similarly to wild-type mice while and after weaning (Li et al., 1997). After weaning weight gain was more slow in homozygous VDR knockout mice compared to wild-type and heterozygous VDR knockout mice (Figure 7.1), and VDR ablated animals presented impaired muscle function together with mixed neurological symptoms (Endo et al., 2003).

![Figure 7.1. Phenotype of VDR knock-out mice at 3.5 months of age. The genotypes of the mice, from left to right, are wild type, VDR+/− and VDR−/−. Adapted from Li et al. (1997).]
7.9 Genetic studies in humans: association with growth and bone outcomes

Gene CYP2R1 codes liver enzyme primarily responsible for the hydroxylation of vitamin D. Mutation in CYP2R1 is rare, but it will have profound effects on vitamin D metabolism such as impaired clearance of vitamin D, and severe rickets with multiple endocrine defects (Cheng et al., 2004). Although there is another mitochondrial enzyme capable of hydroxylating vitamin D it is unable to cover the actions of CYP2R1. A recent case report on a family with an autosomal dominant CYP2R1 mutation confirmed that lack of function mutation of CYP2R1 affects growth and predisposes to severe rickets and will results to short stature at adulthood if not treated (Tosson and Rose, 2012).

The function of vitamin D in the body depends on how efficiently it is bound to a specific carrier, vitamin D binding protein, also called GC protein. Interestingly genetic variation in GC is associated with 25(OH)D bioavailability to target cells (Chun et al., 2010). However, in humans GC protein deficiency is not known, but genetic variation in GC might affect metabolic rate and clinical outcomes. In clinical studies GC protein phenotype is associated with circulating 25(OH)D levels and fracture risk (Lauridsen et al., 2004, 2005). Because genetic variation in GC goes together with skin color, this might explain why vitamin D deficiency presents differently in different populations (Berry and Hypponen, 2011), but its relevance on growth or bone mass accrual remains to be discovered.

VDR is recognized as a candidate gene associated with bone outcomes and growth because of its functional relevance, plausible minor allele frequency and its relative position in chromosome 12 is described with sequence variation (Van der Sluis et al., 2003). Frequent polymorphisms in the VDR gene appear to contribute to growth in utero and in early infancy (Bodnar et al., 2010; Keen et al., 1997; Lorentzon et al., 2000; Morley et al., 2009; Suarez et al., 1997), and notably adult height (Fang et al., 2007; Lorentzon et al., 2000; Macgregor et al., 2008). Morley and colleagues observed that low birth weight newborn to vitamin D deficient mothers carried more frequently FF or Ff VDR phenotype (Morley et al., 2009). VDR gene polymorphism was significantly associated with risk of SGA in a recent study (Bodnar et al., 2010), but this differed by race. Furthermore, they gained evidence that VDR genotype and 25(OH)D concentration may have independent contributions to risk of SGA (Bodnar et al., 2010). A Swedish study reported that length at birth, increase in height from birth to early adulthood, stature at 19 years as well as bone size varied by VDR Bsm polymorphisms (Lorentzon et al., 2000). Also in a study of obese children BsmI was associated with differences in average height, with the effect being most pronounced in postpubertal children (Ferrarezi et al., 2012). Association was independent of 25(OH)D or insulin sensitivity, which in some other studies has been related to VDR polymorphism (Jain et al., 2012; Oh and Barrett-Connor, 2002). A recent meta-analysis with over 14,000 subjects suggested that carriers of Bsm ‘BB’ genotype were approximately 0.6 cm (CI: 0.2–1.1 cm) taller than those with the ‘bb’ genotype; the effect of allelic variant was independent of age, sex or age related height loss (Fang et al., 2007). These findings were partly supported by McGregor et al. (2008), but they concluded that the effect size explains approximately 0.1% of the phenotypic variance in height, thus probably hundreds of such variants are responsible for the observed...
genetic variation in height. VDR polymorphisms have also been related to BMD in some (Arabi et al., 2009; Laaksonen et al., 2004; Strandberg et al., 2003; Viitanen et al., 1996) but not in all studies (Gunnes et al., 1997; Lei et al., 2005), and the results tend to vary according to gender, and age. Higher stature together with greater lumbar spine width, but no difference in BMD in lumbar spine or tibia were observed in carriers of particular haplotype (created by three adjacent restriction enzymes, ‘bAT’) in Caucasian children and young adults in Netherlands (Van der Sluis et al., 2003). Correspondingly, increase in BMC in response to vitamin D supplementation was in one study observed to be dependent on VDR Bsm genotype (Arabi et al., 2009). Because bone gain was affected by genotype only in supplemented groups authors suggested that VDR polymorphism does not affect bone mass accrual per se, but modifies the response to vitamin D supplementation (Arabi et al., 2009).

7.10 Insights into mechanisms of action

Calcitriol is an endocrine hormone and known to influence bone mineral accrual by contributing to calcium homeostasis. Calcium is the major constituent of skeleton, but its actions (absorption, resorption and excretion) in the body are regulated by vitamin D either directly or indirectly (Norman, 2008). However, the action of calcitriol in body may be quite complex as illustrated by Norman (2008) (Figure 7.2). Furthermore, a feedback loop through FGF-23 is identified. After mineralization of collagen matrix, mature osteocytes produce FGF-23 to down-regulate 1-α-hydroxylation of 25(OH)D (Bouillon and Decallonne, 2010). Besides, in bone vitamin D up-regulates osteocalcin production which is identified as an endocrine regulator of glucose metabolism. Secondly, paracrine action of vitamin D exists. Vitamin D affects cell cycle and cell differentiation; whether a mesenchymal stem cell differentiates to osteoblast, muscle cell, neuron, or adipocyte depends on availability of 25(OH)D. At the moment there are about 40 target organs for vitamin D. This means that 25(OH)D can enter these cells and transform to calcitriol locally and influence gene expression of selected genes. Zhou et al. (2010) demonstrated in human bone marrow stromal cells, that vitamin D metabolites (25(OH)D) upregulate the production of IGF-1 which allows the differentiation of stromal cells into osteoblasts. Similarly, IGF-1 was found to promote periosteal growth and bone mass accrual indirectly through muscle growth in a seven year long follow-up (Xu et al., 2011). In addition, studies on cell cultures have proved that IGF-1 is produced locally by osteoblasts and muscle cells under the regulation of vitamin D (Gurlek et al., 2002). Regardless of the cell type the functions of vitamin D in different target cells are anabolic.

In addition, genetic variation may involve lower copy numbers of VDR (in carriers of risk haplotypes) which could explain differences observed in stature or BMD, but more likely it seems that response to vitamin D supplementation is more distinctive according to genotype (Arabi et al., 2009; Bodnar et al., 2010).
Although there are only few high quality RCTs on vitamin D and childhood bone outcomes, evidence is broadly supportive of a beneficial effect, with further enforcements coming from observational studies and animal models. The most pronounced effects appear to be seen in prepubertal girls with high habitual calcium intake and on cortical rich sites as total hip or whole body and to lesser extent in lumbar spine. There is an amounting body of evidence suggesting that maternal vitamin D status is positively related to bone size and bone mass at birth, and this association may remain throughout childhood. However, the association between higher intake of vitamin D and higher axial growth needs to be confirmed in further studies. Little is also known on potential threshold effects, and it is possible that the positive associations between vitamin D and bone mass/density are most pronounced in initially deficient populations. Correspondingly the long-term effects of vitamin D supplementation on bone outcomes will need to be confirmed in future studies.

Evidence on the effects of vitamin D on linear growth is surprisingly limited and relies largely on observational studies which are unable to prove the causality. Some effects may be indirect, for example vitamin D deficient children are likely to be more prone to illnesses such as acute...
7. Vitamin D and growth

respiratory infections (Karatekin et al., 2009; Wayse et al., 2004) and some chronic diseases (Hollams, 2012; Hypponen, 2010; Hypponen et al., 2001) which through influences on general well-being of the child may affect growth (Wayse et al., 2004). In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth (Kumar et al., 2011). However, more studies are needed to establish the effect of vitamin D supplementation on linear growth during childhood, and whether benefits will be seen beyond with the effects on the correction of severe vitamin D deficiency.

References


Institute of Medicine, F.a.N.B., 2011. Dietary Reference Intakes for Calcium and Vitamin D, Institute of Medicine, Washington, D.C.


7. Vitamin D and growth


7. Vitamin D and growth


Key facts

• Rickets, a disease causing soft bones and severe growth retardation in children, was rampant in the industrialized cities of Europe and North America.
• Elmer McCollum at Johns Hopkins University, discovered Vitamin D in 1922 from cod liver oil as a dietary substance that could prevent rickets.
• In the last decade, there has been a resurgence of rickets in the developed world, leading to renewed interest in vitamin D.
• We now recognize that vitamin D has several non-calcemic biological functions and its impact on several chronic diseases, including arthritis, is an area of expanding research.

Summary points

• Vitamin D deficiency is a global epidemic.
• The major source of vitamin D synthesis in humans is cutaneous exposure to the sunlight.
• Vitamin D is a steroid hormone and exerts its diverse biological effects through the vitamin D receptor.
• Vitamin D is an immunomodulator and its role is initiation and exacerbation of autoimmune diseases is an area of ongoing research.
• Epidemiologic data indicate higher prevalence of autoimmune diseases in northern compared to southern latitudes, including inflammatory arthritis.
• Preliminary cross-sectional data suggest increased disease activity in rheumatoid arthritis patients with low vitamin D levels – these findings are not replicated in all studies.
• Low vitamin D levels may increase the risk of incident and progressive osteoarthritis – other studies do not confirm these findings.
• Direct causative role of vitamin D deficiency in arthritis is unclear.
• We await prospective long term interventional trials evaluating the role of vitamin D therapy in arthritis.
8. Vitamin D and arthritis

U.J. Haque
Johns Hopkins School of Medicine, Division of Rheumatology, 5200 Eastern Avenue Mason F. Lord Building, Center Tower, Suite 4100, Baltimore, MD 21224, USA; uhaque@jhmi.edu

Abstract

Vitamin D is a pleiotropic steroid molecule that exerts its physiological effects through the ubiquitous vitamin D receptor. Vitamin D is formed from its precursor in the skin upon exposure to the sunlight. Its importance in calcium homeostasis has been well known for a century, but recently several non-calcemic effects have been recognized. Vitamin D regulates several genes involved in cellular differentiation, apoptosis and has immunomodulatory effects. Epidemiologic data suggest that vitamin D deficiency may increase the risk of common cancers and autoimmune diseases. In experimental animal models, autoimmune diseases can be ameliorated by treatment with active vitamin D metabolites. Potential use of vitamin D supplementation to modify these diseases would certainly be a novel approach. Preliminary data from cross-sectional studies show that low vitamin D levels may be associated with increased disease activity in rheumatoid arthritis. Other studies show a possible increased risk of osteoarthritis with low vitamin D levels. These findings, while interesting, are not confirmed by all studies. Moreover, the cross-sectional design of these studies does not establish causality. Currently, the optimal and minimal level of vitamin D required for its physiological functions in humans is unknown. We, thus, await more research and randomized controlled interventional trials to answer these critical questions.

Keywords: steroid hormone, rheumatoid arthritis, osteoarthritis, disability, disease activity
8.1 Introduction

Vitamin D is a pleiotropic steroid hormone and exerts its physiological effects through the ubiquitous VDR (Bikle, 2010). Activated vitamin D, via its interaction with VDR, is implicated in regulating several genes with diverse biological functions (Dong et al., 2003; Sutton and MacDonald, 2003) which may explain its possible epidemiological association with several chronic diseases (Holick, 2007; Ponsonby et al., 2002). The recent interest in vitamin D is driven by the recognition of its non-calcemic functions beyond its role in bone and calcium homeostasis. Vitamin D deficiency is endemic (Holick, 2007) and its potential impact on health is an area of expanding research. 25(OH)D, a circulating metabolite, can be measured in serum and is considered the best indicator of vitamin D status.

Vitamin D has known immunomodulatory effects (Maruotti and Cantatore, 2010) and its likely role in the initiation or exacerbation of autoimmunity is a topic of ongoing scientific study. In this chapter, we will explore potential association between vitamin D and arthritis. There are observational, epidemiological, experimental and clinical data that suggest a possible link between vitamin D and arthritis. However, the definite causative role of vitamin D deficiency in arthritis has not been established. We will review available data with particular reference to RA, the most common inflammatory arthritis characterized by erosive disease, increased disability and mortality. We will also briefly review the data regarding OA and vitamin D.

8.2 Vitamin D and rheumatoid arthritis

Although not found in all studies, one large observational study has shown increased risk of development of RA in women with lower vitamin D intake. In the Iowa Women’s Health Study,
8. Vitamin D and arthritis

prospective follow up of 29,368 women revealed an inverse association between total vitamin D intake and the risk of RA (RR 0.67, 95% CI 0.44-1.00, \( P = 0.05 \) for trend) (Merlino et al., 2004). In this study, vitamin D levels were estimated using a self-administered dietary questionnaire only and 25(OH)D levels were not measured. This likely resulted in poor correlation with actual vitamin D status in this study and is its major limitation. Nielen et al. (2006) measured 25(OH)D levels in stored sera in a cohort of RA patients who had donated blood prior to onset of symptoms and compared these to matched healthy control. They did not observe any relationship between low levels of vitamin D and future risk of RA (Nielen et al., 2006). Other studies also have observed no association between vitamin D intake and risk of RA (Costenbader et al., 2008; Racovan et al., 2011). In these studies, dietary vitamin D intake and solar irradiation were used to assess vitamin D status and actual 25(OH)D levels were not measured.

Epidemiological and observational evidence suggest a greater incidence of autoimmune diseases (Cantorna et al., 2004; Ponsonby et al., 2002), including inflammatory arthritis, with increasing latitude, similar to the amplified risk of cancer (Apperly and Cary, 1945; Grant, 2002). Higher prevalence of RA was noted in Northern vs. Southern Europe (Sokka and Pincus, 2005). Residents of northern climates are at a greater risk of vitamin D deficiency (Holick, 2007), as solar exposure of the skin is the main source of vitamin D synthesis in humans. Whether this solar gradient, via vitamin D deficiency, plays a causative role in conferring increased risk of arthritis, is unknown.

Data from animal models have also suggested a link between vitamin D and autoimmune diseases. In a study on collagen induced arthritis, mice fed a diet supplemented with 1,25(OH)\(_2\)D\(_3\) had 50% less incidence of collagen induced arthritis compared to the control mice (Cantorna et al., 1998). 1,25(OH)\(_2\)D\(_3\) treatment also halted the progression of severe arthritis compared to the controls. Human TNF transgenic mouse is another animal model of spontaneous chronic arthritis. When these mice were crossed with VDR deficient mice, clinical symptoms of arthritis were aggravated. Absent VDR signaling was associated with increased synovial inflammation and macrophage influx into the inflamed joint (Zwerina et al., 2011).

The mechanisms through which vitamin D potentially influences the immune system have been well documented in \textit{in vitro} studies. VDR are present on primary lymphoid organs. Active vitamin D analog inhibits adaptive immunity by suppressing B cell, dendritic cell maturity and T cell proliferation (Maruotti and Cantatore, 2010). Vitamin D has been shown to down regulate selective chemokines and cytokines (Th1 and Th17 cytokines) and to induce T regulatory cells, shifting Th1 responses towards Th2 responses (Bikle, 2010), potentially beneficial for Th1 driven immune diseases. This topic is discussed in greater detail in other chapters of this book. The pathogenesis of RA is complex and involves both T cells and B cells. Antigen presentation to T cells results in their differentiation into Th1 and Th17 cells with secretion of proinflammatory cytokines such as TNF-alpha and IL-17. Studies on healthy controls have shown that 1,25(OH)\(_2\)D\(_3\) inhibit the production of TNF-alpha and IL-17 by peripheral blood monocytes (Rausch-Fan et al., 2002). These results were replicated in patients with early RA where presence of activated vitamin D analog resulted in suppression of IL-17 in stimulated PBMC of patients with early RA.
Thus, vitamin D has the potential to possibly modulate effector pathways that may be critical in pathogenesis of autoimmune arthritis.

Given these findings, it is plausible to hypothesize that vitamin D, through its immunosuppressive effect, may prevent autoimmunity. The simplistic paradigm of initiation of autoimmunity recognizes a ‘multi-hit’ process, with genetic predisposition resulting in aberrant immune response to an environmental trigger. Is it possible that vitamin D deficiency, via its interaction with the immune system, predisposes to aberrant immune response to an external trigger leading to autoimmune diseases/arthritis? And once autoimmunity is established, can vitamin D deficiency cause exacerbation of inflammatory diseases through its modification of B and T cells and cytokine responses? While epidemiologic, clinical and mechanistic observations point towards a possible role of vitamin D in autoimmunity, conclusive evidence is missing. Moreover, optimal vitamin D levels required to prevent or treat autoimmune disease is unknown.

Clinical studies have shown that low levels of vitamin D are common in RA. While the percentage may vary in different studies depending on the cut-off value used for 25(OH)D levels, several studies indicate greater than 50% prevalence of suboptimal vitamin D levels in this population (Kerr et al., 2011; Rossini et al., 2010). Our data suggest that up to 60% of RA patients have vitamin D deficiency (25(OH)D <30 ng/ml) in the mid-Atlantic region (Haque and Bartlett, 2010). In most studies that compared vitamin D levels in RA patients with controls, no difference was seen between the two groups (Cutolo et al., 2006; Rossini et al., 2010; Turhanoglu et al., 2011), suggesting that RA does not confer an additional risk for vitamin D deficiency.

Patel et al. (2007) were the first to report an inverse relationship between vitamin D levels and disease activity and disability in inflammatory arthritis. In a cohort of 206 patients with early inflammatory arthritis (synovitis ≥2 peripheral joints, symptom duration ≥4 weeks), 25(OH)D levels were inversely associated with clinical measures of RA disease activity (tender joint count, CRP and DAS 28). Patients with lower vitamin D levels also had greater disability, as measure by health assessment questionnaire score.

Since then several studies from different parts of the world have evaluated this relationship in patients with RA. In the largest study from Italy, 1,191 patients with RA were examined (Rossini et al., 2010). This sample concerned mostly female patients (85%), with a mean age of 58.9±11.1 years and a disease duration of 11.5±8.7 years. 45% of the study patients were on vitamin D supplements. Prevalence of vitamin D deficiency (25(OH)D <20 ng/ml) was 43% in the entire cohort. One third of patients taking >800 IU vitamin D on enrollment were still deficient. Low 25(OH)D levels were associated with high disease activity (DAS 28) and disability index (health assessment questionnaire), in non-supplemented RA patients. This remained significant even after adjusting for BMI and sun exposure. Similar results were noted in several smaller studies (Cutolo et al., 2006; Haque and Bartlett, 2010; Turhanoglu et al., 2011).

Not all studies, however, have confirmed these results. Kerr et al. (2011) evaluated this relationship in 850 men (76% Caucasian) enrolled in the Veterans Affairs Rheumatoid Arthritis Registry.
Similar to earlier studies, 43% of the cohort was vitamin D deficient [25(OH)D <20 ng/ml]. They noted an inverse relation between vitamin D deficiency and tender joint count [OR 1.02, (1.01-1.040, \( P = 0.001 \)] and high sensitive CRP [OR 0.99 (0.99-1.00), \( P = 0.008 \)] and anti-ccp positivity [OR 1.55, (1.18-2.05), \( P = 0.002 \)]. However, no correlation of low vitamin D levels with composite scores of disease activity or disability was observed. Craig et al. (2010) also did not observe any relationship between 25(OH)D levels and RA activity in African American patients with early RA.

While these preliminary studies point towards a possible link between vitamin D and RA, it is important to recognize that these are all cross-sectional analyses. This precludes inference about causality. Thus, we are unable to determine whether vitamin D deficiency directly causes increased disease activity in RA or whether the reverse is true. These studies also rely on vitamin D levels measured at one point only. This solitary measure may not be a true reflection of long term vitamin D status in these individuals, as 25(OH)D levels can fluctuate (Dawson-Hughes et al., 1991). Regardless, these data suggest need for larger, prospective interventional studies to replicate these findings and to explore the possible therapeutic role of vitamin D repletion in RA.

Two recent publications have evaluated this question. In a double blind study, patients with active RA (DAS 28 >3.2) on stable disease modifying anti-rheumatic drugs were randomized to 50,000 IU vitamin D weekly or placebo for 12 weeks, on background MTX (Salesi and Farajzadegan, 2011). The primary endpoint was decrease in DAS 28 of 0.6 at week 12. The two groups were similar in demographic and RA related characteristics at baseline. 98 patients completed this study. At week 12, both treatment and placebo groups achieved similar decline in DAS 28, tender joint counts, swollen joint counts and ESR. The only statistical difference observed between the control and active treatment groups was the 25(OH)D levels before and after intervention. It is however, unclear why these two groups showed a robust DAS 28 response rate (placebo group = 64% response rate, vitamin D group = 76%) if no adjustments of disease modifying anti-rheumatic drugs or other medications were allowed that could potentially reduce disease activity, during the study period.

In a larger open label trial, 121 patients with early RA (duration <2 years) were randomized to receive 500 IU 1,25(OH)\(_3\)D\(_3\) + 1000 mg calcium carbonate vs. 1000 mg calcium carbonate daily (Gopinath and Danda, 2011). In addition, both groups also received triple therapy (MTX, sulfasalazine, hydroxychloroquine) for 3 months. Baseline demographics and RA characteristics were comparable in the two groups. Primary outcome was minimum time required for onset of pain relief. At study completion, median time to first achieve pain relief was equal in both groups (\( P = 0.4 \)). However, patients in the treatment group achieved higher pain relief as measured by visual analog score at the end of 3 months (50% vs. 30%, \( P = 0.006 \)). 25(OH)D levels were not measured in all patients and thus the direct correlation of vitamin D levels with pain relief could not be established.

With existing data, the role of vitamin D supplementation in RA, if any is uncertain. Clearly larger, randomized placebo controlled trials of longer duration are needed to evaluate the role of
vitamin D therapy in RA, with assessment of 25(OH)D levels as well as measures of RA disease activity, inflammatory marker and cytokines, at multiple points throughout the study period. This will help us better understand the true relationship of 25(OH)D levels with RA disease activity and its correlation with markers of inflammation and inflammatory cytokines. It may also shed light on the greatest challenge in vitamin D research – the optimal level of 25(OH)D in autoimmune diseases.

Currently there is no expert consensus on the minimum physiologic 25(OH)D levels required for our species. It is difficult to establish a ‘norm’ as we are evaluating a relatively sun-deprived population. The threshold level for vitamin D deficiency has focused on its skeletal benefits and the need to prevent secondary hyperparathyroidism (Chapuy et al., 1997; Dawson-Hughes et al., 2005). The Institute of Medicine, after evaluating data regarding bone health and vitamin D, proposed a cut-off level of 20 ng/ml. Other experts do not concur with this assessment (Dawson-Hughes et al., 2005). Similarly, the optimal levels of vitamin D required to achieve its possible benefits in autoimmune and other diseases are unknown.

8.3 Vitamin D and osteoarthritis

OA is the most common form of arthritis associated with musculoskeletal pain and disability. It is degenerative arthritis, characterized by loss of articular cartilage with concurrent changes in the subchondral bone (Mansell et al., 1997). Bone mineral density and other characteristics of the subchondral bone may influence the expression and progression of OA (Westacott et al., 1997). Vitamin D influences bone quality by its effect on calcium metabolism, bone density and matrix ossification (Parfitt et al., 1982). Thus, it has been hypothesized that vitamin D can impact development or progression of OA. Several longitudinal studies have explored this possibility, with conflicting results. In the Framingham Heart Study, risk of knee OA progression was three times higher in participants in the middle and lower tertiles for vitamin D intake and 25(OH)D levels compared with the upper tertile (McAlindon et al., 1996). However, no effect on incident knee OA was observed. On the other hand, in the Study of Osteoporotic Fractures, lowest tertile of 25(OH)D was associated with risk of incident hip OA (OR 3.3, 95% CI 1.13-9.86), defined as development of joint space loss. A follow up study evaluating two longitudinal cohorts, reported no association of 25(OH)D levels with progression of knee OA (Felson et al., 2007). Another prospective study evaluated the association of serum 25(OH)D levels, sunlight exposure and knee cartilage loss by MRI and noted that baseline 25(OH)D level were positively associated with cartilage volume and predicted knee cartilage loss over 3 years of follow up (Ding et al., 2009). A recent cross-sectional study noted an association between knee pain and low levels of vitamin D but not with radiographic knee OA (Muraki et al., 2011). These studies present interesting associations but other studies have not replicated these findings (Kalichman and Kobyliansky, 2012; Konstari et al., 2012). Most of these studies used only one point assessment of 25(OH)D levels over several years of follow up, which may not be a true indicator of vitamin D status over years. Several of these studies also used X-rays to evaluate incident or progressive OA, which may be insensitive and slow to change. Thus, at this point, the causal role of vitamin D in the
development and progression of OA is unclear. Further studies are required to assess the potential therapeutic role of vitamin D in OA.

8.4 Conclusion

In conclusion, there is emerging evidence regarding several non-calcemic biological functions of vitamin D, including its immunomodulatory effects. There is epidemiologic, experimental and clinical data that suggest a possible link between vitamin D and arthritis, both inflammatory and degenerative arthritis. While data from studies evaluating the relationship of vitamin D with disease activity in RA and incident and progressive OA are intriguing, the causative role of vitamin D in initiation or exacerbation of arthritis is yet to be established. Most of these studies are cross-sectional and thus the direction of association cannot be determined. It is possible that vitamin D is a surrogate marker of health. Currently, there is no consensus regarding the optimal 25(OH)D levels required to achieve beneficial effects of vitamin D in autoimmune diseases. The role of vitamin D as a preventive or therapeutic agent in chronic arthritis, while a novel prospect, is currently unclear. We await larger, prospective controlled studies to further explore these preliminary findings.

References


8. Vitamin D and arthritis


Sutton, A.L. and MacDonald, P.N., 2003. Vitamin D: more than a ‘bone-a-fide’ hormone. Molecular Endocrinology (Baltimore, Md.) 17, 777-791.


Key facts

• The ability of aging skin to produce vitamin D is reduced by more than half in 80 year olds compared to adolescents, due to reduced epidermal production efficiency.

• Exposure of the body in a bathing suit to sunlight for around 6-9 minutes (assuming a fair-skinned person exposed to midday summer sun in temperate regions) is equivalent to an oral dose of around 75 μg (3,000 IU) vitamin D.

• Vitamin D that is not attained from sunlight cannot realistically be replaced by a diet that includes only normal foodstuffs.

• Following an oral dose of vitamin D, 25-hydroxyvitamin D [25(OH)D] reaches peak circulating level in 2-3 weeks, and disappears with a half-life of around 1-3 months.

• The dose-response of 25(OH)D to vitamin D is often estimated as being around 1 nmol/l per μg/day vitamin D (2.5 nmol/l per 100 IU/day or 0.4 ng/ml per μg/day) but is highly variable and depends on a range of factors.

Summary points

• Elderly people often have low vitamin D status due to reduced sunlight exposure and physiological changes in epidermal vitamin D production.

• Research surrounding the use of high-dose vitamin D has expanded since the mid-2000s.

• Moderate- and high-dose interventions include sustained doses ≥25 μg/day (1000 IU/day) or equivalent administered weekly or monthly and higher seasonal, annual or single doses.

• Efficacy of moderate- and high-dose vitamin D depends on factors affecting the pharmacodynamic response of 25(OH)D to vitamin D supplementation, including initial vitamin D status, body composition, form of vitamin D and the dose itself.

• Studies investigating efficacy of moderate- and high-dose vitamin D interventions on musculoskeletal (fractures, falls and their determinants), and other outcomes are inconclusive.

• Sustained daily doses of almost 250 μg/day (10,000 IU/day) and single doses up to 15 mg (600,000 IU) vitamin D have been used in trials involving elderly participants.

• Two studies of daily doses above 160 μg/day (6,500 IU/day) report elevated calcium and episodes of hypercalcaemia.

• Two recent studies suggest that large annual doses of 7.5-12.5 mg (300,000-500,000 IU) may increase risk of fractures and falls.

• Other extra-skeletal benefits of high vitamin D status are proposed but not well supported by data from randomized-controlled trials.
9. High-dose vitamin D supplementation in the elderly

C.J. Bacon
Department of General Practice and Primary Health Care, School of Population Health, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; c.j.bacon@fitkiwi.co.nz

Abstract

The vast majority of studies assessing the effect of vitamin D on a range of outcomes, provide doses of <25 μg/day to older adults. Because of the potential for enhanced convenience or compliance, there has been an interest in higher doses, though availability of high dose products in many parts of the world, particularly Australia and North America, has limited their clinical applicability. Since the middle of the last decade, there has been a rapid expansion of data surrounding efficacy and safety of high-dose interventions, in elderly and in other population groups. Such regimens include weekly or monthly doses, and larger doses administered seasonally, annually, or as loading doses to rapidly boost vitamin D status. The efficacy of high-dose vitamin D supplementation over lower-dose preparations depends on the 25-hydroxyvitamin D response to vitamin D supplementation. An examination of data surrounding the pharmacodynamic effect of oral vitamin D shows that the dose-response is highly variable and depends on factors such as basal vitamin D status, body composition, the type of vitamin D provided and the dose itself. Controlled interventions examining the efficacy of high doses in elderly have mainly investigated musculoskeletal outcomes. Evidence of high-dose vitamin D efficacy is inconclusive for fracture- and falls-risk, their musculoskeletal determinants in this population group, and for other outcomes. Whilst most studies that provide high doses of vitamin D and report follow-up measurements of calcaemic effects find little evidence of increased hypercalcemia, hypercalciuria or renal stones, relative to control groups, there are a lack of adverse effect data from large controlled investigations. A cautious approach to administering loading doses ≥7.5 mg of vitamin D might be warranted since two recent studies have shown modestly increased risk of falls and hip fractures.

Keywords: calciferol, cholecalciferol, ergocalciferol, aged
Abbreviations

25(OH)D  25-hydroxyvitamin D
MED  Minimal erythemal dose
UL  Tolerable upper intake level

Unit conversions

25(OH)D concentration  1 nmol/l = 0.40 μg/ml
Vitamin D dose  1 μg = 40 IU (100,000 IU = 2.5 mg)

9.1 Introduction

Low levels of circulating 25(OH)D are common in frail elderly people (Lips, 2007; McKenna, 1992) for a combination of physiological and behavioral reasons. The ability of aging skin to produce vitamin D is reduced by more than half in 80 year olds compared to adolescents, due to lowered epidermal 7-dehydrocholesterol concentrations (MacLaughlin and Holick, 1985) and increased skin thickness (Need et al., 1993). The effects of aging may also reduce mobility, and physical activity levels are inversely related to vitamin D status in elderly (Bolland et al., 2006; Lucas et al., 2005). Low vitamin D status associated with inactivity is likely to result primarily from reduced outdoor time and increased clothing necessary for warmth as a consequence of impaired mobility (Holick, 1995). Other behavioral factors may also contribute, including deliberate avoidance of direct sun exposure, for example to reduce the risk or exacerbation of skin cancer or for cosmetic protection (Armstrong, 2004; Gilchrest, 2008; Webb, 2006).

Replacement of vitamin D that is not attained from sunlight by consuming normal foodstuffs is not realistic. Exposure of the body in a bathing suit to a modest 0.3 MED of ultra-violet, equivalent to one third the amount required to cause minimal redness of the skin, or 6-9 minutes of sun exposure for a fair skinned person in midday summer sun in temperate regions (Ebeling and Eisman, 2005), is equivalent to an oral dose of around 75 μg vitamin D (Holick, 2001, 2007). By comparison, this dose would be obtained from approximately ten 100 g cans of sardines, 100 eggs, 300 litres unfortified, or 7 litres fortified full-cream milk (Holick, 2007; New Zealand Institute for Crop and Food Research Ltd., 2004). Even for the richest natural source of vitamin D, cod liver oil, 15 teaspoons would be required. Vitamin D fortification of food is also rare in Southern Europe, the United Kingdom, Australia and New Zealand. Consequently, because minimal vitamin D is obtained from any normal diet, it is clear that individuals who do not obtain exposure to outdoor sun require pharmacologically administered vitamin D.

There is substantial uncertainty about what dose to prescribe elderly people who may have low vitamin D status due to lack of sunshine exposure. This uncertainty comes about mainly because of disagreement about what an optimal target level of circulating 25(OH)D should be (Bouillon,
9. High-dose vitamin D supplementation in the elderly

2011; Dawson-Hughes et al., 2005; Lanham-New et al., 2011; Vieth, 2011) and what is the safe upper limit for vitamin D dose (Aloia, 2011; Vieth, 2007). There is no clearly defined threshold level that constitutes a high-dose of vitamin D, and the term high-dose has been variously used to describe daily doses of between 50-2,200 μg (Grimnes et al., 2012; Hackman et al., 2010; Hypponen et al., 2011; Kimball and Vieth, 2008); weekly doses of 35-5,000 μg (Maalouf et al., 2008; Wasse et al., 2012); monthly doses of 1,250 μg (Bacon et al., 2009); and single or annual doses of 7,500-15,000 μg (Bacon et al., 2009; Cesur et al., 2003; Sanders et al., 2010; Von Restorff et al., 2009; Wu et al., 2003). Analysis of studies from a number of meta-analyses show that the vast majority of randomized controlled trials of vitamin D2 or D3, alone or in combination with calcium, to prevent osteoporosis, fractures or falls in elderly, have provided daily doses of 7.5-20 μg (Bischoff-Ferrari et al., 2009; Cranney et al., 2008; Tang et al., 2007). There has been recent interest in studies reporting on higher and/or less frequent vitamin D dose regimens given to this population group. Trials administering vitamin D in this way could be broadly classified into five groups:

1. Daily moderate dose: 25-50 μg/day (1000-2,000 IU/day);
2. Daily high dose: >50 μg/day (>2,000 IU/day);
3. Weekly moderate dose: 250-375 μg/day (10,000-15,000 IU/week);
4. Weekly high dose >375 μg/day (>15,000 IU/week);
5. Loading dose, monthly or seasonal dose.

The aim of this chapter is to address the evidence surrounding moderate- and high-dose vitamin D administration in elderly including the pharmacodynamics, efficacy, potential benefits and possible risks.

9.2 Pharmacodynamics of vitamin D

Circulating 25(OH)D has a relatively lengthy half-life, variably estimated between 10 and 27 days following its own oral or intravenous administration. This half-life depends upon its initial levels (Haddad and Rojanasathit, 1976), dietary fiber intake (Batchelor and Compston, 1983), method of determination (Vicchio et al., 1993), and potentially levels of vitamin D binding protein (Christakos et al., 2003). It is important to recognize that the pharmacokinetics of administered 25(OH)D is not equivalent to the time-course of 25(OH)D following an oral dose of vitamin D3. The latter depends on the absorption of vitamin D3, the hepatic conversion of vitamin D3 to 25(OH)D, the degree of initial accumulation or storage of vitamin D3 in body tissues and, in addition, to the catabolic processes responsible for 25(OH)D break-down, which determine its disappearance half-life. Based on the earlier physiological studies of Mawer et al. (1972), Vieth (2008) has estimated a half-life of combined vitamin D metabolites of around 2 months, somewhat longer than for 25(OH)D half-life.

Until quite recently, there has been little empirical data to assess the pharmacodynamic effect on 25(OH)D of different vitamin D doses. Whilst a number of studies report baseline and single follow-up measurements of 25(OH)D following vitamin D administration in elderly, (for
example, Aloia et al., 2005; Guillemant et al., 1989; Khaw et al., 1994; von Hurst et al., 2010), fewer have reported serial follow-up measures of 25(OH)D (Table 9.1). Data from these studies can be used for two purposes relevant to evaluating the effects of high-dose vitamin D administration. Firstly, those assessing 25(OH)D concentration following cessation of vitamin D or after the administration of a single high dose can confirm the half-life for disappearance of 25(OH)D in the clinical situation. Secondly, data from studies measuring the rise in 25(OH)D resulting from sustained doses of vitamin D, of sufficient duration to attain a new steady-state, can be used to estimate doses required to attain or maintain target levels of 25(OH)D.

### 9.2.1 Clinical half-life of 25-hydroxyvitamin D

Vieth’s (2008) estimate of 2 months for the half-life of combined vitamin D metabolites was confirmed in a US study of healthy American adults aged 27-84 years following a 2.5 mg acute dose of vitamin D3 (Ilahi et al., 2008) which showed that peak circulating 25(OH)D occurred at 7 days with an ensuing linear decline that displayed a half-life of approximately 50 days. Two New Zealand studies report data from similar investigations administering vitamin D3 to elderly men and women recruited from hospital wards (Bacon et al., 2009; Wu et al., 2003). One of these studies noted a longer 25(OH)D half-life of 90 days following a peak response to 7.5 mg vitamin D3 that occurred between 13 and 21 days (Wu et al., 2003) and the other reported a half-life of 1.3 months after a peak level observed at the first follow-up visit at 1 month, in a group who received a single dose of 12.5 mg vitamin D3 (Bacon et al., 2009). Since the actual peak 25(OH)D in the latter study (Bacon et al., 2009) might have occurred 1-3 weeks earlier than the 1-month measurement, the observed half-life is not inconsistent with that observed in the US study (Ilahi et al., 2008). The longer half-life in the former New Zealand study (Wu et al., 2003) may simply reflect greater levels of sun exposure in the study group during follow-up. Whilst these data suggest a clinically-applicable 25(OH)D half-life of around 2-3 months, it is possible that the half-life for these large acute doses is longer than it would be for smaller doses. Higher doses may result in greater accumulation and storage of circulating vitamin D3 and more sustained subsequent release from storage depots in concentrations below the saturation level of hepatic hydroxylation enzymes, and consequently greater overall conversion to 25(OH)D.

### 9.2.2 25-hydroxyvitamin D response to sustained doses

Data from studies that measure 25(OH)D concentration before and after sustained vitamin D supplementation have been used to calculate a response per unit dose (dose-response). An approximate figure of 1 nmol/l increase for every 1 μg vitamin D (2.5 nmol/l per 100 IU) has been suggested (Holick, 2008; Vieth, 2007), but the 25(OH)D dose-response varies widely (Barger-Lux et al., 1998; Binkley et al., 2011; Gallagher et al., 2012) ranging from 0.22-3.1 nmol/l per μg for dose comparison studies in Table 9.1. The actual dose-response may be underestimated for most studies in Table 9.1 since vitamin D treatment periods are relatively short (≤6 months) and a plateau in 25(OH)D may not have occurred. Normally, a duration of 4.5 times the half-life, perhaps 9 months according to Vieth’s (2008) estimate above would be considered adequate for attaining a new steady-state (Buxton, 2006).
Table 9.1. Response of 25-hydroxyvitamin D following vitamin D supplementation in adults. Data are mean ± SD unless indicated otherwise.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention(s) (µg)</th>
<th>Duration (weeks)</th>
<th>Participants: n; age (y)</th>
<th>Baseline 25(OH)D (nmol/l)</th>
<th>Peak 25(OH)D response² (nmol/l) or dose-response (nmol/l per µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (single dose) – Studies reporting serial 25(OH)D measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Davies et al. (1985)</td>
<td>2,500 D2/6 mth</td>
<td>12</td>
<td>128 elderly</td>
<td>6 est. (sample n=20)</td>
<td>not reported</td>
</tr>
<tr>
<td>Wismann et al. (1986)</td>
<td>2,500 D2/y</td>
<td>17-22</td>
<td>27 elderly; range: 72-94</td>
<td>56</td>
<td>peak at 2 weeks</td>
</tr>
<tr>
<td>Wu et al. (2003)</td>
<td>7,500 D3</td>
<td>≤102</td>
<td>49 elderly; 84±5 (range: 69-94)</td>
<td>17±10</td>
<td>62 (est. from figure)</td>
</tr>
<tr>
<td>Ilahi et al. (2008)</td>
<td>2,500 D3</td>
<td>16</td>
<td>30 adults; 20 older 71±6 (range: 61-84)</td>
<td>68±19</td>
<td>peak at 13-21 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 younger 38±8 (range: 27-47)</td>
<td></td>
<td>half-life 90 days</td>
</tr>
<tr>
<td>Premaor et al. (2008)</td>
<td>7,500 D3</td>
<td>39</td>
<td>14 elderly; 79±8 whole cohort (n=28): 81±9 (range: 65-102)</td>
<td>31±17</td>
<td>37 peak at 7 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>half-life³ approx. 60 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 (est. from figure)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>peak between 0-2 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>half-life not reported</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60 peak between 0-3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>half-life 1.3 months</td>
</tr>
<tr>
<td>Steady state (sustained dose) – Studies comparing different doses or dose regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacLennan &amp; Hamilton (1977)</td>
<td>12.5 D/d</td>
<td>4</td>
<td>elderly hospital patients</td>
<td>7 est. from figure (&lt;15)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>50 D/d</td>
<td></td>
<td></td>
<td>14 est. from figure (&lt;30)</td>
<td>0.32</td>
</tr>
<tr>
<td>Stamp et al. (1977)</td>
<td>20-2,500 D</td>
<td>≥17</td>
<td>128 adults mixed ages</td>
<td>not reported</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td>250 D3/d</td>
<td></td>
<td></td>
<td></td>
<td>0.62⁴</td>
</tr>
<tr>
<td></td>
<td>1,250 D3/d</td>
<td></td>
<td></td>
<td></td>
<td>0.51⁴</td>
</tr>
<tr>
<td>Vieth et al. (2001)</td>
<td>25 D3/d</td>
<td>9-22</td>
<td>61 adults; 41±9</td>
<td>43±17</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>100 D3/d</td>
<td></td>
<td></td>
<td>38±13</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 9.1. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention(s) (μg)</th>
<th>Duration (weeks)</th>
<th>Participants: n; age (y)</th>
<th>Baseline 25(OH)D (nmol/l)</th>
<th>Peak 25(OH)D dose-response (nmol/l per μg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state (sustained dose) - Studies comparing different doses or dose regimens (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heaney et al. (2003)</td>
<td>25 D3/d</td>
<td>≈20</td>
<td>67 men; 39±11</td>
<td>70±20</td>
<td>0.574</td>
</tr>
<tr>
<td></td>
<td>125 D3/d</td>
<td></td>
<td></td>
<td></td>
<td>0.664</td>
</tr>
<tr>
<td></td>
<td>250 D3/d</td>
<td></td>
<td></td>
<td></td>
<td>0.584</td>
</tr>
<tr>
<td>Viljakainen et al. (2006)</td>
<td>5 D3/d</td>
<td>6</td>
<td>49 elderly women; 71±4 (range: 65-85)</td>
<td>46±14</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>10 D3/d</td>
<td></td>
<td></td>
<td>47±10</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>20 D3/d</td>
<td></td>
<td></td>
<td>44±13</td>
<td>1.19</td>
</tr>
<tr>
<td>Chel et al. (2008)</td>
<td>15 D3/d</td>
<td>17</td>
<td>338 elderly; 84±6</td>
<td>24±9</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>105 D3/wk</td>
<td></td>
<td></td>
<td>27±13</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>450 D3/mth</td>
<td></td>
<td></td>
<td>24±9</td>
<td>1.985</td>
</tr>
<tr>
<td>Ish-Shalom et al. (2008)</td>
<td>37.5 D3/d</td>
<td>8</td>
<td>48 elderly women; 81±8</td>
<td>38±17</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>262.5 D3/wk</td>
<td></td>
<td></td>
<td>39±25</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1,125 D3/28 d</td>
<td></td>
<td></td>
<td>40±25</td>
<td>1.30</td>
</tr>
<tr>
<td>Sneve et al. (2008)</td>
<td>500 D3/wk</td>
<td>52</td>
<td>296 overweight &amp; obese adults; 47 (range: 20-71) whole cohort (n=445): 48±11</td>
<td>51±18</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>500 D3/2×wk</td>
<td></td>
<td></td>
<td>55±17</td>
<td>0.42</td>
</tr>
<tr>
<td>Pekkarinen et al. (2010)</td>
<td>20 D3/day</td>
<td>52</td>
<td>40 elderly women; 74 (median) (range: 69-79)</td>
<td>54 (median)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>2,433 D3/4 mth</td>
<td></td>
<td></td>
<td>57.5 (median) (range: 17-84)</td>
<td>0.235</td>
</tr>
<tr>
<td>Van Groningen et al. (2010)</td>
<td>625 D3/wk</td>
<td>8</td>
<td>208 adults; 55±17 (range: 18-88)</td>
<td>21±8</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>625 D3/wk</td>
<td>6</td>
<td></td>
<td>20±10</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>625 D3/2 wk</td>
<td>8</td>
<td></td>
<td>19±7</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(whole cohort &lt;50)</td>
<td></td>
</tr>
<tr>
<td>Binkley et al. (2011)</td>
<td>40 D2/d</td>
<td>52</td>
<td>64 elderly; 73±6 est. from table (range: 65-88)</td>
<td>87±24</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>40 D3/d</td>
<td></td>
<td></td>
<td>75±27</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>1,250 D2/mth</td>
<td></td>
<td></td>
<td>81±24</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>1,250 D3/mth</td>
<td></td>
<td></td>
<td>87±23</td>
<td>0.54</td>
</tr>
<tr>
<td>Study</td>
<td>Intervention(s) (μg)</td>
<td>Duration (weeks)</td>
<td>Participants: n; age (y)(^1)</td>
<td>Baseline 25(OH)D (nmol/l)</td>
<td>Peak 25(OH)D dose-response (nmol/l per μg/day)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------</td>
<td>------------------</td>
<td>---------------------------------</td>
<td>--------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Steady state (sustained dose)</strong> – Studies comparing different doses or dose regimens (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verschueren et al. (2011)</td>
<td>20 D3/d</td>
<td>26</td>
<td>113 elderly women; 80 ±5 (all &gt;70)</td>
<td>52 ±32</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>40 D3/d</td>
<td></td>
<td></td>
<td>55 ±36</td>
<td>1.18</td>
</tr>
<tr>
<td>Grimnes et al. (2012)</td>
<td>10 D3/2× d</td>
<td>52</td>
<td>297 postmenopausal women; 63±7 (range: 50-80)</td>
<td>71±22</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>10 D3/2× d +</td>
<td></td>
<td></td>
<td>71±23</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>500 D3/2× wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallagher et al. (2012)</td>
<td>10 D3/day</td>
<td>26 &amp; 52</td>
<td>163 postmenopausal women; 67±7</td>
<td>38±11</td>
<td>2.61(^7)</td>
</tr>
<tr>
<td></td>
<td>20 D3/day</td>
<td>(range 56-91)</td>
<td></td>
<td>39±10</td>
<td>1.68(^7)</td>
</tr>
<tr>
<td></td>
<td>40 D3/day</td>
<td>26 &amp; 52</td>
<td></td>
<td>37±10</td>
<td>1.25(^7)</td>
</tr>
<tr>
<td></td>
<td>60 D3/day</td>
<td>26 &amp; 52</td>
<td></td>
<td>38±10</td>
<td>1.02(^7)</td>
</tr>
<tr>
<td></td>
<td>80 D3/day</td>
<td>26 &amp; 52</td>
<td></td>
<td>40±8</td>
<td>0.85(^7)</td>
</tr>
<tr>
<td></td>
<td>100 D3/day</td>
<td>26 &amp; 52</td>
<td></td>
<td>37±9</td>
<td>0.76(^7)</td>
</tr>
<tr>
<td></td>
<td>120 D3/day</td>
<td>26 &amp; 52</td>
<td></td>
<td>39±9</td>
<td>0.64(^7)</td>
</tr>
<tr>
<td></td>
<td>(all &lt;50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) For all subjects taking all vitamin D doses indicated unless otherwise stated.

\(^2\) For acute (single dose) studies, peak response indicates the change in 25(OH)D (nmol/l) from baseline to peak levels with time to reach peak levels and subsequent disappearance half-life. For steady state (sustained dose) studies, dose-response indicates the dose-adjusted response or rise in 25(OH)D per IU/day vitamin D.

\(^3\) Half-life from these data reported in later analysis by common authors Heaney et al. (2008).

\(^4\) Corrected for results of analysis of actual vitamin D levels in tablets.

\(^5\) Different preparation of vitamin D compared to other doses in the same study.

\(^6\) Same cohort as Jorde et al. (2010b).

\(^7\) Dose response calculated from quadratic function curve fitted to all data.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; im = intramuscular; est. = estimated; /2× wk = twice weekly; /2 wk = fortnightly; /4 mth = 4 monthly; /6 mth = 6 monthly.
9.2.3 Determinants of 25-hydroxyvitamin D response

The 25(OH)D response to vitamin D supplementation may depend on initial 25(OH)D concentration, body mass or composition, the dose quantity or frequency, and whether vitamin D2 or D3 is used. An inspection of steady-state studies in Table 9.1 suggests that the dose-response varies according to baseline 25(OH)D, with greater increments in 25(OH)D for the same vitamin D dose when initial levels are lower. For example, six studies administer daily doses of 25–40 μg D3 (Barger-Lux et al., 1998; Binkley et al., 2011; Gallagher et al., 2012; Heaney et al., 2003; Ish-Shalom et al., 2008; Vieth et al., 2001). Those with starting 25(OH)D levels below 50 nmol/l show dose-responses of 1.2-1.25 nmol/l per μg/day (Gallagher et al., 2012; Ish-Shalom et al., 2008; Vieth et al., 2001) whereas lower dose-responses (0.6-0.8 nmol/l per μg/day) were obtained in those starting from higher baseline levels (Barger-Lux et al., 1998; Binkley et al., 2011; Heaney et al., 2003). The relation between baseline 25(OH)D and dose-response was also statistically confirmed in three studies in Table 9.1 (Barger-Lux et al., 1998; Gallagher et al., 2012; Viljakainen et al., 2006). Because initial levels of 25(OH)D affect dose-response, the efficacy of vitamin D supplementation is likely to depend upon season of delivery. Seasonal effects have been reported in studies concerning regular vitamin D intake and doses. In one study, self-reported vitamin D supplementation was shown to raise 25(OH)D levels relative to no supplementation in winter but not summer (Rapuri et al., 2004) and, in another randomized controlled trial, 10 μg/day vitamin D reduced bone loss in postmenopausal women (Dawson-Hughes et al., 1991).

Similarly, body mass or adiposity may affect the 25(OH)D response to supplementation, since increases in serum vitamin D3 following a standardized dose of ultra-violet radiation in obese people have been shown to be half that of matched lean individuals (Wortsman et al., 2000) and body mass index is inversely associated with 25(OH)D levels and their change in response to supplementation (Jorde et al., 2010a). Barger-Lux et al. (1998) reported independent effects of body mass index, in addition to initial 25(OH)D levels, on 25(OH)D response to vitamin D3 doses of 25-1,250 μg/day. In contrast, Vieth et al. (2001) reported no effect of either initial 25(OH)D concentration or body weight on 25(OH)D change following 5 months of 25-100 μg vitamin D3/day. Similarly, although Gallagher et al. (2012) noted lower 25(OH)D levels resulting from supplementation in those with body mass index ≥25 kg/m² compared to those below, the difference was consistent across the doses administered, indicating that the dose did not affect the 25(OH)D response. Because 25(OH)D levels are inversely cross-sectionally related to indices of adiposity (Arunabh et al., 2003; Hyppönen and Power, 2007; Liel et al., 1988; Wortsman et al., 2000), it is difficult to determine the relative contribution of lower vitamin D status versus lesser adiposity on elevated 25(OH)D response to vitamin D dose, since low vitamin D status is associated with greater adiposity and they are likely to counteract each other.

Vitamin D dose may also influence 25(OH)D response per unit dose. Heaney et al. analyzed data from five studies reporting 25(OH)D response to 18-20 weeks of vitamin D3 treatment (Heaney et al., 2008). Their data suggest that below circulating vitamin D3 levels of 15 nmol/l, estimated as being equivalent to a vitamin D3 input of 50 μg/day, hepatic conversion to 25(OH)D3 depends only on vitamin D3 concentration, and synthesis is close to complete since levels of vitamin D3
remain very low despite increasing 25(OH)D3 levels (Heaney et al., 2008). Above vitamin D3 concentrations of 15 nmol/l, the increase in corresponding steady-state 25(OH)D3 was linear, which the authors attributed to saturation of and rate dependence on hepatic enzymes, combined with a greater degree of production than metabolic consumption. If these data depict the true steady-state relation between circulating vitamin D3 and 25(OH)D3, and equivalent absorption efficiency is assumed, one might expect to observe a lesser 25(OH)D dose-response only when daily vitamin D doses above approximately 50 μg are compared with those below. In contrast to this theoretical expectation, inspection of studies in Table 9.1 suggests that vitamin D dose over a wide range of doses may in fact influence dose-response of 25(OH)D. Of the seven studies in Table 9.1 from which a comparison of the response of different steady-state doses of vitamin D can be made (Barger-Lux et al., 1998; Gallagher et al., 2012; Heaney et al., 2003; Sneve et al., 2008; Van Groningen et al., 2010; Vieth et al., 2001; Viljakainen et al., 2006), all but one (Heaney et al., 2003) demonstrate a trend for a lesser dose-response at higher compared to lower doses. The differences in 25(OH)D dose-response for doses within the range of 5-1,250 μg/day might suggest a continuous reduction in efficiency of absorption of vitamin D or completeness of hepatic synthesis of 25(OH)D3 from vitamin D with increasing doses. Nonetheless, the observed differences in dose-response are likely to be relatively small in relation to variability between individuals and in the only of these studies to provide statistical reporting of such a comparison, differences between doses did not attain statistical significance (Viljakainen et al., 2006).

The response of 25(OH)D may also be affected by the frequency of delivery of vitamin D. Theoretically, vitamin D3 doses administered at approximately the frequency of the half-life for vitamin D metabolites would be expected to have similar effects to same cumulative dose administered daily (Buxton, 2006). If the half-life of 25(OH)D following vitamin D administration is in the order of 2 months as suggested by Vieth (2008), then very little difference in 25(OH)D response would therefore be expected when equivalent doses of vitamin D are administered daily, weekly or monthly. Two recent studies addressing whether this is so provide conflicting data. Confirming the theoretical expectation, Ish-Shalom et al. (2008) administered daily, weekly and monthly (28-day) vitamin D3 at doses equivalent to 37.5 μg/day to elderly women and demonstrated no appreciable difference in the rise in 25(OH)D levels over 56 days. In contrast, Chel et al. (2008), who also compared the effects on 25(OH)D of 15 μg/day vitamin D3 and equivalent weekly or monthly doses, found progressively smaller rises in 25(OH)D, after 4 months, from daily to weekly to monthly dosing. Though statistically significant, 25(OH)D response for daily dosing was only 6.5 nmol/l greater than for weekly dosing, a difference which is probably clinically unimportant. Reasons for the different results between the two studies are unclear. In a follow-up comment on the Chel et al. (2008) study, Vieth (an author in the Ish-Shalom et al., 2008 study) suggested that differences could have resulted from the use of a powdered preparation for the monthly dose only, which may have been incompletely consumed, or from lower ‘trough’ 25(OH)D concentrations for higher, less frequent doses (Vieth, 2008).

The form of administered vitamin D, that is whether D2 (ergocalciferol) or D3 (cholecalciferol) is taken, may be another determinant of 25(OH)D dose-response. Based on the treatment of rickets from the 1930s, vitamins D2 and D3 have traditionally been considered to be equally
effective in humans (Armas et al., 2004) and nutritional guidelines do not differentiate between the two (Vieth, 2004). However, more recent work has suggested a quantitative difference in 25(OH)D rise following supplementation. A number of studies have shown that vitamin D3 compared to D2 results in 0.5-2.5 fold greater increases in 25(OH)D levels (Binkley et al., 2011; Tjellesen et al., 1986; Trang et al., 1998) or up to 9.5 times greater when the area under the curve is considered (Armas et al., 2004). Confirming these findings, a South American study reported 3-month 25(OH)D responses to 125-250 μg/day vitamin D2 supplementation relative to a control group of 0.2 nmol/l per μg/day, or around a third of that expected for comparable trials of vitamin D3 (Mastaglia et al., 2006). On the other hand, recent data reported by Holick and coworkers introduce some uncertainty about the relative advantage of vitamin D2 or D3, as these authors failed to find any difference in 25(OH)D response between daily 250 μg doses of either form (Holick et al., 2008). More uncertainty arises from assay-related difficulties, when comparing 25(OH)D response to vitamin D2 and D3 therapies, since some assays unevenly measure 25(OH)D2 and 25(OH)D3 (Hollis, 2000; 2004).

In summary, surprisingly few data inform clinicians about the likely time-course of 25(OH)D response to vitamin D supplementation. Whilst pragmatic figures have been suggested for calculating the likely 25(OH)D response to specific vitamin D doses, these are likely to be highly variable and depend on a range of factors, including vitamin D status, body composition, the form of vitamin D and dose administered. It is possible that high doses of vitamin D (above 50 μg/day or equivalent) result in a different equilibrium relationship between circulating vitamin D and its hydroxylated metabolites. It is important to consider determinants of the 25(OH)D response to vitamin D supplementation since any factor that affects dose-response will also affect its efficacy for health outcomes.

### 9.3 Efficacy of high-dose vitamin D in elderly

Although the vast majority of studies determining the efficacy of vitamin D supplementation have provided low (<25 μg) daily doses (Avenell et al., 2005; Bischoff-Ferrari et al., 2004, 2005, 2009; Boonen et al., 2006; Cranney et al., 2008; Latham et al., 2003b; Papadimitropoulos et al., 2002; Tang et al., 2007), some contend that this dose is not high enough to meet the needs of elderly people who have limited outdoor sun exposure (Bischoff-Ferrari, 2009; Holick, 2011; Vieth et al., 2007). Indeed, assuming a dose-response of 1 nmol/l per μg/day (a typical value from Table 9.1), a dose of 50 μg/day would be required for an individual with no outdoor sun exposure at all to attain a steady state 25(OH)D level of 50 nmol/l. Furthermore, compliance to daily vitamin D medication may be low in elderly people, particularly if it is deemed to be prophylactic and nutritional in nature, and less essential for health than other medications. Less frequent dose regimens might be more conveniently administered and therefore improve compliance and cost-effectiveness, particularly in hospital or care situations. For these reasons, the data surrounding the efficacy of daily moderate and high doses, and of monthly, seasonal or single loading doses has expanded since the middle of the last decade (Vieth, 2011). One of the challenges for clinicians wishing to adopt these dosing strategies is that Vitamin D3 preparations delivering more than 25
High-dose vitamin D supplementation in the elderly

μg per dose have not been readily available in Australia, Europe or North America. Consequently, evaluation of their efficacy when compared to daily low-dose vitamin D has been difficult because whilst most low-dose large studies delivered vitamin D3, until recently the few sizeable high-dose studies that existed were mainly of vitamin D2 (Dhesi et al., 2004; Flicker et al., 2005; Harwood et al., 2004; Heikinheimo et al., 1992; Law et al., 2006; Nordin et al., 1985; Smith et al., 2007).

9.3.1 Musculoskeletal efficacy

Given the preponderance of studies of vitamin D supplementation, relatively few have administered moderate- or high-dose vitamin D to elderly people. A modest number of studies investigating the effects of vitamin D on various musculoskeletal outcomes in elderly people have administered daily or weekly doses equivalent to 25 up to 100 μg/day (Table 9.2). In addition, several studies have administered larger doses, either as a single loading dose, seasonally or annually (Table 9.3). Controlled investigations of the ability of moderate and high doses of vitamin D to reduce fractures in elderly are inconclusive, variously reporting reductions in fractures (Sato et al., 2005; Trivedi et al., 2003), or a reduction in females only (Heikinheimo et al., 1992); no effect on fractures (Law et al., 2006); or, in two recent sizeable studies, an increase in hip (Smith et al., 2007) or all fractures (Sanders et al., 2010). All except one of these studies, which provided 25 μg vitamin D3/day (Sato et al., 2005), administered large infrequent doses ranging from 3.75-12.5 mg/year without additional calcium compared to the control group.

Data determining falls risk-reduction studies recruiting similar populations are also equivocal. Studies that administer moderate daily or weekly vitamin D doses have reported reductions in falls (Flicker et al., 2005; Sato et al., 2005) or in seasonal (winter and spring only) falls (Prince et al., 2008). However, of those giving elderly people large seasonal or annual doses only one study has noted a reduction in falls (Harwood et al., 2004), with others reporting no effect (Latham et al., 2003a; Law et al., 2006; Smith et al., 2007), or increased falls risk (Sanders et al., 2010).

Findings from randomized controlled trials reporting the effects of moderate or high-dose vitamin D on changes in bone mineral density are also inconsistent. Two smaller studies report reduced hip bone mineral density loss following either daily 25 μg vitamin D2 and 1.2 g calcium for 5 years, compared to placebo (Zhu et al., 2008), or 1 year following either a single dose of 7.5 mg vitamin D2 or 20 μg vitamin D3 daily combined with 1 g calcium, with larger effects noted for the latter preparation (Harwood et al., 2004). In contrast, another study investigating the effects on older African American women of 20 μg vitamin D3, which was increased to 50 μg for the third year of the trial, found no effect on either changes in bone mineral density at any site or in bone turnover markers (Aloia et al., 2005). The lack of effect of 15 μg/day vitamin D3 on bone turnover markers was confirmed by Chel et al. (2008). Three studies have also failed to show an effect of moderate- (Haworth et al., 2004; Verschueren et al., 2011) to high-dose (Grimnes et al., 2012) vitamin D compared to low doses (20-22.5 μg/day) on bone loss.

A number of studies have assessed the effect of vitamin D on muscle function or functional performance, again with mixed results. Improvements, relative to placebo, in muscle strength and
Table 9.2. Controlled trials with musculoskeletal outcomes administering vitamin D at daily doses ≥1000 IU to elderly people. Data are mean ± SD unless indicated otherwise.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention(s) (μg)</th>
<th>Duration (months)</th>
<th>Participants: n; age (y)</th>
<th>Baseline 25(OH)D (nmol/l)</th>
<th>Main treatment outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson et al. (1980)</td>
<td>50 D/d</td>
<td>6</td>
<td>120 elderly; (≥65)</td>
<td>not reported</td>
<td>prevented fall in serum phosphate; no effect on muscle function or mental function</td>
</tr>
<tr>
<td>Corless et al. (1985)</td>
<td>225 D2/d</td>
<td>2.9</td>
<td>65 elderly; 82±37</td>
<td>17±12</td>
<td>no effect on activities of daily living or mental function</td>
</tr>
<tr>
<td>Nordin et al. (1985)</td>
<td>375 D2/wk</td>
<td>24</td>
<td>109 elderly women; 70±2 (range: 65-74)</td>
<td>22±15 ^3</td>
<td>reduced rate of cortical bone loss</td>
</tr>
<tr>
<td>Honkanen et al. (1990)</td>
<td>45 D3/d + 1 g calcium</td>
<td>2.5 (11 wk)</td>
<td>139 elderly women; 77±8 (≥65)</td>
<td>22±16</td>
<td>no effect on grip strength in either group</td>
</tr>
<tr>
<td>Kenny et al. (2003)</td>
<td>25 D3/d + 0.5 g calcium</td>
<td>6</td>
<td>65 elderly men; 76±4 (range: 65-87)</td>
<td>home living: 40±16 hospital: 24±12</td>
<td>no effect on muscle strength, power or physical performance</td>
</tr>
<tr>
<td>Aloia et al. (2005)</td>
<td>20 D3 then 50 D3 for 3rd year + calcium to 1.2 - 1.5 g total intake versus calcium</td>
<td>36</td>
<td>208 older African American women; 61±6 (range 50-75)</td>
<td>46±19</td>
<td>no effect on bone loss or bone turnover markers</td>
</tr>
<tr>
<td>Flicker et al. (2005)</td>
<td>250 D2/wk then 25 D2/d</td>
<td>24</td>
<td>625 elderly; 83±8</td>
<td>42 est. from frequency distribution (range: 25-90)</td>
<td>reduced falls rate</td>
</tr>
<tr>
<td>Sato et al. (2005)</td>
<td>25 D2/d</td>
<td>24</td>
<td>96 elderly women; 74±4</td>
<td>24±3 (all &lt;25)</td>
<td>reduced falls and fractures; increased muscle hypertrophy &amp; strength</td>
</tr>
<tr>
<td>Chel et al. (2008)</td>
<td>15 D3/d</td>
<td>4</td>
<td>338 elderly; 84±6</td>
<td>25±11</td>
<td>no effect on bone turnover markers</td>
</tr>
<tr>
<td></td>
<td>105 D3/wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>450 D3/mth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Intervention(s)</td>
<td>Duration (months)</td>
<td>Participants: n; age (y)</td>
<td>Baseline 25(OH)D (nmol/l)</td>
<td>Main treatment outcome(s)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Prince et al. (2008) &amp; Zhu et al. (2010)</td>
<td>25 D2/d + 1 g calcium vs. calcium</td>
<td>12</td>
<td>302 elderly women; 77±5 (range: 70-90) 45±13 (all &lt;60)</td>
<td>reduced first fall risk in winter &amp; spring; improved timed up-and-go &amp; hip muscle strength for those in lowest outcome tertiles reduced hip BMD loss especially in those ≤68 nmol/l</td>
<td></td>
</tr>
<tr>
<td>Zhu et al. (2008)</td>
<td>25 D2/d + 1.2 g calcium vs. 2 control groups</td>
<td>60</td>
<td>120 elderly women; 75±3 (range: 70-80) 68±29</td>
<td>no difference between doses in hip BMD or in muscle strength or mass</td>
<td></td>
</tr>
<tr>
<td>Verschueren et al. (2011)</td>
<td>40 D3/d + 1 g calcium vs. 800 D3/d + calcium</td>
<td>6</td>
<td>113 elderly women; 80 ±5 (all &gt;70) 53±34</td>
<td>no difference between doses in BMD change; bone formation marker (P1NP) reduced more in low compared to high dose.</td>
<td></td>
</tr>
<tr>
<td>Grimnes et al. (2012)</td>
<td>1,140 D3/wk + 0.5 g calcium vs. 20 D3/d + calcium</td>
<td>12</td>
<td>297 postmenopausal women; 63±7 (range: 50-80) 71±23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Placebo-controlled unless indicated otherwise.
2 For all enrolled subjects taking all vitamin D doses and control group(s) unless otherwise stated.
3 Reported values corrected for assay methodological error used for this calculation.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; vs. = versus; /d = daily; /wk = weekly; /mth = monthly.
Table 9.3. Controlled trials with musculoskeletal outcomes administering vitamin D in monthly, seasonal or single high doses. Data are mean ± SD unless indicated otherwise.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention(s) (mg)¹</th>
<th>Duration or follow-up (months)</th>
<th>Participants: n; age (y)²</th>
<th>Baseline 25(OH)D (nmol/l)²</th>
<th>Main treatment outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heikinheimo et al. (1992)</td>
<td>3.75 D2/y im</td>
<td>24-60</td>
<td>799 elderly; 84 (all &gt;75)</td>
<td>20 (subgroup est. from figure in previous report (Heikinheimo et al., 1991))</td>
<td>fewer fractures in females only; fewer upper limb and rib fractures</td>
</tr>
<tr>
<td>Latham et al. (2003a)</td>
<td>7.5 D3 single</td>
<td>6</td>
<td>243 elderly; 80</td>
<td>42 median est.</td>
<td>no effect on falls of physical health or performance</td>
</tr>
<tr>
<td>Trivedi et al. (2003)</td>
<td>2.5 D3/4 mth</td>
<td>60</td>
<td>2686 elderly; 75±5 (range: 65-85)</td>
<td>not reported</td>
<td>reduced risk of fractures (especially osteoporotic sites)</td>
</tr>
<tr>
<td>Dhesi et al. (2004)</td>
<td>1.5 D2 single</td>
<td>6</td>
<td>139 elderly past fallers; 26 77±6 (all &lt;65)</td>
<td>(all &lt;30)</td>
<td>improved functional performance, reaction time and balance but not strength</td>
</tr>
<tr>
<td>Harwood et al. (2004)</td>
<td>7.5 D2 single</td>
<td>52</td>
<td>111 elderly women; 81 whole cohort (n=150); 81 (range: 67-92)</td>
<td>29 (range: 10-85)</td>
<td>increased hip BMD and reduced falls</td>
</tr>
<tr>
<td>Law et al. (2006)</td>
<td>2.5 D2/3 mth</td>
<td>10 median</td>
<td>3717 elderly; 85</td>
<td>59; 47 median (in sample, n=18) not reported</td>
<td>no effect on fall or fracture risk</td>
</tr>
<tr>
<td>Smith et al. (2007)</td>
<td>7.5 D2/y im</td>
<td>36</td>
<td>9440 elderly; 79 median (all &gt;75)</td>
<td>not reported</td>
<td>increased hip fracture risk; no effect on other fractures or falls</td>
</tr>
<tr>
<td>Sanders et al. (2010)</td>
<td>12.5 D3/y</td>
<td>36-60</td>
<td>2256 elderly women; 76 median (all &gt;70)</td>
<td>49 median est. (53 median in treatment group)</td>
<td>Increased risk of falls and fractures especially in the first 3 months after dose</td>
</tr>
</tbody>
</table>

¹ Placebo-controlled unless indicated otherwise.

² For all enrolled subjects taking all vitamin D doses and control group(s) unless otherwise stated.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; im = intramuscular; est. = estimated; BMD = bone mineral density; /mth = monthly; /y = yearly.
function with 25 μg/day vitamin D2 and 1.2 g calcium were noted for a subgroup of participants with baseline levels in the lowest tertile (Zhu et al., 2010) and in functional performance tests but not strength, following a single 15 mg vitamin D2 dose, in another study (Dhesi et al., 2004). However, for the majority of similar investigations, no effect on muscle strength or functional performance has been noted for a range of daily moderate-, high- or loading-dose vitamin D preparations, either compared to placebo (Corless et al., 1985; Honkanen et al., 1990; Johnson et al., 1980; Kenny et al., 2003; Latham et al., 2003a; Witham et al., 2010a), or compared to lower doses of vitamin D (Grimnes et al., 2012; Verschueren et al., 2011).

9.3.2 Other effects

A few studies have also investigated the effect of moderate-, high- or loading-dose vitamin D supplementation on a range of non-musculoskeletal indices in elderly, sometimes as secondary or tertiary outcome variables, notably cardiovascular-metabolic variables such as blood pressure, cholesterol, endothelial function, B-type natriuretic peptide, and glucose metabolism (Scragg et al., 1995; Witham et al., 2010a,b); mortality from chronic diseases (Trivedi et al., 2003); mental function and depression (Corless et al., 1985; Jorde et al., 2008); and inflammatory cytokines (Jorde et al., 2010c; Schleithoff et al., 2006). Data from these studies cumulatively note effects in favor of vitamin D only for colon cancer mortality (Trivedi et al., 2003), blood pressure and B-type natriuretic peptide (Witham et al., 2010a,b), cytokines (Schleithoff et al., 2006) and depression (Jorde et al., 2008).

9.4 Safety of high-dose vitamin D in elderly

9.4.1 Vitamin D toxicity

High doses of vitamin D are toxic and may result in hypercalcaemia, renal stones and the calcification of soft tissue and blood vessels (Cranney et al., 2007; Vieth, 2007). Because high doses may accumulate in fat, or possibly muscle, tissue (Heaney et al., 2008; Jones, 2008), the effects of toxicity may be long-lasting. Hypercalcaemia comes about via increased intestinal calcium absorption and induction of bone resorption (Suda et al., 2003). Although it was earlier assumed that rises in 1,25(OH)₂D were responsible for the toxicity-associated pathology, the expected substantial elevations in this metabolite have not been consistently demonstrated in cases of toxicity (Jones, 2008). It is now believed that pharmacological concentrations 25(OH)D and/or other vitamin D metabolites exceed the vitamin D binding protein capacity, causing free 25(OH)D or displaced 1,25(OH)₂D to enter cells and stimulate gene transcription (Jones, 2008). The lowest levels of 25(OH)D associated with toxicity are 221 nmol/l, although they are normally higher than 500-700 nmol/l (Jones, 2008; Vieth, 1999). By comparison, the highest recorded levels associated with natural sun exposure are 225-274 nmol/l following deliberate ultraviolet exposure (Krause et al., 1998; Vieth, 1999). Vieth (1999) has suggested that homeostatic control mechanisms, potentially hepatic, prevent the large increases in 25(OH)D necessary for
hypercalcaemic toxicity with long term (>1 year) vitamin D intakes less than 250 μg/day, or shorter-term doses of 500-1,250 μg/day.

The maximum daily intake of a nutrient deemed unlikely to pose a risk of adverse health events to almost all individuals is known as the UL (Grosvenor and Smolin, 2002). In 2011, the UL was raised from the 1997 recommendation of 50 μg/day to 100 μg/day (Aloia, 2011; Ross et al., 2011). Some prominent researchers have argued for an upward revision of UL to 250 μg/day (Giovannucci, 2011; Hathcock et al., 2007; Heaney, 2005; Holick, 2011; Vieth, 2007). The basis of these arguments are, firstly, that the UL is not supported by evidence of toxicity with serum 25(OH)D levels less than 250 nmol/l (equivalent to continuing oral daily intakes of 250 μg/day). Secondly, it is argued that the UL is too restrictive to allow research into the efficacy of high doses of vitamin D, which is desirable in the light of evidence showing that optimal levels are higher than previously accepted (Bischoff-Ferrari et al., 2006; Dawson-Hughes et al., 2005; Gorham et al., 2007) considered in conjunction with data showing that prevailing vitamin D status in relation to these levels is too low (Calvo et al., 2005; Rockell et al., 2006; Ruston et al., 2004).

9.4.2 Calcaemic effects of high vitamin D doses

Most evaluations vitamin D safety report incidence of hypercalcaemia, hypercalciuria and kidney stones, though most of the data comes from high dose studies of young healthy adults, children or recently in adults diagnosed with multiple sclerosis (Aloia et al., 2008; Burton et al., 2010; Ilahi et al., 2008; Kimball et al., 2007; Maalouf et al., 2008). In a large commissioned systematic review, Cranney et al. (2007, 2008) identified 22 trials which focused on incidence of these adverse events in postmenopausal women and elderly men, following supplementation with doses of vitamin D3 ranging from 10 to 100 μg/day and vitamin D2 ranging from 12.5 to 250 μg/day. From meta-analysis, they noted no statistical difference in the incidence of hypercalcaemia or hypercalciuria between treatment and control groups. Reporting of hypercalcaemia was most common and was often explained by other uncovered medical conditions (Cranney et al., 2007). In one study neither serum calcium nor urinary calcium-creatinine excretion ratios changed from baseline in healthy adults provided 100 μg vitamin D3 daily for 6 months (Vieth et al., 2001). Similarly, a later study noted no cases of hypercalcaemia following 6 months of increasing vitamin D3 doses, starting at 50 to 100 μg/day and adjusted at 2-month intervals according to assayed 25(OH)D, to reach a median daily dose of 95 μg (Aloia et al., 2008). Although these authors identified four cases of elevated calcium/creatinine ratio, there was no difference in the number of cases between treatment and placebo groups, and in three cases the elevation was temporary and within the normal range 1 week later (Aloia et al., 2008).

A few studies document adverse events in large vitamin D supplementation studies in postmenopausal women. Some prominence is given to the Women’s Health Initiative trial finding of an increased 7-year occurrence of renal stones amongst the treatment group (2.7%) compared to controls (2.3%) from a total of 36,282 postmenopausal women participants (Jackson et al., 2006). However, in addition to 10 μg/day vitamin D3, this study also provided 1 g/day supplemental calcium to participants, so it is not possible to distinguish between the effects
of the two nutrients. In more recent trials of high dose (25 μg/day) vitamin D, hypercalcaemic episodes were transient (Prince et al., 2008) and not different between treatment and control groups (Prince et al., 2008; Zhu et al., 2008).

Despite the lack of documentation of any evidence of adverse calcaemic effects from moderate or high vitamin D doses, frail elderly people may be more at risk from small fluctuations in calcium homeostasis, as a consequence of hepatic, renal or skeletal stress. In particular, there is some concern about the increased potential for adverse effects from large infrequent, or single doses. An Australian study which treated 50 men and women who had low vitamin D status (25(OH)D <50 nmol/l) with intramuscular 15 mg vitamin D3, found that at 12 months, 20% had hypercalciuria (urine calcium/creatinine excretion index of 0.6 or greater), and 4% had mild hypercalcaemia (mean level 2.67 mmol/l compared to upper reference range of 2.65 mmol/l) (Diamond et al., 2005). Nonetheless, it is not clear whether these observations resulted from the vitamin D intervention. Because it would have been unethical to fail to provide vitamin D to individuals with pre-established insufficiency, there was no control group in this study. Furthermore, three individuals who developed hypercalciuria (6% total) also had elevated values at baseline, and primary hyperparathyroidism was uncovered in an additional person. Finally, since the time to peak 25(OH)D after oral administration of a similarly high dose (Wu et al., 2003) is in the region of 2-3 weeks, it is unclear why calcium levels at 12 months but not at 4 months were higher than baseline (Diamond et al., 2005).

9.4.3 Other risks for elderly people

The additional possibility of increased fracture rates following high-dose vitamin D is suggested by the results of a large (n=9,440) intervention in which annual 12.5 mg intramuscular vitamin D2 was provided for 3 years (Smith et al., 2007). In this study, a 49% increased rate of hip or femur fractures was found for those randomized to vitamin D, an effect that extended to any non-vertebral fracture in women (Smith et al., 2007). More recently, an Australian group confirmed these concerns reporting a 4-year 15% increase in falls risk and a 26% increased risk of fractures following annual 15 mg vitamin D3 (Sanders et al., 2010). These effects remained when risk was adjusted for calcium intake, although the adjusted risk of fractures marginally failed to attain statistical significance. Risks of falls and fractures compared to placebo were higher in the first 3 months after each treatment (31% and 53% respectively) than in the subsequent 9 months. The physiological mechanism behind an effect of large doses of vitamin D on falls and fractures is unclear. It may be that the effects on skeletal bone resorption dominate in response to very large single vitamin D doses. In an editorial that accompanied the Australian study report Dawson-Hughes and Harris (2010) speculate that the high dose may have up-regulated 1,25(OH)2D catabolic enzyme as has been observed for 1,875 μg/week vitamin D3 doses in rats (Beckman et al., 1995). It should be noted that this dose in rats is probably equivalent to a per-kg dose in humans approximately 10-fold greater than that delivered to participants in the Australian study (Sanders et al., 2010). Dawson-Hughes and Harris alternatively suggest that the high dose vitamin D may have transiently improved mobility, perhaps via its effect on muscle function, physical performance, pain or mood, which may in turn have increased opportunities for falls to
occur (Dawson-Hughes and Harris, 2010). Whatever the mechanism by which increased falls and fractures in response to very high single doses of vitamin D might occur, it would seem prudent to avoid such high doses, at least until more data documenting adverse events in controlled trials of high-dose vitamin D are available.

9.5 Summary

There has been recent interest in data surrounding the efficacy and safety of vitamin D administered in sustained doses of 25 μg/day and above, equivalent weekly or monthly doses, or as higher seasonal, annual or loading doses. Seasonal or annual doses may be particularly appealing for treatment for frail or institutionalized elderly people because they may be more convenient or improve compliance. The efficacy of these doses is largely dependent on the physiological response of 25(OH)D to vitamin D. The time-course of 25(OH)D changes in response to vitamin D is highly variable and depends on a number of factors that probably include initial vitamin D status, body composition, and the form of vitamin D provided, and potentially include the quantity and frequency of the dose. Whilst many contend that doses up to 10,000 IU/day and equivalent weekly or monthly doses, which probably have a comparable physiological effect, are safe in that the hypercalcaemia of vitamin D toxicity is avoided, limited adverse event data are available from large placebo-controlled studies of elderly people. Caution might be warranted for large single doses of 12.5 mg and above since two recent studies have shown small but statistically significant increases in fall and fracture risk.

References


9. High-dose vitamin D supplementation in the elderly


Bouillon, R., 2011. Why modest but widespread improvement of the vitamin D status is the best strategy? Best Practice & Research Clinical Endocrinology & Metabolism 25, 693-702.


C.J. Bacon


9. High-dose vitamin D supplementation in the elderly


Handbook of vitamin D in human health


9. High-dose vitamin D supplementation in the elderly


Vieth, R., 2008. Comment on Chel et al.: efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporosis International 19, 721-722; author reply 723.

Vieth, R., 2011. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/l (30 ng/ml). Best Practice & Research Clinical Endocrinology & Metabolism 25, 681-691.


Vitamin D and chronic disease
Key facts

- Mean global vitamin D status is close to the insufficiency range.
- Several experimental studies show that a complete or partial lack of vitamin D action results in premature death.
- Many cells (e.g. monocytes, dendritic cells, B-lymphocytes, colonocytes, smooth muscle cells, endothelial cells) also possess the 25(OH)D-1α-hydroxylase (CYP27B1) which is required for the ability to synthesize the active form 1,25(OH)₂D, suggesting important regulatory properties of the active vitamin D metabolite on a cellular level.
- Some prospective cohort studies found a U-shaped association between circulating 25(OH)D levels and cancer morbidity and mortality with an increased risk at both, low and high levels.
- Adequately powered RCTs with all-cause mortality as primary endpoint are still needed.

Summary points

- There is likely evidence from prospective cohort studies and meta-analyses of randomized controlled trials where mortality was a secondary endpoint that adequate vitamin D status reduces all-cause mortality risk.
- Large randomized controlled trials with all-cause mortality or cause-specific mortality as primary endpoint are still lacking.
- Evidence from prospective cohort studies is accumulating that vitamin D deficiency increases the risk for cardiovascular diseases.
- Meta-analyses of cohort studies suggest that an increase of vitamin D reduces all-cause mortality in chronic kidney disease patients.
- For cancer mortality, available data does not allow final conclusions.
10. Vitamin D deficiency and premature death

A. Zittermann and S. Prokop
Clinic for Thoracic and Cardiovascular Surgery of the Heart and Diabetes Centre North Rhine-Westphalia, Georgstr. 11, 32545 Bad Oeynhausen, Germany; azittermann@hdz-nrw.de

Abstract

This chapter gives an overview of the current knowledge about vitamin D deficiency and premature death and discusses possible life-prolonging mechanisms of vitamin D. Globally, vitamin D status is low and the prevalence of vitamin D deficiency is common. Historically, rickets was associated with a high mortality risk in Europe and Northern America. In frail elderly people, both mortality risk and the prevalence of vitamin D deficiency increase exponentially. Likewise, prospective cohort studies indicate an elevated mortality risk in vitamin D deficient people in the general population. Results from two meta-analyses that have summarized available data from randomized studies where all-cause mortality was a secondary endpoint indicate a 6-7% reduction in all-cause mortality by vitamin D supplementation in frail elderly people. Studies with experimental animals lacking vitamin D action support the assumption that vitamin D deficiency results in premature death. Possible beneficial vitamin D actions include important regulatory properties of 1,25-dihydroxyvitamin D on a cellular level as well as higher telomerase activity, leading to maintained telomere length. Data from prospective cohort studies on cardiovascular mortality concur with those on all-cause mortality. For cancer mortality, data from epidemiological studies are less convincing and the evidence from randomized controlled trials is inconsistent. In conclusion, there is likely evidence that vitamin D reduces all-cause mortality in elderly people. However, adequately powered randomized controlled trials with all-cause mortality as primary endpoint are still needed.

Keywords: mortality, survival, premature death, cancer, cardiovascular disease, chronic kidney disease
10.1 Introduction

It has long been known that vitamin D is essential for human calcium and phosphorus homeostasis. These vitamin D effects are important for bone mineralization and bone health. This also explains why vitamin D deficiency causes rickets in infants and small children and osteomalacia in adults.

In recent decades, however, it became increasingly clear that besides its classical actions on mineral and bone metabolism, vitamin D also has a broad range of nonclassical actions in the human body. Experimental studies brought evidence forward that vitamin D is essential for muscle function, cardiovascular homeostasis, nervous function and the immune response. It is easy to imagine that disturbances in these biological systems lead to clinically relevant diseases, which may be life-threatening if the disturbances are severe. Low vitamin D status has indeed been associated with a high incidence of various chronic diseases such as CVD, various types of cancer, multiple sclerosis, and type 1 and 2 diabetes. This chapter gives an overview of the current knowledge about vitamin D deficiency and premature death. In addition, data on vitamin D deficiency and disease-specific mortality is presented. Since morbidity and mortality is sometimes closely linked with each other, e.g. in specific types of cancer, the association of vitamin D status with disease morbidity are also briefly elucidated. Possible life-prolonging mechanisms of vitamin D are discussed as well.

10.2 Human vitamin D status

Circulating 25(OH)D is the generally accepted indicator of human vitamin status. This parameter reflects the sum of vitamin D synthesis in the skin and oral vitamin D intake from dietary and supplemental sources. Recently, the IOM has stated that 25(OH)D levels below 30 nmol/l and between 30 and 50 nmol/l bear the risk of vitamin D deficiency and inadequacy, respectively.
is noteworthy, that this statement is exclusively based on beneficial vitamin D effects on fracture risk. The IOM considers levels above 125 nmol/l as potentially harmful. While there is general agreement that both vitamin D deficiency and excess can be harmful, the cut-offs presented by the IOM have been criticized by many researchers. An international expert panel, for example, has recommended a target 25(OH)D range of at least 75 to 100 nmol/l and has proposed an upper safety limit for circulating 25(OH)D of 250 nmol/l (Souberbielle et al., 2010), a value which is considerably higher than the upper safety limit of the IOM. Others consider values up to 372 nmol/l as safe (Holick, 2007). It is however also noteworthy that almost all classifications definitively consider circulating 25(OH)D levels <50 nmol/l and <25 nmol/l as inadequate and deficient, respectively. Table 10.1 presents a classification by the authors where the different stages of vitamin D status are associated to circulating 25(OH)D concentrations and are also related to possible biochemical and clinical alterations.

It is important to mention that the estimated mean global vitamin D level is 54 nmol/l (Hagenau et al., 2009) and thus close to the insufficiency range. In apparently healthy adults in North America and Central Europe the prevalence of 25(OH)D levels in the deficiency range is 8% and 16%, respectively (Looker et al., 2011; Zittermann, 2010), with a prevalence of up to 32% in African-Americans. Levels <25 nmol/l are also widespread in regions such as South Asia and the Middle East. In the Middle East, for example, 25(OH)D levels that are lying in the deficiency range have been reported in up to 60% of elderly people and up to 80% of young women. Older age, female sex, higher geographic latitude, winter season, darker skin pigmentation, less sunlight exposure, avoidance of vitamin D-rich foods such as fish, and absence of vitamin D fortification are the main factors that are significantly associated with lower 25(OH)D levels (Mithal et al., 2009).

### Table 10.1. Vitamin D status classified according to circulating 25(OH)D concentrations (Zittermann and Gummert, 2010).

<table>
<thead>
<tr>
<th>Stage</th>
<th>25(OH)D (nmol/l)</th>
<th>Clinical/biochemical alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>&lt;25</td>
<td>Rickets, osteomalacia, myopathy, calcium malabsorption, severe hyperparathyreodism, low 1,25(OH)2D levels, impaired immune and cardiovascular function, death</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>25-49.9</td>
<td>Reduced bone mineral density, impaired muscle function, elevated parathyroid hormone levels, slightly reduced 1,25(OH)2D levels</td>
</tr>
<tr>
<td>Hypovitaminosis D/</td>
<td>50-74.9</td>
<td>Low bodily stores of vitamin D, slightly elevated parathyroid hormone levels</td>
</tr>
<tr>
<td>suboptimal supply</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequacy</td>
<td>75-372</td>
<td>No disturbances of vitamin D-dependent functions</td>
</tr>
<tr>
<td>Intoxication</td>
<td>&gt;372</td>
<td>Intestinal calcium hyperabsorption, hypercalcemia, soft tissue calcification, death</td>
</tr>
</tbody>
</table>

Handbook of vitamin D in human health 177
10.3 Vitamin D deficiency and all-cause mortality in the general population

10.3.1 Infants

In Europe and North America, rickets was historically associated with a high mortality risk. A century ago, for example, autopsies of infants who died at 18 months of age or less found histopathological evidence of rickets in 96% (214 of 221 cases) (Rajakumar, 2003). Unfortunately, randomized controlled trials highlighting the relation of rickets to mortality risk are scarce. In a recently published Indian study (Kumar et al., 2011), 2079 low birthweight infants received 35 μg vitamin D or placebo weekly for 6 months. Primary endpoint was admission to hospital or death. Adjusted rate ratio for death or hospital admission was 0.93 (95% CI: 0.68, 1.29) and thus not different between the two study groups. However, it is noteworthy that at 6 months only 57% of infants in the vitamin D group had 25(OH)D levels lying in the adequate range (>50 nmol/l), whereas in 27% of infants in the placebo group values were also lying in the adequate range. Thus, the study was probably underpowered to find statistically significant differences between the vitamin D and placebo group in admission to hospital or death.

10.3.2 General adult population

Mortality risk increases exponentially in frail elderly people. These people also have a high risk for deficient 25(OH)D levels (Zittermann et al., 2009). A recent prospective cohort study has demonstrated that deficient vitamin D status is independently associated with an elevated mortality risk in frail elderly individuals (Pilz et al., 2012). Unfortunately, randomized controlled trials examining the effect of vitamin D on all-cause mortality as primary endpoint are completely lacking in the adult population. Nevertheless, results from two meta-analyses that have summarized available data from randomized studies where all-cause mortality was a secondary endpoint are available. The meta-analysis by Autier and Gandini (2007) included 18 RCTs with an overall sample of 57,113 individuals. The majority of these RCTs were originally undertaken to determine the effect of a vitamin D supplement on fracture risk in frail elderly people. Daily vitamin D intake varied from 300 to 2,000 IU yielding in a mean daily vitamin D dose of 528 IU. A total of 4,777 deaths occurred during a mean follow-up of 5.7 years. Combined estimates resulted in a decreased risk of total mortality of 7% (HR=0.93, 95% CI: 0.87, 0.99) in the vitamin D group during follow-up. In trials for which baseline 25(OH)D concentrations were available, covering 15,979 participants, values ranged from 22.0 to 47.0 nmol/l at baseline and increased to 62.0-105.0 nmol/l in vitamin D-supplemented individuals.

A more recent meta-analysis of the Cochrane Library by Bjelakovic et al. (2011) compromised of 50 randomized trials with 94,148 participants comparing a randomly assigned vitamin D supplement to a placebo- or non-interventional group. The vast majority of the trial population consisted of women older than 70 years. The Cochrane Review found that a supplementation with vitamin D3 decreases mortality significantly by about 6% (RR=0.94, 95% CI: 0.91, 0.98; 74,789 participants, 32 trials) whereas vitamin D2, alfacalcidol, or calcitriol (1,25(OH)2D3) did not (Bjelakovic et al., 2011). Alfacalcidol and calcitriol increased the risk of hypercalcaemia
10. Vitamin D deficiency and premature death

(RR=3.18, 95% CI: 1.17, 8.68). Adverse effects such as renal stone formations were also identified in case vitamin D supplements were given in combination with calcium supplements (RR=1.17, 95% CI: 1.02, 1.34). In some of these studies, renal stone formation can reliably be attributed to supplementary calcium and not to the relatively low amount of supplementary vitamin D. Nevertheless, it cannot be definitely ruled out that frailty itself may increase the risk of renal stone formation after combined calcium and vitamin D supplementation. In physically active individuals similar amounts of calcium and vitamin D may lead to calcium excretion via sweat or calcium deposition in the skeleton.

Data on the association of circulating 25(OH)D serum concentrations with the mortality risk in the general adult population has been summarized in a recent meta-analysis of prospective cohort studies (Zittermann et al., 2012). The meta-analysis consisted of two models: a nonparametric model and a parametric analysis to assess the dose-risk relation. The nonparametric model compared the highest level of 25(OH)D with the lowest level. Fourteen studies met the criteria for this model. The analysis included 62,584 individuals of whom 5,562 individuals died. The mean follow-up ranged from 1.3 to 27 years. In the nonparametric analysis, the summary estimate for highest compared with lowest categories of 25(OH)D showed a significant mortality risk reduction of 29% (95% CI: 0.50, 0.91). In the parametric model, eleven studies with a total of 59,231 individuals were analyzed. For this approach the lowest quantile (mean 25(OH)D value of 27.5 nmol/l) was used as a reference category. Findings from this analysis indicate a nonlinear decline in overall mortality with an individual RR of 0.86 (95% CI: 0.82, 0.91), 0.77 (95% CI: 0.70, 0.84) and 0.69 (95% CI: 0.60, 0.78) for increasing 25(OH)D serum levels of 12.5, 25.0 and 50.0 nmol/l, respectively. There was, however, no significant decrease in mortality when an increase of approximately 87.5 nmol/l above the reference category occurred. Data are in line with the suggestion of an exponential increase in morbidity and mortality at deficient 25(OH)D levels.

10.3.3 Critically ill patients

Vitamin D deficiency should also be a point of concern in critically ill patients. In an Australian study (Lee et al., 2009), a total of 17% of intensive care unit patients had undetectable (<15 nmol/l) levels of 25(OH)D and additional 38% had levels below 30 nmol/l. The Simplified Acute Physiology Score II was highest in patients with deficient 25(OH)D levels, indicating more severe organ dysfunction than in patients with sufficient 25(OH)D levels. In addition, mortality rates were highest in vitamin D deficient patients. Similar results were reported from two hospitals in Boston (Braun et al., 2011). Among a population of 1,325 critically ill patients, deficiency of 25(OH)D at the time of critical care initiation was a significant predictor of all-cause patient mortality.

10.3.4 Possible preventive mechanisms

Experimental studies support the assumption that vitamin D deficiency results in premature death. It has been described in several studies that a complete or partial lack of vitamin D action (VDR<sup>-/-</sup> mice and CYP27B1<sup>-/-</sup> mice) results in premature death. VDR mutant mice have growth
retardation, osteoporosis, kyphosis, skin thickening and wrinkling, alopecia, ectopic calcification, progressive loss of hearing and balance as well as a short life span (Tuohimaa, 2009). The active vitamin D metabolite, 1,25(OH)₂D, functions through the VDR, which is expressed in almost all human cells and tissues. There are several known genes whose expression is influenced by the vitamin D-hormone 1,25(OH)₂D (Nagpal et al., 2005). It is estimated that 3% of the genome are regulated directly or indirectly by vitamin D (Bouillon et al., 2008). Furthermore, antiproliferative effects and effects on cell differentiation and angiogenesis are mechanisms discussed for beneficial effects of vitamin D. Many cells (e.g. monocytes, dendritic cells, B-lymphocytes, colonocytes, smooth muscle cells, endothelial cells) also possess the 25(OH)D-1α-hydroxylase (CYP27B1) which is required for the ability to convert 25(OH)D to the active form 1,25(OH)₂D, suggesting important regulatory properties of 1,25(OH)₂D on a cellular level. A population-based cohort study in female twins highlighted a more general mechanism (Richards et al., 2007). This study reported longer leukocyte telomere length with higher circulating 25(OH)D concentrations. Leukocyte telomere length is a predictor of aging-related disease and is positively related to longevity. The difference in leukocyte telomere length between the highest and lowest tertiles of circulating 25(OH)D (mean values: 41.0 and 124.3 nmol/l, respectively) was 107 base pairs, which is equivalent to 5 years of telomeric aging. A more recent study (Zhu et al., 2011) has demonstrated that oral supplementation with 60,000 IU vitamin D3 per month (equivalent to approximately 2,000 IU per day) significantly increased peripheral blood monocyte cell telomerase activity in overweight African Americans. Telomerase is an essential enzyme for maintaining telomere length.

10.4 Vitamin D deficiency and cardiovascular mortality

Globally, CVD is the number one cause of mortality. In 2005, CVD was responsible for approximately 30% deaths worldwide. CVD includes various illnesses such as coronary heart disease, peripheral arterial diseases, cerebrovascular diseases such as stroke, and congestive heart failure (Zittermann and Gummert, 2010).

10.4.1 General adult population

Ecological studies have reported higher rates of coronary heart disease with increasing distance from the equator, a phenomenon that can be attributed to the higher prevalence of vitamin D deficiency in regions with less exposure to sunlight (Zittermann and Koerfer, 2008). Research on vitamin D for CVD has increased more rapidly in the recent past years than for any other disease. A substantial number of observational cohort studies are available and several possible mechanism by which vitamin D may affect the outcome of CVDs have been discussed (see below).

A meta-analysis of prospective cohort studies by Grandi et al., (2010) evaluated the association of vitamin D status with cardiovascular morbidity and mortality. The analysis included four studies on cardiovascular incidence and 5 studies on cardiovascular mortality. Data were presented as HR for the lowest 25(OH)D quantile. The highest quantile served as reference category. For incidence
10. Vitamin D deficiency and premature death

studies, the HR was 1.53 (95% CI: 1.22, 1.95) and for mortality studies the HR was 1.83 (1.19, 2.80). However, it was mentioned by the authors of that study themselves that heterogeneity and the small number of studies which could be included in the data analysis limits the interpretation and generalization of the results (Grandi et al., 2010).

Meanwhile, various other prospective cohort studies evaluating the association between circulating 25(OH)D and cardiovascular mortality have been published. Results of 13 individual studies that were published until 2011 are illustrated in Figure 10.1. In summary, the data indicate an increased cardiovascular mortality risk if 25(OH)D levels are lying in the deficiency range.

In 2010, Wang et al. published a meta-analysis of RCTs on vitamin D and cardiovascular risk. All studies were performed in postmenopausal women or elderly people with preserved kidney function. The vitamin D dose ranged between 10 μg daily and 2.5 mg every 4 months, the latter being equivalent to approximately 20 μg vitamin D daily. The CVD risk was slightly (RR=0.90 and 0.83, respectively) but not significantly lower in the two studies testing solely the effect of vitamin D. In 1 of these 2 studies, CVD mortality was also assessed and was 0.84 (95% CI: 0.65, 1.10) in the supplementation group. In the 2 studies that used a combination of vitamin D and calcium, CVD risk was 1.21 and 1.04 in the supplementation group. The pooled CVD risk for the two vitamin D studies and the two vitamin D plus calcium studies was 0.90 (0.77, 1.05) and 1.04 (0.92, 1.18), respectively in the supplemented groups (Wang et al., 2010). In total, results are in gross agreement with the observation that supplemental calcium increases CVD risk. It was concluded

![Figure 10.1. Relative risk of fatal cardiovascular events according to 25(OH)D levels; results from prospective cohort studies; data are adopted from Zittermann et al. (2012). Each point stands for a single study. Results represent the multivariable adjusted RR of a fatal cardiovascular event for the lowest 25(OH)D quantile compared to the respective highest 25(OH)D quantile. Circles indicate significant differences whereas triangles indicate no significant differences (95% CI includes 1).](image-url)
by the authors of that meta-analysis that vitamin D supplements at moderate to high doses may reduce CVD risk. In a more recent study by Avenell et al. (2012), the incidence of vascular disease death in participants allocated to vitamin D compared to participants not allocated to vitamin D was very similar to the results of the meta-analysis by Wang et al. (2010) (HR=0.91; 95% CI: 0.79, 1.05). In post hoc analyses adjusted for compliance with vitamin D, vascular disease mortality (HR=0.76; 95% CI: 0.49, 1.40) also did not differ significantly in response to vitamin D, although all trends were accentuated compared to the intention to treat analysis. Again, the study was probably underpowered to find statistically significant differences between the vitamin D and placebo group.

10.4.2 Chronic kidney disease and cardiovascular mortality

CKD is an illness that predisposes patients to CVD. CKD is frequently associated with vascular calcification. Vascular calcification is an important predictor of cardiovascular and all-cause mortality in CKD patients (Rodriguez et al., 2011). In parallel with the decline in kidney function, there is a progressive deterioration in mineral homeostasis including secondary hyperparathyroidism and low 25(OH)D and 1,25(OH)₂D levels.

Deficient levels of 25(OH)D are very prevalent in CKD (LaClair et al., 2005; Naveneethan et al., 2011). A recent meta-analysis of 10 prospective cohort studies (Pilz et al., 2011a) with an overall sample of 6,853 patients found that an increase per 25 nmol/l of 25(OH)D reduces all cause-mortality by 14% (RR=0.86; 95%CI: 0.82, 0.91). There were no differences among patients with or without dialysis. Some more recently published studies (Pilz et al., 2011b; Santoro et al., 2011; Naveneethan et al., 2011; Inaguma et al., 2008) support the results of that earlier meta-analysis.

The 2009 'Kidney Disease: Improving Global Outcomes' clinical practice guidelines (KDIGO, 2009) include a weak recommendation to test for and treat vitamin D deficiency in patients with CKD stages 3-5D. However, the recommendations are based on the treatment strategies for the general population and detailed information about the amount and form of vitamin D supplementation is missing.

10.4.3 Possible preventive mechanisms of vitamin D against cardiovascular disease

There is experimental and clinical evidence for a cardio-protective role of vitamin D through anti-hypertensive, anti-inflammatory and anti-diabetic effects and through the control of parathyroid hormone and the renin-angiotensin aldosterone system (Pilz et al., 2011c). Vitamin D also regulates intracellular calcium metabolism (Santillan and Boland, 1998), which plays a pivotal role in cardiac muscle contraction. In experimental studies, VDR knockout mice develop myocardial hypertrophy, arterial hypertension, and increased blood clotting tendency, nephropathy, and overexpression of the renin-angiotensin-aldosterone system (Bouillon et al., 2008). Deletion of the cardiomyocyte-specific VDR gene results in a reduction in end-diastolic and end-systolic volume and increased atrial natriuretic peptide expression (Chen et al., 2011).
10.5 Vitamin D deficiency and cancer mortality

10.5.1 Ecological studies

In the early eighties, Garland and Garland (1980) hypothesized that the impact of UVB radiation due to geographical differences correlates with cancer mortality (Garland and Garland, 1980). Since then, a number of ecological studies have shown that UVB radiation is inversely associated with cancer incidence as well as cancer mortality (Boscoe and Schymura, 2006; Grant, 2002; Grant and Mohr, 2009).

Sunlight exposure is the major vitamin D source in humans. Therefore, the effect of UVB irradiation concerning vitamin D supply may explain the results of the ecological studies. Due to the study design, reliability of geological studies is however limited. Nevertheless, data are in line with a death certificate case-control study that analyzed the relation between breast, ovarian, colon and prostate cancers and residential and occupational exposure to sunlight. In this exploratory study, residential exposure to sunlight was significantly associated with a reduced mortality from breast (OR=0.74; 95% CI: 0.72, 0.76), ovarian (OR=0.84; 95% CI: 0.81, 0.88), prostate (OR=0.84; 90% CI: 0.87, 0.93), and colon cancer (OR=0.73; 95% CI: 0.71, 0.74). Sunlight from occupational exposure played a minor role for cancer mortality. Non-melanoma skin cancers served as a positive control. It was positively associated with both residential (OR=1.23; 95% CI: 1.14, 1.33) and occupational (OR=1.15; 95% CI: 1.00, 1.32) skin cancer deaths (Freedman et al., 2002).

10.5.2 Prospective cohort studies

Since vitamin D status depends on multiple factors such as skin pigmentation, actual UVB exposure and oral vitamin D intake, Giovannucci et al., (2006) calculated a score that reflects long term 25(OH)D level due to the aforementioned vitamin D sources. The score was implemented in the ongoing Health Professionals Follow-up Study. Among 47,800 men, cancer mortality increased significantly in relation to predicted low vitamin D levels, whereas an increment of 25 nmol/l was associated with a 29% reduction in total cancer-mortality (RR=0.71; 95% CI: 0.60, 0.83) and 45% reduction in digestive-system cancer (RR=0.55, 95% CI: 0.41, 0.74).

The NHANES III was one of the first prospective studies that examined the relationship of low serum vitamin D levels and cancer mortality. A total number of 16,819 male and female participants were followed up from 1988-1994 through 2000. Among the study participants, 536 cancer deaths were identified during 146,578 person-years of observation. Because season and latitude are related to serum 25(OH)D levels and these parameters were linked in the dataset, the opportunity to assess each variable independent of the other was limited. Therefore, two subgroups were built ‘winter/lower latitude’ and ‘summer/higher latitude.’ However, season and latitude were unrelated to cancer mortality. High 25(OH)D levels were also not associated with a decline in total cancer mortality risk, but were significantly associated with colorectal cancer risk. In detail, 25(OH)D levels of 80 nmol/l or higher were associated with a decreased risk by 72% (HR=0.28, 95% CI: 0.11, 0.68; \( P=0.02 \)) compared to levels less than 50 nmol/l (Freedman et al., 2007). In an
additional analysis, the observation period of this trial was expanded over a period of six years from 2000 through 2006. In total, 884 cases of cancer deaths occurred during the entire follow-up. Again, overall cancer mortality was not related to vitamin D status. In addition, colorectal cancer mortality risk showed only a tendency for a decrease with higher 25(OH)D levels. However, the long follow-up of 16 years from initial 25(OH)D measurements is an important limitation of this study. Note that the correlation coefficient between 2 measurements of 25(OH)D taken 3 years apart is moderately high (0.70) (Platz et al., 2004). On the one hand, this suggests that a single 25(OH)D measurement is a useful tool in epidemiologic studies with a medium follow-up. On the other hand, it also suggests that initial 25(OH)D measurement may not reliably reflect vitamin D status over a very long period. It is therefore important that a meta-analysis of eight prospective cohort studies including data from the Physician’s Heath Study yielded a significant inverse association of colorectal cancer with 25(OH)D levels, regarding highest and lowest quartiles with pooled multivariate OR of 0.66 (95% CI: 0.54, 0.81) (Lee et al., 2011). Another meta-analysis (Gandini et al., 2011) identified 9 prospective studies with 2,630 study participants with data on serum 25(OH)D and colorectal cancer risk. The summary RR for a 25 nmol/l increase in serum 25(OH)D was 0.85 (95% CI: 0.79, 0.91). For breast cancer (6,175 cases in 10 studies) and prostate cancer (3,956 cases in 11 studies) no significant association with serum 25(OH)D was found. In a meta-analysis by Touvier et al. (2011), not only serum 25(OH)D levels but also dietary vitamin D intake was inversely associated with colorectal cancer risk. In detail, in 10 studies with daily vitamin D intakes ranging from 39 to 719 IU, RR of colorectal cancer decreased by 5% per 100 IU vitamin D intake, yielding a summary RR of 0.95 (95% CI: 0.93, 0.98). In 6 studies, an increase in serum 25(OH)D by 6.2 nmol/l was associated with a RR of 0.96 (95% CI: 0.94, 09.7).

The NHANES III study has also highlighted an important other issue concerning vitamin D and cancer risk: a subgroup analysis revealed a decreased cancer risk among women at higher altitude and who had blood drawings in summer and therefore mean 25(OH)D levels >100 nmol/l (RR=0.52; 95% CI: 0.25, 1.15). For men, however, there was an increased risk of overall cancer mortality with higher vitamin D levels (≥100 nmol/l) compared to the group with vitamin D levels lower 37.5 nmol/l (RR=1.85; 95% CI: 1.02, 3.35) concluding a sex specific impact of cancer incidence and mortality. Similar results were observed in a longitudinal nested case-control study of Nordic men (Ahn et al., 2008). There was a U-shaped curve with both high (>80 nmol/l) and low (<19 nmol/l) levels of 25(OH)D associated with increased risk of prostate cancer. An increased risk at very high levels (≥100 nmol/l) was also noted for pancreatic cancer (Hetzlsouer, 2010). The causes for an increased cancer risk at 25(OH)D levels >100 nmol/l are unclear at present. Interestingly, experimental data demonstrate that high 25(OH)D levels can also be the result of low 1,25(OH)2D levels (Vieth et al., 1987). Thus, due to the lack of 1,25(OH)2D measurements in the aforementioned studies it remains unclear whether excess vitamin D or deficient vitamin D action was responsible for the excess cancer risk in individuals with high 25(OH)D levels.

Currently, there is still inconsistency about the interpretation of epidemiologic data on vitamin D and cancer risk. The authors of the NHAMES III data recommend caution before encouraging vitamin D supplements for cancer preventing as the relationship of cancer and mortality might
be more difficult than suggested (Freedman et al., 2010). These findings are in great contrast to the conclusion from Garland et al. in 2009 who estimated that raising the minimum year-around serum 25(OH)D level to 100-150 nmol/l has no unreasonable risk for the population, and would prevent approximately 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer each year in the United States and Canada (Garland et al., 2009).

10.5.3 Randomized controlled trials

The present literature includes only very few randomized placebo-controlled trials that address cancer mortality in relation to vitamin D status. One of these RCTs is the three year long-term RECORD trial (Randomized Evaluation of Calcium Or vitamin D), involving 5,292 participants, mainly women, who were randomly assigned to receive either a daily supplement of 800 IU, 1000 mg calcium, both, or placebo. Cancer mortality did not differ significantly among the vitamin D and control group (HR=0.85; 95% CI: 0.68, 1.03). In a post hoc statistical analysis adjusting for compliance, trends for reduced mortality with vitamin D and increased mortality with calcium were accentuated, although all results remain non-significant (Avenell et al., 2012).

In the WHI study, the largest clinical trial of vitamin D (400 IU/day) and calcium (1000 mg/day) so far, there was a nonsignificant (P=0.17) reduction of cancer mortality with supplementation during a mean follow-up of 7 years (Brunner et al., 2011). Some studies have also analyzed cancer morbidity in subgroups of the WHI participants. Women concurrently treated with estrogen therapy had a decreased risk of colorectal cancer (Ding et al., 2008). In another subgroup analysis of the WHI study (Bolland et al., 2011) in 15,646 women who were not taking personnel calcium or vitamin D supplements at randomization, calcium plus vitamin D significantly reduced total, breast and invasive breast cancer by 14-20%, respectively, and nonsignificantly reduced the risk of colorectal cancer by 17%. In a 4-year randomized double-blind, placebo-controlled trial 1,179 community dwelling women received 1,400-1,500 mg supplemental calcium alone, supplemental calcium plus 1,100 IU vitamin D or placebo daily. The RR of incident cancer in the calcium plus vitamin D and calcium alone groups were 0.402 and 0.532, respectively. When analysis was confined to cancers diagnosed after the first 12 months, RR for the calcium plus vitamin D group fell to 0.232 (95% CI: 0.09, 0.60) but did not change significantly for the calcium alone group.

It is noteworthy that hitherto no data are available for men. Since observational studies found sex-specific differences in cancer mortality, randomized trials including an adequate number of men are needed.

10.5.4 Possible anticancer actions of vitamin D

Several anticancer actions have been attributed to the vitamin D hormone 1,25(OH)₂D. These actions include anti-proliferative properties. The molecular mechanisms by which 1,25(OH)₂D may mediate anti-cancer effects have been shown to involve multiple pathways and in some cases be cell-type specific. Nevertheless, in most cell types that express functional VDR, exposure to 1,25(OH)₂D results in the accumulation of cells in the G0/G1 phase of the cell cycle. In breast,
colon, and prostate cancer cells, it has been demonstrated that 1,25(OH)$_2$D has the ability to induce apoptosis. One mechanism of apoptosis induction is the down regulating of mitogenic pathways, such as those induced by insulin-like growth factors. Additionally, 1,25(OH)$_2$D sensitizes cancer cells to different cytotoxic substances and radiation. Both, in vitro and in vivo experiments showed that 1,25(OH)$_2$D can also modulate angiogenesis, a key step in continues tumor growth and progression. In addition to the activation of genomic pathways, 1,25(OH)$_2$D also triggers rapid, non-genomic responses that activate a transmembrane signaling cascade. 1,25(OH)$_2$D also has anti-inflammatory properties. Since inflammation contributes to the development and progression of many cancers, the suppressive vitamin D effects on inflammatory processes might contribute to its beneficial effects in multiple cancers (Vanoirbeek et al., 2011).

10.6 Conclusion

In adults, there is likely evidence from prospective cohort studies and especially from meta-analyses of RCTs where mortality was a secondary endpoint that vitamin D deficiency is associated with an elevated mortality risk. A similar statement concerning vitamin D and mortality risk has recently been made by the German Nutrition Society (DGE, 2012).

Data on cardiovascular mortality concur with results on all-cause mortality. Prospective cohort studies indicate that the risk of fatal events is highest when 25(OH)D levels are lying in the deficiency range. Although first RCTs indicate that risk reduction is probably small, the effects on the population's morbidity and life expectancy may be profound due to the large number of patients who are affected by CVD.

For cancer mortality, data from epidemiological studies are less convincing and the evidence from RCTs is inconsistent. There is also an urgent need to clarify the etiology of the U-shaped association between circulating 25(OH)D levels and cancer morbidity and mortality in some prospective cohort studies.

In summary, adequately powered RCTs with all-cause mortality as primary endpoint are still needed.

References


10. Vitamin D deficiency and premature death


10. Vitamin D deficiency and premature death


**Key facts**

- Circulating vitamin D must be hydroxylated by 1α-hydroxylase in the kidney to become active.
- The activity of 1α-hydroxylase is reduced as kidney function declines.
- Reduced levels of active vitamin D leads to secondary hyperparathyroidism in patients with chronic kidney disease (CKD).
- Secondary hyperparathyroidism induces bone disturbances and increased fracture risk.
- Since the 1970s vitamin D analogs have been used for treatment of secondary hyperparathyroidism in patients with CKD.

**Summary points**

- With declining renal function an increased prevalence of disturbances in the mineral metabolism (secondary hyperparathyroidism) and bone (renal osteodystrophy) develops.
- Calcitriol and active vitamin D analogs are used for treatment of secondary hyperparathyroidism and bone disease in patients with CKD.
- High levels of calcium and phosphate are associated with extra-osseous calcification.
- New active vitamin D analogs with selective properties are developed in order to suppress secondary hyperparathyroidism and avoid the concomitant high calcium and phosphate levels.
- Patients treated with calcitriol or active vitamin D analogs seem to have an improved survival independent of the mineral metabolism, although interventional trials should confirm this survival advantage.
11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

D. Hansen
Department of Medicine, Roskilde Hospital, Koegevej 7-14, 4000 Roskilde, Denmark and Department of Nephrology, Rigshospitalet, Blegdamsvej 9, 2100 København Ø, Denmark; dittehansen@dadlnet.dk

Abstract

Secondary hyperparathyroidism is a common clinical problem in patients with chronic kidney disease (CKD). It causes disturbances in bone metabolism and increased risk of bone fracture. Secondary hyperparathyroidism develops as a consequence of reduced renal phosphate excretion and thereby elevated phosphate levels, hypocalcaemia, increased fibroblast growth factor 23 levels and reduced levels of calcitriol due to decreased renal 1α-hydroxylation of vitamin D. Treatment with calcitriol or its analogs suppress the secondary hyperparathyroidism. But increasing doses of calcitriol induces elevated levels of calcium and phosphate. Elevated calcium and phosphate levels are associated with extra-vascular calcification, cardiovascular disease and increased mortality. Active vitamin D analogs have been developed in order to increase the therapeutic window for parathyroid hormone suppression, without increasing phosphate and calcium levels. Observational studies have demonstrated an improved survival in patients with CKD treated with calcitriol or active vitamin D analogs. Both observational and animal studies suggest an effect of vitamin D and vitamin D analogs through mechanisms that are independent of the mineral metabolism.

Keywords: mineral metabolism, renal osteodystrophy, mortality, cardiovascular disease
11.1 Vitamin D in patients with chronic kidney disease

Vitamin D deficiency is common in patients with CKD. The prevalence increases as the kidney function declines. In patients with CKD5 (Table 11.1) around 70% has 25(OH)D deficiency (25(OH)D <50 nmol/l) and around 70% has 1,25(OH)2D deficiency (1,25(OH)2D <22 pg/ml) (Hansen, 2012; Levin et al., 2007).

The low levels of 25(OH)D are due to many factors. This population has an older age and increased prevalence of diabetes compared to the general population, factors known to be associated with decreased levels of 25(OH)D. CKD is often a disarming disease and these patients may have the skin less exposed to the sun. A decreased vitamin D3 synthesis in the skin of the uremic patient has been shown. In order to avoid uremic symptoms, hyperphosphatemia and elevated potassium, patients with CKD are holding a restrictive diet which can exaggerate 25(OH)D deficiency. Proteinuria may decrease the level of VDBP and thereby the level of circulating 25(OH)D. In patients with disturbed renal function, the activity of 25-hydroxylase (CYP27A1 and CYP2R1) in the liver is decreased and may decrease 25-hydroxylation of vitamin D2 and D3. Furthermore,


<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (ml/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD1</td>
<td>Kidney damage with normal or elevated GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>CKD2</td>
<td>Kidney damage with mild decrease in GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>CKD3</td>
<td>Moderate decrease in GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>CKD4</td>
<td>Severe decrease in GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>CKD5</td>
<td>Kidney failure</td>
<td>&lt;15 or dialysis (CKD5D)</td>
</tr>
</tbody>
</table>

Abbreviations

1,25(OH)2D 1,25-dihydroxyvitamin D
25(OH)D 25-hydroxyvitamin D
CaSR Calcium-sensing receptor
CKD Chronic kidney disease
FGF-23 Fibroblast growth factor 23
FGFR Fibroblast growth factor 23 receptor
GFR Glomerular filtration rate
PTH Parathyroid hormone
VDBP Vitamin D binding protein
VDR Vitamin D receptor
11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

treatment with activated vitamin D (calcitriol or its analogs), has been shown to decrease the activity of 25-hydroxylase in the liver (Armas and Heaney, 2011).

The vitamin D deficiency in patients with reduced renal function differs from other populations, because the 1α-hydroxylation of 25(OH)D in the kidney is disturbed. Even patients with sufficient nutritional supply or production in the skin of vitamin D2 and vitamin D3, may still be deficient in circulating active 1,25(OH)₂D.

As the kidney mass declines the amount of 1α-hydroxylase (CYP27B1) decrease, and as glomerular filtration rate declines the delivery of vitamin D to 1α-hydroxylation via glomerular filtration declines.

FGF-23 is a recently discovered hormone synthesised by the bone osteocytes. FGF-23 inhibits the renal phosphate reabsorption, inhibits the renal 1α-hydroxylase, and increases the activity of 24-hydroxylase (CYP24). FGF-23 increases as renal function declines. The deficiency of 1,25(OH)₂D may be induced by FGF-23, as FGF-23 begins to rise already in CKD3 (Isakova et al., 2011).

Phosphate is retained due to decreased excretion with declining renal function, and has the potential to further decrease the activity of 1α-hydroxylase.

11.2 Secondary hyperparathyroidism in patients with chronic kidney disease

Secondary hyperparathyroidism occurs with increasing prevalence as kidney function declines. Secondary hyperparathyroidism develops due to disturbances in the mineral metabolism (Figure 11.1). First, phosphate levels increase due to decreased renal phosphate excretion. Phosphate increases the stability of PTH mRNA. Second, calcium levels are lowered due to hyperphosphatemia and low levels of vitamin D. Hypocalcaemia is an important regulator of PTH levels. A decrease in calcium levels inactivates the CaSR of the parathyroid cell and PTH secretion is increased. Hypocalcaemia retards the PTH degradation in the parathyroid glands and increases the parathyroid cell division. Third, the low levels of 25(OH)D and 1,25(OH)₂D decrease the vitamin D induced suppression of PTH synthesis. Fourth, uremia increases the stability of PTH mRNA. Fifth, the expression of the CaSR and the synthesis of the VDR declines as kidney function decreases. Finally, FGF-23 has been shown to suppress PTH secretion and parathyroid cell proliferation. However, in uremic animals a reduced expression of the FGFR and the obligate FGFR cofactor klotho has been demonstrated.

Chronic stimulation of the parathyroid gland leads to diffuse hyperplasia which may progress into nodular formation with autonomous monoclonal growth and PTH production. Monoclonal proliferation is present in more than 50% of parathyroid glands removed by parathyroidectomy in uremic patients (Cunningham et al., 2011).
Several interventions are applied to prevent the development and treat the condition of secondary hyperparathyroidism.

Phosphate load is sought to be decreased by dietary advice. In dialysis patients phosphate levels may be reduced through increased dialysis dose, and calcium-load may be regulated by different concentrations of calcium in the dialysate. Phosphate binders are used to reduce the phosphate absorption from the intestine by binding the ingested phosphate. Two types of phosphate binders are available. Calcium-containing phosphate-binders increase calcium levels, while reducing phosphate levels. Non calcium-containing phosphate-binders reduce phosphate levels without an increase in calcium levels.

Vitamin D supplementation both as nutritional and active vitamin D analogs are used for suppression of PTH, and reduces hypocalcaemia (further discussed in the following section).

Calcimimetics increases the calcium sensibility of the CaSR in the parathyroid gland, thereby decreasing the PTH level. Calcimimetics are used in patients with concomitant hyperparathyroidism and elevated ionised calcium levels.

Finally if hyperparathyroidism remains uncontrolled despite intensive medical strategies, parathyroidectomy may be needed.

11.3 Chronic kidney disease mineral and bone disorder

CKD mineral and bone disorder (Moe, 2006) is a systemic disorder of mineral and bone metabolism due to CKD manifested as either one or a combination of:
1. abnormalities of calcium, phosphate, PTH or vitamin D metabolism;
2. abnormalities of bone turn over, volume, mineralisation, linear growth or strength;
3. vascular or other soft tissue calcification.
11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

The first has been described in the previous section.

Bone fracture occurs with a prevalence as high as 40-50% in a hemodialysis population. Furthermore, bone fracture in CKD patients is associated with a 2-2.5 times increased mortality compared to patients without CKD.

The abnormal bone histology in patients with CKD is termed renal osteodystrophy. The gold standard for diagnosis of renal osteodystrophy is a bone biopsy. Imaging methods and biochemical abnormalities as surrogate markers of histological bone abnormalities have been sought.

Measurement of PTH is at the moment the single most useful biomarker to predict bone histology. The three main conditions of renal osteodystrophy are (1) osteitis fibrosa cystica or high turnover bone disease associated with elevated PTH; (2) adynamic bone disease or low turnover bone disease associated with low PTH; (3) mixed disease.

Patients with CKD have increased mortality compared to the general population. In a hemodialysis population the annual mortality rate is around 20%. The risk of cardiovascular disease and cardiovascular mortality increase as kidney function declines.

The increased cardiovascular morbidity and mortality is only partially explained by traditional risk factors such as hypertension, diabetes and dyslipidemia.

The increased risk of cardiovascular disease and mortality is associated with disturbances in the mineral metabolism such as elevated calcium, phosphate and PTH levels. Calcium and phosphate stimulates vascular calcification \textit{in vitro}. Observational studies have demonstrated an association between elevated calcium and phosphate levels and vascular calcification in coronary arteries. In CKD patients a high prevalence of calcification of the arterial smooth muscle layer (media calcification) is present, and associated with elevated calcium and phosphate levels. It gives rise to great concern that the bone minerals apparently deposits in the vasculature.

Like in the vasculature, elevated calcium and phosphate can induce soft tissue calcification in a wide variety of organs. Calcific uremic arteriolopathy with painful necrotic skin ulcerations is a particularly feared form of extra-osseous calcification. The exact interaction between calcium, phosphate and calcification inducers and inhibitors are under investigation.

Clinical guidelines propose target levels for ionised calcium, phosphate and PTH. These are based on the association between PTH and bone histology data, and based on cut-off values from epidemiologic studies describing the association between mineral metabolism and clinical outcomes such as bone fracture, cardiovascular disease and mortality. It is unknown whether reaching these targets improve clinical outcome, as no randomised clinical trial has addressed this question (Anonymous, 2009).
11.4 Treatment of vitamin D deficiency in patients with chronic kidney disease

In the 1970s 1α-hydroxylated metabolites of vitamin D were synthesised. Treatment with these active vitamin D analogs made it possible to bypass the problem concerning lack of 1α-hydroxylation in the kidney in patients with reduced renal function.

First, calcitriol (1,25(OH)₂D₃) and alfacalcidol (1α(OH)D₃) were provided to the uremic patients, in order to increase intestinal calcium absorption and thereby improve skeletal abnormalities. Later, the direct suppressive effect of 1,25(OH)₂D₃ on PTH synthesis in the parathyroid gland was discovered.

The ability of vitamin D analogs to suppress PTH decreases as CKD progress. This is caused by the progressive down regulation of the VDR and the CaSR in the parathyroid gland. Therefore increasing doses of vitamin D analogs are needed.

The use of vitamin D analogs is limited by their tendency to induce hypercalcemia and hyperphosphatemia. Vitamin D analogs increase the intestinal calcium and phosphate absorption, and enhance the PTH induced renal calcium reabsorption. Especially with the concomitant use of calcium-containing phosphate-binders, hypercalcemia is often an encountered problem.

New vitamin D analogs has been developed to increase the potency of PTH suppression without inducing hypercalcemia and hyperphosphatemia: doxercalci ferol (1α-hydroxyvitamin D₂), paricalcitol (19-nor-1α,25-dihydroxyvitamin D₂), maxacalcitol (22-oxa-1α,25-dihydroxyvitamin D₃) and falecalcitriol (26,27-F₆-1,25-dihydroxyvitamin D₂).

The different analogs are briefly described in the following sections.

There are no clinical trials comparing the vitamin D analogs and their effect on clinical end points such as bone fracture, cardiovascular morbidity or mortality in CKD patients.

Alfacalcidol and doxercalci ferol are classically considered pro-hormones to 1,25(OH)₂D₃ and 1,25(OH)₂D₂, which become active after 25-hydroxylation in the liver. However, alfacalcidol and doxercalci ferol may have an intrinsic effect. In bovine parathyroid cells alfacalcidol and doxercalci ferol suppressed PTH production and the PTH suppression persisted after 25-hydroxylase was blocked (Brown et al., 2006).

11.4.1 Alfacalcidol

Alfacalcidol suppresses PTH with a concomitant increase in ionised calcium. The use of low-calcium in dialysis fluids makes it possible to induce a long-term suppression of PTH by alfacalcidol in dialysis patients (Brandi, 2008). Long-term comparative studies of oral and intravenous alfacalcidol and calcitriol in hemodialysis patients found an equal suppression of PTH with
similar changes in calcium and phosphate levels (El-Reshaid et al., 1997; Kiattisunthorn et al., 2011). Alfacalcidol is widely used in Europe (Etalpha, Leo Pharma A/S, Ballerup, Denmark).

11.4.2 Paricalcitol

Paricalcitol lacks the exocyclic C19 in calcitriol, and has a vitamin D2 side chain. In animal studies paricalcitol was found to suppress PTH, while paricalcitol induced less increase in calcium and phosphate levels than calcitriol (Slatopolsky et al., 1995). In hemodialysis patients a randomised study comparing calcitriol and paricalcitol found a faster PTH suppression in the paricalcitol treated patients, although no difference in the number of patients achieving PTH suppression after 32 weeks of treatment. However, the paricalcitol treated patients presented less episodes of persistent hypercalcemia (Sprague et al., 2003). Paricalcitol was compared to alfacalcidol in a 16 week interventional study in hemodialysis patients, and both analogs suppressed PTH with similar increases in calcium and phosphate levels (Hansen et al., 2011). Paricalcitol is widely used in America, Europe and Asia (Zemplar, Abbott Laboratories, Chicago, IL, USA).

11.4.3 Doxercalciferol

Doxercalciferol suppress PTH in CKD 3-5D in placebo controlled studies. The PTH suppression is at least partly induced by a concomitant increase in calcium levels (Coburn et al., 2004; Frazao et al., 2000; Tan, Jr. et al., 1997). Doxercalciferol has been compared to calcitriol in a randomised 8 month trial in a paediatric peritoneal dialysis population. Both analogs suppressed PTH similar, with no differences in calcium or phosphate levels (Wesseling-Perry et al., 2011). Doxercalciferol is available in the USA (Hectorol, Genzyme, Cambridge, MA, USA).

11.4.4 Maxacalcitol

A substitution of carbon 22 by an oxygen atom makes 22-oxacalcitriol differ from calcitriol. In 91 hemodialysis patients intravenous maxacalcitol and calcitriol was compared during 1 year in a randomised study. There was no difference between the number of patients reaching a PTH less than 150 pg/ml, and the final level of phosphate and calcium was similar between groups (Hayashi et al., 2004). Intravenous maxacalcitol and oral calcitriol was compared in 46 hemodialysis patients in a randomised 24 week intervention study. Whereas calcitriol initially suppressed PTH faster accompanied by an increased calcium level, there was no difference in the final levels of PTH, phosphate and calcium, although hypercalcemia was more frequent in the calcitriol treated patients (Tamura et al., 2005). Maxacalcitol is available in Japan (Oxarol, Chugai Pharmaceuticals CO., Tokyo, Japan).

11.4.5 Falecalcitriol

Falecalcitriol differs from calcitriol in carbon 26 and 27 where hydrogen atoms are substituted by fluoride atoms. Falecalcitriol was compared to alfacalcidol in a cross-over design in 25 hemodialysis patients. Oral doses were adjusted to maintain initial calcium levels during the
24 week treatment periods. Falecalcitriol tended to inhibit an increase in PTH, and the control of phosphate levels were better (Akiba et al., 1998). When oral falecalcitriol was compared to intravenous calcitriol in 22 patients in a randomised cross-over trial, similar suppression of PTH was attained, with equal incidence of hypercalcemia and hyperphosphatemias and similar final calcium and phosphate levels (Ito et al., 2009). Oral Falecalcitriol is available in Japan (Hornel, Taisho Pharmaceuticals Co., Tokyo, Japan, and Fulstan, Kissei Pharmaceuticals Co., Matsumoto, Japan).

11.4.6 Mechanisms for vitamin D analog selectivity

Continuous research tries to develop effective vitamin D analogs with selectivity for PTH suppression without calcium and phosphate level elevation. There are several possible interactions that may be modified in order to change the biological activity of the vitamin D analogs (Brown and Slatopolsky, 2008):

1. Binding to the VDBP. The VDBP carries 99% of calcitriol in the circulation. Binding to VDBP increases the circulating half life of and decreases the tissue accessibility of the vitamin D analog. Maxacalcitol has a 500 times lower affinity for VDBP than calcitriol, leading to faster clearance from the circulation, but a higher level in the intestine and parathyroid glands.

2. Metabolism. Metabolism of the vitamin D analogs may influence on their activity. As discussed above alfacalcidol and doxercalciferol are classically considered to need 25-hydroxylation before they become active. However before they are 25-hydroxylated their affinity for VDBP is reduced, which may lead to increased accessibility into peripheral tissue as discussed in the proceeding section. The catabolism of vitamin D analogs by 24-hydroxylation attenuates the activity of the compounds. The 24-hydroxylase synthesis is induced by calcitriol and its analogs, and this induction may differ between the analogs. Modification of the vitamin D analog site chain affects their rate of catabolism, as Falecalcitriol which undergoes slower metabolic inactivation. Furthermore, as demonstrated for maxacalcitol, the catabolism may differ between cell-types. Maxacalcitol was faster degraded in keratinocytes than in parathyroid cells (Bikle et al., 1995). The vitamin D analogs may be metabolised into active metabolites. Falecalcitriol are metabolised into an active 23-hydroxylated metabolite (Komuro et al., 2003).

3. Interaction with VDR. After binding of the vitamin D analog to the VDR, the VDR undergoes conformational changes and interacts with the retinoid X receptor and co modulators and regulates gene transcription. The affinity for the VDR is important for the effect of the vitamin D analog. The binding of the vitamin D analog to the VDR may differ and induce differential conformational changes of the receptor and differential interaction with the co activators and co repressors of gene transcription. Differential recruitment of co activators has been described between maxacalcitol and calcitriol (Takeyama et al., 1999).

4. Interactions with cell-surface receptor. It appears that calcitriol may act at the cell membrane through a rapid non-genomic pathway. The function of this pathway is still not fully understood. This pathway may induce rapid calcium and phosphate absorption in the enterocytes. It may be a potential target for new vitamin D analogs (Farach-Carson and Nemere, 2003).
11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

5. Intracellular VDBPs. Intracellular proteins (HSC-70 and Bag-1) bind vitamin D analogs with high affinity and may thereby influence their activity and metabolism.

11.4.7 Native vitamin D

The native form of vitamin D; cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), are increasingly used in daily nephrology practice. The presence of the wide distribution of the VDR and 1α-hydroxylase in the body, makes a local synthesis of 1,25(OH)_2D possible, and native vitamin D may be needed for paracrine or autocrine functions in a wide range of cellular systems. Whether these local functions can be satisfied by supplementation with active vitamin D analogs are unknown. It may be necessary to substitute with both native and active vitamin D in CKD patients.

In parathyroid glands 1α-hydroxylase are present and can produce calcitriol (Ritter et al., 2006). Treatment of low 25(OH)D levels has been shown to prevent the development and improve established hyperparathyroidism in CKD2-3, although the results in patients with more advanced kidney disease has not shown convincing evidence of PTH suppression (Al-Aly et al., 2007; Shroff et al., 2012; Zisman et al., 2007).

11.5 Clinical outcomes and treatment with vitamin D in patients with chronic kidney disease

Multiple observational studies have shown an improved survival in CKD patients treated with activated vitamin D (Hansen, 2012). A survival advantage as high as 24% has been reported. An observational study even found an improved survival in patients treated with paricalcitol compared to calcitriol, although others found that this difference disappeared after adjustment for several variables.

The survival advantage linked to the use of active vitamin D has been shown to be present at different levels of calcium, phosphate and PTH, pointing towards an influence of vitamin D through other mechanisms than the mineral metabolism.

The survival benefit of vitamin D is further stressed by observational studies showing low levels of vitamin D both as 25(OH)D and 1,25(OH)_2D are associated with increased mortality in CKD patients (Pilz et al., 2011).

No randomised controlled trial has addressed whether vitamin D treatment of any kind improve survival in CKD patients. Meta-analysis of vitamin D treatment and survival in predialysis and dialysis patients also found data too sparse to address this question (Palmer et al., 2009a,b).

Possible mechanisms for a survival benefit in CKD patients treated with vitamin D are being explored. Especially the influence on renal function and cardiovascular disease in CKD patients.
D. Hansen

has been paid attention. Vitamin D may influence many cellular systems and organs in the CKD patients as well as in the general population (Holick, 2007), this is discussed in other chapters.

11.5.1 Renal function

Treatment with vitamin D analogs has been shown to decrease progression of renal insufficiency in observational studies (Kovesdy et al., 2008; Shoben et al., 2008).

Recently, the results of a multinational randomised trial found a decrease in albuminuria by paricalcitol compared to placebo in 281 type 2 diabetic patients with nephropathy (De Zeeuw et al., 2010). Whether this decrease in albuminuria can be translated into a reduced progression in renal insufficiency is unknown.

Vitamin D down regulates the renin-angiotensin system by inhibiting the renin synthesis in animal studies (Li, 2003). Down regulation of the renin-angiotensin system by ACE-inhibitors and angiotensin receptor blockers has been shown to preserve kidney functions, therefore a renal protective function of vitamin D seems possible.

Vitamin D has anti-inflammatory properties (Mathieu and Adorini, 2002) and has been demonstrated to attenuate renal inflammation. This is a possible mechanism for preserving renal function.

11.5.2 Cardiovascular disease

The high mortality in CKD patients are at least in 50% caused by cardiovascular disease. Observational studies have demonstrated an association between cardiovascular mortality and both vitamin D deficiency and lack of treatment with vitamin D (Petchey et al., 2011).

VDR knockout mice develops hypertension and left ventricular hypertrophy. Animal studies have pointed towards a reduction in left ventricular hypertrophy by vitamin D analogs. Like the reduction in albuminuria this may be mediated through an inhibition of the renin-angiotensin system. The results of a randomised placebo controlled trial in 227 CKD3-4 patients evaluating the change in left ventricular mass index during paricalcitol treatment are awaited.

Vascular calcification is common in CKD patients. High doses of vitamin D induce vascular calcification in experimental models. Vitamin D increases calcium and phosphate levels which are linked to increased vascular calcification. In both animal and observational studies a U-shaped curve between vitamin D dose and calcification was demonstrated pointing towards a therapeutic interval for vitamin D should be targeted.
11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

References


11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease


Key facts

- Over millennia, skin pigmentation adapts to where people live, dark enough to reduce free radical production and folate destruction, light enough to permit production of vitamin D.
- Sunlight reduces the risk of many types of disease primarily through production of vitamin D in the skin from the ultraviolet B (UVB) spectral region (290-315 nm).
- Ecological studies (population studies comparing geographical variation or seasonal variation of disease outcome with indices of risk factors) are often the first type of study to identify and quantify links to various risk-modifying factors.
- Vitamin D3 supplements are bioidentical to vitamin D produced by solar or artificial UVB.

Summary points

- Solar UVB and vitamin D reduce the risk of about 20 types of cancer.
- Solar UVB and vitamin D reduce the risk of several types of bacterial infectious diseases, including bacteremia, dental caries, pneumonia, sepsis, and tuberculosis.
- Solar UVB and vitamin D reduce the risk of several types of viral infectious diseases, including Epstein-Barr virus diseases (Hodgkin’s lymphoma, multiple sclerosis, and infectious mononucleosis) and type A influenza.
- Solar UVB and vitamin D reduce the risk of several autoimmune diseases, including type 1 diabetes mellitus, multiple sclerosis, and rheumatoid arthritis.
- Hill’s 1965 criteria for causality in a biological system can be used to assess whether links between solar UVB and/or vitamin D are causal. The criteria are strength of association, consistency, specificity (not applicable to vitamin D), temporality, biological gradient, plausibility (e.g. mechanisms), coherence, experiment (e.g. randomized controlled trial), and analogy. Later work added accounting for confounding factors and avoidance of bias.
12. Diseases affected by vitamin D: sun exposure

W.B. Grant
Sunlight, Nutrition and Health Research Center, P.O. Box 641603, San Francisco, CA 94164-1603, USA; wbgrant@infionline.net

Abstract

Solar ultraviolet B (UVB) irradiance, through production of vitamin D, is important in human health. In response to solar UV doses, skin pigmentation adapted over millennia to where people lived, changing from dark in the African plains to light in Northern Europe. Many ecological (geographical) studies have inversely correlated about 20 types of cancer with solar UVB doses. Observational studies along with empirical data from two randomized controlled trials (RCTs) largely support these ecological findings. Research has generally revealed the mechanisms whereby vitamin D can reduce cancer risk. By inducing cathelicidin and defensins, solar UVB reduces the risk of several bacterial and viral infections. Bacterial infections with strong evidence of UVB/vitamin D effects include dental caries, periodontal disease, pneumonia, sepsis, and tuberculosis. Viral infectious diseases with similar evidence include Epstein-Barr virus-linked diseases such as Hodgkin’s lymphoma, multiple sclerosis, and infectious mononucleosis, as well as type A influenza. Solar UVB and vitamin D also reduce risk of other autoimmune diseases, including inflammatory bowel disease/Crohn’s disease, rheumatoid arthritis, and type 1 diabetes mellitus. Good evidence for beneficial roles of UVB/vitamin D in reducing risk of cardiovascular disease (CVD) comes from observational studies of prediagnostic serum 25-hydroxyvitamin D and ecological studies of the seasonality of CVD incidence or mortality rates. That cold temperature also appears to be an important risk factor for CVD tends to somewhat cloud the seasonality studies. However, the same effect applies for type A influenza, yet two RCTs found beneficial effects of vitamin D supplementation. Although RCTs are often called for as definitive proof that vitamin D reduces the risk of various types of disease, the criteria for causality in a biological system that A.B. Hill established in 1965 can serve as an alternative approach.

Keywords: autoimmune disorders, cancer, cardiovascular disease, infections, ultraviolet B
12.1 Introduction

Over millennia, skin pigmentation varies throughout the world in response to ambient solar UV doses where people live. Those living in the tropical plains have dark skin, whereas those living in Northern Europe have pale skin. Skin pigmentation varies in response to two driving forces: (1) protection against the adverse effects of solar UV irradiance, free radical production and alteration of DNA, and destruction of folate; and (2) production of vitamin D (Jablonski and Chaplin, 2000, 2010). As people moved out of Africa to higher latitudes, those with lighter skin pigmentation had better bone development – improving the likelihood that the pelvic opening would be well formed to permit babies to exit the birth canal – as well as reduced risk of infectious diseases such as tuberculosis. Thus, we could expect that solar UVB doses, skin pigmentation, and time spent in the sun would have a major impact on general health and risk of many diseases. We also expect that health disparities between dark- and light-skinned people living outside the tropics could be due in part to differences in vitamin D production – which seems to be the case (Grant and Peiris, 2010, 2012).

The first disease linked to sunlight was rickets, for which Trousseau in France realized that lack of sunlight was a risk factor in 1861 (Rajakumar, 2003; Rajakumar et al., 2007). However, not until 1922 did McCollum et al. demonstrate that vitamin D prevented or cured rickets. Rickets still occurs today, especially among breast-fed dark-skinned infants living in the midlatitudes (Rajakumar and Thomas, 2005).

Many more diseases are linked to low solar UVB doses and serum 25(OH)D concentrations. This chapter will highlight some of these diseases, presenting the evidence that both solar UVB irradiance and vitamin D reduce risk.
12. Diseases affected by vitamin D: sun exposure

12.2 Diseases

12.2.1 Cancer

Many types of cancer are more common in regions with lower solar UVB doses. A paper published in 1937 noted that those who developed ‘skin irritation’ (probably NMSC and actinic keratoses) had a reduced risk of cancer incidence (Peller and Stephenson, 1937). A paper published in 1941 noted that US cancer mortality rates increase with latitude (Apperly, 1941). Not until 1980 did the brothers Cedric and Frank Garland propose the UVB-vitamin D-cancer hypothesis to explain the geographical variation of US colon cancer mortality rates (Garland and Garland, 1980). Higher dietary vitamin and calcium levels were associated with reduced risk of colorectal cancer (Garland et al., 1985), and prediagnostic serum 25(OH)D concentration inversely correlated with colon cancer (Garland et al., 1989). The geographical variations of mortality rates also supported the UVB-vitamin D-calcium hypothesis for breast (Garland et al., 1990) and ovarian (Lefkowitz and Garland, 1994) cancer. Another group proposed that solar UVB reduced the risk of prostate cancer (Schwartz and Hulka, 1990). Pancreatic cancer increased with latitude in Japan (Kato et al., 1985), and non-Hodgkin’s lymphoma inversely correlated with solar UVB doses in contrast to the direct US association between solar UVB and melanoma and NMSC (Hartge et al., 1996).

Although additional work supporting the UVB-vitamin D-cancer hypothesis emerged, the number of cancers with mortality rates inversely correlated with solar UVB doses did not reach 15 until 2002 (Grant, 2002). That study compared cancer mortality rates by state economic area with solar UVB doses for July 1992 (Leffell and Brash, 1996). US summertime solar UVB doses are highest in the Southwest, lowest in the Northeast (Leffell and Brash, 1996). The reasons include that the surface elevation is generally higher in the West and that the ozone layer is thinner as the westerly winds push the tropopause higher as the air masses prepare to cross the Rocky Mountains. Wintertime solar UVB doses vary directly with latitude (Grant, 2008b). That study led to other studies supporting the UVB-vitamin D-cancer hypothesis, such as an observational study from Harvard (Giovannucci et al., 2006) and an RCT of vitamin D and calcium supplementation from Creighton University (Lappe et al., 2007). That study received criticism for not having included other cancer risk-modifying factors, so the work was extended by including indices for alcohol consumption, ethnic background, poverty, smoking, and urban/rural residence, with similar results for solar UVB doses (Grant and Garland, 2006). A similar study was also published that year (Boscoe and Schymura, 2006). Several papers have reviewed ecological studies (Grant, 2012a,b; Grant and Mohr, 2009; Mohr, 2009). The most recent review ‘consistently found strong inverse correlations with solar UVB for 15 types of cancer: bladder, breast, cervical, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal, renal, and vulvar cancer; and Hodgkin’s and non-Hodgkin’s lymphoma. Weaker evidence exists for nine other types of cancer: brain, gallbladder, laryngeal, oral/pharyngeal, prostate, and thyroid cancer; leukemia; melanoma; and multiple myeloma’ (Grant, 2012a: 223).

Looking at cancer diagnosis or death related to incidence or death from NMSC is another way to show that solar UVB reduces cancer risk. A review of cohort studies reporting cancer incidence
rates with respect to NMSC diagnoses used this approach: ‘For NMSC, relative risks for cervical, esophageal, gastric, and rectal cancer were significantly reduced; those for colon and gallbladder cancer were marginally insignificant, while those for female breast, laryngeal, ovarian, renal, and uterine corpus cancers were insignificantly reduced; relative risks for lip and salivary gland cancers and melanoma were significantly increased’ (Grant, 2007a: 668). That same year, a record linkage study of cancers after diagnosis of NMSC in sunny countries (Australia, Singapore, and Spain) found significant inverse correlations with liver, pancreatic, and prostate cancer and found insignificant inverse correlations with bladder, colon, gastric, ovarian, rectal, and renal cancer (Tuohimaa et al., 2007). In northern mid- and high-latitude countries, however, direct correlations emerged between diagnosis of NMSC and incidence of solid tumors. I attributed this finding to such factors as less body surface area exposed owing to colder weather (Grant, 2008a). Also, my ecological study of cancer mortality rate by province in Spain inversely correlated about 15 types of cancer with NMSC mortality rates (Grant, 2007b).

Using 1950-1964 cancer mortality rate data for California, Devesa et al. (1999) analyzed NMSC mortality rates as an index of solar UVB irradiance at the population level. NMSC rates were lower along the US West Coast than inland during 1950-1969, whereas mortality rates for internal cancers were often higher along the West Coast than inland, especially for males. Data for those older than 40 years in 5-year increments for the 19 state economic areas of California were obtained from the National Cancer Institute (http://ratecalc.cancer.gov/ratecalc/). Significant inverse correlations with NMSC mortality rate in multiple linear regression analyses emerged for 1950-1964 for eight types of cancer for males: bladder, brain, colon, gastric, prostate, and rectal cancer; multiple myeloma; and non-Hodgkin’s lymphoma. No similar results emerged for females with respect to the other two UVB indices. Their NMSC mortality rates averaged 60% lower than those for males. Lung cancer mortality rates were directly correlated with three types of cancer for males: laryngeal, oral, and renal. No significant correlations with NMSC mortality rates appeared for later periods. Reasons for this finding may include reduced NMSC mortality rates, high immigration rates, and movement from rural to urban locations (Grant, 2012b).

As mentioned, most studies finding inverse correlations between solar UVB and cancer risk come from low and mid latitudes. Cancer survival after diagnosis is reportedly higher for summer or fall diagnosis than winter diagnosis for breast, colon, prostate cancer, and Hodgkin’s lymphoma (Porojnicu et al., 2007). Cancer incidence rates by occupation can show an effect of solar UVB in reducing cancer risk at higher latitudes. Data on cancer SIRs by sex and 54 occupation categories based on 1.4 million male and 1.36 million female cancer cases for 1961-2005 in the five Nordic countries undergird an ecological study of solar UVB’s role in the risk of many types of cancer at high latitudes (Pukkala et al., 2009). Lip cancer SIRs less lung cancer SIRs for men were the best index of solar UVB dose; these SIRs were inversely correlated with SIRs for both melanoma and NMSC. Lung cancer SIRs were used as the index of the effects of smoking. For men, the UVB index was significantly inversely correlated with 15 types of cancer: bladder, breast, colon, gallbladder, kidney, laryngeal, liver, lung, oral, pancreatic, pharyngeal, prostate, rectal, small intestine cancer, and melanoma. For women, the same UVB index was inversely correlated with
12. Diseases affected by vitamin D: sun exposure

bladder, breast, colon, and rectal cancer. These results generally agree with findings from other studies (Grant, 2012c).

The inverse correlation between the UVB index in my study can be explained by how UVB irradiance protects against melanoma and basal cell carcinoma, the most common but least deadly of all skin cancers. Three ways exist: (1) increased pigmentation (tanning) and thickening of the stratum corneum; (2) vitamin D production (Field and Newton-Bishop, 2011); and (3) elastosis, or skin aging. Both solar UV and smoking lead to cross-linking of collagen in the skin. Skin elastosis reduces the risk of melanoma (Berwick et al., 2005) and basal cell carcinoma (Walther et al., 2004). Also, melanoma develops later in life for regions of the body chronically exposed to solar UV than to regions exposed only sporadically (Dal et al., 2007). Elastosis might protect against melanoma because melanoma reaches out into the surrounding epidermis for fibroblasts (Flach et al., 2011), which is harder to accomplish with elastosis.

The mechanisms whereby vitamin D reduces the risk of cancer are generally well known and include effects on cellular differentiation and proliferation, cellular adhesion, calcium absorption, metastasis, and angiogenesis (Fleet et al., 2012; Garland et al., 2009; Krishnan and Feldman, 2011; Lamprecht and Lipkin, 2003).

12.2.2 Infectious diseases

Solar UVB doses are associated with reduced risk of several bacterial and viral infectious diseases. Bacterial diseases include dental caries (Grant, 2011), periodontal disease (Grant and Boucher, 2010), pneumonia (Grant and Giovannucci, 2009), sepsis (Grant, 2010), and tuberculosis (Liu et al., 2006, 2007). Viral infectious diseases included type A influenza (Cannell et al., 2006, 2008), Epstein-Barr virus-related diseases such as Hodgkin's lymphoma (Douglas et al., 1996; Hjalgrim et al., 2007), infectious mononucleosis (Douglas et al., 1996; Hjalgrim et al., 2007), and MS (Grant and Holick, 2005; Orton et al., 2011). Solar UVB reduces risk of these diseases through producing vitamin D and then inducing cathelicidin and defensins (Gombart, 2009). Cathelicidin has antimicrobial and antiendotoxin properties (Mookherjee et al., 2007). Of these diseases, this chapter discusses two bacterial diseases, dental caries and sepsis, and two viral diseases, type A influenza and MS.

12.2.3 Dental caries

Solar UVB doses inversely correlated with dental caries among US male adolescents in the 1930s (East, 1939). However, evidence of a role of solar UVB in reducing risk of dental caries goes back to the Civil War, on the basis of a report that those trying to enlist in the Union Army from the Northeast had more missing teeth than those from as far south as Kentucky (Lewis, 1865). In 1928, an experiment with children found that oral vitamin D intake reduced the risk of dental caries (Mellanby and Pattison, 1928). Researchers of the era thought that vitamin D’s role in increasing calcium absorption and putting it in the hard tissues explained the findings. However, 21st-century researchers found that cathelicidin killed the bacteria responsible for caries (Dale...
and Fredericks, 2005). I published a unifying review of the literature in 2011 (Grant, 2011). A similar review was made for periodontal disease (Grant and Boucher, 2010), for which the role of solar UVB is less evident.

### 12.2.4 Type A influenza and pneumonia

Influenza is largely seasonal, with highest rates in winter, lowest rates in summer (Hope-Simpson, 1981). Cannell and colleagues hypothesized that the seasonality was due largely to annual variations in solar UVB doses and vitamin D production (Cannell et al., 2006), with this hypothesis updated in 2008 (Cannell et al., 2008). RCTs (Aloia and Li-Ng, 2007; Urashima et al., 2010) and observational studies (Sabetta et al., 2010) have supported this hypothesis. However, an opposing point of view is that influenza is largely seasonal because of changes in temperature and absolute humidity: the influenza virus survives longer outside the body when temperatures are cold and absolute humidity is low (Shaman et al., 2010, 2011a,b). Both hypotheses seem to explain features of the annual cycle of influenza infections. Nature largely controls temperature and humidity; oral vitamin D intake can compensate for low solar UVB doses in winter.

One effect of influenza is rapid increase of proinflammatory cytokines, giving rise to a cytokine storm (Cheng et al., 2011), which can disrupt the cells lining the lungs, thereby increasing the risk of bacterial or viral pneumonia. Most people who die soon after developing influenza die from pneumonia, approximately 10 days after the initial influenza infection. During the 1918-1919 pandemic, US rates of A/H1N1 influenza were much higher in the Northeast than in the South and West (Britten, 1932). An ecological study on case-fatality rates found that indices for summer and winter solar UVB doses explained about half of the variance (Grant and Giovannucci, 2009).

A study in Philadelphia significantly inversely correlated solar UVB doses with infection by invasive pneumococcal disease, a common cause of community-acquired pneumonia (White et al., 2009). An RCT found that vitamin D supplementation reduced the risk of pneumonia in children (Manaseki-Holland et al., 2010).

### 12.2.5 Multiple sclerosis

Prevalence of MS increases with latitude (Kurtzke, 1993). However, prevalence in the Nordic countries, where more fish is consumed, is lower than in European countries just to the south (Kurtzke, 1993). The US prevalence of MS (on the basis of men enlisting in the armed forces during World War II and the Korean War (Wallin et al., 2004)) shows a pronounced latitude dependence that indicates wintertime serum 25(OH)D concentrations rather than summertime concentrations (Grant and Holick, 2005). Other studies have also reported low solar UVB doses in winter as a risk factor (Orton et al., 2011; van der Mei et al., 2003). In the UK, seasonal UVB doses and infectious mononucleosis prevalence explained 72% of the variance of MS prevalence (Ramagopalan et al., 2011).
12. Diseases affected by vitamin D: sun exposure

12.2.6 Inflammatory bowel disease

IBD occurs in two forms: CD and UC. Two studies reported higher rates of IBD for higher latitudes than for lower latitudes, one in Europe (Shivananda et al., 1996) and the other in France (Nerich et al., 2006, 2010); one study also reported a latitudinal gradient for CD in Scotland (Armitage et al., 2004). The geographical variation for CD in France is similar to that for breast cancer (Engel et al., 2011; Grant, 2010) and other cancers (Grant, 2010); where this variation was linked to solar UVB doses and vitamin D production. However, the geographical variation of UC in France does not show a similar latitudinal gradient.

US rates for CD and UC are highest in the Northeast (Kappelman et al., 2007; Sonnenberg, 2010). US summertime solar UVB doses are highest in the Southwest, lowest in the Northeast (Leffell and Brash, 1996). An ecological study using summertime UVB, wintertime UVB, smoking, and obesity analyzed the data, both prevalence and mortality rates by state, for CD, UC, and Clostridium difficile colitis in the US (Sonnenberg, 2010). For CD, summertime UVB (inverse association or risk reduction) and smoking (risk) were significantly correlated. For UC, UVB was significantly inversely correlated, but not as strongly as for DC. Smoking was correlated with C. difficile colitis (Grant, unpublished data). A study from India reported lower serum 25(OH)D levels in those with CD with lower sun exposure (Joseph et al., 2009).

12.2.7 Type 1 diabetes mellitus

The seasonality of birth for those diagnosed with T1DM generally has higher rates in summer and lower rates in winter than all birth rates in the population. Studies have reported this trend for several countries, including New Zealand (Willis et al., 2002), the United States (peak from March to June) (Kahn et al., 2009), but not most European countries (McKinney, 2001). Higher rates in summer suggest that low serum 25(OH)D levels in late winter or early spring are a risk factor for T1DM. The 3- to 5-month lag between minimum serum 25(OH)D levels and spring or summertime birth corresponds to the effect of low serum 25(OH)D having greatest impact in the fourth to sixth month of gestation. This time may be when the pancreas develops. Pancreas A cell density was greatest in the 6-month fetus (Wirdnam and Milner, 1981).

Those with T1DM with pronounced β-cell autoimmunity born in Sweden or Berlin had a different seasonality of birth rate from that of others (Lewy et al., 2008). The authors suggested that this finding was consistent with perinatal viral infections. Wintertime is the season of highest risk for developing T1DM in New Zealand (Willis et al., 2002). That is when respiratory viral infections are most common, due largely to lower solar UVB doses (Cannell et al., 2006).

An ecological study of T1DM incidence rates for 51 countries found a significant inverse correlation between solar UVB irradiance adjusted for both cloud cover and per capita health expenditure (Mohr et al., 2008). A plot of T1DM incidence rate versus latitude produced a ‘smiley’ curve, with low rates near the equator and high rates at higher latitudes.
12.2.8 Rheumatoid arthritis

Evidence exists that RA is linked to low solar UVB/vitamin D. For example, a US study found highest rates of RA in the Northeast, where solar UVB doses in summer are lowest (Vieira et al., 2010). A study in Italy inversely related RA disease activity and disability scores with 25(OH)D concentrations (Rossini, et al., 2010).

12.2.9 Cardiovascular disease

For CVD, three lines of evidence suggest that solar UVB reduces risk. One is the seasonality of CVD mortality rates, highest in winter, lowest in summer (Scragg, 1981). The second comes from observational studies reporting reduced risk of CVD incidence or increased survival for those with higher serum 25(OH)D concentrations. The third is from direct correlations with some measure of UVB irradiance.

The period of peak CVD mortality rates in the northern hemisphere is December-March (Crawford et al., 2003; Douglas et al., 1991; Kendrovski, 2006; Tanaka et al., 2000) and in the southern hemisphere, May-September (Barnett et al., 2008; Weerasinghe et al., 2002). These are the periods of lowest temperatures (Kendrovski, 2006) and lowest solar UVB doses and serum 25(OH)D concentrations (Hypponen and Power, 2007; Livesey et al., 2007). Thus, separating the effects of temperature from those of UVB/vitamin D is difficult. An analogous situation exists for influenza: peak infection rates occur near December or January (Hope-Simpson, 1981), and studies have implicated both temperature/relative humidity (Shaman, 2011a,b) and solar UVB/vitamin D (Cannell et al., 2006, 2008). Low temperature and relative humidity permit the influenza virus to survive longer outside a body, thereby increasing person-to-person transmission. Vitamin D reduced risk of type A influenza in one RCT (Urashima et al., 2010). UV has one further effect on risk of CVD: lowering of blood pressure due to UV A-induced release of nitric oxide from cutaneous photolabile nitric oxide derivates (Oplander et al., 2009).

Several studies have inversely correlated prediagnostic serum 25(OH)D concentrations with CVD incidence. A meta-analysis of 17 studies found an odds ratio of 0.67 (95% confidence interval, 0.55-0.81) for high versus low serum 25(OH)D for CVD incidence (Parker et al., 2010).

An observational study from the Intermountain Healthcare system (based on 41,497 subjects with at least one vitamin D measurement from 2000 to 2009) examined the prevalence of CVD diagnoses for those older than 50 years for three serum 25(OH)D concentration ranges: less than 15 ng/ml, 16-30 ng/ml, and greater than 30 ng/ml. The study found statistically significant differences for all types of CVD: coronary heart disease, heart failure, atrial fibrillation, peripheral vascular disease, previous myocardial infarction, previous stroke, previous transient ischemic attack, and ventricular tachycardia (Anderson et al., 2010).

Another observational study associated vitamin D supplementation with an odds ratio for death after diagnosis with CVD of 0.44 (95% confidence interval, 0.34-0.59) (Vacek et al., 2011).
12. Diseases affected by vitamin D: sun exposure

The mechanisms whereby vitamin D reduces the risk of CVD appear to include reduced risk of diabetes (Parker et al., 2010), hypertension (Vaidya and Forman, 2012), infection and inflammation (Guillot et al., 2010), and vascular calcification (Chang et al., 2012; Rodriguez et al., 2011; Zagura et al., 2011).

Some studies have correlated higher altitude with lower risk of CVD. A Swiss study found that mortality rates for coronary heart disease decreased by 22% per 1000 m in altitude, whereas stroke mortality rates decreased by 12% per 1000 m in altitude (Faeh et al., 2009). A Swedish study found reduced risk of venous thrombotic events for women by about 30% for several measures of UVB irradiance (Lindqvist et al., 2009).

12.2.10 Anaphylaxis

The epidemiological evidence for a role of vitamin D comes largely from studies of the geographical variations in anaphylactic symptoms and seasonality of births among children with food allergies. The first epidemiological study was ecological, using regional differences in 2004 US EpiPen prescriptions (Camargo et al., 2007). The highest rates were in the Northeast (8-12 prescriptions/1000 people), whereas the lowest rates were in the Southwest (2-3 prescriptions/1000 people). According to Figure 2 in that paper, the variation seems related to summertime solar UVB doses, which are highest in the Southwest and lowest in the Northeast (Fioletov et al., 2010; Leffell and Brash, 1996). The distribution is highly asymmetric for two reasons: higher surface elevations in the western states and thinner stratospheric ozone layer in the West. The thin ozone layer is due to the prevailing westerly winds crossing the Rocky Mountains and pushing the tropopause higher. Solar UVB is the primary source of vitamin D for most Americans.

A similar study in Australia found a significant increase in EpiPen prescription rates going from 20°S to 45°S latitude (Mullins et al., 2009). Because Australia has no mountain ranges, solar UVB doses decrease with increasing latitude. Other factors did not significantly affect the finding. A related study in Australia also found more use of hypoallergenic formula for infants in the southern and eastern regions (Mullins et al., 2010).

A study of US emergency department hospital visits for acute allergic reactions found the highest rates in the Northeast, with a stronger association seen when the reactions were limited to those caused by food allergy (Rudders et al., 2010).

Two studies of season of birth and food allergy in Boston children found higher rates for those born in fall or winter than in spring or summer. The first such study was conducted in the United States. It found a seasonal pattern, with lowest rates for emergency department patients younger than 5 years born in April to July and highest rates in December and January (Vassallo et al., 2010). No seasonality was apparent for older children or adults. A second study involving 3,792 patients in Boston found the lowest rate for those younger than 18 years born in spring, with increasing relative ratio for summer, fall, and winter births (Vassallo and Camargo, 2010).
12.3 Causality

Much of the evidence that UV reduces the risk of disease comes from ecological and observational studies, with support from ecological, observational, and clinical studies on vitamin D. Many in the health system are used to having RCTs demonstrate efficacy and lack of harm for pharmaceutical drugs. Some have questioned this requirement for cancer prevention (Kristal, 2008). In fact, carrying out RCTs with UV irradiance would be difficult because solar UV is the primary source, and the lag time between exposure and disease outcome for chronic diseases is many years. Even for melanoma, which is linked to solar and artificial UV irradiance – more to sporadic than chronic irradiance (Gandini et al., 2005; Chang et al., 2009) – no RCTs have been conducted.

An alternative approach is to apply A.B. Hill’s criteria for causality in a biological system (Biesalski et al., 2011; Hill, 1965): strength of association, consistency, specificity (not applicable to vitamin D), temporality, biological gradient, plausibility (e.g. mechanisms), coherence, experiment (e.g. RCT), and analogy. Later work added accounting for confounding factors and avoidance of bias (Potischman and Weed, 1999). Several types of cancer (Grant, 2009a,b, 2012a,b), periodontal disease (Grant and Boucher, 2010), and MS (Hanwell and Banwell, 2011) generally satisfy these criteria.

The ecological approach is often considered the weakest type of epidemiological study. However, well-conducted ecological studies have often been the first type of study to identify and quantify a link between risk factors and disease outcome. Examples include dietary risks for cancer (Armstrong and Doll, 1975) and Alzheimer’s disease (Grant, 1997), as well as UVB-vitamin D and cancer risk reduction (Garland and Garland, 1980). It is amusing that the finding of dietary fat as an important risk factor for many types of cancer (Armstrong and Doll, 1975) was opposed for many years until the realization that the effect of diet could occur early in life. When cohort studies included younger women, that meat and fat were risk factors for breast cancer (Cho et al., 2006) became apparent.

12.4 Summary and conclusion

This chapter has summarized evidence that solar UVB, through production of vitamin D, reduces the risk of many types of chronic and infectious diseases. The research and journal literature on vitamin D is growing rapidly, so consider the discussion in this chapter an overview of the field as of early 2012. One can follow the literature as it develops by regularly searching the National Library of Medicine’s PubMed database (http://www.pubmed.gov/).
12. Diseases affected by vitamin D: sun exposure

Disclosure

I receive funding from the UV Foundation (McLean, VA, USA), Bio-Tech Pharmacal (Fayetteville, AR, USA), the Vitamin D Council (San Luis Obispo, CA, USA), and the Vitamin D Society (Canada).

References


12. Diseases affected by vitamin D: sun exposure


Grant, W.B., 2008b. Hypothesis-Ultraviolet-B irradiance and vitamin D reduce the risk of viral infections and thus their sequelae, including autoimmune diseases and some cancers. Photochemistry and Photobiology 84, 356-365.

Grant, W.B., 2009a. How strong is the evidence that solar ultraviolet B and vitamin D reduce the risk of cancer?: An examination using Hill’s criteria for causality. Dermato-Endocrinology 1, 17-24.

Grant, W.B., 2009b. Solar ultraviolet-B irradiance and vitamin D may reduce the risk of septicemia. Dermato-Endocrinology 1, 37-42.


Grant, W.B., 2012c. Role of solar UV irradiance and smoking in cancer as inferred from cancer incidence rates by occupation in Nordic countries. Dermato-Endocrinology 4, 203-211.
Grant, W.B. and Boucher, B.J., 2010. Are Hill's criteria for causality satisfied for vitamin D and periodontal disease? Dermato-Endocrinology 2, 30-36.


12. Diseases affected by vitamin D: sun exposure


Key facts

- Multiple sclerosis, allergic asthma and type 1 diabetes are diseases driven by active immune processes, and downregulated by anti-inflammatory medications.
- There is a greater incidence of multiple sclerosis, allergic asthma and type 1 diabetes in populations living at higher latitudes (i.e. a positive latitude gradient).
- There are seasonal effects in expression of multiple sclerosis, allergic asthma and type 1 diabetes with highest incidence in winter.
- UV irradiation of skin stimulates suppression of immune responses both locally at the irradiated site and systemically with reduced responses to antigens administered to non-irradiated sites.
- All immune cells express a vitamin D receptor and can respond to vitamin D.

Summary points

- Ultraviolet radiation (UVR) exposure reduces immune responses to antigens, allergens and tumour antigens in humans.
- The immunosuppressive effects of UVR are used in phototherapy for treatment of skin inflammatory diseases such as psoriasis and atopic dermatitis.
- The extent of involvement of UVR-induced vitamin D in the immunosuppressive effects of UVR is unknown.
- Responses to vitamin D in vitro by immune cells are associated with reduced immunity.
- Inverse associations have been reported between incidence of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes and serum vitamin D levels (i.e. increased disease if reduced vitamin D levels, reduced disease if higher vitamin D levels).
- Some but not all studies in mice made vitamin D deficient by dietary intervention have increased expression of symptoms associated with chronic immune diseases.
- Trials of vitamin D supplementation for chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes have been inconclusive.
- In murine studies, complementary as well as overlapping pathways are stimulated by vitamin D-dependent and vitamin D-independent pathways after UVR exposure.
- The importance in humans of UVR-induced pathways of immunosuppression that are not vitamin D-dependent remain unknown.
- Decisions on immunotherapeutic approaches for chronic immune diseases that target vitamin D-dependent or vitamin D-independent pathways must await the outcomes of several ongoing multi-centre large trials of vitamin D supplementation for disease control.
13. Immune modulation by UV: role of vitamin D

P.H. Hart and S. Gorman
Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, P.O. Box 855, West Perth 6872 Australia; prueh@ichr.uwa.edu.au

Abstract

A positive latitude gradient, namely a greater incidence of disease for individuals living at higher latitudes away from the equator, has been reported for chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes. Lower circulating levels of vitamin D have also been associated with increased prevalence of these diseases. As humans obtain more than 80% of their vitamin D by exposure of skin to the ultraviolet B (UVB) component of sunlight, it has been proposed that the two observations are linked. In this chapter, evidence for and evidence against the involvement of vitamin D in the immunoregulatory properties of UV radiation is presented. There are multiple photoreceptors in skin that absorb UVB photons, of which 7-dehydrocholesterol, the vitamin D precursor, is one. There may be many interacting and/or redundant pathways stimulated by ultraviolet radiation (UVR) exposure resulting in reduced immune responses. In mouse models both UVR and vitamin D are able to regulate immune responses but the potency of these effects in vivo is not fully understood. It is possible that levels of vitamin D are a biomarker of sun exposure and associations of disease incidence with circulating vitamin D levels have been incorrectly interpreted as dependent on the biological actions of vitamin D. Both vitamin D-dependent and vitamin D-independent pathways may account for the immunoregulatory effects of UVR. More research is needed. The most important test will be whether vitamin D supplementation can reduce the risk of diseases positively associated with latitude.

Keywords: multiple sclerosis, allergic asthma, type 1 diabetes, immunotherapy, latitude gradient
13.1 Evidence for ultraviolet radiation-induced immunosuppression

The best evidence for UVR-induced immunosuppression comes from epidemiological observations of increased disease prevalence of chronic immune diseases with increasing latitude of residence, and by inference, less sun (UVR) exposure. Further, support for UVR-induced immunosuppression has come from seasonal changes in expression of immune diseases with greater symptoms of disease during winter when less sun exposure occurs. The expression of the debilitating autoimmune disease, multiple sclerosis, is an excellent example. The concordance rate for identical twins is 30% and suggests important environmental influences on disease development. Both seasonal variation in disease expression (Van der Mei et al., 2011) and a positive latitude gradient have been reported for multiple sclerosis (Simpson et al., 2011). Further, greater amounts of sun exposure may benefit patients of all ages with multiple sclerosis, although exposure during childhood may have particularly enhanced benefits on expression of disease.

Using latitude as a proxy measure for sun exposure, positive latitude gradients have also been reported for other chronic diseases characterised by overzealous immune reactivity. These include allergic asthma (Krstic, 2011) and type 1 diabetes. A positive latitude gradient may account for up to 40% of the variation in type 1 diabetes (Ponsonby et al., 2005). Further, seasonal variation of diagnosis of type 1 diabetes with peaks in winter and troughs in summer has been reported (Moltchanova et al., 2009).

In phototherapy, UVR is used to treat patients with inflammatory skin conditions, including psoriasis and atopic dermatitis. To complement the improvement of symptoms in summer, exposure to narrow band UVB (311-313 nm) can improve the symptoms of these conditions (Milliken et al., 2012).

UVR-induced immunosuppression has also been implicated in the development of UVR-induced skin cancers. UVR-induced skin cancers are immunogenic and will be destroyed by a competent immune system. However, because UV is immunosuppressive, skin cancers continue to grow and develop. This has been highlighted by the increased skin cancer development in human patients on immunosuppressive therapy, for example in transplant patients. Supporting these findings is the evidence that exposure to UVR can interfere with outcomes of vaccination against poliovirus,
influenza virus, tuberculosis, hepatitis B virus, measles virus and rubella virus (reviewed by Norval and Woods, 2011). Seasonal effects of recall immune responses to vaccine antigens have been reported. After a holiday involving summer sun exposure, recall immune responses to standard multitest antigens were reduced when they were applied to both the sun-exposed and to the non-exposed skin (Norval and Woods, 2011).

Studies in mice have allowed a clearer dissection of the mechanisms of UVR-induced immunosuppression with measurement of cells, cytokines and other soluble mediators in UVR-exposed skin, the draining lymph nodes and in distant lymph nodes. Further, responses to UVR may depend on variables that relate to each individual (genetic makeup, skin colour, age, amount of skin exposed) as well as environmental factors other than season and latitude that may alter the intensity and spectral range of UV (time of day, pollution, cloud cover, ozone layer properties). The immunoregulatory properties of different wavelengths are a matter of much debate with UV A wavelengths (320-400 nm) reported to be both immunosuppressive and immunoprotective, and with different effects measured in response to different doses of UVR delivered. Upon measuring responses to nickel challenge in nickel-allergic individuals, UVB at 300 nm and UVA at 370 nm were immunosuppressive (Halliday et al., 2012). UVB wavelengths (290-315 nm) however, remain the most potent and UVR-induced immunosuppression can be measured in the absence of erythema (redness of the skin and sunburn) (Hart et al., 2011; Norval and Halliday, 2011; Ullrich and Byrne, 2012).

13.2 Ultraviolet radiation photoreceptors in skin

13.2.1 7-dehydrocholesterol

Upon absorption of UVB photons, 7-dehydrocholesterol of keratinocyte membranes is converted to pre-vitamin D3 which then thermally isomerises to vitamin D3 (cholecalciferol) (reviewed by Bouillon et al., 2008). Much of the vitamin D3 is stepwise hydroxylated in the liver to 25(OH)D3, and in the kidney to 1,25(OH)2D3, the active form of vitamin D. In addition to this route, keratinocytes and immune cells in the skin form 1,25(OH)2D3 within 16 hours of UVR exposure. 25(OH)D3 is the storage form of vitamin D and representative of the vitamin D ‘status’ of an individual; it is the level typically used in studies investigating associations of serum vitamin D with disease outcomes. The amount of 25(OH)D3 produced upon UVB irradiation of skin varies for different individuals and can depend on your pre-exposure vitamin D levels; if you have a lower starting level, sunlight will stimulate greater increases in 25(OH)D3. Dark-skinned individuals are less efficient at making vitamin D. For most individuals, diet contributes in a minor way to vitamin D levels.

13.2.2 DNA, urocanic acid and membrane phospholipids

DNA changes characteristic of UVB exposure include the formation of cyclobutane pyrimidine dimers and pyrimidine(6-4)photoproducts. The UVA signature includes both cyclobutane...
pyrimidine dimers as well as 8-oxo-7,8-dihydroguanine caused by UV-induced reactive oxygen species (Tewari et al., 2012). The involvement of UV-induced DNA damage in the mechanisms of UVR-induced immunosuppression was supported by studies in which UV-induced suppression of contact hypersensitivity responses was not detected if the irradiated mice were treated with liposomes containing T4 endonuclease V, an excision repair enzyme. Further, the application of cytokines that can activate nucleotide excision repair can reduce UVR-induced immunosuppression (Schwarz and Schwarz, 2011).

Trans-urocanic acid in the stratum corneum isomerises to its cis isomer upon UVR exposure. Cis-urocanic acid initiates signalling pathways involving receptors for platelet activating factor, serotonin and histamine. Soluble mediators from keratinocytes, nerves, skin dendritic cells and mast cells have been implicated in the immunosuppressive events following cis-urocanic acid formation. The production of reactive oxygen species by cis-urocanic acid, resulting in oxidised DNA and protein has also been reported.

UVR may stimulate a stress response in the membranes of keratinocytes and other skin cells. This can be characterised by absorption of UV photons by tryptophan and the formation of ligands for the cytoplasmic aryl hydrocarbon receptor. Activation of this receptor increases the transcription of many genes, and increased production of immunoregulatory cytokines and mediators such as PGE2 (Krutmann et al., 2012).

13.2.3 Concluding statement

The extent of overlap and redundancy of the signalling pathways initiated by UV photoreceptors is not known. The purpose of this chapter is to discuss the relative importance of UVR-induced vitamin D in UVR-induced immunosuppression, particularly as it relates to the perceived control by UVR, as suggested by latitude gradients, of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes.

13.3 Ultraviolet-induced vitamin D as a major contributor

13.3.1 Vitamin D associations and increased immune disease

For patients with multiple sclerosis, low serum 25(OH)D3 levels have been associated with increased risk of disease, progression of disease, and increased relapse rate (Holick, 2007). A recent meta-analysis of case-control studies concluded that polymorphisms in the vitamin D receptor gene are not associated with multiple sclerosis risk (Huang and Zie, 2012) although VDR variants have been reported to moderately modulate the risk of disease conferred by HLA-DRB1*15:01, a strong risk factor in females (Irizar et al., 2011).

For paediatric and adult patients with allergic asthma, low serum 25(OH)D3 levels have correlated with increased allergen sensitivity (high IgE levels), bronchial hyperresponsiveness,
poor lung function and reduced responses to steroids (reviewed by Dimoloe et al., 2010; Hollams et al., 2011). In a community-based cohort study in Perth, Australia, the transition of children to an allergic asthma phenotype was studied; 25(OH)D3 levels at ages 6 and 14 were negatively associated with concurrent allergic phenotypes, particularly in boys (Hollams et al., 2011). Vitamin D levels at age 6 were also significant predictors of subsequent atopy/asthma-associated phenotypes at age 14.

Similar negative associations of lower serum 25(OH)D3 levels and increased risk of diabetes in both children and adults have been published (Baeke et al., 2010). For both asthma and type 1 diabetes, vitamin D receptor polymorphisms have been linked with risk of disease.

13.3.2 Use of mouse models of disease

The associations between vitamin D insufficiency and measures of prevalence and intensity of multiple sclerosis, allergic asthma and type 1 diabetes have not confirmed vitamin D insufficiency as the cause, rather than a consequence, of the disease. For this reason, regulation of murine models of these diseases has been studied. Mice genetically deficient in the vitamin D receptor or Cyp27b1, the enzyme responsible for hydroxylation of 25(OH)D3 to 1,25(OH)2D3, are available. However, these mice have serious developmental problems that lead to skeletal, reproductive and immune system dysfunction and abnormal skin physiology (Bouillon et al., 2008). As they are unsuitable for studies of administration of UVR to skin, disease models have been studied in mice deficient or supplemented by dietary means with vitamin D.

The results from murine studies have not been definitive. UVR at a dose that stimulated a just perceptible erythema, delivered either before or after allergen sensitisation, reduced allergic airways disease in mice (Scott et al., 2011). This supported the finding that there was increased expression of many aspects of allergic disease in mice made vitamin D deficient by dietary intervention (Gorman et al., 2012). Thus, sufficient vitamin D and UVR-induced vitamin D may control disease expression. Similarly, vitamin D deficiency by dietary control early in life increased the incidence of diabetes in nonobese diabetic mice (Giulietti et al., 2004) whilst chronic administration of 1,25(OH)2D3 induced suppressor cells and reduced disease outcomes (Mathieu et al., 1994). With the murine model of multiple sclerosis based on autoimmune responses to myelin oligodendrocyte glycoprotein, chronic UVR, 25(OH)D3 and 1,25(OH)2D3 were all immunosuppressive, even if hypercalcaemia was induced by vitamin D (Becklund et al., 2010). It is notable that in these studies, UVR was immunoregulatory of the multiple sclerosis model without induction of significant vitamin D above baseline levels.

13.3.3 Similar pathways of immunoregulation by UVR and vitamin D

If mechanisms of immunoregulation by UVR and vitamin D are similar, UVR may signal in large part by production of vitamin D. Alternatively, UVR may signal immunosuppressive pathways independently of vitamin D. Many of the cells and mediators involved in UVR-
induced immunosuppression will be discussed below and will be directly compared with those characterised in immunomodulation by $1,25(\text{OH})_2\text{D}3$ binding to the vitamin D receptor.

13.3.4 **Dendritic cells**

Dendritic cells are very important sentinel cells that capture and process antigen and present it to T lymphocytes. Both UVR and topically applied vitamin D target dendritic cells of the skin. In local UVR-induced immunosuppression, it has been postulated that UVR-damaged dendritic cells with oxidative damage to their DNA, membrane lipids and protein, ‘limp’ to the draining lymph nodes and present antigen in an altered way such that T regulatory cells are induced (Schwarz and Schwarz, 2011). Similarly upon application of $1,25(\text{OH})_2\text{D}3$ to skin, the priming ability of draining lymph node dendritic cells is subverted (Gorman et al., 2010a). In this latter study, dendritic cells from the draining lymph nodes of $1,25(\text{OH})_2\text{D}3$-treated mice induced significantly smaller ear-swelling responses in a contact hypersensitivity reaction. Further, T regulatory CD4+CD25+ cells isolated from the auricular lymph nodes of mice that received ear injections of dendritic cells from donor mice topically treated with $1,25(\text{OH})_2\text{D}3$ more potently suppressed effector cell proliferation. Finally, dendritic cells from the draining lymph nodes of $1,25(\text{OH})_2\text{D}3$-treated mice expressed increased levels of indoleamine 2,3-dioxygenase mRNA, and suggested that pathway by which $1,25(\text{OH})_2\text{D}3$ may regulate dendritic cell function (Gorman et al., 2010a).

13.3.5 **Increased expression of the receptor activator of NFkB ligand**

RANKL expression in keratinocytes increases upon UVR exposure or after topical application of vitamin D (Ghoreishi et al., 2009) and has been implicated in expansion of antigen specific T regulatory cells. In response to a UVR-induced, vitamin D-dependent process, it is proposed that dendritic cells migrate to the draining nodes and augment the number and suppressive activity of lymph node resident regulatory T lymphocytes. Ghoreishi and colleagues were unable to detect this effect of UVR in mice without a functional vitamin D receptor (Ghoreishi et al., 2009) which strongly implicated a vitamin D-dependent pathway. In another model, UVR-induced PGE$_2$ also stimulated epidermal RANKL expression and in turn, increased numbers of both dendritic cells and T regulatory cells in the draining nodes (Soontrapa et al., 2011). It is plausible that UVR-induced vitamin D and PGE$_2$ have synergistic functions.

13.3.6 **Induction and activation of T regulatory cells**

It is generally proposed that UVR-damaged dendritic cells are tolerogenic and induce antigen-specific T regulatory cells (Schwarz and Schwarz, 2011). However, not all UVR-induced CD4+ T regulatory cells are antigen specific. CD4+CD25+ cell from the nodes of UV-irradiated skin can suppress immune responses to new antigens both *in vitro* and *in vivo* (Gorman et al., 2007a). As topically applied $1,25(\text{OH})_2\text{D}3$ led to the same outcome (Gorman et al., 2007b), this activation of naturally occurring T regulatory cells in the draining lymph nodes of UVR-treated skin is probably due to UVR-induced vitamin D3. It is of note that dendritic cells may not always be the
conduit by which 1,25(OH)$_2$D$_3$ increases T regulatory cell activity. It has recently been reported that 1,25(OH)$_2$D$_3$ can directly promote FOXP3 expression, an important functional aspect of T regulatory cells, by binding to vitamin D response elements in its conserved noncoding sequence region (Kang et al., 2012).

13.3.7 Activation of mast cells

Dermal mast cells are important to the mechanisms by which UVR causes a systemic immunosuppression. Murine strains that are very sensitive to the immunoregulatory properties of UVR (e.g. C57BL/6) have a high mast cell prevalence in skin whilst those that require larger doses of UVR to be immunosuppressive (e.g. BALB/c) have a relatively low mast cell prevalence in their skin (Hart et al., 1998). Further, mast cell-deficient mice are unable to be immunosuppressed by UVR unless mast cell progenitors are transplanted into skin that is subsequently UV irradiated (Hart et al., 1998). Most importantly, if the mast cell progenitors are derived from mice genetically deficient in the vitamin D receptor, they are unable to respond to UVR (Biggs et al., 2010). The ability of mast cells to produce immunoregulatory IL-10 in response to 1,25(OH)$_2$D$_3$ was also demonstrated (Biggs et al., 2010). Thus, UVR-induced mast cell activation may depend on UVR-induced vitamin D production.

13.3.8 Local skin effects and responses to vaccine antigens

The efficacy of topical vaccination may be reduced if applied to UV-irradiated skin. Of note, responses to immunisation were more reduced if the antigen was applied to irradiated skin 24 hours after UVR exposure, and was associated with UVR-induced activation of Cyp27b1, the enzyme responsible for hydroxylation of 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$ (Enioutina et al., 2002). Similarly, delivery of 1,25(OH)$_2$D$_3$ at the time of allergen sensitisation can reduce later responses to allergen challenge (Taher et al., 2008).

13.3.9 Summary

Negative associations of serum 25(OH)D$_3$ levels with disease prevalence, and intensity of disease, suggest UVR-induced vitamin D may be responsible in large part for the latitude gradients reported for chronic immune diseases such as multiple sclerosis, asthma and type 1 diabetes. Many common pathways exist by which UVR and vitamin D can regulate immune processes in human and murine skin. Thus, one could propose that vitamin D may provide an inexpensive mediator to control chronic immune diseases. However, as will be discussed in the next section, supplementation of patients with vitamin D has not yet endorsed this conclusion.
13.4 Ultraviolet-induced vitamin D as a minor contributor

13.4.1 Outcome of vitamin D supplementation

In discussion of the outcome of any trials of vitamin D supplementation, the dose of vitamin D used, and whether supplemented with calcium, must be considered. In November 2010, the Institute of Medicine (USA) recommended a daily vitamin D allowance of 600 IU/day until 71 years of age, and 800 IU/day when older (Ross et al., 2011). However, members of the Endocrine Societies have recommended vitamin D supplements of up to 4,000 IU/day (Holick et al., 2011). It has been calculated that assuming a linear relationship at least up to 2,000 IU/day, 600 IU/day and 2,000 IU/day should increase 25(OH) vitamin D3 levels by 10 nmol/l (4 ng/ml) and 32 nmol/l (12.8 ng/ml), respectively (Agency for Healthcare Research and Quality, 2007).

For treatment of patients with multiple sclerosis, there are many ongoing trials with vitamin D. The low incidence rate of the disease necessitates multi-centre participation and many years to complete. In one trial, some benefit was reported when vitamin D was given as an add-on therapy with interferon β-1b. However, generally the benefits of vitamin D therapy for multiple sclerosis have been modest (Smolders et al., 2011). For asthma, some encouraging but small supplementation studies have been reported for both newly diagnosed patients (Majak et al., 2011) and for patients already on steroids and other medications (Urashima et al., 2010; Xystrakis et al., 2006). Several investigators have supplemented pregnant women with vitamin D; however, reduced allergic asthma in the children has not been consistently measured (Wjst, 2012). For control of type 1 diabetes in children, variable responses have been measured. Similar limited success has been measured in adult patients with type 2 obesity-related diabetes. In post hoc analyses from eight trials among participants with normal glucose tolerance at baseline and in three small underpowered trials of patients with established type 2 diabetes, there was no effect of vitamin D supplementation on glycaemic outcomes (Mitri et al., 2011).

13.4.2 Further discordance between ultraviolet radiation and vitamin D

A recent study in patients with skin disease examined the effects of narrowband UVB light (311 nm) on human serum 25(OH)D3 and systemic immune function (Milliken et al., 2012). In the 24 patients studied, UV treatment increased T regulatory cell numbers and was associated with reduced proliferative and IL-10 responses to anti-CD3/CD28. UV also increased serum vitamin D levels but the increases were independent of the immune changes. The authors concluded that UVB light and serum vitamin D levels may affect particular immune functions independently (Milliken et al., 2012).

In the murine models of multiple sclerosis, both 25(OH)D3 and 1,25(OH)2D3 regulated disease expression (Becklund et al., 2010). However, the hypercalcaemia induced would be unacceptable in human trials. Further there was no suppression of the disease outcomes if the hypercalcaemia was controlled (Cantorna et al., 1999). In contrast, UVR was immunosuppressive without altering serum calcium levels. If vitamin D deficiency is part of the pathogenesis of multiple sclerosis, the
13. Immune modulation by UV: role of vitamin D

disease model should be worse in vitamin D-deficient mice. Contrary results, namely less disease in vitamin D-deficient animals, have been published (De Luca and Plum, 2011; Fernandes de Abreu et al., 2010).

In models of asthma, a conclusion that UVR-induced vitamin D is responsible for UVR-induced regulation of disease outcomes may be premature. Mice without functional vitamin D receptors, express no overt disease under conditions in which wild type mice develop experimental allergic airways disease (Wittke et al., 2007). Further, vitamin D may have a greater effect on lung development than on the immunological processes associated with disease expression upon allergen exposure (Zosky et al., 2011).

Topically applied 1,25(OH)\(_2\)D\(_3\) can decrease UVR-induced DNA damage, and repair the cyclobutane pyrimidine dimers. It is a little controversial however, as to whether this repair of DNA reverses UVR-induced systemic immunosuppression. A reduction of the extent of suppression of contact hypersensitivity responses by UVR by topically applied 1,25(OH)\(_2\)D\(_3\) was reported by one group (Mason et al., 2010) whereas in our laboratory, a consistent augmentation of the extent of suppression is measured if the shaved dorsal skin of mice is given both UVR and 1,25(OH)\(_2\)D\(_3\) (Gorman et al., 2010b). It is possible that 1,25(OH)\(_2\)D\(_3\) is reversing the effects of UVR by neutralising some of the effects of UV-induced nitric oxide (Mason et al., 2010). In human skin topical 1,25(OH)\(_2\)D\(_3\) does not reverse UVR-induced immunosuppression (Damian et al., 2010). Although we do not fully understand the biochemical processes, all the results (antagonism or synergy) suggest that the pathway by which UVR suppresses contact hypersensitivity responses is not largely dependent on formation of 1,25(OH)\(_2\)D\(_3\).

There is a further newly recognised pathway by which UVR may be immunoregulatory and may be important in UVR control of multiple sclerosis, allergic asthma and type 1 diabetes. In these studies, erythemal UVR stimulated the production of poorly immunogenic dendritic cells from bone marrow (Ng et al., 2010). This may reflect a more sustained response to UVR compared with other immune alterations as the bone marrow is the site of development of many haemopoietic lineages for subsequent migration out to peripheral organs and for the rejuvenation and replacement of immune cells during inflammation. This depends on the inflammatory nature of erythemal UVR on skin and would be independent of the actions of UVR-induced vitamin D. This process is dependent on the production of prostanoids, particularly PGE\(_2\) (Ng et al., 2010; Scott et al., 2012).

Further research is required but not all UV wavelengths that can signal UVR-induced immunosuppression can induce vitamin D production in skin. The universally recognised action spectrum for the UV-induced conversion of 7-dehydrocholesterol to pre-vitamin D3 in human skin indicates a maximum at about 297 nm with essentially no production above 315 nm. Although the validity of this action spectrum is being questioned (Norval et al., 2010), UVR can be immunosuppressive at larger wavelengths that would not involve vitamin D production (Halliday et al., 2012).
13.5 Conclusion

A strong case has been outlined above suggesting that vitamin D may be responsible for many of the immunosuppressive properties of UVR, particularly in response to UVB wavelengths. Many similar and over-lapping pathways are stimulated in UVB-treated skin and skin topically applied with 1,25(OH)₂D₃. Many cells implicated in UVR-induced immunosuppression have similar properties in response to UVR and 1,25(OH)₂D₃. Further, there are examples where UVR was not effective in mice engrafted with cells not expressing the vitamin D receptor. On the other hand, there are other immune pathways that are proposed important in UVR-induced immunosuppression that are not stimulated by 1,25(OH)₂D₃. Although there is no evidence that similar pathways do not occur in human skin, it is of note that many of the studies of the functional properties of UVR and 1,25(OH)₂D₃ have been completed in mice. It is also possible that some of the benefits attributed to UVR exposure and/or higher vitamin D levels in patients with multiple sclerosis, allergic asthma and type 1 diabetes may be due to the exercise that they receive outside and vitamin D levels are a proxy measure of this exercise in the sunshine (Maxwell and Wood, 2011).

It is reasonable to conclude that both complementary as well as over-lapping pathways are stimulated by vitamin D-dependent and vitamin D-independent pathways after UVR exposure. It is a matter of debate and ongoing research as to where the balance lies. It is notable that both sun exposure and vitamin D are independent risk factors for demyelination of the central nervous system (Lucas et al., 2011). Perhaps more importantly, the balance relates to the identification of the pathways that are responsible for UVR-regulation, and by proxy the latitude gradients, for suppressing the incidence and progression of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes. It is also important in formulating advice that can be given by physicians to their patients with these diseases. Should they recommend vitamin D supplementation or extended periods of moderate sun exposure?

To answer this question, large multi-centre randomised controlled trials are required of patients given vitamin D3. The dose of supplemental vitamin D3 will be important, as well as the stage and intensity of the disease during which the vitamin D3 supplements are given. Trials to date may not have been powered sufficiently for measures of change in these complex immune diseases. The next few years will be very important for determining an answer to the title of this chapter, namely the role of vitamin D in immune modulation by UV. If trials suggest that vitamin D can efficiently regulate chronic immune diseases, immune modulation by UVR indicated by latitude gradients and disease relief in summer may reflect, at least in part, UVR control by a vitamin D-dependent pathway.

Acknowledgements

Our research has been supported by the National Health and Medical Research Council of Australia, the Cancer Council Western Australia and the Asthma Foundation of Western
Australia. PHH is supported by a Principal Research Fellowship from the National Health and Medical Research Council of Australia. SG is supported by a Fellowship from the Brightspark Foundation (Australia).

References


13. Immune modulation by UV: role of vitamin D

Majak, P., Olszowiec-Clebna, M., Smejda, K. and Stelmach, I., 2011. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. Journal of Allergy and Clinical Immunology 127, 1294-1296.


Key facts

- Periodontitis is an inflammatory disease of the supporting tissues of the teeth, caused by microorganisms, resulting in progressive destruction of the periodontal ligament and bone loss.
- Data on the prevalence of periodontal disease show that 5% to 20% of any population suffers from generalized severe periodontitis.
- Periodontitis requires treatment and may be costly and uncomfortable for the patient.
- Vitamin D supplementation reduced tooth loss and alveolar ridge resorption.
- There are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis.

Summary points

- The pathogenesis of periodontal disease involves activation of host-defense cells by bacterial release of inflammatory mediators, which results in the destruction of connective tissue and alveolar bone.
- Vitamin D increases calcium absorption and stimulates osteoblasts to enable normal bone growth and preservation.
- Vitamin D may be also beneficial for the treatment of periodontal disease, because of its anti-inflammatory effect through inhibition of cytokine production and stimulation of immune cells to secrete peptides with potent antibiotic activity.
- Vitamin D induces the formation of cathelicidin and other defensins that combat bacterial infection. Polymorphisms of the vitamin D receptor gene have been associated with periodontitis.
- The research to date suggests the beneficial effects of vitamin D on periodontal disease. Vitamin D supplementation regimen may positively affect periodontal disease, above and beyond standard periodontal care; however, further studies are needed.
14. Vitamin D and periodontal disease

N. Garcia1,2, D. Miley1,2 and D.A. Dixon2

1Saint Louis University Center for Advanced Dental Education, 3320 Rutger Street, St. Louis, MO 63104, USA; 2Department of Applied Dental Medicine, Southern Illinois University School of Dental Medicine, 2800 College Avenue, Alton, IL 62002, USA; garciann@slu.edu

Abstract

Periodontal disease is an inflammatory condition, characterized by alveolar bone loss induced by the host immune response to bacterial attack. Because of vitamin D is associated with some inflammatory diseases and plays a significant role in bone homeostasis and immunity, vitamin D deficiency could negatively affect the periodontium and increase tooth loss. In spite of the limitations of previous studies, there are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis. Vitamin D promotes calcium absorption and protects bone strength; the active form of vitamin D, 1a,25-dihydroxyvitamin, function as an immunomodulator because of its anti-inflammatory and antimicrobial properties that can inhibit the inflammatory response through modulation of cytokine production by immune cells and stimulation of monocytes and macrophages to secrete peptides with potent antibacterial action; another potential function of vitamin D in periodontal health is supported by the finding that polymorphisms of the vitamin D receptor gene are associated with periodontitis, clinical attachment loss, bone loss, and/or tooth loss. These multiple actions of vitamin D are potentially interesting for the treatment of patients with periodontitis. If increased vitamin D intake can reduce alveolar bone loss, oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy for the prevention and treatment of periodontal disease. The research to date suggests the beneficial effects of vitamin D on periodontal disease; however, further studies are needed.

Keywords: periodontitis prevention and treatment, alveolar bone, tooth loss
Periodontitis is an inflammatory disease of the supporting tissues of the teeth, caused by specific microorganisms, where bacterial biofilms adhere to the gingival tissue leading to a host response and resulting in progressive destruction of the periodontal ligament and alveolar bone (Newman et al., 2011). According to the Research, Science and Therapy committee of the American Academy of Periodontology (2005), data on the prevalence of periodontal disease show that 5% to 20% of any population suffers from generalized severe periodontitis. Mild or moderate forms of the disease affect the majority of all adults.

Tooth loss is the result of lack of support from bony and connective tissues, and periodontitis is the primary cause of this condition. Even though there has been a decrease in edentulism (total tooth loss) during the last several years, almost one out of every four individuals over 65 have lost all of their teeth. This results in negative esthetic and functional consequences that affect speech and chewing capacity (Bailey et al., 2005). Edentulism reduces the quality of life, affects nutrition, and requires costly treatments. Specific factors responsible for alveolar bone loss and continued resorption of the residual alveolar ridge (subsequent to tooth loss) are not well understood, nor have treatments to prevent alveolar bone loss been universally successful.

Any form of periodontitis requires treatment and may be costly and uncomfortable for the patient. In many cases tooth loss is inevitable, requiring far more complex and expensive options. Reducing periodontal disease-induced oral bone loss would lessen the need for periodontal surgical treatments, reduce tooth loss, and improve the outcome of interventions, such as tooth implants, when they cannot be avoided.

### 14.2 Periodontal disease pathogenesis and the relationship with vitamin D

The pathogenesis of periodontal disease involves a bacteria-host interaction, with microorganisms and their byproducts inducing tissue destruction indirectly by activating host defense cells including lymphocytes, macrophages and PMNs, which in turn produce and release mediators that result in connective tissue breakdown and alveolar bone resorption (Page and Kornman, 1997).
As part of this process, components of microbial plaque have the capacity to induce the initial infiltrate of inflammatory cells including lymphocytes, macrophages, and PMNs. Bacterial components, especially lipopolysaccharide, have the capacity to activate macrophages to synthesize and secrete different molecules like cytokines, cell proteins that are the cause of lymphocytic infiltration, that result in bone resorption and dissolution of the extracellular matrix. Cytokines regulate the body's inflammatory response by transmitting signals between cells. The contribution of interleukin-1, interleukin-6 and tumor necrosis factor-α to periodontal tissue destruction, is associated with the resorption of alveolar bone because of their potent osteoclastogenic features (Graves and Cochran, 2003; Offenbacher, 1996). Interleukin-1 also stimulates the release of metalloproteinases which degrade the extracellular matrix and prostaglandin E2 which is responsible for vasodilatation, edema, and bone loss. Antigen-stimulated lymphocytes (B and T cells) are also important in this process. These locally produced cascade of events finally lead to connective tissue breakdown and bone loss via activation of osteoclast-mediated bone resorption (Cochran, 2008).

It has been suggested that vitamin D and proinflammatory cytokines as well as periodontal disease are associated with diabetes mellitus and cardiovascular diseases (Grossi and Genko, 1998; Page, 1998; Zitterman, 2003). These conditions are also linked with obesity and because vitamin D and 25(OH)D are stored in adipose tissue, obese subjects have low serum levels of 25(OH)D. Moreover, specific vitamin D-receptor genotypes have been shown to be associated with localized aggressive periodontal disease, oral bone loss, clinical attachment loss, and tooth loss (Hildebolt, 2005).

### 14.3 The effect of vitamin D on oral health

Many studies have investigated the effect of vitamin D on various aspects of oral health. Krall et al. (2001) examined tooth loss in elderly patients. During the first three years of their study, 27% of the placebo group (no supplementation) lost one or more teeth. Only 13% of the subjects in the supplement group (500 mg calcium and 700 IU cholecalciferol) lost one or more teeth. Since the supplement group also received calcium, it is not possible to determine the sole effect of vitamin D on these subjects.

Dietrich et al. (2004) evaluated whether serum 25-hydroxyvitamin D concentrations were associated with periodontal disease in the third National Health and Nutrition Examination Survey. They analyzed data on attachment loss, which is the clinical diagnostic criteria for periodontal disease. They found that 25(OH)D concentrations were significantly and inversely associated with attachment loss in men and women ages 50 years and older.

Calcium and vitamin D supplementation may have an effect of alveolar ridge resorption. 60 subjects who received immediate dentures were given either placebos or 750 mg calcium and 375 IU vitamin D per day. Bone loss was measured with panoramic radiographs over a one year period. Subjects that completed the study and received the supplementation had 36% less
bone loss than subjects taking the placebo (Wical and Brussee, 1979). Calcium and vitamin D supplementation has also been shown to increase mandibular bone mass. As part of a study on estrogen/hormone replacement therapy, Hildebolt et al. (2004) found that 17 of 19 women who took calcium and vitamin D supplementation for 3 years had increases in mandibular bone mass.

A recent study (Jabbar et al., 2011) examined women with osteoporosis. Their subjects with osteoporosis had lower 25(OH)D levels. Women with osteoporosis also exhibited a greater incidence of periodontal disease. They concluded that 25(OH)D levels were significantly lower in subjects with either past or active periodontal disease. Periodontal disease was best predicted by vitamin D status along with receptor activator of nuclear factor kappa-B ligand, serum C-terminal telopeptide and weight. Vitamin D insufficiency has also been associated with maternal periodontal disease in pregnancy. Boggess et al. (2011) examined the relationship between maternal vitamin D status and periodontal disease. They concluded that the adjusted odds ratio for moderate to severe periodontal disease among women with vitamin D insufficiency was 2.1.

Not all studies are in agreement. Liu et al. (2009) examined patients with chronic periodontitis and aggressive periodontitis. They conducted full periodontal examinations and determined plasma calcifediol levels. These levels were significantly higher in the patients with aggressive periodontitis as compared to healthy controls. The plasma calcifediol levels were not significantly different between patient with chronic periodontitis and healthy controls. The levels of calcifediol were also significantly correlated with the bleeding index in the aggressive periodontitis patients. It is important to note that the authors indicate that nearly 60% of their subjects had lower levels of plasma calcifediol when considering 27 nmol/l as the lower limit of the normal hematic range, therefore, most of these subjects were vitamin D deficient to begin with. This may explain the contradictory results from this study.

14.4 The potential benefit of vitamin D on periodontal disease

Only a few studies have evaluated the function of vitamin D on periodontal disease. Some information has been published using the Third National Health and Nutrition Examination Survey data to analyze the association between serum 25(OH)D levels and attachment loss and gingival inflammation (Dietrich et al., 2004, 2005). These epidemiological studies found more attachment loss and gingival inflammation in subjects with lower the levels of 25(OH)D. These associations were independent of factors such as smoking and diabetes. While useful, these cross-sectional, survey type studies do not provide any significant insights on possible physiologic mechanisms of the potential protective effect of vitamin D on periodontal disease, because no analyses of local bacterial and cytokine production were performed.
14. Vitamin D and periodontal disease

14.4.1 Effects of vitamin D supplementation on periodontal disease

The effects of vitamin D supplementation on periodontal disease have not been totally elucidated. Some early reports suggested that vitamin D supplementation reduced tooth loss and alveolar ridge resorption (Baxter, 1984; Habets et al., 1988a,b; Krall et al., 2001; Renner et al., 1984; Wical and Brussee, 1979; Wical and Swoope, 1974), but most of these studies did not directly assess periodontal disease status.

The available experience on dietary supplementation is not very extensive but hopeful. A two-year program of dietary supplementation with 400 IU per day of vitamin D and increased calcium intake to 1000 mg per day resulted in either maintenance or an increase of mandibular bone mass in 83% of a study population (Kribbs, 1992). Unfortunately, data on periodontal disease status was not given and 20% of these patients were edentulous. The results of this study are comparable to those that were reported by Civitelli et al. (2002), in three-year hormone replacement study, where women who received 1000 mg of calcium and 400 IU of vitamin D per day showed significant increases in both alveolar crest height and alveolar bone mass. Women in this study were, however, periodontally healthy and there was no control group in which women received only a placebo.

A recent survey to determine the frequency of vitamin D and calcium oral supplementation use by patients in periodontal maintenance programs at two dental schools in Missouri and Illinois, found that only 7% had vitamin D and calcium levels that met the Food and Nutrition Board’s recommended intakes (Dixon et al., 2009). These results suggest that even though the use of vitamin D and calcium supplementation has been widely promoted for years, the knowledge of the benefits of supplementation needs to be better disseminated and research needs to continue in order to determine optimal levels of supplementation.

A cross-sectional pilot study which followed the survey analysis, evaluated the differences in alveolar crest heights between subjects who were taking calcium and vitamin D supplementation and those who were taking neither vitamin D nor calcium supplements and had dietary intakes of calcium <1000 mg/day and of vitamin D <400 IU/day (non-takers). All eight clinical parameters of periodontal health were better at baseline in subjects who took supplementation relative to non-takers, (Miley et al., 2009). A second goal of this study was to determine the changes in periodontal disease measurements for a one year period. The results supported the concept that a regular vitamin D supplementation regimen may positively affect periodontal disease, above and beyond standard periodontal care, despite the small number of subjects and the fact that the participants’ periodontal health was generally good and the intakes of vitamin D were relatively low (which may have reduced the potential benefits from vitamin D and calcium supplementation) (Garcia et al., 2011).

A recent case-control study, examined the relationship between vitamin D status and periodontal disease among pregnant women, measuring serum 25(OH)D levels. Despite some limitations, this study suggested that vitamin D supplementation could improve maternal oral health (Boggess...
et al., 2011). According to the authors, the relationship between maternal vitamin D status, periodontal disease, and adverse pregnancy outcomes requires further studies before definitive conclusions could be stated. However, the data suggested that improvement of vitamin D status is a potential intervention to improve oral health among pregnant women.

In summary, all of the currently available studies on vitamin D supplementation and oral health have some methodological weaknesses. Since both vitamin D and calcium were used in most of the previous studies, it is difficult to distinguish between the effects of the two supplements. For the effects of vitamin D on periodontitis, there has never been a clinical trial in which randomization and masking were carefully controlled, the periodontal disease and serum 25(OH)D status of patients were known, and periodontal disease measures were the primary outcomes. Therefore, there is sufficient observational and preliminary evidence supporting the rationale for a prospective, controlled evaluation of the effects of oral supplementation on periodontal disease.

14.4.2 Mechanisms to explain the role of vitamin D in reducing the risk of periodontitis

In spite of the limitations of previous studies, there are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis. First, it is well known that vitamin D increases calcium absorption and protects bone strength. Second, vitamin D has anti-inflammatory properties that can inhibit the inflammatory response associated with periodontal disease. Third, vitamin D induces the formation of cathelicidin and other defensins that combat bacterial infection. Fourth, polymorphisms of the VDR gene have been associated with periodontitis, alveolar bone loss, and clinical attachment loss.

Vitamin D’s anti-inflammatory properties

Besides its role in bone and calcium homeostasis, nonskeletal (autocrine/paracrine) actions of vitamin D have been described. The biologically-active hormonal form of vitamin D [1,25-dihydroxyvitamin D] is a potent immunomodulator (De luca, 2004, Penna et al., 2005). In this form, 1,25(OH)₂D inhibits T cell proliferation, alters cytokine production by Th1 lymphocytes, and is a powerful inhibitor of B cell-mediated antibody production and dendritic cell differentiation, while it stimulates macrophage phagocytosis of bacteria (Arnson et al., 2007; Boonstra et al., 2001; Mattner et al., 2000; Rigby et al., 1987; Van Etten and Mathieu, 2005). It can also modulate monocyte and macrophage differentiation and the release of inflammatory cytokines and chemokines (Abu-Amer and Bar-Shavit, 1993, 1994; Arnson et al., 2007; Cohen et al., 1986; Helming et al., 2005; Hewison et al., 2004). Therefore, it is reasonable to expect that local production of 1,25(OH)₂D can inhibit the inflammatory response associated with periodontal disease and could result in a lowering of blood levels of C-reactive protein, which is the most widely used biomarker of inflammation (Paraskevas et al., 2008, Ridker and Silvertown, 2008). Dietrich et al. (2005) provide some evidence of the anti-inflammatory properties of vitamin D. They examined 77,503 gingival units (teeth) in 6,700 non-smoking subjects. Compared with sites in subjects in the lowest 25(OH)D quintile, sites in subjects in the highest 25(OH)D quintile were
20% less likely to bleed upon gingival probing. This is significant due to the fact that bleeding upon probing is the best measure of clinical gingival inflammation.

**Stimulation of defensins and cathelicidins**

One of the mechanisms that is proposed to explain the role of vitamin D in reducing the risk of periodontal disease, is the stimulation of α- and β-defensins and cathelicidins, proteins with broad-spectrum antimicrobial activity (Cannell et al., 2008). Beyond vitamin D's anti-inflammatory effect, monocytes and macrophages exposed to lipopolysaccharide or *Mycobacterium tuberculosis* up-regulate the VDR gene and the 25-hydroxyvitamin D-1α-hydroxylase gene (which converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D). Increased production of 1,25-dihydroxyvitamin D results in the synthesis of cathelicidin, a peptide capable of destroying *M. tuberculosis* as well as other infectious agents (Liu et al., 2006). Vitamin D is thus capable of stimulating the production of a potent natural antibiotic. When serum levels of 25-hydroxyvitamin D fall below 20 ng per milliliter (50 nmol per liter), the monocyte or macrophage is prevented from initiating the innate immune response (Liu et al., 2006). This concept may explain why groups who are vitamin D-deficient tend to have a more aggressive form of tuberculosis and are more prone to contracting the disease. This, in turn, suggests that differences in serum 25-hydroxyvitamin D levels may contribute to the susceptibility to microbial infections (Liu et al., 2006).

**Vitamin D receptors**

The importance of vitamin D's role in periodontal disease, is supported as well by findings that suggest genetic polymorphisms of the VDR gene are associated with periodontitis, alveolar bone loss, clinical attachment loss, and/or tooth loss (Inagaki et al., 2003; Yoshie et al., 2007). VDRs are found in nearly every cell of the body. Recently, 1,25(OH)\(_2\)D\(_3\) has been shown to have anticancer, immune modulatory and cardiovascular regulator effects by activating the VDR, a transcription factor of the nuclear receptor superfamily. The 1,25(OH)\(_2\)D\(_3\)-VDR system plays a role in maintaining oral health through its effects on bone and mineral metabolism and innate immunity, and its dysfunction have been reported to be associated with periodontal disease (Amano et al., 2009). Deng et al. (2011) conducted a meta-analysis to investigate the association of VDR gene polymorphisms with susceptibility to aggressive and chronic periodontitis. Their analysis of 15 studies included 1,338 cases and 1,302 controls and confirmed that the VDR gene is a candidate gene for periodontitis.

**14.5 The potential impact of vitamin D on periodontal disease treatment**

The standard of care for periodontal disease treatment is mostly local (meticulous oral hygiene and scaling and root planing). Although scrupulous oral hygiene has long been accepted as a successful method for preventing and treating periodontal disease, patients are often not able to maintain these practices. Scaling and root planing and surgical options (root debridement,
Resective and regenerative procedures are well-accepted treatments for periodontal disease. However, many patients do not have access to, cannot afford, or cannot tolerate the discomfort associated with such therapies. Systemic/antimicrobial and pharmacologic treatments overcome some of these restrictions, but there are limitations associated with most of these options. Oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy for the prevention and treatment of periodontal disease.

If increased vitamin D and calcium intake can demonstrate reduced alveolar bone loss, oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy, resulting in a significant impact on the quality of life by leading to: (1) a reduction in surgical treatments aimed at repairing or minimizing alveolar bone damage caused by periodontal disease; (2) a decrease in tooth loss attributable to alveolar bone loss; (3) a decline in the placement of implants or prosthetic devices (bridges and partial dentures) to replace lost teeth; and (4) a reduction in the number of denture remakes and relines required because of alveolar bone resorption.

Vitamin D supplementation could also impact periodontal disease treatment outcomes. Bashutski et al. (2011) evaluated the vitamin D status and the results from periodontal surgery. They found improved clinical attachment level gain and probing depth reduction for patients that had sufficient vitamin D levels prior to surgery. Patients that had insufficient vitamin D levels prior to surgical treatment lost clinical attachment after treatment. This patient population all exhibited severe periodontal disease. Vitamin D supplementation could prove to be a valuable adjunctive treatment for those requiring extensive surgical procedures.

**14.6 Conclusions**

Vitamin D is antibacterial, anti-inflammatory, bone protective, safe, inexpensive, available over the counter and easy to use. The research to date suggests the beneficial effects of vitamin D on periodontal disease; however, further studies are needed. There is no randomized controlled trial on vitamin D’s effect on alveolar bone loss in periodontal disease. Vitamin D supplementation in deficient patients could potentially result in less non-surgical and surgical periodontal treatment, less tooth loss, fewer denture remakes and relines and fewer dental implant placements. This dietary supplement could have a major impact on periodontal disease prevention and treatment.

**References**


14. Vitamin D and periodontal disease

Key facts

• The prevalence of vitamin D insufficiency in cystic fibrosis (CF) is very high but only recently the topic has received increased attention.
• Bisphosphonates improve bone mineral density (BMD) in CF patients. Due to a lack of adequate studies it remains unclear whether vitamin D supplementation has a positive effect on BMD.
• Despite the lack of evidence, the current European and US guidelines for vitamin D supplementation recommend relatively high doses of colecalciferol and aim for s25(OH)D>50 nmol/l (Europe) and s25(OH)D>75 nmol/l (US) in all CF patients.
• Ultraviolet lamps have high theoretical potential in successfully improving vitamin D status in CF patients, but this alternative and its safety have been addressed by a limited number of studies.
• Because of its immunomodulatory and glucose-lowering effects, vitamin D may have a broad potential in CF patients, who often suffer from chronic inflammation and CF-related diabetes. Caution should however be exercised as dampened inflammation may not only be of benefit.

Summary points

• CF is a common hereditary disease; CF patients suffer mainly from recurrent lung infections and defective food nutrient uptake.
• Many patients with CF are deficient in vitamin D, despite daily vitamin supplementation.
• There are several known reasons behind the tendency for low vitamin D levels in CF.
• Vitamin D insufficiency is associated with bone disease in CF, but it has not been clearly proven that supplementation improves bone health in CF.
• The US guidelines for vitamin D supplementation in CF published in 2005 were shown to be ineffective at improving vitamin D status of the patients. The latest European (2011) and US (2012) guidelines are mainly consensus based due to lack of evidence and low number of relevant studies available.
• Vitamin D levels in CF patients exhibit strong seasonality pattern, and use of ultraviolet lamps might be an alternative to vitamin D supplementation in patients who are willing to stay adherent with the therapy.
• Test tube experiments and studies describing CF populations have recently created hypotheses that vitamin D may have beneficial effect on the CF patients’ immunity, lung function and blood glucose regulation.
• There is need for well-performed vitamin D supplementation trials to show whether the hypotheses are true and whether vitamin D is beneficial or harmful to CF patients.
• There are a few registered current/future trials that might answer some of these questions, but more studies are clearly needed.
15. Vitamin D and cystic fibrosis

T. Pincikova¹ and M. Flodström-Tullberg²

¹Stockholm CF Center, Karolinska Institutet, Karolinska University Hospital Huddinge, 14186 Stockholm, Sweden; ²Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital Huddinge, 14186 Stockholm, Sweden; terezia.pincikova@ki.se

Abstract

Cystic fibrosis (CF) patients suffer from malnutrition and lung infections, both acute and chronic. The majority are also vitamin D insufficient despite vitamin D supplementation. With better medical care the life expectancy for CF patients has increased and so has the prevalence of long-term complications, such as bone disease and CF-related diabetes. Vitamin D insufficiency is associated with bone disease in CF, but evidence that vitamin D supplementation improves bone mineral density in CF patients is missing. The pathophysiology of vitamin D insufficiency in CF is multifactorial. Serum 25-hydroxyvitamin D (s25(OH)D) levels in CF patients fluctuate throughout the year, depending on the amount of UV exposure, with lowest values at the end of the winter. The current European and US guidelines for vitamin D supplementation for CF patients prefer colecalciferol over ergocalciferol, consider age of the patients, endorse individualisation of the supplementation regimen and are mostly consensus based due to lack of CF-specific evidence. In all age groups, s25(OH)D >50 nmol/l (Europe) or s25(OH)D >75 nmol/l (US) should be achieved and maintained. The current literature is missing CF-specific hard evidence on many issues, such as the optimal s25(OH)D level, the relative benefit of colecalciferol vs. ergocalciferol and daily vs. weekly supplementation regimen. The use of UV lamps is a promising alternative, but has so far been ineffective in raising s25(OH)D level in CF patients due to poor adherence with the treatment. In vitro experiments and human descriptive studies indicate that vitamin D might have beneficial immunomodulatory and glucose-lowering effects in CF patients, but a careful approach is to be recommended given the absence of well-designed intervention trials proving causality, safety and clinical relevance of the amplitude of the potential effects. There are a few registered trials currently running or planned to start soon, which address some of these questions.

Keywords: bone mineral density, guidelines, immunomodulation, CF-related diabetes, registered trials
15.1 Cystic fibrosis

CF is the most prevalent life-shortening disease with autosomal-recessive heredity pattern in Caucasians (FitzSimmons, 1993). More than 1,500 disease-causing mutations have been described so far. The most common mutation is F508del. The cfr gene encodes a chloride channel located in the membranes of epithelial cells. Mutations in the cfr lead to abnormally thick mucus and dysfunction of epithelial organs. Thus, symptoms of the disease mainly come from the pulmonary and gastrointestinal tracts, including the liver and pancreas.

The major underlying reason for morbidity and premature death is the progressive pulmonary disease. CF patients have an increased overall infection susceptibility and particularly enhanced susceptibility to contracting chronic lung Pseudomonas aeruginosa infection/colonisation. Moreover, some CF patients develop ABPA, i.e. lung colonisation with Aspergillus species and allergic reactions directed against the fungi. The chronic infection/colonisation and recurrent exacerbations are associated with chronic inflammation and a decline in lung function. The majority of the CF patients suffer from pancreatic insufficiency, resulting in malabsorption and insufficiency of fat soluble vitamins. Therefore, pancreatic enzyme preparations have to be taken with every meal by patients who are pancreatic insufficient, and fat soluble vitamin supplements are recommended for all CF patients (Peters and Rolles, 1993).

The therapy available today is symptomatic and requires a multifaceted approach given that multiple organs are affected. The main focus of the therapy is strict infection control and tight nutritional follow-up. Advances in the CF disease management have been increasing the life-span of the CF patients worldwide, and it has been calculated that 95% of the CF patients born in Scandinavia today will live longer than 25 years (Hjelte, 2008). However, this has led to increased prevalence of long-term complications of the disease, such as CFRD and CF-related bone disease,
and the focus of the therapy has slightly shifted towards these complications. Sadly enough, the potential causative therapy for the majority of the CF patients still mainly remains at the research level, and the only available treatment of the end-stage pulmonary disease is lung transplantation.

## 15.2 Cystic fibrosis-related low bone mineral density and vitamin D

With improved medical care and increased life expectancy of patients with CF, bone disease has become a common complication (Aris et al., 2005). The prevalence of low BMD and its complications in CF is high. Observational studies show pooled prevalence of osteoporosis and osteopenia in adults with CF being as high as 23.5% and 38.0%, respectively (Paccou et al., 2010). Even adolescents with CF were shown to have decreased total body BMD and cortical wrist BMD (Buntain et al., 2004), and some studies report low BMD also in young children with CF (Sermet-Gaudelus et al., 2007).

The underlying reasons for poor bone health in CF include pancreatic insufficiency leading to malabsorption and low BMI, delayed puberty, glucocorticoid use (Aris et al., 1996), immunosuppression after lung transplantation (Spira et al., 2000), CFTR dysfunction (Le Heron et al., 2010), the systemic response to infection (Shead et al., 2010), vitamin K insufficiency (Nicolaidou et al., 2006), compromised calcium balance (Schulze et al., 2003), CFRD (Levin et al., 1976) and reduced daily physical activity (Tejero-Garcia et al., 2011). Vitamin D insufficiency has been associated with poor bone health (Stead et al., 1988, Shane et al., 1996). PTH correlates with 25(OH)D (West et al., 2011) and it responds to vitamin D administration in CF (Brown et al., 2003; Khazai et al., 2009).

The complications of low BMD in CF include vertebral and non-vertebral fractures with a high prevalence (Paccou et al., 2010), and severe kyphosis (Aris et al., 1998), which can secondarily lead to severe morbidity with diminished lung function (O’Reilly et al., 2009; Legroux-Gerot et al., 2012) and deformity (Elkin et al., 2001). In the long run, this may accelerate the progression of CF disease; therefore, the maintenance of bone health in CF patients is crucial.

Current prevention and treatment recommendations for CF-related low BMD are primarily based on targeting the known risk factors for poor bone health in CF (Aris et al., 2005; Sermet-Gaudelus et al., 2011; UK CF Trust, 2007). With vitamin D insufficiency being one of the observed risk factors associated with poor bone health in CF (Shane et al., 1996; Stead et al., 1988) the UK CF Trust, the European CF guidelines and the US Cystic Fibrosis Foundation Consensus Panel recommend routine vitamin D supplementation for CF patients (Aris et al., 2005; Sermet-Gaudelus et al., 2011; Tangpricha et al., 2012; UK CF Trust, 2007). Secondarily, in patients with very low BMD (T/Z score ≤-2.0), with history of fragility fractures or after lung transplantation, bisphosphonates should be considered, but owing to several potential safety concerns and common adverse events their broader use cannot be recommended (Aris et al., 2005; Sermet-Gaudelus et al., 2011; UK CF Trust, 2007).
Interestingly, while oral and intravenous bisphosphonates have been proven to increase BMD in CF patients (Conwell and Chang, 2009), no relevant studies have managed to prove that vitamin D supplementation improves BMD (Ferguson and Chang, 2009). Whether the lack of hard evidence for vitamin D supplementation improving BMD is the reason why vitamin D supplementation in CF received little attention in the past remains unclear.

15.3 Vitamin D insufficiency in cystic fibrosis

Due to lack of attention to vitamin D in CF in the past, patients used to be given multi-vitamin supplements in uniform dose and s25(OH)D level would not be monitored (Hahn et al., 1979). As a consequence, vitamin D insufficiency has been a common phenomenon among both the paediatric and adult CF patients over the last few decades (Boyle et al., 2005; Donovan et al., 1998; Hanly et al., 1985; Henderson and Lester, 1997; Khazai et al., 2009; Pincikova et al., 2011a; Shane et al., 1996; Solomons et al., 1981; Stead et al., 1988; Stephenson et al., 2007; Wolfenden et al., 2008); despite routine vitamin supplementation, vitamin D and/or its metabolites studied in various CF patient populations were lower than in control non-CF subjects (Congden et al., 1981; Hahn et al., 1979; Reiter et al., 1985; Rovner et al., 2007). Interestingly, one third of CF infants detected by newborn screening were reported to have low s25(OH)D level at initial evaluation (Sokol et al., 1989), implying that vitamin D insufficiency occurs already early in life in CF patients. Luckily, the data on vitamin D concentrations suggest a trend toward higher s25(OH)D levels over recent years, which may well reflect somewhat improved attention to vitamin D by CF centres (Hall et al., 2010). Indeed, the frequency of s25(OH)D measurements in patients followed at the Stockholm CF Centre increased steadily during years 2007-2009, which complies with the suggested hypothesis that the attention to vitamin D in the field of CF is continuously improving (Pincikova and Hjelte, 2010).

The underlying reasons for the high prevalence of vitamin D insufficiency in CF include pancreatic exocrine insufficiency, little body fat, low serum vitamin D binding protein (DBP) concentrations (Coppenhaver et al., 1981), impaired absorption of vitamin D2 despite pancreatic enzyme supplementation and hypothesised accelerated excretion of vitamin D before hepatic hydroxylation of the vitamin (Lark et al., 2001), liver disease causing impaired hepatic hydroxylation (Wills and Savory, 1984), low sunlight exposure (Thompson, 1987), and low adherence with vitamin D supplement prescriptions (Hollander et al., 2010). Several studies have highlighted sun exposure as an important determinant of s25(OH)D levels in CF patients. Moreover, s25(OH)D values were shown to exhibit a clear season-dependent pattern (Pincikova and Hjelte, 2010; Robberecht et al., 2011; Thompson, 1987). Robberecht et al. (2011) showed that s25(OH)D concentrations are significantly higher during ‘Months with high UVB exposure’ (May–October) than during ‘Months with low UVB exposure’ (November–April). This confirmed the results of our retrospective study of Stockholm CF patients (Pincikova and Hjelte, 2010). In line with this, the vitamin D supplementation recommendations published very recently by the CFF recommend the yearly screening for vitamin D status to be done preferably at the end of winter (Tangpricha et al., 2012).
15.4 Current vitamin D supplementation guidelines in cystic fibrosis and future challenges

Because vitamin D in CF has only recently received attention, the current recommendations for vitamin D supplementation for CF-related bone disease are ranked as ‘C’ (UK CF Trust, 2007), due to absence of directly applicable studies of good quality (Petrie et al., 1995). The Cochrane Cystic Fibrosis and Genetic Disorders Group assessed the content of the Intervention Review ‘Vitamin D supplementation for cystic fibrosis’ as up-to-date in August 2011. Here, the authors concluded that there is no evidence of benefit or harm for vitamin D supplementation in CF and suggested to consider adherence to relevant CF guidelines before further evidence appears (Ferguson and Chang, 2009).

The current UK CF Trust guidelines are based on the Report of the UK CF Trust Bone Mineralisation Working Group published in February 2007. The report recommends daily doses of ergocalciferol or colecalciferol of 400 IU for infants, 400-800 IU for 1-12 years old patients, 800-2,000 IU for patients older than 12 years (UK CF Trust, 2007). The US CFF consensus panel recommends ergocalciferol due to low cost, higher safety and wide variety of dosing alternatives, as compared with colecalciferol. The recommended routine dosing here is 400 IU for infants and 800 IU for patients older than 1 year of age, and if this supplementation fails to achieve or maintain optimal s25(OH)D levels, a high-dose ergocalciferol repletion regimen should be initiated, where the unresponsive individuals are administered 50,000 IU ergocalciferol weekly for 8 weeks. The patients who still fail to achieve optimal s25(OH)D levels should be given 50,000 IU ergocalciferol biweekly for 8 weeks (Aris et al., 2005). Both the UK and the US guidelines endorse individualisation of the vitamin D supplementation, aiming for s25(OH)D levels 75-150 nmol/l. Here, the maximum recommended dosing is 50,000 IU biweekly for patients ≥5 years in the US guidelines (Aris et al., 2005), whereas the UK guidelines do not specify maximum dosing (UK CF Trust, 2007). Moreover, both of these guidelines underline that some patients may require aggressive supplementation with higher dosing, and they recommend even more polar vitamin D analogues (calcitriol, alfacalcidol or calcifediol) or phototherapy in cases where the patients do not respond adequately to more aggressive supplementation with ergocalciferol or colecalciferol. Furthermore, they highlight the need for additional research to optimise dosing and choice of the proper vitamin D analogue, and to retest the response to intramuscular ergocalciferol, which might be another potential way of vitamin D supplementation in nonresponsive patients despite that the single study done in this area has failed to prove it (Ontjes et al., 2000).

Comparing these guidelines with the vitamin D intake recommendations for the healthy, non-CF, population, they endorse somewhat higher vitamin D dosing for routine supplementation (IOM, 2010). In addition, they are very flexible, recommending an individualised approach to dosing and to the choice of vitamin D analogue. Thus, one would expect these guidelines to be sufficient and well-working. Therefore, it is striking that the majority of CF patients worldwide continue to have insufficient s25(OH)D levels (Boyle et al., 2005; Khazai et al., 2009; Pincikova et al., 2011a; Stephenson et al., 2007; Wolfenden et al., 2008). This might be partly caused by low patient adherence with vitamin prescriptions (Hollander et al., 2010), but a number of studies have found
the currently recommended vitamin D supplement doses in CF ineffective. Already in year 1985 it was shown that supplementation with 800 IU per day in adolescents and adults with CF was unsuccessful in raising $s_{25}(OH)D$ levels (Hanly et al., 1985). Kelly et al. (2002) demonstrated that 95% of CF patients needed to be administered 1,800 IU ergocalciferol daily in order to achieve the target $s_{25}(OH)D$ level 50 nmol/l, implying that the dose of 800 IU is inadequate. The recommended high-dose aggressive repletion regimen with oral ergocalciferol endorsed in the US recommendations for individuals unresponsive to the routine dosing has been tested in a study by Boyle et al. (2005). They reported that treatment with 50,000 IU ergocalciferol weekly for 8 weeks resulted in 8% patients achieving optimal $s_{25}(OH)D$ levels, and that none of those patients who went on to receive 50,000 IU ergocalciferol biweekly for 8 weeks corrected their vitamin D insufficiency. Haworth et al. (2004) evaluated the effect of two pills Calcichew D3 Forte per day (1000 mg calcium and 800 IU colecalciferol daily) compared with placebo, in CF patients with low BMD, all of them getting their standard ergocalciferol dose of 900 IU per day. Here again, there was no change in $s_{25}(OH)D$ levels between the treatment and the placebo group after 1 year of intervention (Haworth et al., 2004). Clearly, the guidelines developed by UK CF Trust in 2007 and by the US Cystic Fibrosis Foundation consensus panel in 2005 seem to be inadequate. The first study showing that achieving $s_{25}(OH)D$ levels 75 nmol/l in all treated subjects is possible was published in 2009, where 100% of the adult CF patients who ingested 50,000 IU of vitamin D3 weekly for 3 months reached $s_{25}(OH)D$ levels above 75 nmol/l. Patients who received 50,000 IU of vitamin D2 weekly for 3 months also increased their $s_{25}(OH)D$ significantly at 3 months compared with baseline, and 60% of them reached $s_{25}(OH)D >75$ nmol/l (Khazai et al., 2009).

Collectively, the studies and observations reviewed above highlighted that the recommendations for prevention and treatment of CF-related bone disease clearly needed an update. Thus, EuroCareCF funded a work package in order to develop the recently published European CF bone mineralisation guidelines (Sermet-Gaudelus et al., 2011). These guidelines recommend higher doses of vitamin D than the older US or UK guidelines, but on the other hand they did not find enough support in the CF-specific literature for the benefit and safety of $s_{25}(OH)D$ levels $>75$ nmol/l in the CF population. Thus, they recommend $s_{25}(OH)D$ levels above the threshold 50 nmol/l. Vitamin D2 or D3 should be administered in the starting dose of 1000-2,000 IU/day in infants and 1000-5,000 IU/day in patients above 1 year of age. The dose should later be adjusted individually in order to keep $s_{25}(OH)D$ level above the threshold of 50 nmol/l (Sermet-Gaudelus et al., 2011). The publishing of the updated European guidelines was followed by the development of new guidelines for the management of vitamin D deficiency in CF patients by the CFF Vitamin D Evidence-Based Review Committee, which were published online on March 7 this year (Tangpricha et al., 2012). Here again, the committee had to provide consensus recommendations on most of the issues because of very limited CF-specific evidence. In brief, the committee recommends colecalciferol over ergocalciferol, the dosage from previous recommendations (Aris et al., 2005) has been increased, age and season are given consideration and the recommended $s_{25}(OH)D$ level is 75-125 nmol/l for all age groups (Tangpricha et al., 2012). Whether application of the recently published European guidelines (Sermet-Gaudelus et al., 2011) and the just published CFF guidelines (Tangpricha et al., 2012) leads to better vitamin D status in CF patients remains to be evaluated.
Another important issue that remains to be solved is the choice of the most proper first-hand vitamin D analogue. Due to lack of relevant CF-specific studies in the area at the time when the US (2005) or UK (2007) guidelines were structured, the recommendation of the first hand ‘analogue-of-choice’ was based on local circumstances such as availability of the various vitamin D analogues on the local market, price and dosing possibilities (Aris et al., 2005; UK CF Trust, 2007). This may however decrease the possibility for individualised dosing and aggressive supplementation regimens. However, already in 2001 it was demonstrated that ergocalciferol is absorbed less efficiently in CF than in control subjects (Lark et al., 2001), and more recently, Khazai et al. (2009) showed that high-dose colecalciferol treatment was more efficacious than high-dose ergocalciferol treatment in raising s25(OH)D levels in 30 adult CF patients, where 100% of patients treated with colecalciferol reached s25(OH)D level of 75 nmol/l, whereas only 60% of patients treated with ergocalciferol in the same dose achieved these concentrations. Not surprisingly, this finding in the CF population confirms findings in various non-CF populations, where high-dose colecalciferol was more effective than high-dose ergocalciferol in raising s25(OH)D levels (Armas et al., 2004; Glendenning et al., 2009; Leventis and Kiely, 2009; Romagnoli et al., 2008; Trang et al., 1998). Thus, the recently published European guidelines favour colecalciferol over ergocalciferol (Sermet-Gaudelus et al., 2011).

Yet it has to be noted that the study performed in CF patients by Khazai et al. (2009) demonstrated that there was a significant decrease in PTH from baseline to final measurements in both the colecalciferol and ergocalciferol treatment groups and there was no significant difference in the change in PTH concentrations between the two treatment groups. A similar finding was obtained in non-CF population where despite significant difference in achieved s25(OH)D levels, no difference in PTH concentrations was noted, comparing groups treated with ergocalciferol versus colecalciferol (Glendenning et al., 2009). This questions the clinical importance of the difference in efficacy in solely raising s25(OH)D levels. Moreover, the results of the trial by Khazai et al. (2009) might have been confounded by the fact that the ergocalciferol contained an oil-based carrier and the colecalciferol capsules contained a powder-based carrier. The choice of carrier may be of relevance in the pancreatic-insufficient CF population suffering from fat malabsorption. In addition, several studies performed in non-CF subjects, to whom lower daily doses of supplemented vitamin D were administered, failed to show any difference between the efficacy of ergocalciferol and colecalciferol (Biancuzzo et al., 2010; Gordon et al., 2008; Holick et al., 2008). Since low daily dosing rather than high-dose once-weekly dosing is the most common way of vitamin D supplementation in the majority of CF populations, these findings in non-CF populations might be of relevance. Thus, one could postulate that the suggestion that colecalciferol is of preference in the CF population is preliminary, underlining the need for more therapeutic trials in this area.

Last but not least, another question that remains unanswered is the optimal s25(OH)D level to be recommended for CF patients. The current European guidelines differ from the current US guidelines, endorsing a lower threshold of 50 nmol/l compared to the 75 nmol/l in the US guidelines (Aris et al., 2005; Sermet-Gaudelus et al., 2011; Tangpricha et al., 2012). The lower threshold of s25(OH)D 75 nmol/l for optimal vitamin D status has been proposed (Holick,
2009) based on the finding of PTH levels beginning to plateau when s25(OH)D concentration is around 75 nmol/l (Chapuy et al., 1997; Holick, 2005; Thomas et al., 1998) and the s25(OH)D >75 nmol/l has been referred to as optimal even in relation to other endpoints (Bischoff-Ferrari et al., 2006). However, the European experts interested in both CF and bone metabolism reached consensus on not recommending s25(OH)D >75 nmol/l for CF patients yet, because the long term consequences of these s25(OH)D levels have not been extensively explored (Sermet-Gaudelus et al., 2011). They also referred to the study by Chapelon et al. (2009) who suggested that PTH levels in the high normal range may promote bone formation in children and reasoned that too low PTH levels may block bone formation. Indeed, persistently low intact PTH levels have recently been demonstrated to be independently related to aortic arch calcification and mortality in incident hemodialysis patients (Rhee et al., 2012). Moreover, Malabanan et al. (1998) showed that healthy adults who had s25(OH)D >50 nmol/l had no significant change in their PTH level after receiving 50,000 IU of vitamin D once a week for 8 weeks, which indicates that the threshold of 75 nmol/l may be unnecessarily high. On the other hand, West et al. (2011) recently examined PTH concentrations as the function of s25(OH)D levels in CF patients and concluded that the optimal s25(OH)D levels in CF in relation to PTH are higher than those in healthy population, recommending s25(OH)D >87.5 nmol/l in CF. This might imply that s25(OH)D levels of 75 nmol/l recommended by the US CFF consensus panel do not keep PTH in CF patients at the very minimum, which questions the reasoning of the experts forming the European guidelines and their fear of blocking bone formation by too low PTH if patients get supplemented to reach levels of 75 nmol/l. Moreover, Heaney et al. (2003) reported calcium absorption from the gut to be 65% higher at s25(OH)D concentrations averaging 86.5 nmol/l than at s25(OH)D levels averaging 50 nmol/l. However, this study was done in non-CF postmenopausal women and studying calcium absorption in CF patients might give different results. Indeed, Hillman et al. (2008) studied true calcium absorption in children with CF and concluded that it was in the normal range at baseline. Treatment with 2,000 IU colecalciferol with or without 1g calcium for 6 months increased neither s25(OH)D nor true calcium absorption significantly. However, the study might have been underpowered and there was a slight trend towards increase in true calcium absorption in the group receiving both colecalciferol and calcium. With all of this information collectively, one could summarise that the issue of optimal s25(OH)D levels for preventing CF-related low BMD and for maximising calcium absorption in CF patients remains controversial, which highlights acute need for studies in this area.

Considering the problem of intestinal malabsorption in the vast majority of CF patients, an interesting issue which might be worth further exploration is the potential use of UVB radiation for increasing or maintaining s25(OH)D levels. A non-randomised trial (Gronowitz et al., 2005), a case-series (Chandra et al., 2007) and an RCT (Khazai et al., 2009) showed that UVB radiation for 2 to 3 months does increase s25(OH)D significantly in CF patients who are adherent to the treatment. Unfortunately, all of these studies report low adherence to the UVB radiation treatment (Chandra et al., 2007; Gronowitz et al., 2005; Khazai et al., 2009). Due to non-adherence, the RCT published by Khazai et al. (2009) failed to show any increase in s25(OH)D in patients receiving the UVB treatment, but after excluding the 4 non-adherent patients from the analysis, the rise in s25(OH)D was similar to the rise in s25(OH)D in patients receiving vitamin D2 per os. This
suggests that if one could make the CF patients adherent with the UVB treatment, the UVB may well become a successful treatment option for the vitamin D insufficiency in CF.

### 15.5 Extra-skeletal effects of vitamin D in cystic fibrosis

While the importance of vitamin D in maintaining bone health has been known for a long time, more recent studies have suggested that vitamin D also has extraskeletal effects. In 1979 and a few years after that it was discovered that several tissues and cells such as pancreas, skin, placenta, and immune cells express the VDR (Bhalla et al., 1983; Stumpf et al., 1979). Around the same time it was also found that the enzyme responsible for the second hydroxylation step in the production of active vitamin D, 1α-hydroxylase (Cyp27B), is expressed not only in the kidneys but also by other types of cells and tissues (Adams et al., 1983; Weisman et al., 1979). Collectively, these observations suggested that active vitamin D is not produced solely in the kidneys and that vitamin D might have biological functions besides maintaining calcium homeostasis. Today we know that vitamin D has an impact also on the skin, the cardiovascular system and the immune system, etc (recently reviewed in (Christakos and DeLuca, 2011; Schwalfenberg, 2011).

The immunomodulatory effects of vitamin D have received great attention lately as data suggests that vitamin D has important functions both in host defense and in dampening inflammation. By regulating the production of tight junction proteins vitamin D may contribute to the intactness (and thereby permeability) of epithelial barriers covering the skin, gut, urinary and respiratory tracts (Clairmont et al., 1996; Gniadecki et al., 1997; Palmer et al., 2001). Vitamin D is also important for the production of antimicrobial peptides at such sites (Wang et al., 2004), suggesting important functions in host defense. Low vitamin D levels have been associated with autoimmune and allergic diseases (Van Belle et al., 2011). This, together with the observations that VDR and enzymes involved in the generation of biologically active vitamin D are expressed in numerous types of immune cells, clearly point to that vitamin D also has effects on the immune system. The immunomodulatory actions of vitamin D have recently been covered in excellent reviews (Baeke et al., 2010; Van Belle et al., 2011), and the emerging picture is that vitamin D stimulates the actions of cells associated with innate immunity (e.g. macrophages), but dampens the adaptive immune response at least in part by reducing the production of Th1 and Th17-associated cytokines (e.g. IL-2, IFNγ, IL-17 and IL-21) and decreasing plasma cell differentiation and the production of IgM and IgG.

VDR is expressed in murine and human respiratory epithelial cells (Menezes et al., 2008; Yim et al., 2007; Zhong, et al., 1994), and human lung epithelial cells can convert inactive 25-dihydroxyvitamin D3 to active 1,25-dihydroxyvitamin D3 (Hansdottir et al., 2008). Human tracheobronchial epithelial cells express VDR-regulated genes including the antimicrobial peptide cathelicidin upon stimulation with 25-dihydroxyvitamin D3 (Hansdottir et al., 2008), suggesting that vitamin D is produced locally in the lung and that epithelial cell derived vitamin D contributes to the host innate immune defense.
CF has been associated with increased inflammation as well as impaired ability to clear viral and bacterial infections. As reviewed above, CF is often associated with vitamin D deficiency or insufficiency (Boyle et al., 2005; Khazai et al., 2009; Pincikova et al., 2011a; Stephenson et al., 2007; Wolfenden et al., 2008). Based upon the bulk of data supporting an important role for vitamin D in regulating immune- and epithelial cell functions more studies on the effect of vitamin D insufficiency on host defense and inflammation in CF is clearly warranted. Recent studies have demonstrated that human bronchial and tracheal cell lines harboring mutations in CFTR respond to active vitamin D by increasing the expression of cathelicidin mRNA levels (McNally et al., 2011; Yim et al., 2007). Moreover, the production of pro-inflammatory cytokines (IL-6 and IL-8) was reduced when such cells were pre-treated with active vitamin D prior to challenge with LPS or conditioned medium from cultures with Pseudomonas aeruginosa (McNally et al., 2011). On top of that, heightened Th2 reactivity in cohort of CF patients with ABPA correlates with lower mean s25(OH)D, and in vitro addition of 1,25(OH)2-D3 substantially reduces dendritic cell expression of the costimulatory molecule OX40L, increases dendritic cells expression of TGF-beta, increases Treg TGF-beta expression and reduces Th2 responses by CD4+ T cell from CF patients with ABPA (Kreindler et al., 2010). Collectively, these studies indicate that vitamin D may have a therapeutical potential in CF. Indeed, by stimulating the production of antimicrobial peptides it may promote antibacterial defense in the lung. At the same time it may dampen excessive inflammatory responses and prevent/treat ABPA. Clinical trials are clearly warranted to investigate the potentially beneficial effects of vitamin D supplementation on antimicrobial defense and inflammation in the CF lung.

These CF-specific in vitro findings and hypotheses they created are in agreement with our human epidemiological study results. We demonstrated that serum total IgG levels are negatively associated with s25(OH)D (P<0.001) and supplemented and total vitamin D intake per kilogram bodyweight (P<0.001 and P=0.002 respectively). Moreover, s25(OH)D is positively associated with FEV1 (P=0.025) in a large, well-described cohort of Scandinavian CF children and adults (n=896). This cross-sectional multi-centre study used robust multiple linear regression models, which enabled us to correct for potential confounders and explained 37.5-39.8% of variation in IgG and 30.8% of variation in FEV1 (Pincikova et al., 2011a). A significant positive association between s25(OH)D and FEV1 (R^2=0.30, P<0.001) in a CF cohort was also described in a separate study. This study had a descriptive retrospective design and evaluated data from 185 adult CF patients collected form medical records over 2 years. Here, the use of specific vitamin D supplements provided protection against vitamin D insufficiency whereas use of multivitamins did not (Wolfenden et al., 2008). On the other hand, Sagel et al. (2011) demonstrated that daily supplementation with AquADEKs® softgels over 3 months in fourteen CF patients does improve vitamin D status, albeit it does not completely normalise it. Moreover, they observed modest increase in both weight percentile and lung function (Sagel et al., 2011).

Even post-transplant CF patients might be affected by the immunomodulatory function of vitamin D. In a cohort of 41 lung transplanted CF patients, the cumulative dose of hydrocortisone needed during the first week after transplantation was inversely related to their cumulative vitamin D supplement dose (r=-0.52; P<0.05). Additionally, 1,25(OH)2D was positively correlated with
cumulative dose of cyclosporine A one year after transplantation \((r=0.93; P<0.01)\) (Pincikova et al., 2011b). This confirms findings in renal transplant recipients (Sato et al., 2009), and suggests that awareness of vitamin D and its complex effects is to be recommended in order to optimise the management of lung transplanted CF patients.

Not only immunomodulatory effects but also glucose-lowering properties of vitamin D have been described. Several outstanding reviews cover this topic (e.g. Takiishi et al., 2010). Clearly, vitamin D may very well play role in the pathogenesis of diabetes mellitus and have a therapeutic potential in the prevention and/or treatment of both type 1 diabetes and type 2 diabetes. The reported positive associations of \(s25(OH)D\) concentration with insulin secretion and peripheral insulin sensitivity in type 2 diabetes (Chiu et al., 2004), and the reported ability of vitamin D to decrease the incidence of type 1 diabetes (Hyppönen et al., 2001) may theoretically benefit CF patients as well. This hypothesis is supported by results of our cross-sectional multi-centre study where \(s25(OH)D <30 \text{ nmol/l (OR}=1.79)\) and vitamin D insufficiency degree \((\text{OR}=1.36)\) were significant independent risk factors for CFRD \((P<0.05 \text{ in both})\) in 898 Scandinavian CF patients. Moreover, vitamin D status was associated with \(\text{HbA}_1c\) values, and the associations were stronger in CF children than in CF adults (Pincikova et al., 2011c). Given the increasing prevalence of CFRD and its profound impact on CF patients’ clinical status (Lanng et al., 1992), one might hypothesise that the described associations between vitamin D and CFRD may well be clinically relevant. Thus, further studies testing the causality of vitamin D insufficiency and the amplitude of clinical benefit of vitamin D supplementation are strongly warranted.

All the findings mentioned above suggest beneficial effects of vitamin D for CF patients. However, a careful approach is to be recommended. The CF-specific findings are either demonstrated \textit{in vitro} only, or they present pure associations without any evidence of causality. The general ‘publication bias’, with negative studies not being published easily has to be kept in mind. Moreover, the CF population differs from non-CF populations in many aspects. For instance, it is debated whether dampening the chronic lung inflammation in patients chronically infected with \(P.\ aeruginosa\) is beneficial in the long run (Elston and Geddes, 2007). Maybe the inflammation keeps the bacteria in check, preventing their spread and septicaemia? Maybe CF patients down-regulate vitamin D when needed, such as in some cases of infection when increased immune responses are required? Is aggressive vitamin D treatment in these cases not harmful? Adequately powered, well-designed long-term trials with hard endpoints are crucial before we say definitely yes to vitamin D for CF patients.

\section*{15.6 Ongoing research}

The results of the research papers available today consistently suggest that improved attention to vitamin D in CF is likely to reduce morbidity through improved bone health, lung function, glucose homeostasis, and modulation of inflammation. However, the current true knowledge only allows us to create hypotheses. Before any conclusions about the benefit and safety of vitamin
D supplementation in CF patients can be drawn, we need to await the conclusions of the current and future clinical trials, RCTs being most important.

We searched for trials registered at Clintrials.gov and found five clinical trials exploring the effects of vitamin D supplementation *per os* in patients with CF, which have not yet published their results (Table 15.1). We hope that these registered trials together with other ongoing trials will succeed in answering the vitamin-D-and-CF-specific questions asked. Thereby, they will contribute each with their own unique piece to the puzzle and throw more light into this yet unexplored but relevant research area. Then maybe the ‘vitamin D and CF’-mystery will once be solved.

**Acknowledgements**

T.P. and M.F-T. are supported by Karolinska Institutet, Stockholm, Sweden. Studies on Cystic Fibrosis in the M.F-T. laboratory are supported by The Swedish Cystic Fibrosis Association and Erica Lederhausen’s Minnesstiftelse. Professor Lena Hjelte is acknowledged for consultation.
Table 15.1. Trials (unpublished) registered at clinicaltrials.gov under the identifier number studying vitamin D supplementation effects in cystic fibrosis patients.

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Trial design</th>
<th>Principal investigator</th>
<th>Study population</th>
<th>Dose and analogue</th>
<th>Study duration</th>
<th>Primary outcome(s)</th>
<th>Secondary outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00685971</td>
<td>double-blinded placebo-</td>
<td>Stephenson</td>
<td>adult patients with s25(OH)D &lt;75 nmol/l on stable</td>
<td>5,000 IU per day colecalcifer</td>
<td>12 weeks</td>
<td>change in mean s25(OH)D and LL-37</td>
<td>calcium, FEV1, PTH, creatinine, percentage of patients</td>
</tr>
<tr>
<td></td>
<td>controlled RCT</td>
<td>Tangpricha</td>
<td>vitamin D supplementation</td>
<td></td>
<td></td>
<td></td>
<td>reaching s25(OH)D &gt;75 nmol/l, subgroup analyses by pancreatic sufficiency</td>
</tr>
<tr>
<td>NCT00788138</td>
<td>double-blinded placebo-</td>
<td>Tangpricha</td>
<td>adult patients admitted to hospital for acute lung</td>
<td>250,000 IU colecalcifer in single dose</td>
<td>3 months</td>
<td>change in mean s25(OH)D</td>
<td>FEV1, days of hospitalisation, days on antibiotic</td>
</tr>
<tr>
<td></td>
<td>controlled RCT</td>
<td></td>
<td>exacerbation</td>
<td></td>
<td></td>
<td></td>
<td>therapy</td>
</tr>
<tr>
<td>NCT01426256</td>
<td>double-blinded placebo-</td>
<td>Hjelte</td>
<td>patients older than 16 years</td>
<td>250,000 IU (bolus dose) + 50,000 IU (maintenance dose every other week)</td>
<td>12 months</td>
<td>composite number of deaths and re-hospitalisations</td>
<td>inflammation, antimicrobial peptides, antibiotic use</td>
</tr>
<tr>
<td></td>
<td>controlled RCT</td>
<td></td>
<td></td>
<td>colecalcifer</td>
<td></td>
<td></td>
<td>FEV1, mortality and re-hospitalisations as single</td>
</tr>
<tr>
<td>NCT01321905</td>
<td>open-label RCT</td>
<td>Pilewski</td>
<td>patients older than 5 years</td>
<td>5,000 IU per day (if 6-15 years of age) or 7,150 IU per day (if older than 15</td>
<td>3 months intervention</td>
<td>Aspergillus-induced IL-13 responses in CD4+ T-cells</td>
<td>calcium, PTH, inflammation, infection, lung function,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>years) colecalcifer or ergocalcifer</td>
<td>followed by 2 months</td>
<td></td>
<td>quality of life, adherence, safety</td>
</tr>
<tr>
<td>NCT01222273</td>
<td>open-label non-randomised</td>
<td></td>
<td></td>
<td>4,000 IU per day colecalcifer</td>
<td>6 months</td>
<td></td>
<td>IgE and IgG, vitamin D levels, FEV1, cytokine production by Aspergillus-</td>
</tr>
<tr>
<td></td>
<td>trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>stimulated peripheral blood T-cells, urine/creatinine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ratio</td>
</tr>
</tbody>
</table>
References


15. Vitamin D and cystic fibrosis


15. Vitamin D and cystic fibrosis


15. Vitamin D and cystic fibrosis


Vitamin D and infectious disease
Key facts

- Vitamin D deficiency is a result of inadequate sun exposure and/or poor dietary intake.
- This chapter addresses the global high prevalence of vitamin D deficiency among women of childbearing age and infants and toddlers.
- Vitamin D status (nutritional and sun exposure) is assessed by measuring the transport form of vitamin D, 25-hydroxyvitamin D (25(OH)D).
- Active hormonal form is synthesized in most tissues from 25(OH)D.
- Vitamin D status is associated with immune development and lung function.
- Clinical trials are needed to establish the link between vitamin D deficiency and susceptibility to respiratory tract infections.

Summary points

- Vitamin D status of mothers and infants is poorest in darker skinned individuals, particularly those living in Northern latitudes.
- The poorest vitamin D status occurs in exclusively breast fed infants who are not supplemented with vitamin D. In contrast, formula fed infants have lower prevalence of vitamin D deficiency; however, older toddlers have limited access to vitamin D-rich foods.
- Children with Fitzpatrick skin types II to IV, in the northern and southern United States can only achieve a minimum amount of vitamin D from sun exposure during the summer months if they do not wear sunscreen at all except during beach vacations. Dark-skinned (skin types ≥ V), children fall short of achieving their needs for vitamin D from sun exposure year round.
- In vivo animal and in vitro models provide evidence that vitamin D deficiency in utero is associated with altered lung development and immune functions, and increasing postnatal susceptibility to respiratory infections.
- Adequate vitamin D is critical for the production of anti-microbial peptides such as cathelicidin and Beta-defensins that are key protective mechanisms in preventing respiratory infections in children under five years of age.
- Clinical trials are currently underway to determine if vitamin D supplementation reduces respiratory tract infections in infants and toddlers and during gestation in supplemented pregnant or lactating mothers.
16. Vitamin D and respiratory infections in infants and toddlers: a nutri-shine perspective

K.V. Balan¹, U.S. Babu¹, D.E. Godar² and M.S. Calvo¹
¹Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 8301 Muirkirk Road, HFS-025/MOD1, Laurel, MD 20708, USA; ²Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA; kannan.balan@fda.hhs.gov

Abstract

There is compelling evidence of a global problem of poor vitamin D status in expecting mothers and postnatal life; and even more critical, is the evidence showing the association of vitamin D deficiency with increased morbidity and mortality risks from respiratory infections. Viral and bacterial pneumonia kills more children than any other illness, accounting for 19% of all deaths in children less than five years of age worldwide; and under-nutrition, which includes vitamin D insufficiency/deficiency, has been implicated in 53% of all these deaths. Poor vitamin D status is a result of insufficient sunlight exposure and/or poor dietary intake. Greater understanding of the role of vitamin D deficiency in precipitating lung infections grew from the use of rodent models and observational and intervention studies in infants and toddlers. Vitamin D adequacy is important to maintaining the key protective mechanism of developing lungs since it mediates the synthesis of antimicrobial peptides, the lungs strongest defense against viral and bacterial pathogens. If vitamin D intervention currently under study in several clinical trials is proven successful, then implementation of new fortification practices, revised guidelines for healthy sun exposure and public health programs for vitamin D supplementation of pregnant/lactating women and their infants may be effective strategies to aide in preventing neonates and children under five from developing pneumonia. Globally, there is potential to save more than a million young lives with preventive treatment, a compelling reason why the efficacy of optimizing vitamin D mediated defense against respiratory pathogens in infants and children merits further study.

Keywords: vitamin D deficiency, infants, vitamin D supplementation, wheezing, skeletal health, respiratory infections, breast fed infants

16.1 Introduction

Vitamin D is a secosteroid hormone that is synthesized in humans upon solar UVB (290-315 nm) mediated conversion of endogenous skin 7-dehydrocholesterol to cholecalciferol (vitamin D3) (MacLaughlin et al., 1982), and is also derived from food and dietary supplements. During the winter when adequate sunlight is limited for synthesis, there is greater dependency on dietary intake and thus the term ‘vitamin’ meaning essential nutrient was applied to this sunshine-derived hormone. Since vitamin D occurs naturally in a limited number of foods, fortified foods such as milk and milk products, margarines, and breakfast cereals constitute the major dietary sources of the two forms of vitamin D, cholecalciferol or ergocalciferol (vitamin D2) in the US and Canada (Calvo et al., 2004). After absorption or synthesis, vitamin D2 or D3 is transported by vitamin D binding protein to the liver where the enzyme 25-hydroxylase converts it to 25(OH)D. This intermediary metabolite circulates and delivers the precursor to the active or hormonal form of vitamin D to different tissues. In this capacity, plasma 25(OH)D serves as the main status indicator for vitamin D. The mitochondrial enzyme, 1α-hydroxylase converts 25(OH)D to the active form of vitamin D, 1,25(OH)_{2}D (Holick, 2007). Renal tissues are thought to be the major source of circulating levels of 1,25(OH)_{2}D, but most extra-renal tissues including the immune and airway epithelial cells constitutively or upon activation express 1α-hydroxylase and can produce 1,25(OH)_{2}D (Hansdottir and Monick, 2011).

Vitamin D has pleiotropic functions, and is involved in the regulation of approximately 1000 human genes (Tavera-Mendoza and White, 2007), including genes associated with immune responses and lung development. While its classical role in bone mineralization is well documented, the interplay of non-rachitic vitamin D status to in utero and post-natal health outcomes such as susceptibility to early-life respiratory infections continues to evolve. In this chapter, we review the current public health concern about vitamin D status in infants and toddlers in North America, the dietary guidelines and Federal regulations governing the level of vitamin D in foods and

Abbreviations

25(OH)D  25-hydroxyvitamin D
CAMP    Cathelicidin antimicrobial peptide
NHANES  National Health and Nutrition Examination Survey
IOM     US Institute of Medicine
EAR     Estimated average intake
RDA     Recommended dietary allowance
RTI     Respiratory tract infection
LRTI    Lower respiratory tract infection
RSV     Respiratory syncytial virus
URTI    Upper respiratory tract infection
UVB     Ultraviolet B
supplements during pregnancy and early childhood, and examine the evidence for association between poor vitamin D status and risk of respiratory infections in infants and toddlers.

16.2 Vitamin D status and dietary guidelines during pregnancy and early childhood

Plasma 25(OH)D is considered the best biomarker for vitamin D status as it is relatively stable and reflects contributions from all sources of vitamin D (diet and endogenous synthesis). The IOM, an independent non-governmental body, recently defined adequate vitamin D status as having serum 25(OH)D concentrations greater than 50 nmol/l (or 20 ng/ml; 1 ng/ml = 2.5 nmol 25(OH)D/l) in both the general population and pregnant women, and concentrations between 30-49 nmol/l (or 12-20 ng/ml) or <30 nmol/l (or <12 ng/ml) were considered to be inadequate or deficient respectively (IOM, 2011). The IOM cutoff levels defining adequate, insufficient and deficient plasma vitamin D status are specific to bone health and do not consider other systems that are affected by circulating levels of 25(OH)D such as the immune system and respiratory health (IOM, 2011). Although serum 25(OH)D levels of at least 25 nmol/l prevents rickets, it has been proposed by some investigators that concentrations around 80 nmol/l (32 ng/ml) are optimal, since these levels lead to the greatest calcium absorption and the highest bone mass (Bischoff-Ferrari et al., 2006; Dawson-Hughes, 2008) and are associated with better health outcomes involving non-skeletal tissue (Hollis, 2011).

An accurate determination of the overall dietary requirements for maintaining vitamin D adequacy is impossible because of substantial variation in skin synthesis due to season, location, age, duration of exposure, pollution, skin pigmentation and exposed area, and use of sun screen. Consequently, assuming minimal sun exposure and after thorough review of literature on skeletal health, the IOM established a RDA of 600 IU (15 μg; 1 μg=40 IU vitamin D2 or D3) vitamin D for pregnant and lactating women and toddlers. With insufficient evidence to develop an RDA in infants, an adequate intake of 400 IU (10 μg) was proposed to ensure vitamin D nutritional adequacy in this population. The IOM derives the RDA from an EAR, which was determined for vitamin D for the first time in 2011. From a public health perspective the EAR (400 IU or 10 μg/d for individuals >1 year) is used to evaluate the nutrient intake adequacy of a population at the 50th percentile (median) level of intake for all age groups (Whiting and Calvo, 2011). There appears to be global variation in recommended vitamin D intake guidelines for pregnant women, infants and toddlers (Table 16.1). This variation could be a result of differences due to season, demographics of light versus dark skinned individuals, national supplementation and fortification policies, and more importantly skepticism associated with non-skeletal health benefits from basic science experiments and observational studies as compared to randomized clinical trials.

16.2.1 Maternal status of vitamin D levels

Maternal vitamin D deficiency during pregnancy is global and widely prevalent, and can be attributed to inadequate vitamin D intake and restricted sunlight exposure during winter months
compared to summer months, northern latitudes, and higher melanin levels in dark-skinned or veiled women. Based on a National Human Activity Pattern Survey it has been estimated from the outdoor-sunlight data that US women including those of child-bearing age (22-40 years) get lower annual UVB doses than males because they spend less time outdoors (Godar, 2001; Godar et al., 2001). In the US, 29% of African-American pregnant women and 5% of Caucasian pregnant women residing in the northern latitudes are vitamin D deficient (defined as serum 25(OH)D less than 37.5 nmol/l); whereas 54% of African-American participants and 47% of Caucasian participants are vitamin D insufficient (defined as serum 25(OH)D 37.5-80 nmol/l) (Bodnar et al., 2007). Moreover, 18% of pregnant women in the United Kingdom, 25% in the United Arab Emirates, 80% in Iran, 42% in northern India, 61% in New Zealand and 60-84% of pregnant non-Western women in the Netherlands have been shown to have serum 25(OH)D concentrations <25 nmol/l (Dawodu and Wagner, 2007). Thus, there is concern that in utero vitamin D deficits could lead to developmental re-programming of body functions and long-term morbidity in infants, children and adults.


<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>3 months</th>
<th>9 months</th>
<th>5 years</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>United Kingdom</td>
<td>340</td>
<td>280</td>
<td>_3</td>
<td>_3</td>
</tr>
<tr>
<td>1996</td>
<td>Italy</td>
<td>400</td>
<td>700</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>1997</td>
<td>IOM (United States/Canada)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2001</td>
<td>France</td>
<td>800-1000</td>
<td>200-1000</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2001</td>
<td>World Health Organization (WHO)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2004</td>
<td>Nordic countries</td>
<td>400</td>
<td>400</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>2004</td>
<td>Germany/Austria/Switzerland</td>
<td>400</td>
<td>400</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2005</td>
<td>Australia and New Zealand</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2011</td>
<td>IOM (United States/Canada)</td>
<td>400</td>
<td>400</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>2011</td>
<td>The Endocrine Society – United States</td>
<td>400-1000</td>
<td>400-1000</td>
<td>600-1000</td>
<td>1,500-2,000</td>
</tr>
<tr>
<td>2012</td>
<td>France</td>
<td>1000-1,200</td>
<td>1000-1,200</td>
<td>2 doses4 of 80,000- or 100,000-IU in winter – Nov &amp; Feb</td>
<td>1 dose4 of 80,000- or 100,000-IU at start of 3rd trimester</td>
</tr>
</tbody>
</table>

1 Breastfed infants.
2 Below 50 yrs of age.
3 400 IU/d in cases of insufficient UVB exposure.
4 Bolus dose.
Cumulative intake data from the nationally representative NHANES survey conducted from 2007-2008 show that US women of childbearing age have significantly lower ($P<0.01$) vitamin D intakes than their age-matched male counterparts (Figure 16.1) (USDA, 2010). A comprehensive assessment of 25(OH)D serum data from participants in NHANES 2001-2006, one year of age and over, after adjusting for age and season, showed that males are less likely to be at risk of deficiency (<30 nmol/l) than females (Figure 16.2) and the prevalence of those at risk of inadequacy (30-49 nmol/l) did not differ by sex. However, in the 2001-2006 NHANES cohort, among women of childbearing age, those who were pregnant or lactating were less likely to be at risk of deficiency compared to women who were not pregnant or lactating (Figure 16.2) and no differences in prevalence of vitamin D inadequacy were observed between the two groups (Looker et al., 2011). These prevalence data reflect 24 hr recall levels of vitamin D intake, which suggests that pregnant and lactating women in general in the US are in compliance with the current dietary guidelines for maintaining vitamin D status or have adequate sun exposure. A rigorous analysis of current NHANES data is needed to determine the association of vitamin D status with sun exposure and total ‘usual’ dietary intake for individual race/ethnicities, since smaller cross-sectional studies have shown association of maternal deficiency, race (African-American), winter birth, and a BMI of $\geq 35$ as risk factors for newborn vitamin D deficiency.

**Figure 16.1.** Vitamin D intake in the United States (NHANES, 2007-2008). Mean vitamin D intake ($\pm$SE) from food per individual, by gender and age of participants in the 2007-2008 NHANES survey of US population. * Estimated average requirement (EAR) for vitamin D intake established in 2011 (IOM, 2011). The differences in vitamin D intake between males and females were significant across all age groups ($P<0.01$) (USDA, 2010).
During pregnancy the fetus is wholly dependent on the mother for vitamin D, and 25(OH)D readily crosses the placenta such that cord blood 25(OH)D levels are between 80% and 100% of maternal concentrations (Fleischman et al., 1980; Gertner et al., 1980; Hillman and Haddad, 1974). There is a strong correlation between maternal and newborn 25(OH)D circulating levels as the half-life of 25(OH)D is approximately 2 to 3 weeks, and a low maternal status often results in the infant being deficient in vitamin D during the first weeks post-partum (Hollis and Wagner, 2004a; Yu et al., 2009). Because vitamin D secretion in breast milk is limited (Kovacs, 2008), lactating women require robust serum 25(OH)D levels to support vitamin D status in nursing infants (Hollis and Wagner, 2004b). The World Health Organization recommends supplementation with 400-IU daily vitamin D to all pregnant women; however, because of lack of awareness and poor compliance, 46% of newborns in industrialized countries are born with insufficient serum levels of 25(OH)D (Belderbos et al., 2011).

More recently Balk and the Council on Environmental Health and Section on Dermatology recommended that children younger than 1-year of age avoid direct sunlight and also use sunscreen (Balk, 2011). Furthermore, for reasons that are not entirely clear, data suggest that the relationship between vitamin D intake and serum 25(OH)D levels is non-linear (Hypponen et al., 2009), and current guideline levels for vitamin D supplementation (200-600 IU/d) are unlikely to achieve optimal serum 25(OH)D levels (IOM, 2011). In a study of 40 mother-infant pairs, 76% of mothers and 81% of newborns had a 25(OH)D level below 50 nmol/l at the time...
of birth, despite the fact that during pregnancy the mothers ingested about 600 IU/d of vitamin D from a prenatal supplement and consumed two glasses of milk (Lee et al., 2007). Thus, it is understandable that infants who are fed only human breast milk are prone to developing vitamin D deficiency leading to a Federal mandate both in the United States and Canada to fortify infant formula with vitamin D at 40-100 IU/kcal and 40-80 IU/kcal, respectively. The effectiveness of infant formula fortification was evident in a cross-sectional study of 247 healthy infants in a primary care setting in Boston as breastfeeding without supplementation markedly increased the odds of vitamin D deficiency compared with infants who were exclusively formula (bottle)-fed (Gordon et al., 2008). Interestingly, although maternal supplementation of 200-IU/d vitamin D was available to all nursing mothers, there may be barriers to obtaining the supplement or lack of awareness of the need to supplement, which resulted in poor compliance. Thus, with emerging data on insufficiency in vitamin D levels with the IOM 1997 recommendation of 200 IU/d, the revised recommendation is now set at 600 IU/d for infants in the United States and Canada (IOM, 2011).

16.2.3 Vitamin D status in toddlers

Most American children may not be going outside enough to meet their vitamin D3 needs from sun exposure (Godar et al., 2012). Recent estimates from everyday outdoor exposure suggest that children (≤5 years) in the northern (45°N) (Figure 16.3a) and southern (35°N) (Figure 16.3b) United States with Fitzpatrick Type II skin (Caucasian) (Fitzpatrick, 1988) can have adequate skin synthesis of vitamin D3 during the summer and children living in the south during the spring as well but only if they do not wear sunscreen at all except during beach vacations. Children with Fitzpatrick Type III and IV skin (olive tone-Hispanic/Asian or brown tone-Indian, respectively)
can only synthesize the suggested minimum daily recommendation during the summer months, while those with the darkest pigmentation (skin type V and higher, usually African-Americans) never make the suggested minimum amount of vitamin D3 from sun exposure (Godar et al., 2012). These vitamin D3 estimates from solar exposures assume certain skin types, liberal clothing scenarios during each season and that sunscreens are not worn except during beach vacations. These could be overestimates for infants and toddlers since mothers tend to be overly protective of their children to avoid skin cancer later in life or could be a result of children spending more time indoors watching television. Despite the fact that younger children are spending more time indoor, children in the younger age group (≤5 years) have more cutaneous vitamin D synthesis than those in the older age groups (Godar, 2001).

With children falling short of the daily needed levels of vitamin D3 from sunshine exposure especially in the winter months, fortified foods are key to maintain adequate vitamin D status in toddlers, and increasingly, the manufacturers of foods targeted to toddlers are recognizing the need to fortify with vitamin D. Levels of vitamin D fortification range from 1 μg (40 IU) per regulatory serving for ready-to-eat cereals to 2.5 μg for fluid milk servings (Calvo and Whiting, 2013). It is evident from reviewing the vitamin D content of the ‘baby foods’ listed in the recent USDA database, Standard Release-24 for nutrient composition of foods that there is a need for newer fortified baby foods. A review of 223 foods showed vitamin D contents ranged from 0 to 0.8 μg vitamin D per 100 g, and approximately 90% had no listed vitamin D content. With the availability of limited fortified baby foods, in a population study of 750 healthy children aged 6-36 months, the mean overall vitamin D intake from food plus supplements was 290±124 IU/d in 6-12 month age group and 240±128 IU/d in 1-3 years age group (Figure 16.4), and group corresponding serum 25(OH)D levels decreased with age. A quite concerning trend in this cohort

![Figure 16.4](image.png)

**Figure 16.4.** Effect of lack of vitamin D-fortified foods on the daily dietary intake in a cohort of healthy infants and toddlers. Vitamin D daily dietary intake (±SD) in 6-12 months (n=131) and 13-36 months old (n=645) healthy children. Significant age differences in vitamin D intake, P<0.001. Figure drawn from Carpenter et al. (2012).
was that children discontinued breastfeeding by 5 months of age and most children (84%) were no longer formula-fed at 5 months (Carpenter et al., 2012). Thus, with the IOM recommended adequate intake of 400 IU/d for infants and 600 IU/d for toddlers approximately 82% of infants and 2% of toddlers met the age-specific dietary intake recommendation. These findings suggest that vitamin D-fortified infant formulas provide a positive effect on vitamin D levels and similar supplementary practices are likely to be effective in increasing 25(OH)D status in young children (Carpenter et al., 2012). The mean intake data for toddlers (2-5 years age) from the NHANES 2007-2008 cohort shows a significant difference in vitamin D intake among the racial and ethnic groups with Non-Hispanic blacks consuming less than Non-Hispanic whites, who consume less than Mexican Americans (Figure 16.5) (USDA, 2010). Correspondingly, the prevalence of vitamin D insufficiency and deficiency in children (1-5 years) during the 2001-2004 NHANES was highest among Non-Hispanic blacks followed by Mexican Americans and Non-Hispanic whites (Figure 16.6) (Kumar et al., 2009).

There is clear evidence for global and widespread vitamin D deficiency or insufficiency due to various risk factors including lack of sunlight exposure, seasonal variation, suboptimal dietary intake, and dark skin pigmentation. More importantly, we have shown the greatest barriers to adequate vitamin D from sun exposure or diet occur in individuals with the poorest vitamin D status. How this widespread deficiency relates to the risk of respiratory tract infections in infants and toddlers is less clear and merits further study.

Figure 16.5. Vitamin D intake among toddlers in the United States, NHANES, 2007-2008. Mean intake (±SE) of vitamin D (D2 + D3) from NHANES 2007-2008 for toddlers 2-5 years, presented by race and ethnic group. a EAR for vitamin D intake established in 2011 (IOM, 2011). NH: Non-Hispanic. * Indicates significant difference in vitamin D intake between NH Black and Mexican Americans, P<0.01. Figure drawn with data from USDA (2010).
16.3 Fetal and postnatal outcomes associated with vitamin D status

The fetal origins hypothesis, first articulated by David Barker, postulates that in utero epigenetic fetal programming, as a result of environmental events during pregnancy, induces specific genes and genomic pathways that control fetal development and subsequent disease risk (Barker et al., 2002). The biological effects of active vitamin D are achieved through the regulation of gene expression in a cell and tissue specific manner. Briefly, active vitamin D binds to the vitamin D nuclear receptor, and initiates dimerization with the retinoic X receptor. This active complex binds to the nuclear vitamin D responsive elements within the promoter regions of vitamin D-specific responsive genes and initiates gene expression (MacDonald et al., 1993). The developmental periods of in utero and infancy represent critical periods of dynamic development and maturation of key processes. Therefore, in the following sections, we focus on the role of vitamin D in modulating lung structure and innate immune functions, a primer for susceptibility to viral respiratory tract infections during infancy and early childhood (Figure 16.7).

16.3.1 Lung development

Lung development occurs predominantly before birth (Stick, 2000) with extensive interactions between epithelial and mesenchymal tissue beginning by the fourth week of gestation and continuing for years after birth, and factors that impair fetal and early childhood lung development have the potential to exert major effects on lung function during childhood and adulthood. Despite differences in the epithelial growth and differentiation during lung development in rodents and humans, rodent models are widely used for lung developmental studies. Rodent models act as a bridge between studies in the laboratory and studies in humans, and have been used to study pulmonary infections (Balan et al., 2011). In rodents, it has been demonstrated that vitamin
16. Vitamin D and respiratory infections in infants and toddlers

D modulates key alveolar epithelial-mesenchymal interactions, such as type II pneumocyte and lipofibroblast proliferation and differentiation which are critical for alveolar development and septal thinning during perinatal pulmonary maturation (Sakurai et al., 2009). Vitamin D-deficient rodent models provide mechanistic evidence for a causal link between vitamin D deficiency and deficits in lung function and altered lung structure. Specifically, offspring of vitamin D-deficient mice showed no effect on the overall somatic growth, had marginal reduced numbers of alveoli, and exhibited physiologically significant decreases in lung volume and altered lung mechanics when compared with offspring of vitamin D-replete mice (Zosky et al., 2011). This mouse study complements data showing decreased lung compliance in 50-d-old rats born to vitamin D-deficient mothers (Gaultier et al., 1984), confirming the relationship between vitamin D deficiency and lung function. In addition, as vitamin D deficiency has the potential for premature births in humans (Dawodu and Nath, 2011), studies have shown that prematurity leads to diminished lung function and may predispose premature infants to severe viral lower respiratory tract infections in infancy (Drysdale et al., 2011).

Active vitamin D synthesis has been shown in rodent fetal lung fibroblasts, whereas adjacent alveolar type II pneumocytes express vitamin D receptors and respond to the hormone by undergoing differentiation and maturation resulting in decreased cell glycogen content, and increased surfactant synthesis and secretion (Nguyen et al., 1996, 2004). Surfactant proteins were initially identified as a lipoprotein complex that reduced surface tension at the air-liquid interface of the lung, but recent studies have identified surfactant proteins as components of the lung innate and adaptive immune system with novel roles in the direct killing of inhaled microorganisms and

Figure 16.7. Role of nutrition and sunshine in maintaining vitamin D adequacy and reducing incidence of respiratory tract infections in postnatal life.
viruses, and control of pulmonary inflammation (Wright, 2005). The vitamin D-mediated rodent type II pneumocyte maturation data looks intriguing but the relevance to humans is uncertain. Regulation of surfactant protein gene expression in human fetal type II pneumocytes by active vitamin D is not coordinated as it is in rodents, but vitamin D receptor immuno-staining is observed in human fetal fibroblasts and type II pneumocytes (Phokela et al., 2005; Stio et al., 1997).

Thus, there is evidence for in utero vitamin D deficiency and association with altered epithelial-mesenchymal maturation, lung mechanics, and immunoregulatory surfactant production. While the association between surfactants in providing innate immunity against respiratory viral infections is conceivable, the role of altered pulmonary function with vitamin D deficiency could also indicate an association with asthma, a chronic inflammatory condition that is described in detail in a separate chapter.

### 16.3.2 Vitamin D and innate immune protection

The fetus and neonate face a complex set of immunologic demands, and vitamin D has been shown to have an important role in the innate immune system, which helps to prevent infection without the need for immunologic memory from previous exposure to the pathogen (Adams and Hewison, 2008). Innate immunity includes the production of antimicrobial peptides such as Beta-defensins and CAMP by epithelial cells and circulating leukocytes, which are capable of killing a variety of respiratory pathogens including viruses, bacteria, and fungi (Hiemstra, 2007). In early gestation, human decidual cells have been shown to synthesize active vitamin D, which may exert autocrine or paracrine effects on the developing fetal immune system. Vitamin D mediates expression of mRNA for CAMP in decidual cells (Evans et al., 2006), and has been linked to intrauterine immunity (Singh et al., 2005). Human monocytes in an in vitro model, when supplemented with 25(OH)D-deficient cord blood plasma showed a significant decrease in CAMP expression, thus correlating with increased susceptibility to newborn infections (Walker et al., 2011).

In postnatal life, respiratory epithelial cells provide a barrier between the outside environment and internal parenchyma, and are primary targets of respiratory pathogens. The respiratory epithelial cells constitutively activate vitamin D, and are capable of creating a microenvironment that has high levels of active form of the vitamin, resulting in the activation of downstream genes such as those for CAMP. Moreover, viral RNA increases the expression of 1α-hydroxylase, leading to increased activation of vitamin D and further increases in CAMP mRNA (Hansdottir et al., 2008). Hansdottir and colleagues (2010) have shown in an in vitro human tracheo-bronchial epithelial cell model for respiratory syncytial virus infection, that vitamin D attenuates inflammatory cytokine and chemokine response, while maintaining the antiviral activity. This local vitamin D-mediated anti-viral, anti-inflammatory immune response could result in decreased disease severity and morbidity from this common infection (Hansdottir et al., 2010). Thus, vitamin insufficiency and notably, a seasonal decrease of vitamin D-dependent epithelial and leukocyte innate host defense could contribute to increased susceptibility to respiratory infections during
winter. A prospective descriptive study of outcomes associated with vitamin D deficiency and pneumonia reported 25(OH)D deficiency associated with increased mortality but not associated with levels of cathelicidin or Beta-defensin (Leow et al., 2011). Clinical trials are underway to assess CAMP expression as a biomarker for fetal-neonatal immune function following antenatal vitamin D supplementation (ClinicalTrials.gov identifier: NCT01126528) and in a separate trial for Intensive Care Unit-associated lung failure, CAMP and Beta-defensin concentrations will be evaluated as a secondary outcome following high-dose vitamin D regimen (ClinicalTrials.gov identifier: NCT01372995).

While we limit our review to the role of innate immune antiviral functions to respiratory infections, vitamin D status has been associated with other prenatal and postnatal immunomodulatory effects, such as promoting peripheral tolerance by rendering antigen presenting dendritic cells tolerogenic and development of T-regulatory cells. Dendritic and T-regulatory cells serve as pivotal links between innate and adaptive immunity, and play a key role in the protection against the inflammatory sequela of airway infections and in the protection against induction and expression of atopic disease (Chambers and Hawrylowicz, 2011; Holt et al., 2008).

16.4 Evidence for vitamin D status and risk of respiratory infections & childhood wheezing

There is an association of wintertime peaks in respiratory infections especially in the higher latitudes to vitamin D status as the cutaneous synthesis of vitamin D is naturally blunted during that time of the year (Cannell et al., 2006). Respiratory tract infections in the neonatal and pediatric populations are normally viral in origin with accompanying wheezing, pneumonia or bronchiolitis. The infections can be classified into URTIs and LRTIs with the URTI a primary site of contact for inhaled agents, and LRTIs as infections of the intra-thoracic airways and/or lung parenchyma with severe cases leading to bronchiolitis and pneumonia. The main etiological agents include human rhinovirus, RSV, human coronavirus, adenovirus, parainfluenza virus and influenza virus. Cases of bronchiolitis associated with human rhinovirus (46-49%), RSV (11-27%), and parainfluenza viruses (5-13%) account for most of the outpatient sampling in infants with URTIs and LRTIs (Camargo et al., 2011a). Viral infections including rhinovirus and RSV could lead to bronchiolitis and early episodic wheezing in infants (Gern and Busse, 2002), symptoms which are commonly associated with the likelihood of developing reactive airway disease or asthma in early childhood (Asher et al., 2006; Litonjua, 2012; Wu et al., 2008). Large cohort studies, however have demonstrated that many children who wheeze in early childhood during acute respiratory infections do not go on to develop asthma (Camargo et al., 2011b; Martinez et al., 1995; Stein and Martinez, 2004). In addition, accumulating evidence implicates a background of atopy as a leading cause of asthma associated with airway inflammation from respiratory infections (Holt et al., 2012). Nevertheless, asthma is a heterogeneous disease and since clinical diagnosis of asthma in children remains a challenge, epidemiological studies of children often focus on childhood wheezing; and in this chapter, we present selected epidemiological studies
on vitamin D deficiency in children and associated respiratory infections and wheezing but not related to asthmatic conditions.

16.4.1 Observational prospective serum 25(OH)D birth cohort studies

Circulating 25(OH)D level provides a distinct advantage to assess vitamin D status than self-reported dietary intake, and cord-blood 25(OH)D concentration is strongly associated with maternal concentration during pregnancy. In a birth cohort study of 922 children cord-blood levels of 25(OH)D had inverse associations with the risk of respiratory infection by three months of age (OR: 1.00 for ≥75 nmol/l, 1.39 for 25-74 nmol/l, and 2.16 for <25 nmol/l). Likewise, cord-blood 25(OH)D levels were inversely associated with risk of wheezing by 15 months, 3 years, and 5 years of age (all P<0.05) and no association to incident asthma by the age of 5 years. Additional adjustment for potential confounders including seasons of birth did not materially change these results (Camargo et al., 2011b). A recent birth cohort study of 156 healthy neonates showed an association of vitamin D deficiency with increased risk of RSV LRTIs in the first year of life. Neonates born with 25(OH)D concentrations <50 nmol/l had a sixfold (95% CI=1.6-24.9; P=0.01) increased risk of RSV LRTI in the first year of life compared with those with 25(OH)D concentrations ≥75 nmol/l (Belderbos et al., 2011). Similarly, in a population based mother-child cohort study there was a trend of independent association between higher levels of maternal circulating 25(OH)D in pregnancy and decreased odds of LRTIs in offspring (for cohort- and season-specific quartile Q4 vs. Q1, OR: 0.67 (95% CI=0.50-0.90); test for trend, P=0.016). No association was found between 25(OH)D levels in pregnancy and risk of wheezing at age 1 year or 4 years, or asthma at age 4-6 years (Morales et al., 2012).

16.4.2 Case-control studies

Nutritional rickets due in part to vitamin D deficiency is a major health problem in developing countries. It is associated with respiratory muscle weakness and increased risk of respiratory infections. The first association of sub-clinical nutritional rickets to respiratory infections was observed in a case-control trial among Indian children (3-12 years) with multiple episodes of respiratory infections. Administration of oral vitamin D, 60,000 IU/wk and 650 mg of calcium/d for 6 weeks decreased the incidence of respiratory infections in the test population (Rehman, 1994). Similarly in a case-control study, 13-fold higher incidence of nutritional rickets was observed among toddlers (<5 years) with pneumonia than among controls (OR=13.37; P<0.001) (Muhe et al., 1997). More recently, significant associations have been identified with non-rachitic vitamin D levels and respiratory tract infections suggesting that vitamin D insufficiency is enough to trigger respiratory infection rather than a secondary manifestation of acute deficiency typically leading to nutritional rickets.

In a hospital-based case-control study of non-rachitic Indian children age 2-60 months, serum 25(OH)D levels of <22 nmol/l had more than 10-fold higher odds of acquiring severe acute LRTIs (OR: 0.09; P<0.001) (Wayse et al., 2004), and in a similar study design in rural Bangladesh, 25(OH)D levels in children (1-18 months) hospitalized with acute lower respiratory infections
16. Vitamin D and respiratory infections in infants and toddlers

(ARLIs) were significantly lower (29.1 nmol/l) than case matched controls (39.1 nmol/l). The unadjusted odds ratio of ARLIs was halved for each 10 nmol/l increase in 25(OH)D (OR=0.53; 95% CI=0.3, 0.96) (Roth et al., 2010). Furthermore, in a neonatal case-control study of ARLIs in Turkey, mean serum level of 25(OH)D was 22.7±22.2 nmol/l compared to age-matched control levels, 40.6±33.6 nmol/l (P=0.011) and corresponding 25(OH)D levels in mothers of the study group were lower than those in the mothers of the control group (33.4±42 nmol/l and 57±42.3 nmol/l respectively; P=0.012). These findings suggest that newborns with subclinical vitamin D deficiency may have an increased risk of suffering from acute lower respiratory infection and the strong positive correlation between newborns and mothers 25(OH)D concentrations shows that adequate vitamin D supplementation of mothers should be emphasized during pregnancy (Karatekin et al., 2009).

In contrast, two case-control studies in Canadian children did not reveal an association between vitamin D deficiency and respiratory infections as virtually all children consumed vitamin D fortified infant formula or supplements (Roth et al., 2009). While no difference was observed in vitamin D levels between the entire acute lower respiratory infection group and control group, approximately 50% of the patients admitted to the pediatric intensive care unit were vitamin D deficient (<50 nmol/l) compared to only 20% on the general medical floor (OR: 8.23; 95% CI: 1.4, 48.0; P=0.02) suggesting that low levels of vitamin D predispose to greater acute LRTI severity (McNally et al., 2009).

16.4.3 Interventional trials

Published data from randomized control clinical trials to evaluate the effects of vitamin D on reducing RTIs in children are limited. Despite mixed results because of study design, lack of measurement of 25(OH)D serum levels and poor compliance, four studies have shown potential for vitamin D intervention to help control respiratory tract infections in the adult population (Aloia and Li-Ng, 2007; Avenell et al., 2007; Laaksi et al., 2007; Li-Ng et al., 2009).

A supplementation of vitamin D (1,200 IU/d) for four winter months among 334 schoolchildren in Japan showed a reduction in influenza A (10.8%) children in the vitamin D group compared with (18.6%) children in the placebo group [relative risk (RR), 0.58; 95% CI: 0.34, 0.99; P=0.04]. In a sub-group analysis significant reductions of influenza A were more prominent in children taking additional vitamin D supplements (RR 0.36; 95% CI: 0.17-0.78). The study, however, lacked measurements of serum 25(OH)D and serum antibody concentrations to influenza A (Urashima et al., 2010). A current search of the ClinicalTrials.gov database lists the Vitamin D Outcomes and Interventions In Toddlers (DO IT Trial: NCT01419262, Primary Outcome: Respiratory Infection) and Maternal Vitamin D Supplementation to Prevent Childhood Asthma (VDAART Trial: NCT00920621, Secondary Outcome: LRTIs in the first 3 years of life) as ongoing trials to evaluate the effects of vitamin D on reducing RTIs in infants and toddlers. The gestational supplementation of 4,000 IU/d in the VDAART trial is noted as a recent trial on evaluating clinical safety and effectiveness of vitamin D supplementation during pregnancy concluded that
supplementation of 4,000 IU/d is safe and most effective in achieving sufficiency in all women and their neonates regardless of race (Hollis et al., 2011).

16.5 Summary and conclusion

We have presented what we believe is compelling evidence of a global problem of poor vitamin D status experienced in utero and perinatally; and even more critical, is the evidence showing the association of vitamin D deficiency during this dynamic period of development with increased morbidity and mortality risks from respiratory infections. Worldwide recognition of this problem is growing and this awareness is stimulating changes in dietary guidelines (Table 16.1), as well as advice on safe sun exposure (Godar et al., 2012) and strategies for national fortification policies (Babu and Calvo, 2010). Viral and bacterial pneumonia kills more children than any other illness, accounting for 19 per cent of all under five deaths worldwide (UNICEF, 2006). According to the 2006 UNICEF/WHO report, an estimated 26% of neonatal deaths are caused by severe infections during the neonatal period, the majority of which are caused by pneumonia/sepsis. Undernutrition, which includes vitamin D insufficiency/deficiency, has been implicated in 53% of all deaths among children under five (UNICEF, 2006). If vitamin D intervention currently under study in the clinical trials described in this chapter is proven successful, then implementation of new fortification practices, revised guidelines for healthy sun exposure and public health programs for vitamin D supplementation of pregnant/lactating women and their infants may be effective strategies to aid in preventing neonates and children under five from developing respiratory infections. Globally, there is potential to save more than a million young lives with preventive treatment, a compelling reason why the efficacy of optimizing vitamin D-mediated defense against respiratory pathogens in infants and children merits further study.

Disclaimer

The findings and conclusions presented in this review are those of the authors and do not necessarily represent the views, opinions or policies of the US Food and Drug Administration.

References

16. Vitamin D and respiratory infections in infants and toddlers


16. Vitamin D and respiratory infections in infants and toddlers


Hollis, B.W. and Wagner, C.L., 2004b. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. American Journal of Clinical Nutrition 80, 1752S-1758S.


16. Vitamin D and respiratory infections in infants and toddlers


Key facts

- Vitamin D deficiency is frequent among HIV-infected persons.
- HIV disease or treatment-related factors have been associated with vitamin D deficiency.
- Vitamin D deficiency has been associated with unfavorable HIV disease evolution.
- Causal relationships should be explored in controlled trials.
- Vitamin D supplementation seems effective and safe in HIV-infected persons.

Summary points

- Vitamin D deficiency has been associated, in the general population with conditions that are becoming of concern in HIV-infected persons, which recently generated considerable interest in vitamin D and HIV infection.
- Although vitamin D deficiency does not generally appear more frequent in HIV-infected persons, specific disease-related factors (immune deficiency) and the use of certain antiretroviral drugs have been associated with it in this population.
- Vitamin D deficiency has been associated with long term complications and comorbidities of treated HIV-infection.
- Vitamin D deficiency has been associated with a pejorative evolution in both untreated and treated HIV infection.
- Establishing vitamin D deficiency as a new, modifiable cofactor in HIV disease evolution, requires interventional studies.
17. Vitamin D in HIV/AIDS: a role?

J.-P. Viard\textsuperscript{1} and J.-C. Souberbielle\textsuperscript{2}

\textsuperscript{1}Unité Fonctionnelle de Thérapeutique en Immuno-infectiologie, Centre de Diagnostic et de Thérapeutique, Hôtel-Dieu, 1 Place du Parvis Notre-Dame, 751781 Paris Cedex 04, France; 
\textsuperscript{2}Laboratoire d'explorations fonctionnelles, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75783 Paris Cedex 15, France; jean-paul.viard@htd.aphp.fr

Abstract

Vitamin D deficiency is frequent in the general population, where it has been associated with a variety of conditions. While it does not seem to be more frequent in persons living with HIV, factors related to HIV disease or its treatment probably contribute to vitamin D deficiency in this population. Two studies, one in an African, untreated, population, and one in a European population largely treated with antiretrovirals, show that vitamin D deficiency is associated with the onset of clinical events and all-cause mortality in persons living with HIV. The role of vitamin D in T cell activation and the links established between vitamin D deficiency, inflammation, and coagulation activation could explain the clinical associations. These hypotheses should be tested in intervention studies.

Keywords: HIV infection, vitamin D deficiency, antiretroviral drugs, all-cause mortality, vitamin D supplementation
17.1 Introduction

Vitamin D is now extensively studied for its extra-osseous effects. $1,25(\text{OH})_2\text{D}$ is a steroid hormone that binds a cytosolic receptor regulating gene function in many different cell types, including innate and adaptive immunity cells. Mice in which the VDR gene has been invalidated present with multiple dysfunctions (Bouillon et al., 2008) and, in humans, vitamin D deficiency has been epidemiologically associated, in the general population, with a wide variety of conditions: infections (particularly tuberculosis), cancers (particularly breast and colon cancers), coronary artery disease and hypertension, insulin resistance and diabetes, and autoimmune diseases (Souberbielle et al., 2010). In addition, in older persons or in patients with chronic condition (diabetes, high cardiovascular risk, cancer, heart transplant, renal failure, etc.) vitamin D deficiency has been associated with a higher risk of all-cause or cardiovascular death (Dobnig et al., 2008).
Considering first the frequency of vitamin D deficiency in the general population, second the frequency of lower bone mineral content in the HIV-infected population (Childs et al., 2011), and third the large overlap between vitamin D-associated conditions (in the general population) and the emerging long-term comorbidities/complications of antiretroviral-treated HIV infection, the question of the relationships between vitamin D status and HIV infection had to be addressed (Giusti et al., 2011). Five main issues can be raised: (1) What is the frequency of vitamin D deficiency in HIV-infected persons? (2) Are there specific disease- or treatment-related factors associated with vitamin D deficiency in HIV-infected persons? (3) Is vitamin D status associated with HIV disease course or complications? (4) If yes, what could be the reasonable explanations and testable hypotheses to demonstrate causal relationships? (5) Is vitamin D supplementation warranted in persons living with HIV?

### 17.2 Vitamin D insufficiency in HIV-infected persons

Vitamin D insufficiency or deficiency is widespread in the general population, and its prevalence has augmented over the last decade, in men and women, across age and ethnic categories, which has been mainly explained by changes in life style and by the increased prevalence of obesity. In the US, the prevalence of severe deficiency (25(OH)D <10 ng/ml) has increased from 2% to 6% from 1988-1994 to 2001-2004 (Ginde et al., 2009). In Europe, general population studies found severe deficiency in 2-30% of adults (Mithal et al., 2009). In cross-sectional studies of HIV-infected persons, the prevalence of vitamin D insufficiency (25(OH)D <30 ng/ml) varied from 39.3% in Tanzania (Mehta et al., 2010) to 93% in France (Legeai et al., 2012); the prevalence of levels <20 ng/ml varied from 26.8% in Thailand (Wiboonchutikul et al., 2012) to 67% in France (Legeai et al., 2012); and the prevalence of severe deficiency varied between 0% in Hawaii (Shikuma et al., 2012), and 42% in Switzerland during Spring (Mueller et al., 2010).

Studies have compared 25(OH)D levels in HIV-infected and HIV-negative persons (Table 17.1). Studies in the US found that vitamin D deficiency was not more prevalent in HIV-positive patients than in control populations. For example, the prevalence of a 25(OH)D level below 30 ng/ml and below 20 ng/ml were comparable in the SUN cohort (HIV-infected persons) and in the NHANES study (general population) after adjusting for age, gender and race: 70.3% vs. 79.1% and 29.7% vs. 38.8%, respectively (Dao et al., 2011). Vitamin D deficiency therefore appeared rather less prevalent in HIV-infected persons. This could be explained by a smaller body mass index in HIV-infected patients, or by the fact that persons belonging to certain HIV transmission groups may have life styles leading to more sun exposure, or by the fact that being under care for HIV infection may drive people to pay more attention to nutritional efforts, particularly in countries where dairy products are enriched in vitamin D. Two studies, one from Spain (Cervero Jimenez et al., 2012) and one from India (Paul et al., 2010), found vitamin D deficiency to be more prevalent in HIV-infected persons.

Patients enrolled in these studies (Table 17.1) were largely receiving antiretroviral drugs. Fewer studies have been published in untreated HIV-infected persons. Indications can be found in...
Table 17.1. Prevalence of vitamin D deficiency in cross-sectional studies of HIV-infected persons vs. HIV-negative controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Number of participants</th>
<th>HIV-positive</th>
<th>HIV-negative</th>
<th>25(OH)D thresholds</th>
<th>Participants with vitamin D deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HIV-positive</td>
<td>HIV-negative</td>
<td>Mean:</td>
<td>HIV-positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross <em>et al.</em>, 2011</td>
<td>USA (Ohio)</td>
<td>149</td>
<td>100%</td>
<td>39</td>
<td></td>
<td>Mean: 52.1±1.04 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>On antiretrovirals</td>
<td></td>
<td></td>
<td>21%</td>
</tr>
<tr>
<td>Milazzo <em>et al.</em>, 2011</td>
<td>Italy</td>
<td>144 with HIV, 93 with HIV/HCV</td>
<td>100%</td>
<td>76, age, gender and BMI-matched</td>
<td>&gt;30ng/ml</td>
<td>Mean: 63.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;20 ng/ml</td>
<td>Mean: 7.46%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;10 ng/ml</td>
<td>Mean: 70.3%</td>
</tr>
<tr>
<td>Dao <em>et al.</em>, 2011</td>
<td>USA (different states)</td>
<td>672</td>
<td>89.9%</td>
<td></td>
<td>&lt;10-30 ng/ml</td>
<td>29.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;10 ng/ml</td>
<td>Mean: 71.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;30 ng/ml</td>
<td>Mean: 44.0%</td>
</tr>
<tr>
<td>Cervero Jimenez <em>et al.</em>, 2012</td>
<td>Spain</td>
<td>352</td>
<td>89.5%</td>
<td>116 (prior study)</td>
<td>&lt;20 ng/ml</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;30 ng/ml</td>
<td>Mean: 11%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11-29 ng/ml</td>
<td>45%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 ng/ml</td>
<td>Mean: 36%</td>
</tr>
<tr>
<td>Rutstein <em>et al.</em>, 2011</td>
<td>USA (PA)</td>
<td>81 children (mean age: 13.8±4.1)</td>
<td>84%</td>
<td>372 healthy children (mean age: 12.4±3.4)</td>
<td>&lt;30 ng/ml</td>
<td>11%</td>
</tr>
<tr>
<td>Stein <em>et al.</em>, 2011</td>
<td>USA (NY)</td>
<td>89 post-menopausal African American and Hispanic women</td>
<td>80%</td>
<td>94 post-menopausal African American and Hispanic women</td>
<td>&lt;30 ng/ml</td>
<td>74.0%</td>
</tr>
<tr>
<td>Adeyemi <em>et al.</em>, 2011</td>
<td>USA (different states)</td>
<td>1,268 women (WIHS study)</td>
<td>72%</td>
<td>510 women (WIHS study)</td>
<td>&lt;20 ng/ml</td>
<td>60.0%</td>
</tr>
</tbody>
</table>
### Table 17.1. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Number of participants</th>
<th>HIV-positive</th>
<th>HIV-negative</th>
<th>25(OH)D thresholds</th>
<th>Participants with vitamin D deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HIV-positive</td>
<td>HIV-negative</td>
<td></td>
<td>HIV-positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number on anti-retrovirals</td>
<td></td>
<td>On anti-retrovirals</td>
<td>&lt;32 ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;20 ng/ml</td>
<td>69%</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;10 ng/ml</td>
<td>30%</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;20 ng/ml</td>
<td>treated: 74%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>naive: 37%</td>
<td>37%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;30 ng/ml</td>
<td>77.4%</td>
<td>96.2%</td>
<td></td>
</tr>
<tr>
<td>Yin et al., 2010</td>
<td>USA (different states)</td>
<td>82 pre-menopausal women</td>
<td>56%</td>
<td>58 pre-menopausal women</td>
<td>&lt;32 ng/ml</td>
<td>91%</td>
</tr>
<tr>
<td>Paul et al., 2010</td>
<td>India</td>
<td>70 men</td>
<td>50%</td>
<td>35 matched men</td>
<td>&lt;20 ng/ml</td>
<td>69%</td>
</tr>
<tr>
<td>Ornesher et al., 2011</td>
<td>USA (DC)</td>
<td>199 African-American men</td>
<td>Not reported</td>
<td>424 matched African-American men (NHANES Study)</td>
<td>&lt;30 ng/ml</td>
<td>77.4%</td>
</tr>
</tbody>
</table>
a study of untreated Tanzanian women (Mehta et al., 2010) and in baseline data of cohorts or trials initiating antiretroviral therapy (Table 17.2). These data are important because antiretroviral drugs may have an impact on vitamin D metabolism.

17.3 Factors associated with vitamin D insufficiency in HIV-infected persons

Although vitamin D deficiency does not generally appear more prevalent in HIV-infected persons than in the general population, specific disease- or treatment-related factors have been associated with low 25(OH)D levels in this group, in addition to classical risk factors.

Studies (generally multivariate analyses) do not all agree on the factors associated with lower 25(OH)D levels in HIV-infected persons, some of them even present contradictory results. The following traditional risk factors have been evidenced in several studies: older age (Dao et al., 2011; Rutstein et al., 2011; Viard et al., 2011), black ethnicity and season of sampling (Adeyemi et al., 2011; Brown et al., 2010; Cervero Jimenez et al., 2012; Conesa-Botella et al., 2010; Choi et al., 2011; Crutchley et al., 2011; Dao et al., 2011; Kim et al., 2011; Kwan et al., 2012; Mueller et al., 2010; Rutstein et al., 2011; Shikuma et al., 2011; Stein et al., 2011; Van den Bout et al., 2008; Vescini et al., 2011; Viard et al., 2011; Welz et al., 2010), geographical region (Viard et al., 2011). In line with what has been observed in the general population, one study found recent samples to be more at risk for low 25(OH)D than older samples (Viard et al., 2011). In a majority of studies, higher body mass index was associated with higher odds of vitamin D deficiency (Adeyemi et al., 2011; Cervero Jimenez et al., 2012; Crutchley et al., 2011; Choi et al., 2011; Dao et al., 2011), but one found an association with higher 25(OH)D levels. This discrepancy may reflect a complex relationship between 25(OH)D levels, adipose tissue volume and the interference of some antiretrovirals with adipocyte metabolism (lipodystrophy syndrome). Among HIV-related factors, the route of HIV transmission (namely non homosexual, or intravenous drug use) (Mueller et al., 2010; Viard et al., 2011), the current CD4 count or the CD4 count nadir (i.e. the lowest value ever reached by a given individual) (Adeyemi et al., 2011; Kwan et al., 2012; Rutstein et al., 2011; Shikuma et al., 2011; Stein et al., 2011) and the exposure to antiretroviral treatment (Allavena et al., 2012; Paul et al., 2010; Welz et al., 2010) have been reported. Contradictory results exist as regards the association with detectable (Cervero Jimenez et al., 2011; Kim et al., 2011; Kwan et al., 2012) vs. undetectable (Adeyemi et al., 2011) plasma HIV RNA viral load, which may be related to treatment history.

It should be noted that the ‘factors associated with vitamin D deficiency’ or ‘predictors of vitamin D deficiency’ reported in these studies may in fact fall into two categories: some of these factors (e.g. anthropometric, sociodemographic and geographic characteristics, antiretroviral treatment use) are plausible explanatory factors for low 25(OH)D levels, from a mechanistic point of view, while others (i.e. current or nadir CD4 count, viral load) could either be explanatory factors on the path to vitamin D deficiency or a consequence thereof. In the latter category, metabolic parameters and end-organ abnormalities, will be discussed below.
Table 17.2. Vitamin D deficiency in antiretroviral-naïve HIV-infected persons, longitudinal studies and clinical trials.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>Country</th>
<th>Number of participants</th>
<th>Antiretroviral-naïve at baseline</th>
<th>Antiretroviral regime</th>
<th>25(OH)D thresholds</th>
<th>Participants with vitamin D deficiency at baseline</th>
<th>Evolution of 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown and McComsey, 2010</td>
<td>Longitudinal</td>
<td>USA (Ohio)</td>
<td>87</td>
<td>100%</td>
<td>EFV-based (n=51);</td>
<td>&lt;37.5 nmol/l</td>
<td>33%</td>
<td>Decrease in EFV group:</td>
</tr>
<tr>
<td></td>
<td>(6-12 months)</td>
<td></td>
<td></td>
<td>non-EFV-based (n=36)</td>
<td></td>
<td></td>
<td></td>
<td>-12.7 (IQR: 20; 2.7) nmol/l</td>
</tr>
<tr>
<td>Conesa-Botella et al., 2010</td>
<td>Longitudinal</td>
<td>Belgium</td>
<td>87</td>
<td>100%</td>
<td>NNRTI-based (n=43;</td>
<td>&lt;30 ng/ml</td>
<td>70.1%</td>
<td>Stable in non-EFV group:</td>
</tr>
<tr>
<td></td>
<td>(12 months)</td>
<td></td>
<td></td>
<td>EFV=20, NVP=23);</td>
<td></td>
<td></td>
<td></td>
<td>1.0 (IQR: 10.2; 14.5) nmol/l</td>
</tr>
<tr>
<td>Latuuda et al., 2009</td>
<td>Longitudinal</td>
<td>Italy</td>
<td>18</td>
<td>100%</td>
<td>PI-based (n=44)</td>
<td>&lt;20 ng/ml</td>
<td>43.7%</td>
<td>Decrease of (25)OHD:</td>
</tr>
<tr>
<td></td>
<td>(3 years)</td>
<td></td>
<td></td>
<td>Starting AZT+3TC+NVP</td>
<td></td>
<td></td>
<td></td>
<td>81.6%</td>
</tr>
<tr>
<td>Mueller et al., 2010</td>
<td>Longitudinal</td>
<td>Switzerland</td>
<td>211</td>
<td>100%</td>
<td>Mixed</td>
<td>&gt;75 nmol/l</td>
<td>5% (spring)</td>
<td>No significant evolution (means):</td>
</tr>
<tr>
<td></td>
<td>(18 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>baseline: 52.0±28.1 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>year 3: 66.1±21.0 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No change on HAART:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10% (spring)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24% (autumn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23% (autumn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30-74 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>53% (spring)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38% (spring)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62% (autumn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59% (autumn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;30 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42% (spring)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52% (spring)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14% (autumn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18% (autumn)</td>
</tr>
<tr>
<td>Rockstroh et al., 2010</td>
<td>Clinical trial</td>
<td>Europe</td>
<td>157</td>
<td>100%</td>
<td>2 nucleosides plus</td>
<td>&lt;50 nmol/l</td>
<td>26%</td>
<td>Mean 25(OH)D at</td>
</tr>
<tr>
<td></td>
<td>(24 weeks)</td>
<td></td>
<td></td>
<td>etravirine or EFV</td>
<td></td>
<td></td>
<td></td>
<td>week 0 and 24:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;25 nmol/l</td>
<td>2.5%</td>
<td>EFV: 62.4 and 41.4 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ETV: 64.7 and 44.4 nmol/l</td>
</tr>
</tbody>
</table>
### Table 17.2. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>Country</th>
<th>Number of participants</th>
<th>Antiretroviral-naïve at baseline</th>
<th>Antiretroviral regime</th>
<th>25(OH)D thresholds</th>
<th>Participants with vitamin D deficiency at baseline</th>
<th>Evolution of 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wohl et al., 2011</td>
<td>Clinical trial</td>
<td>World</td>
<td>582</td>
<td>100%</td>
<td>Tenoforv/</td>
<td>&lt;75 nmol/l</td>
<td>67.0%</td>
<td>Week 48 decrease:</td>
</tr>
<tr>
<td></td>
<td>(48 weeks)</td>
<td></td>
<td></td>
<td></td>
<td>emtricitabine plus EFV</td>
<td>25-50 nmol/l</td>
<td>24.7%</td>
<td>EFV: -6.2±18.0 nmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>or RPV</td>
<td>&lt;25 nmol/ml</td>
<td>4.6%</td>
<td>RPV: -0.6±17.9 nmol/l</td>
</tr>
<tr>
<td>Van Vonderen et al., 2009</td>
<td>Clinical trial</td>
<td>Europe, Russia,</td>
<td>48</td>
<td>100%</td>
<td>AZT/3TC/RTV/ LPV/RTV</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No change over time in either group</td>
</tr>
<tr>
<td>Legeai et al., 2012</td>
<td>Cohort</td>
<td>Israel, France</td>
<td>355</td>
<td>100%</td>
<td>or NVP/ LPV /RTV Mixed</td>
<td>Not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;30 ng/ml</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;20 ng/ml</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;10 ng/ml</td>
<td>24%</td>
<td></td>
</tr>
</tbody>
</table>
The possible impact of antiretroviral drugs on vitamin D metabolism is an intriguing issue, with some conflicting data between in vitro and in vivo studies. Several reports, including longitudinal studies (Table 17.2), have shown an independent association of lower or decreasing 25(OH)D concentrations with the exposure to EFV, and not NVP (Lattuada et al., 2009) or ETV (Rockstroh et al., 2010) or RPV (Wohl et al., 2011), all four drugs belonging to the NNRTI class. All studies, including longitudinal studies, have shown a neutral, or even protective effect of PI use on vitamin D deficiency, as reflected by blood 25(OH)D levels. One study (Fox et al., 2010) has suggested that being on the nucleosidic reverse transcriptase inhibitor (NRTI) zidovudine was also a risk factor for deficiency, and that stopping this drug was associated with increasing 25(OH)D with time, just as was the case with EFV.

Why should antiretrovirals influence 25(OH)D levels? Several of them are substrates of, and have an impact on, the CYP450 enzyme complex, particularly on the CYP3A4 isoenzyme (Tseng and Foisy, 2012). For instance, NNRTIs are CYP3A4 inducers, while PIs are CYP3A4 inhibitors. This is especially true for the PI RTV, probably the most potent CYP3A4 inhibitor among licensed drugs, which is used at a low, virologically inefficient, dosage as a pharmacological booster for the other PIs. The metabolism of vitamin D relies on hydroxylations through CYP450 enzymes (Holick, 2007): activation through 25- and 1α-hydroxylations (particularly by CYP2R1, CYP27B1), and inactivation through 24- and 4-hydroxylations (by CYP24 and CY3A4). PIs and NNRTIs could therefore modify vitamin D metabolism, the hypothesis being that PIs would inhibit vitamin D activation and NNRTIs would increase its inactivation, since CYP3A4 has been shown to play a direct role in 24-and 4-hydroxylation (Wang et al., 2012; Yang et al., 2006).

As shown in Table 17.3, it has indeed been shown that ‘first generation’ PIs (IDV, NFV, RTV) were able to impair 25(OH)D3 synthesis in hepatocyte and 1,25(OH2D3 synthesis in monocyte cell line cultures, in a reversible, dose-dependent manner (Cozzolino et al., 2003). PIs also inhibited 24-hydroxylation in macrophages, but with a lower potency than that shown on 1α-hydroxylation, resulting in a net reduced 1,25(OH)D3 production in these cells. It has also been shown that, among NNRTIs, both EFV and NVP were able to increase expression of the VDR and the vitamin D-induced upregulation of the CYP24 gene, in human renal clear-cell carcinoma cells (Landriscina et al., 2008). EFV was also shown to suppress the expression of CYP2R1 in fibroblasts (Ellfolk et al., 2009) (Table 17.4).

Data on 1,25(OH)2D are few but they may provide insight in the impact of antiretrovirals on vitamin D metabolism. In the Swiss cohort study, no correlation with NNRTI use was found, but tenofovir use was correlated with higher 1,25(OH)2D, as was body mass index, while HCV seropositivity, previous AIDS and high CD4 counts were correlated with lower 1,25(OH)2D (Mueller et al., 2010). To date, no study examining the effects of antiretroviral drugs on the levels of vitamin D inactive metabolites (e.g. 24- or 4-hydroxylated forms) has been published. This would be of high interest, since there is some discrepancies between the few in vitro studies and the already numerous clinical studies, only based on 25(OH)D measurement (e.g. effect of PIs, differences between NNRTIs).
Table 17.3. Documented effects of antiretroviral drugs on vitamin D metabolism in vitro.

<table>
<thead>
<tr>
<th>Study</th>
<th>Drugs tested</th>
<th>Cell model</th>
<th>Effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cozzolino et al., 2003</td>
<td>RTV, IDV, NFV</td>
<td>THP-1 monocyte cell line-derived macrophages</td>
<td>Inhibition of 1α-hydroxylase (RTV, IDV, NFV)</td>
<td>EFV shows no effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibition of 24-hydroxylase (RTV, UDV, NFV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net effect: decreased 1,5(OH)2D in macrophages</td>
<td></td>
</tr>
<tr>
<td>Landriscina et al., 2008</td>
<td>EFV, NFV</td>
<td>Hep3B hepatocyte cell line</td>
<td>Inhibition of 25-hydroxylase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clear-cell renal carcinoma primary culture</td>
<td>Upregulation of VDR and calbindin 28k genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Enhanced expression of CYP24 in response to vitamin D</td>
<td></td>
</tr>
<tr>
<td>Ellfolk et al., 2009</td>
<td>EFV</td>
<td>BJ dermal fibroblast cell line</td>
<td>60% decrease of CYP2R1 mRNA</td>
<td>No effect on CYP27A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LNCaP prostate cancer cell line</td>
<td>30% reduction of 1,25(OH)D synthesis</td>
<td>EFV and 1,25(OH)2D had similar effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25% decrease of CYP2J2 mRNA</td>
<td>No pregnancy X receptor expression on</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LNCap, may explain differences with BJ</td>
</tr>
</tbody>
</table>

Table 17.4. Incidence rate of events according to 25(OH)D tertile (ng/ml) at baseline in 1,985 patients from the EuroSIDA Cohort (per 100 PY of follow-up) (Viard et al., 2011).

<table>
<thead>
<tr>
<th>Events</th>
<th>Total</th>
<th>25(OH)D≤12</th>
<th>12&lt;25(OH)D≤20</th>
<th>25(OH)D&gt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS-defining events</td>
<td>No. of events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>159</td>
<td>73</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>PY 11,720</td>
<td>3,737</td>
<td>3,654</td>
<td>4,329</td>
</tr>
<tr>
<td></td>
<td>Incidence (95% CI)</td>
<td>1.36 (1.15-1.87)</td>
<td>1.95 (1.51-2.40)</td>
<td>1.07 (0.73-1.40)</td>
</tr>
<tr>
<td>All-cause deaths</td>
<td>No. of events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>188</td>
<td>87</td>
<td>47</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>PY 12,225</td>
<td>3,963</td>
<td>3,780</td>
<td>4,482</td>
</tr>
<tr>
<td></td>
<td>Incidence (95% CI)</td>
<td>1.54 (1.32-1.76)</td>
<td>2.20 (1.73-2.66)</td>
<td>1.24 (0.89-1.60)</td>
</tr>
</tbody>
</table>
17. Vitamin D in HIV/AIDS

The impact of tenofovir, a NRTI has also been examined because it can induce proximal tubulopathy with an increased urinary excretion of phosphorus, which could in turn modify the metabolism of vitamin D. Exposure to tenofovir has not been found to be a risk factor for lower 25(OH)D levels, and some studies even suggested a protective role (Allavena et al., 2012; Dao et al., 2011). There seems to be a complex interaction between tenofovir exposure and vitamin D deficiency. Persons on tenofovir who also have vitamin D deficiency are those showing the highest alkaline phosphatase and PTH levels (Childs et al., 2010; Pocaterra et al., 2011; Rosenvinge et al., 2010). Vitamin D supplementation can lower PTH levels in persons on tenofovir, more efficiently than in those receiving other NRTIs (Havens et al., 2012).

17.4 Vitamin D and long-term non-AIDS defining complications and comorbidities

Due to the efficacy of antiretroviral combinations, HIV infection has become a chronic disease characterized by a life-long treatment and by the emergence of conditions that are considered as age-related in the general population, such as non AIDS-defining cancers (e.g. lung cancer), metabolic disorders (hyperlipemia, insulin resistance and diabetes), cardiovascular disease, osteopenia and osteoporosis, renal impairment, neurocognitive disorders, etc. These conditions appear with a higher incidence, and at an earlier age, in the HIV-infected population than in the general population. This has led to seeing HIV-infected persons as experiencing an ‘accelerated aging’ phenomenon. It is not easy to define the respective responsibility of harbouring the retrovirus (even on antiretrovirals) and of being exposed to drugs (with a toxic potential) for decades, in the pathogenesis of these complications and comorbidities. However, since vitamin D deficiency has been epidemiologically associated with many of these conditions in the general populations, it is no surprise that studies were performed to test whether these associations also hold true in HIV-infected persons.

As reviewed in 2011 (Childs et al., 2011), osteopenia (BMD T-score between -1 and -2.5) and osteoporosis (T-score<-2.5) are frequent in persons living with HIV: they were found respectively in 52% and 15% of patients in a meta-analysis including studies published up to 2005 (Brown and Qaqish, 2006). In a French study that carefully excluded patients with a possible cause for secondary BMD loss (Mary-Krause et al., 2009), among 700 men and 192 women, a T-score ≤2.5 was found in 7.9% of men and 1.1% of women, with a median age of 46 years (IQR, 41-53). In these studies, classical risk factors for bone loss (low body mas index, older age, glucorticoid use) were found to be predictors, but some HIV-related characteristics also emerged, such as duration of HIV infection, CD4 count nadir, previous AIDS-defining conditions, and antiretroviral use (particularly PI use and recent tenofovir exposure). Although not clearly associated with low BMD in all cross-sectional studies (Childs et al., 2011), lower 25(OH)D has been associated with lower hip BMD at baseline and with greater BMD reduction at the femoral neck during prospective follow-up (Dolan et al., 2006; Yin et al., 2010).
Preliminary results in HIV-infected persons indicate that vitamin D deficiency is associated with type 2 diabetes (Szep et al., 2011) and insulin resistance (Hammond et al., 2012), carotid intima-media thickness (Choi et al., 2011; Ross et al., 2011), arterial dysfunction, namely coronary calcification and flow-mediated vasodilation (Shikuma et al., 2011), proteinuria in injection drug users (Estrella et al., 2012), and with a composite endpoint of non-AIDS defining conditions (chronic kidney disease, diabetes, cardiovascular events, non-AIDS defining malignancies) (Vescini et al., 2011). Vitamin D deficiency has also been associated with the severity of liver fibrosis in HIV/HCV coinfected persons with active chronic hepatitis (Terrier et al., 2011). Few of these studies examined correlations of vitamin D status in HAART-naïve patients, which is a problem because antiretrovirals improve immune functions, decrease inflammation to a certain extent, and because some drugs have a negative impact on inflammation, lipid levels, glucose homeostasis, adipocyte and kidney proximal tubule functions. All this makes it difficult to examine the potential impact of vitamin D deficiency independently of the positive or negative effects of drugs. One large study in antiretroviral naïve patients (Legeai et al., 2012) showed an association of low 25(OH)D with lower CD4 counts, higher inflammation markers and lipid parameters (lower low-density lipoproteins-cholesterol), with noticeable differences between black and white patients. Several studies have found a negative association between 25(OH)D and estimated glomerular filtration rate in HIV-infected persons, treated or not (which demonstrates that this is not related to drug toxicity): this intriguing finding may be due to an increase in creatinine production through VDR activation, without modification of creatinine or iothalamate clearance, as suggested in a study where patients with chronic kidney disease received paricalcitol (Agarwal et al., 2011).

17.5 Vitamin D insufficiency and HIV disease clinical progression

More surprisingly, vitamin D deficiency has been associated with a defavourable evolution of HIV disease itself, both in antiretroviral-naïve patients and in the setting of widespread antiretroviral treatment.

In a secondary analysis of a study performed in 1995-1997 in 884 untreated HIV-infected pregnant Tanzanian women, 25(OH)D was measured at enrolment. A median prospective follow-up of 70 months, without vitamin D supplementation was available for the analysis of events (Mehta et al., 2010). 347 women had a 25(OH)D below 32 ng/ml. Compared to those with a level equal or superior to 32 ng/ml, they showed a higher risk of clinical progression, according to the WHO classification, with a HR of 1.25 (95% CI: 1.05-1.50) and a higher risk of anemia, with a HR of 1.46 (95% CI: 1.09-1.96). Furthermore, having a 25(OH)D level in the highest quintile, compared to the lowest quintile was associated with a lower risk of all-cause death, with a HR of 0.58 (95% CI: 0.40-0.84). Women with low vitamin D also had a higher risk of developing wasting syndrome, oral thrush, and acute upper respiratory tract infections (Mehta et al., 2011). The 25(OH)D level in mothers was also associated with a higher risk of morbidity and mortality in children (Mehta et al., 2009). Those born from mothers with a 25(OH)D level below 32 ng/ml had a higher risk of mother-to-child HIV transmission at 6 weeks (HR: 1.50, 95% CI: 1.02-2.20) and during
breastfeeding (HR: 2.03, 95% CI: 1.08-3.82). They also had a higher risk of reporting cough during follow-up, of stunting and being underweight (Finkelestein et al., 2012), and of all-cause death at 24 months (HR: 1.61, 95% CI: 1.25-2.07).

In the EuroSIDA cohort study (Viard et al., 2011), 25(OH)D was measured in 1,985 patients at enrolment and a median 5-year prospective follow-up was available for the analysis of events. 83% of patients were on antiretroviral treatment. A strong and robust association between having a baseline 25(OH)D level in the lowest tertile (<12 ng/ml) and the occurrence of AIDS-defining events and all-cause deaths was found. Persons with baseline 25(OH)D levels in the middle and higher tertiles had a 40% lower incidence rate ratio for these endpoints, which was statistically significant in a multivariate analysis adjusted for demographic characteristics, calendar year, season, geographical region, CD4 count, HIV ARN plasma load and antiretroviral treatment (Table 17.4).

There are major differences between the two studies mentioned above: untreated vs. treated HIV infection, African vs. European setting, nutritional status, and socio-economic level of persons. This raises the interesting question of the relevant threshold that would define a higher risk of HIV disease clinical evolution: in the Tanzanian study, women with a 25(OH)D just below the ‘optimal’ level appeared at a higher risk, while in the EuroSIDA study, the risk was clearly associated with a severely deficient status. One could hypothesize that in the most vulnerable patients (malnourished, with multiple nutrient deficiencies and with no antiretroviral treatment), mild vitamin D insufficiency is sufficient to accelerate disease evolution, while in a more favourable setting, only severe deficiency becomes a cofactor of disease progression.

It should be emphasized that these results, obtained from observational studies, do not allow to draw any firm conclusion on the causal relationship between vitamin D insufficiency or deficiency and the occurrence of events. However, the results appear striking enough as to view vitamin D deficiency as a possible, hitherto unrecognized, cofactor in HIV disease evolution.

17.6 Testable hypotheses towards establishing causal relationships

In the setting of HIV infection, just like in the general population, the key question is the following: how can vitamin D deficiency be associated with such a wide range of conditions? Two series of hypotheses seem particularly interesting in this context, and could be tested in controlled trials, in order to explore causal relationships.

The first one is the role played by vitamin D in the regulation of immune responses. Vitamin D could be instrumental in first-line, innate immunity against a variety of pathogens, including HIV itself. Thus, a study has clearly shown its role in the induction of autophagy (Campbell et al., 2011). Autophagy is a finely regulated mechanism that is important in the defence against infectious agents, particularly viruses, and which is inhibited by HIV. Active vitamin D induced autophagy in monocyte-derived macrophages, in a dose-dependent manner. Moreover, this was associated
with a reduced infectability of the cells with *Mycobacterium tuberculosis* and HIV. It has been also shown that the vitamin D – VDR system is necessary to the TCR-mediated activation of naïve T cells (Von Essen et al., 2010). One can therefore hypothesize that vitamin D deficiency contributes to the abnormalities of cell-mediated immunity that persist in patients, even when they receive fully suppressive antiretroviral drugs (residual immune activation, immune senescence, etc.). Before the active antiretroviral therapy era, low 1,25(OH)D2 has been associated with immune deficiency and poorer survival (Haug et al., 1994). Vitamin D deficiency has also been associated with a smaller CD4 cell gain on antiretroviral therapy (Ross et al., 2011). Whether vitamin D administration improves innate immune functions or CD4 counts and functions in HIV-infected persons should be tested.

The second series of hypotheses pertains to the association between vitamin D deficiency on the one hand, and, on the other hand, the activation of inflammation and coagulation. In patients addressed for a coronary angiography, vitamin D deficiency was associated with mortality but also with high levels of cell adhesion, oxidative stress and inflammation markers (in particular C-reactive protein and interleukin 6) (Dobnig et al., 2008). In a placebo-controlled study (Schleithoff et al., 2006), vitamin D supplementation allowed a significant reduction in the serum level of TNF-α et and a significant rise of the anti-inflammatory cytokine IL-10. In a large general population cohort, low vitamin D levels were associated with higher levels of markers of pre-clinical activation of coagulation (tissue activator of plasminogen and D-dimers) (Hyppönen et al., 2010). This makes particular sense in the setting of HIV infection, because inflammation (CRP and IL-6) and coagulation activation (D-dimers) markers have been proposed as predictors of mortality and opportunistic diseases in HIV-infected patients (Kuller et al., 2008, Rodger et al., 2009). Whether vitamin D administration can lower the level of these and other biomarkers of immune activation, inflammation, and coagulation, should also be tested.

### 17.7 Vitamin D supplementation in HIV-infected persons

A study of 483 HIV-infected patients (93% on antiretrovirals) tested vitamin D supplementation (Pacanowski et al., 2012): median baseline 25(OH)D was 19 ng/ml (IQR: 13-26), 25(OH)D was <30 ng/ml in 81% and <10 ng/ml in 12% of persons, and vitamin D3 (100,000 IU) was administered monthly. After 4 months, median 25(OH)D rised to 36 ng/ml (IQR: 28-43) and 70% of patients reached a 25(OH)D level in the desirable range (≥30 ng/ml). Risk factors for a persistant suboptimal level (<30 ng/ml) at month 4 were a low actual supplementation dosage (<300,000 IU), a low 25(OH)D baseline level (<30 ng/ml), non-white ethnicity, a high body mass index (>25), and being on the NRTI zidovudine. Vitamin D supplementation was well tolerated with no report of hypercalcemia.

Foissac et al. (2012) performed a population pharmacokinetic study, modeling optimal supplementation from observational data of 422 HIV-infected patients starting with a median 25(OH)D of 16 ng/ml (range: 11-23). In order to reach a 25(OH)D level in the 30 to 150 ng/ml
range in 90% of patients, they proposed a dosing scheme of 100,000 IU every month. This scheme resulted in less than 1% of patients above 100 ng/ml 25(OH)D.

17.8 Conclusions

The ultimate question of interest to all clinicians involved in HIV-infected patients care is: ‘should patients with vitamin D deficiency be supplemented?’ In our opinion the answer to this difficult question is twofold.

On the one hand, there is no definitive proof that vitamin D deficiency is causal in the complications of HIV disease. In consequence, with the exception of bone mineral density abnormalities, for which the scheme defined in the general population can reasonably be superimposed to HIV-infected persons, there is no hard evidence that vitamin D supplementation will improve health outcomes in this population.

On the other hand, vitamin D deficiency is frequent in people living with HIV and they resemble, in many aspects, persons weakened by age or underlying conditions such as diabetes, high vascular risk, chronic kidney disease, etc, all settings were vitamin D deficiency has been associated with a higher risk of complications and mortality. Therefore, without any proof of causal relationships, and without any 25(OH)D target value linking vitamin D status with the onset of specific outcomes, we would recommend supplementation, without prior measurements (except when bone disease is diagnosed). According to practice, availability of vitamin D formulations, and patients’ preferences, daily doses of vitamin D2 or 3 around 3,000 UI/day or monthly doses of vitamin D3 around 100,000 IU (possibly after a more intense dosing during the first 6 weeks in patients with severe deficiency) seem adequate.

References


17. Vitamin D in HIV/AIDS


17. Vitamin D in HIV/AIDS


Key facts

- The importance of sunlight has been noted throughout the history of tuberculosis treatment. Solar ultraviolet B radiation is the primary source of vitamin D for most people living on earth.
- Tuberculosis is a major global health problem and often coincides with nutritional deficiency.
- Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors.
- An association between vitamin D deficiency and tuberculosis has been reported in a number of studies.
- High doses of vitamin D were widely used to treat active tuberculosis before the emergency of antibiotics.

Summary points

- Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis.
- Genetic studies provide an excellent opportunity to link molecular variations, such as polymorphisms, with subtle biological effects. Host genetic factors are important determinants of susceptibility to tuberculosis.
- In a meta-analysis study, low vitamin D levels were associated with higher risk of active tuberculosis.
- Bacillus Calmette Guérin (BCG) vaccination was developed as a vaccine to provide protection against tuberculosis.
- BCG-vaccinated infants are almost 6 times more likely to have sufficient vitamin D concentrations than unvaccinated infants 3 months after BCG vaccination, and this association remained strong even after adjusting for season, ethnic group and sex.
- Clinical and radiological improvements were more evident in children with tuberculosis who were taking vitamin D supplements compared to controls.
- Vitamin D supplementation may be beneficial to individuals with insufficient vitamin D levels.
18. Vitamin D and tuberculosis

K. vinh quốc Lương and L.T.H. Nguyễn
Vietnamese American Medical Research Foundation, 14971 Brookhurst St., Westminster, CA 92683, USA; lng2687765@aol.com

Abstract

Tuberculosis is a major global health problem and often coincides with nutritional deficiency. Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors. Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis. Genetic factors, such as mutations (or deletion) in the vitamin D receptor (VDR), major histocompatibility complex regions, chromosome 20, Toll-like receptors, natural resistance-associated macrophage protein 1 and nucleotide-binding oligomerization domain containing proteins 2, contribute to both vitamin D status and tuberculosis. The role that vitamin D plays in tuberculosis has also been demonstrated by its effects on Bacillus Calmette Guérin, vascular endothelial growth factor, matrix metalloproteinases, prostaglandins, reactive oxygen species, and reactive nitrogen intermediates, in addition to its synergistic effects with some anti-tuberculosis medications. Vitamin D plays a definite role in tuberculosis. Vitamin D itself may affect tuberculosis through the VDRs or may influence tuberculosis through indirect effects.

Keywords: vitamin D, calcitriol, tuberculosis, mycobacteria
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)₂D₃</td>
<td>1,25-dihydroxyvitamin D₃</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacillus Calmette Guérin</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D-binding protein</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>IL-12</td>
<td>Interleukin-12</td>
</tr>
<tr>
<td>INF-α</td>
<td>Interferon-alpha</td>
</tr>
<tr>
<td>iNOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug-resistant</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>MyD88</td>
<td>Myeloid differentiation factor 88</td>
</tr>
<tr>
<td>NOD2</td>
<td>Nucleotide-binding oligomerization domain containing proteins 2</td>
</tr>
<tr>
<td>NRAMP1</td>
<td>Natural resistance-associated macrophage protein 1</td>
</tr>
<tr>
<td>PGES</td>
<td>Prostaglandin E synthase</td>
</tr>
<tr>
<td>PGs</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>PZA</td>
<td>Pyrazinamide</td>
</tr>
<tr>
<td>RNI</td>
<td>Reactive nitrogen intermediates</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF-γ</td>
<td>Tumor necrosis factor-gamma</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>

### 18.1 Introduction

Cell-mediated immunity is crucial to the host response to infection with *Mycobacterium tuberculosis* (Lowrie and Andrew, 1988; Rook, 1988a). Upon infection with *M. tuberculosis*, T-cells secrete Th1 cytokines (e.g. INF-γ) and activate macrophages, which enhance the host’s ability to kill *M. tuberculosis* and achieve immune protection (Chang-hong *et al.*, 2008). In contrast, Th2 cytokines can reduce macrophage bactericidal activity of macrophages and weaken the immune response to *M. tuberculosis* (Seah *et al.*, 2000). Moreover, the Th1 cytokine INF-γ up-regulates TLR 2/1 induction of 25-hydroxyvitamin D₃-1α-hydroxylase, leading to the conversion of 25(OH)D to its active metabolite 1,25(OH)₂D₃. In contrast, Th2 cytokines induce the catabolism of 25(OH)D to the inactive metabolite 24,25(OH)₂D (Edfeldt *et al.*, 2010). In a mouse model of tuberculosis, Th1 cells accumulation and the activation of myeloid cells were noted in *M. tuberculosis*-infected lungs, but a decreased accumulation of Th1 cells on day 21 was also noted (Kang *et al.*, 2011). The production of Th2 cytokines increases as a model of...
active pulmonary tuberculosis progresses and worsens, but Th2 cytokine levels decrease when the disease is cured (North, 1988). Patients with MDR display impaired Th1 responses (Geffner et al., 2009) and suppressed Th1 and Th2 cell activation (Tan et al., 2012). On the other hand, calcitriol has some paradoxical effects on host resistance to infection. Treating tuberculosis with calcitriol is associated with decreased Th1 mediated immunity but increased bactericidal activity and tuberculosis clearance in infected human macrophages (Cantorna et al., 2008). Vitamin D supplements influence the development of a Th2 response in murine allergic airway disease (Matheu et al., 2003). Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* (Kreindler et al., 2010). Calcitriol also has anti-inflammatory effects on a Th2-dependent asthma model (Topilski et al., 2004). Furthermore, malnutrition is known to suppress immunity (Good, 1981), and a possible link between vitamin D deficiency and impaired host defense against *M. tuberculosis* has been suggested (Davies, 1985; Lương and Nguyễn, 2011). In the present paper, we will review the role of vitamin D in tuberculosis.

### 18.2 Risk factors for the development of both vitamin D deficiency and tuberculosis

Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors. It has been reported that vitamin D deficiency and tuberculosis share many of the same risk factors, including both environmental and genetic risk factors.

#### 18.2.1 Environmental factors

Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis.

**Tobacco smoking**

Smoking is a major health hazard that exhibits side effects on many organs. Tobacco users are at risk for low bone mineral density, decreased intestinal calcium absorption, and low 25(OH)D plasma levels (Jorde et al., 2005; Krall and Dawson-Hughes, 1999; Lorentzon et al., 2007; Need et al., 2002; Supería et al., 2006). The newborns of smoking mothers have significantly lower anthropometric measurements and serum 25(OH)D levels (Diaz-Gómez et al., 2007). In addition, the VDR *TaqI TT* polymorphism was moderately associated with both the presence and the progression of periodontitis in smokers, while no association was detected in non-smoking individuals (Nibali et al., 2008). On the other hand, both passive and active exposure to tobacco smoke were associated with susceptibility to tuberculosis (Basu et al., 2011; Jee et al., 2009; Leung et al., 2010; Tachfouti et al., 2011; Wang et al., 2009), but this risk was substantially reduced when individuals quit smoking (Awaisu et al., 2011; Wen et al., 2010). Evidence from meta-analysis studies suggested an association between smoking and tuberculosis (Bates et al., 2007; Slama et al., 2007). In addition, the association between active exposure to tobacco smoke and tuberculin
activity has been reported in many studies (Anderson et al., 1997; Nisar et al., 1993). Tuberculosis patients who are smokers are more likely to have more cavity lesions (Abal et al., 2005; Trifunović et al., 2009), and an increased the risk of morbidity and mortality were observed in tuberculosis patients who smoked (Jiang et al., 2009; Racil et al., 2010). These findings suggested that tobacco smoking may contribute to the development of vitamin D deficiency and tuberculosis.

**Geographic factors**

It has been observed that there is a progressive decrease in solar UVB radiation exposure with each 10-degree deviation from the equator (Diffey, 1991). Solar UVB radiation is the primary source of vitamin D for most people living on Earth. As latitude increases, vitamin D-producing UV radiation decreases dramatically, potentially inhibiting vitamin D synthesis in humans (Kimlin et al., 2007). In Boston, USA (42.2° North latitude), human skin does not produce any previtamin D3 when exposed to sunlight on cloudless days from November through February. In Edmonton, Canada (52° North latitude), this ineffective period extends from October through March. In areas further south (between latitudes 34° North and 18° North), sunlight effectively photo-converts dehydrocholesterol to previtamin D3 in the middle of winter (Webb et al., 1988). Similarly, a case-control study in Asian-Indians immigrants living in South London has shown a significant trend of an increased risk of tuberculosis in these immigrants compared with the indigenous UK population (Strachan et al., 1995). Vitamin D deficiency has been suggested as a factor contributing to the increased rates of tuberculosis reactivation among those emigrating from tropical areas (high UVB exposure) to temperate climates (low UVB exposure) (Rook, 1988b). The molecular analysis of isolates has shown that immigrants may acquire tuberculosis after arrival in the host country and that transmission between foreign-born and autochthonous individuals may occur in both directions (Garzelli and Rindi, 2012).

**Seasonal factors**

Significant seasonal vitamin D variations were reported in both southern and northern latitudes (Oliveri et al., 1993; Stryd et al., 1979). Barger-Lux and Heany (2002) confirmed and quantified the relatively large seasonal fluctuations in 25(OH)D levels that are associated with sun exposure in outdoor workers. The median serum 25(OH)D levels of the workers decreased from 122 nmol/l in late summer to 74 nmol/l in late winter. Similarly, a seasonal pattern has been detected in tuberculosis. In Russia, the number of hospitalizations related to tuberculosis was significantly higher in cold months than in warm months (Atun et al., 2005). In the Voronezh region, the highest incidence of tuberculosis was registered in winter months (Shilova and Glumnaia, 2004). In South Africa, there is an increased transmission of childhood tuberculosis in the autumn and winter (Schaff et al., 1996). An inverse relationship between seasonal variations in vitamin D status and tuberculosis diagnosis was observed in Cape Town, South Africa (Martineau et al., 2011). In addition, seasonal variations in tuberculosis rates and a prevalence of tuberculosis in the winter months were reported in South Western Cameroon (Ane-Anyangwe et al., 2006). In the systematic review of peer-reviewed studies, the risk of transmission of M. tuberculosis appears to be the greatest during the winter months (Fares, 2011). Moreover, the seasonality of pulmonary
tuberculosis was also reported in migrant workers entering Kuwait (Akhtar and Mohammad, 2008). The migrants have a very high proportion of tuberculosis and arrive mainly in the winter months, while migrants from Sri Lanka and other low burden countries have a low proportion of tuberculosis and arrive mainly in the summer.

18.2.2 Genetic factors

Genetic studies provide an excellent opportunity to link molecular variations such as polymorphisms to subtle biological effects. Host genetic factors are important determinants of tuberculosis susceptibility.

Chromosome 20

A genomic DNA clone for 1,25(OH)$_2$D$_3$ 24-hydroxylase has been isolated from a human chromosome 20 library (Chen and DeLuca, 1995). In a genome-wide association study, a variant at the 20q locus was identified as a risk factor for vitamin D insufficiency (Wang et al., 2010a). A region of chromosome 20 has been linked to tuberculosis susceptibility in Thais (Mahasirimongkol et al., 2009). However, the co-localization of these loci on the same chromosome does not necessarily that they are related.

Human leukocyte antigen

The HLA system is the major histocompatibility complex in humans. The primary function of the HLA system is to regulate the immune response. This system is involved in conferring susceptibility to or protection from mycobacterial disease in animal and humans. It has been shown that tuberculosis susceptibility can be influenced by MHC haplotype. Knockout mice with defined HLA class II deficiencies were infected with M. bovis BCG, whereas controls were able to inhibit bacterial growth and resist infection (Ladel et al., 1995). A number of studies have reported an association between HLA class II alleles and tuberculosis, but these associations are not consistent across different populations. HLA studies have revealed that the following HLA alleles may be associated with a higher risk of tuberculosis; such as, DQA1*0101 in the Iranian population (Amirzargar et al., 2004), DQB1*0301-0304 in the South African population (53), DQB1*05 in the South African and Polish populations (Lombard et al., 2006; Dubaniewicz et al., 2003), DQB1*0501 in the American Indian and Mexican populations (Teran-Escandon et al., 1999), DQB1*0502 in the Thai population (Vejaesya et al., 2002), DQB1*0503 in the Cambodian population (Goldfeld et al., 1998), DQB1*0601 in the Korean and Asian Indian populations (Kim et al., 2005; Ravikumar et al., 1999), DQB1*0602/3 in the South African population (Lombard et al., 2006), DRB1*07 in the Iranian population (Dubaniewicz et al., 2003), DRB1*0803 in the Korean population (Kim et al., 2005), DRB1*1101-1121 in the South African population (Lombard et al., 2006), DRB1*1101 and DRB1*12 in the Indonesian population with recurrent tuberculosis (Yuliwulandari et al., 2010), DRB1*13 in the Polish population (Dubaniewicz et al., 2000), DRB1*1302 in the South African population (Lombard et al., 2006), DRB1*14 in the Iranian (Mahmoudzardeh-Niknam and Fadavi, 2003), Tyvian (Mattrashkin et al., 2002), and Portuguese
populations (Duarte et al., 2011), DRB1*1501 in the Asian Indian (Mehra et al., 1995; Ravikumar et al., 1999) and Chinese populations (Shi et al., 2011), DR16 in the Chinese population (Liu et al., 2004a), and DRB1*16 in the Polish population (Dubaniewicz et al., 2000). In contrast, other alleles such as the following may be associated with a lower risk of tuberculosis: DQB1*02 and DRB1*13 in the Polish population (Dubaniewicz et al., 2000; Lombard et al., 2006), DQA1*0301 and 0501 in the Iranian population (Amirzargar et al., 2004), DRB1*1202 in the absence of B*1802 in the Indonesian population (Ravikumar et al., 1999), and DRB1*13 in the Chinese population (Liu et al., 2004a). On the other hand, calcitriol is known to stimulate phagocytosis but suppresses MHC class II antigen expression in human mononuclear phagocytes (Tokuda and Levy, 1996; Tokuda et al., 1992), thereby preventing antigen-specific T cell proliferation. In addition, calcitriol exerts effects that oppose the effect that IL-4 has on MHC class-II antigen expression in human monocytes (Xu et al., 1993). Calcitriol specifically modulates human monocyte phenotype and function, altering HLA-DR antigen expression and antigen presentation, while leaving lytic function intact (Rigby et al., 1990). Calcitriol also decreases interferon-gamma-induced HLA-DR antigen expression on normal and transformed human keratinocytes (Tamaki et al., 1990-1991; Tone et al., 1991) and reduces the levels of HLA-DR mRNA in cultured epithelial tumor cell lines (Tone et al., 1993). In addition, 1α-calcidol significantly modulated the expression of HLA-DR in human peripheral blood monocytes (Scherberich et al., 2005). The relationship between allelic variations in HLA and tuberculosis summarized in Table 18.1.

**Vitamin D receptors**

The levels of VDR protein were significantly decreased in tuberculosis (Selvaraj et al., 2009). The VDR genes have also been suggested to influence the cell-mediated immune response to the *M. tuberculosis* antigen in tuberculosis patients (Selvaraj et al., 2000a). Several polymorphisms that may affect the clinical outcome and severity of the disease were found in the VDR gene. VDR polymorphisms have wide ranging roles in innate immunity and are associated with tuberculosis. Four common polymorphisms (TaqI, Apal, BsmI and FokI) in the VDR gene were studied in clinically diagnosed tuberculosis. There is no evidence that TaqI is associated with tuberculosis in Tibetan and Cambodian populations (Chen et al., 2006a; Delgado et al., 2002). However, it has been reported that the TaqI tt genotype occurred more frequently in female tuberculosis (Selvaraj et al., 2000b) and was found to be associated with a higher risk of smear positive tuberculosis in Asian Indians (Sharma et al., 2011). However, this genotype might be associated with resistance to tuberculosis in Africans (Bellamy et al., 1999). The Tt genotype increased the probability of culture conversion during tuberculosis treatment (Roth et al., 2004) and T-containing genotypes predict a faster response to tuberculosis chemotherapy (Babb et al., 2007). In Paraguayans, the TaqI t allele protects against active tuberculosis disease but not infection; individuals with a TT genotype are 42 times less likely to mount a delayed-type hypersensitivity response (Wilbur et al., 2007). It seems that the ApaI Aa and aa genotypes indicate a high of smear positive disease in the general population and in Muslim populations of central India, respectively (Sharma et al., 2011). Apal AA genotypes were predictive of a faster response to tuberculosis treatment (Babb et al., 2007). There is a significantly higher frequency of BsmI Bb in male tuberculosis (Selvaraj et al., 2003). The bb genotype conferred a significant risk for smear positive and MDR
18. Vitamin D and tuberculosis

Table 18.1. The relationship between allelic variations in HLA and tuberculosis.

<table>
<thead>
<tr>
<th>HLA</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher risk of tuberculosis</td>
<td></td>
</tr>
<tr>
<td>DQA1 0101</td>
<td>Iranian</td>
</tr>
<tr>
<td>DQB1 0301-0304</td>
<td>South African</td>
</tr>
<tr>
<td>05</td>
<td>South African and Polish</td>
</tr>
<tr>
<td>0501</td>
<td>American Indian and Mexican</td>
</tr>
<tr>
<td>0502</td>
<td>Thai</td>
</tr>
<tr>
<td>0503</td>
<td>Cambodian population</td>
</tr>
<tr>
<td>0601</td>
<td>Korean and Asian Indian</td>
</tr>
<tr>
<td>0602/3</td>
<td>South African</td>
</tr>
<tr>
<td>DRB1 07</td>
<td>Iranian</td>
</tr>
<tr>
<td>0803</td>
<td>Korean</td>
</tr>
<tr>
<td>1101-1121</td>
<td>South African</td>
</tr>
<tr>
<td>1101 and 12</td>
<td>Indonesian</td>
</tr>
<tr>
<td>13</td>
<td>Polish</td>
</tr>
<tr>
<td>1302</td>
<td>South African</td>
</tr>
<tr>
<td>14</td>
<td>Iranian, Tyvian and Portuguese</td>
</tr>
<tr>
<td>1501</td>
<td>Asian Indian and Chinese</td>
</tr>
<tr>
<td>16</td>
<td>Polish</td>
</tr>
<tr>
<td>Lower risk for tuberculosis</td>
<td></td>
</tr>
<tr>
<td>DQA1 0301 and 0501</td>
<td>Iranian</td>
</tr>
<tr>
<td>DQB1 02</td>
<td>Polish (in the absence of B*1802)</td>
</tr>
<tr>
<td>DQB1 13</td>
<td>Polish and Chinese</td>
</tr>
<tr>
<td>Role of vitamin D</td>
<td>Suppress MHC class II antigen expression</td>
</tr>
<tr>
<td></td>
<td>Reduce the levels of HLA-DR mRNA</td>
</tr>
</tbody>
</table>

tuberculosis in the Muslims of central India (Sharma et al., 2011). In another study, the BsmI BB genotype was reported to increase susceptibility to tuberculosis, while the bb genotype decreased susceptibility to tuberculosis in the Turkish (Ates et al., 2011). It was reported that the FokI ff genotype occurred more frequently in Tibetans and Gujarati Asians in West London (Chen et al., 2006b; Wilkinson et al., 2000). Asian Indians with Ff and ff genotypes were at a high risk of developing MDR and smear positive tuberculosis disease (Sharma et al., 2011). The FokI F allele protects Paraguayans from tuberculosis. FF genotypes are 17 times more likely to test positive for exposure to tuberculosis but are no more likely to have ever been diagnosed with active tuberculosis (Wilbur et al., 2007). FokI polymorphisms in the VDR gene are also associated with susceptibility to extrapulmonary tuberculosis (Motsinger-Reif et al., 2010) and spinal tuberculosis in the Chinese Han population (Zhang et al., 2010). There is no evidence of a FokI association with tuberculosis in the Tibetan population (Chen et al., 2006b). In a family-based
study, the transmission-disequilibrium test analysis showed a significantly greater association between the SNP combinations FokI-BsmI-ApaI-TaqI or FokI-ApaI and tuberculosis than between individual SNPs and tuberculosis (Bornman et al., 2004). A study on host susceptibility confirmed the association of BsmI (BB+bb) SNPs in the VDR and SNPs in the TNF-γ genes with susceptibility to tuberculosis in Iranians (Merza et al., 2009). Moreover, the rate of tuberculosis among Canadian Aboriginals was higher than that among Canadian-born non-Aboriginals (27.4 vs. 2 cases/100,000 individuals); the Canadian Aboriginal populations exhibits a significantly higher frequency of SNPs that are associated with lower expression of VDR, INF-α, and TNF-α, and higher monocyte chemoattractant protein-1 production compared to a Caucasian cohort (Larcombe et al., 2008). In meta-analysis studies, polymorphisms at FokI loci (VDR-ff) showed a statistically significant association with tuberculosis susceptibility; however, a variant in TaqI was not associated with tuberculosis (Zhao et al., 2009). However, Gao et al. (2010) found that VDR polymorphisms (FokI, TaqI, ApaI and BsmI) are associated with tuberculosis in Asians and that none of these polymorphisms were significantly related to tuberculosis in Africans or South Americans. The Cdx-2 GG genotype, which is a SNP in VDR, is associated with protection from tuberculosis (Selvaraj et al., 2008). In a case-control study, the combined VDR genotypes AbfT and AabbFfTT were statistically significant factors that protected Iranian against tuberculosis (Marashian et al., 2010). The VDR haplotype FbAT also significantly protected South Africans from tuberculosis (Lombard et al., 2006). Recent studies have identified methylation variable positions in the 3’ CpG island that distinguish ethnicity and tuberculosis status (Andraos et al., 2011). Moreover, a Gc gene variant of the DBP is correlated with decreased circulating levels of 25(OH)D, 1,25(OH)_{2}D3 and DBP (Lauridsen et al., 2005; Abbas et al., 2008) and was strongly associated with susceptibility to active tuberculosis in Gujarati Asians who are living in London (Martineau et al., 2010). Finally, studies from different populations have determined that VDR polymorphisms may have differential susceptibility or resistance to tuberculosis. The relationship between allelic variations in VDR and tuberculosis is summarized in Table 18.2.

**Human toll-like receptors**

TLRs are a group of glycoproteins that function as surface trans-membrane receptors and are involved in innate immune responses to exogenous pathogenic microorganisms. The production of IL-12, TNF-α, and NO are induced soon after the innate recognition of mycobacteria through TLRs (Underhill et al., 1999). TLR2 expression was up-regulated on monocytes obtained from tuberculous pleural fluid; TLR2 and TLR4 expression were also enhanced on INF-γ secreting CD4+ T cells (Prabha et al., 2008). MyD88-deficient mice, which lack a critical component of TLR signaling, failed to control acute and chronic M. avium growth and succumbed to infection 9-14 weeks post-infection. Infected TLR2^{−/−} mice also showed increased susceptibility but displayed longer survival times and a lower bacterial burden than MyD88^{−/−} mice. Infected TLR4^{−/−} mice were indistinguishable from wild-type animals. The histopathological examination of MyD88^{−/−} mice revealed the massive destruction of lung tissue, which was not present in the wild-type, TLR2^{−/−}, or TLR4^{−/−} mice (Feng et al., 2003). TLR2^{−/−} mice, however, succumbed to M. tuberculosis infection (Drennan et al., 2004) due to compromised IL-23 expression in response to M. tuberculosis (Teixeira-Coelho et al., 2011). The role played by TLR4 in susceptibility to M. tuberculosis varies
### 18. Vitamin D and tuberculosis

#### Table 18.2. The relationship between allelic variations in VDR and tuberculosis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Link to tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR polymorphism</td>
<td></td>
</tr>
<tr>
<td>TaqI</td>
<td></td>
</tr>
<tr>
<td><em>tt</em> genotype</td>
<td>No association with tuberculosis in Tibetan and Cambodian populations. Occurs more frequently in female tuberculosis patients.</td>
</tr>
<tr>
<td><em>t</em> genotype</td>
<td>Associated with high risk of smear positive tuberculosis in Asian Indians. Associated with resistance to tuberculosis in Africans.</td>
</tr>
<tr>
<td><em>Tt</em> genotype</td>
<td>Protects against active tuberculosis in Paraguayans.</td>
</tr>
<tr>
<td><em>TT</em> genotype</td>
<td>Increased probability of culture conversion during tuberculosis treatment. Predictive of a faster response to tuberculosis chemotherapy.</td>
</tr>
<tr>
<td>ApaI</td>
<td></td>
</tr>
<tr>
<td><em>Aa</em> genotype</td>
<td>Forty-two times less likely to mount a delayed-type hypersensitivity response in Paraguayans.</td>
</tr>
<tr>
<td><em>aa</em> genotype</td>
<td>Is at high risk of tuberculosis in general population of central India. Is at high risk of tuberculosis in Muslims of central India.</td>
</tr>
<tr>
<td>BmsI</td>
<td></td>
</tr>
<tr>
<td><em>Bb</em> genotype</td>
<td>Occurs more frequently in male tuberculosis patients.</td>
</tr>
<tr>
<td><em>bb</em> genotype</td>
<td>Increased frequency susceptibility to tuberculosis in Turkish populations. Risk of smear positive and multiple drug resistant (MDR) tuberculosis in Muslims of central India.</td>
</tr>
<tr>
<td>FokI</td>
<td></td>
</tr>
<tr>
<td><em>ff</em> genotype</td>
<td>Associated with extrapulmonary tuberculosis and spinal tuberculosis.</td>
</tr>
<tr>
<td><em>Ff</em> and <em>ff</em> genotypes</td>
<td>High risk of MDR and smear positive tuberculosis in Asian Indians.</td>
</tr>
<tr>
<td><em>F</em> genotype</td>
<td>Protects Paraguayans from tuberculosis.</td>
</tr>
<tr>
<td><em>FF</em> genotype</td>
<td>17 times more likely to test positive for exposure to tuberculosis, but no more likely to have ever been diagnosed with active tuberculosis.</td>
</tr>
<tr>
<td>Combination of SNPs (<em>Fokl-Apal</em> and <em>Fokl-BsmI-Apal-TaqI</em>)</td>
<td>Higher risk of tuberculosis than an individual SNP.</td>
</tr>
<tr>
<td>Association of SNPs in <em>BsmI (BB+bb)</em> and <em>TNF-γ</em> genes</td>
<td>Susceptibility to tuberculosis in Iranians.</td>
</tr>
<tr>
<td><em>Cdx-2</em> GG genotype</td>
<td>Associated with protection against tuberculosis.</td>
</tr>
<tr>
<td>Combined <em>AbfT</em> and <em>AabbFfTT</em></td>
<td>Protects Iranians against tuberculosis.</td>
</tr>
<tr>
<td><em>Haplotype FbAT</em></td>
<td>Protects South Africans from tuberculosis.</td>
</tr>
<tr>
<td>DBP alleles</td>
<td></td>
</tr>
<tr>
<td><em>Gc</em> gene variant</td>
<td>Decreased levels of 25(OH)D, 1,25(OH)D and DBP. Associated with susceptibility to active tuberculosis among Gujarati Asians living in London.</td>
</tr>
</tbody>
</table>

---

*Handbook of vitamin D in human health* 329
between different studies (Branger et al., 2004; Shim et al., 2003). Cooperation between TLR2 and TLR4 mediated signaling is involved in *M. tuberculosis*-induced macrophage death (Sánchez et al., 2010). TLR9 is involved in the control of *M. avium* infection but is not related to the induction of Th1 responses (Carvalho et al., 2011). Changes in the structure of TLR signaling molecules might be associated with susceptibility to various infectious diseases. Variants of TLR1 and TLR6 were associated with an altered-immune response to BCG vaccination in South African infants (Randhawa et al., 2011). TLR2 polymorphisms are associated with susceptibility to tuberculosis in Tunisians (Ben-Ali et al., 2004), Koreans (Yim et al., 2006), Vietnamese (Thuong et al., 2007), Taiwanese (Chen et al., 2010), and Turkish children (Dalgic et al., 2011). The S/M TLR2 genotype may increase susceptibility to tuberculosis in Han Chinese, but the S/L genotype may act as a negative risk factor (Xue et al., 2010a). Variants in TLR2 and 9 might also play important roles in determining tuberculosis susceptibility in Caucasians, African-Americans, and West Africans (Velez et al., 2010). TLR4 polymorphisms influence the susceptibility and severity of tuberculosis in Asian Indians (Najmi et al., 2010), HIV-infected patients in Tanzania (Ferwerda et al., 2007), and the Caucasian population in the Mediterranean (Pulido et al., 2010), but are not linked to the southeastern Chinese (Xue et al., 2010b) and Gambian populations (Newport et al., 2004). TLR6 polymorphisms were associated with altered IL-6 levels in response to *M. tuberculosis* and BCG (Shey et al., 2010). TLR8 polymorphisms are associated with tuberculosis susceptibility in Indonesian males (Davila et al., 2008), and male Turkish children (Dalgic et al., 2011). However, the allele and genotype frequencies of the various TLR genes are not risk factors for tuberculosis in South Indian (Selvaraj et al., 2010). On the other hand, calcitriol suppresses the expression of TLR2 and TLR4 protein and mRNA in human monocytes and triggers hyporesponsiveness to pathogen-associated molecular patterns (Sadeghi et al., 2006). Calcitriol has also been shown to down-regulate intracellular TLR2, TLR4 and TLR 9 expression in human monocytes (Dickie et al., 2010). TLR activation results in the expression of the VDR and 1α-vitamin D hydroxylase in human monocytes, leading to the induction of the antimicrobial peptide cathelicidin and the killing of intracellular *M. tuberculosis* (Liu et al., 2006). Calcitriol can cause the vitamin D-induced expression of cathelicidin in bronchial epithelial cells (Yim et al., 2007) and may enhance the production of cathelicidin LL-37 (Rivas-Santiago et al., 2008). Poor vitamin D intake may increase susceptibility to *M. tuberculosis* infection by inefficiently supporting the induction of cathelicidin mRNA expression in monocytes (Liu et al., 2006; 2007). The addition of a VDR antagonist inhibited the induction of cathelicidin mRNA by more than 80%; consequently, the protein expression of this antimicrobial agent was reduced by approximately 70% (Liu et al., 2006). Furthermore, the knockdown of cathelicidin in primary monocytes results in the loss of TLR-mediated antimicrobial activity against intracellular mycobacteria (Liu et al., 2009). Upon *M. tuberculosis* stimulation, calcitriol modulates cytokine production towards an anti-inflammatory profile by decreasing the expression of TLR2 and TLR4, while increasing cathelicidin production (Khoo et al., 2011). Calcitriol also induces autophagy in human monocytes via cathelicidin and leads to the co-localization of mycobacterial phagosomes and autophagosomes in human macrophages in a cathelicidin-dependent manner (Yuk et al., 2009). These findings suggested that vitamin D down-regulates the pro-inflammatory cytokine responses to *M. tuberculosis* through pattern recognition receptors while inducing the production of antimicrobial cathelicidin.
18. Vitamin D and tuberculosis

The natural resistance-associated macrophage protein 1

Nramp1 (now renamed SLC11a1-solute carrier family 11a member 1) plays a critical role in macrophage defenses against intracellular pathogens. The expression of Nramp1 in pathogen-containing phagosomes is associated with enhanced fusion to lysosomes, increased phagosomal acidification, and greater bactericidal activity. The NRAMP1 gene has been shown to regulate the concentration of divalent cations in the phagosomes of macrophage (Forbes and Gros, 2001). The antibacterial role of NRAMP1 could be a result of the extrusion of protons and divalent metal ions from the phagosomal lumen toward the cytoplasm (Jabado et al., 2000). Polymorphism

NOD2-deficient mice had a higher mycobacterial burden in the lung six months after infection and succumbed to...
The role of vitamin D in tuberculosis

An association between vitamin D deficiency and tuberculosis has been reported in a number of studies. Low vitamin D levels were associated with a five-fold increased risk for progression to tuberculosis (Talat et al., 2010). A vegetarian diet, which is associated with a low plasma vitamin D level, is an independent risk factor for active tuberculosis among Asian immigrants living in South London (Strachan et al., 1995). Tuberculosis patients also have significantly lower mean concentrations of serum 25(OH)D compared with healthy subjects (Davies et al., 1985; Sita-Lunsden et al., 2009). A correlation between serum levels of vitamin D and risk for latent tuberculosis has been noted among African immigrants living in Australia (Gibney et al., 2008). Patients with chronic renal failure are also at an increased risk for developing tuberculosis (Cuss et al., 1986), because certain uremic toxins can suppress 1,25(OH)2D3 synthesis and its biological activity (Hsu and Patel, 1997). In a meta-analysis study, low vitamin D levels were associated with a higher risk of active tuberculosis (Nnoaham and Clark, 2008).

The BCG vaccine was developed to provide protection against tuberculosis. BCG-vaccinated infants are almost 6 times more likely to have sufficient vitamin D concentrations than unvaccinated infants 3 months after BCG vaccination, and this association remains strong even after adjusting for season, ethnic group and sex (Lalor et al., 2011). Among the vaccinated group, there was also a strong inverse correlation between the IFN-γ response to *M. tuberculosis* PPD and vitamin D concentration; infants with higher vitamin D concentrations had lower IFN-γ responses. Similarly, tuberculosis in cattle usually presents with a rapid transient increase in serum calcitriol within the first two weeks following infection (Rhodes et al., 2003). 1,25(OH)2D3-positive mononuclear cells were later identified in all of the tuberculous granulomas. During tuberculosis infection, alveolar macrophage-produced calcitriol plays a beneficial role by limiting inflammation-mediated tissue injury, potentiating NO production by stimulated monocytes/macrophages, inhibiting INF-γ production by stimulated CD4+ cells, and suppressing the growth of *M. tuberculosis* (Ametaj et al., 1996; Rockett et al., 1998). In persons with positive tuberculin test results, calciferol is able to suppress the tuberculin reaction (Huff, 1963). A single dose of vitamin D has been reported to enhance immunity to mycobacteria (Martineau et al., 2007). The
VDR genes have also been suggested to influence the cell-mediated immune response to the *M. tuberculosis* antigen in tuberculosis patients (Selvaraj et al., 2000a).

High doses of vitamin D were widely used to treat active tuberculosis in the preantibiotic era. The importance of sunlight has been noted throughout the history of tuberculosis treatment. In 1854, Herman Brehmer, a Silesian botany student who was suffering from tuberculosis, traveled to the Himalayan mountains to pursue his botanical interests and cured his tuberculosis (Warren, 2006). Vitamin D was used to treat tuberculosis of the bone and caused some improvement (Pattison, 1929). UVB exposure, however, is sufficient to double the circulating 25(OH)D levels (from 11.23 ng/ml to 20.39 ng/ml), but no significant changes have been observed in antimycobacterial immunity (Yesudian et al., 2008), which might be attributed to low vitamin D levels in patients with tuberculosis. Clinical and radiological improvements were more evident in *M. tuberculosis*-infected children taking additional vitamin D than controls (Morcos et al., 1998). Serum 25(OH)D concentrations increased during the first two months of tuberculosis treatment (Tostmann et al., 2010). Vitamin D is known to suppress the intracellular growth of *M. tuberculosis* *in vitro* (Rockett et al., 1998). Calcitriol was shown to inhibit the multiplication of virulent tubercle bacilli in cultured human macrophages *in vivo* (Crowle et al., 1987). Calciferol was reported to dissolve cavities in tuberculosis patients (Brincourt, 1969). In a random trial, the use of multivitamin supplements, including vitamin D, reduced HIV-infected tuberculosis patient mortality by 50% (Range et al., 2006). Moreover, vitamin D supplementation resulted in more rapid sputum clearance of acid-fast bacilli and radiological improvement in Indonesian tuberculosis patients (Nursyam et al., 2006). The correction of vitamin D deficiency and antituberculosis treatments have also resulted in clinical and microbiological improvements in a refractory drug-susceptible tuberculosis patient (Yamshchikov et al., 2009). In another trial, however, vitamin D did not improve the clinical outcomes of tuberculosis patients, demonstrating no overall effect on the mortality of tuberculosis patients (Wejse et al., 2009). This finding may have been due to a suboptimal dosage.

MMPs are proteolytic enzymes that are responsible for extracellular matrix remodeling and the regulation of leukocyte migration through the extracellular matrix, which is an important step in inflammatory processes and infectious diseases. MMPs are produced by many cell types including lymphocytes, granulocytes, astrocytes and activated macrophages. The expression of *MMPs*-1, -2, -3, -7, and -9 was increased in human astrocytes that were stimulated by conditioned medium from *M. tuberculosis*-infected monocytes (Harris et al., 2007). MMP-mediated proteolysis of the mycobacterial heat shock protein 65 was reported to contribute to the complex immunomodulatory interplay during the course of tuberculosis infection (Shiryaev et al., 2011). In tuberculosis patients, the circulating level of MMP-9 is correlated with disease severity (Hrabec et al., 2002) and granuloma formation (Sheen et al., 2009). In addition, infection with *M. tuberculosis* has been shown to decrease the expression of TIMPs-1, -2 and -3 in peripheral blood mononuclear cells and human epithelial cells (Brew et al., 2000). Patients with an *MMP-1* (-1607G) polymorphism are more vulnerable to extensive lung fibrosis one year after antituberculosis treatment (Wang et al., 2010c), and the 1G genotypes of *MMP-1* polymorphisms were associated with a greater risk of developing tracheobronchial stenosis in the Taiwanese (Kuo et
In addition, the mouse MMP-1 ortholog is not expressed in the lung and mice infected with *M. tuberculosis* do not develop tissue destruction equivalent to that in humans. In MMP-1 transgenic mice, however, *M. tuberculosis* infection increased MMP-1 expression, resulting in alveolar destruction in lung granulomas and significantly greater collagen breakdown (Elkington et al., 2011). On the other hand, VDR knockout mice had increased influx of inflammatory cells, phospho-acetylation of nuclear factor-kappaB associated with increased pro-inflammatory cells, and up-regulation of MMP-2, MMP-9, and MMP-12 in the lung (Sundar et al., 2011). The VDR TaqI polymorphism is associated with the decreased production of tissue metalloproteinase inhibitor-1, which is a natural inhibitor of MMP-9 (Timms et al., 2002). Calcitriol modulates tissue MMP expression under experimental conditions (Dean et al., 1996), down-regulates MMP-9 levels in keratinocytes, and may attenuate the deleterious effects caused by the excessive TNF-α-induced proteolytic activity associated with cutaneous inflammation (Bahar-Shany et al., 2010). Calcitriol inhibits both basal and *Staphylococcus*-stimulated production of MMP-9 in human blood monocytes and alveolar macrophages (Lacraz et al., 1994). Moreover, a vitamin D analog was also reported to reduce the expression of MMP-2, MMP-9, VEGF and parathyroid hormone-related peptide in Lewis lung carcinoma cells (Nakagawa et al., 2005). Furthermore, calcitriol significantly attenuated *M. tuberculosis*-induced increases in the expression of MMP-7 and MMP-10, and suppressed the secretion of MMP-7 by *M. tuberculosis*-infected PBMCs. MMP-9 gene expression, secretion and activity were significantly inhibited, irrespective of infection status (Coussens et al., 2009). In another study, calcitriol also suppressed the production of MMPs (MMP-7 and MMP-9) and enhanced the level of TMP-1 in tuberculosis patients (Anand and Selvaraj, 2009). These studies suggested that calcitriol may play an important role in the pathological process of tuberculosis by down-regulating the levels of MMPs and regulating the levels of TIMPs.

Angiogenesis is a complex process involving the coordinated steps of endothelial cell activation, proliferation, migration, tube formation and capillary sprouting, and requires the participation of intracellular signaling pathways. VEGF is a key mediator of angiogenesis. The vascular changes associated with angiogenesis usually occur in cancer, but have also been reported in inflammatory process diseases. VEGF gene expression was reported to be up-regulated in macrophages infected with *M. tuberculosis* (Ragno et al., 2001). The serum levels of VEGF were significantly higher in patients with active pulmonary tuberculosis than in patients with old tuberculosis and acute bronchitis; the decrease in serum VEGF titer paralleled the clinical improvement of pulmonary tuberculosis (Abe et al., 2001; Matsuyama et al., 2000; Alatas et al., 2004). *Mycobacteria* induce pleural mesothelial permeability and release VEGF from mesothelial cells by down-regulating β-catenin expression (Mohammed et al., 2003; Seiscento et al., 2010). The serum and cerebrospinal fluid levels of VEGF were significantly higher in tuberculous meningitis than in other meningitis; the decrease in cerebrospinal fluid VEGF titer paralleled the clinical improvement of tuberculous meningitis (Matsuyama et al., 2001). In these studies, the immuno-histochemical staining of autopsied brains demonstrated the presence of VEGF in the mononuclear cells of the dense fibroconnective tissue in both the subarachnoid space and surrounding the vasculitis lesions. VEGF is a known a marker of disease activity in neurotuberculosis (Husain et al., 2008). In contrast, calcitriol was reported to inhibit angiogenesis *in vitro* and *in vivo* (Mantell et al., 2000).
Calcitriol significantly inhibits VEGF-induced endothelial cell spouting and elongation in a dose-dependent manner and decreases the production of VEGF (Gruber et al., 2008). Calcitriol is a potent inhibitor of retinal neovascularization in mouse model of oxygen-induced ischemic retinopathy (Albert et al., 2007). Vitamin D and its analog also reduce the expression of VEGF in various cancer cell lines (Ben-Shoshan et al., 2007; Nakagawa et al., 2005). Moreover, DBP-macrophage activating factor was reported to inhibit angiogenesis and tumor growth in mice (Kisker et al., 2003) and inhibited the VEGF signaling by decreasing the VEGF-mediated phosphorylation of VEGF-2 and ERK1/2, a downstream target of the VEGF signaling cascade (Kalkunte et al., 2005).

PGs play a role in the inflammatory process. Cyclooxygenase participates in the conversion of arachidonic acid into PGs. PGs regulate the cell-mediated immune response and have a role in the progression of pulmonary tuberculosis. VEGF stimulates the expression of cyclooxygenase-2 and PGES in response to cell activation (Sakurai et al., 2004). During the chronic phase of experimental murine tuberculosis, an inhibitor of PG synthesis enhanced Th1 cytokine levels, decreased Th2 cytokines levels, increased the expression of iNOS and reduced pulmonary inflammatory, fibrosis and mycobacteria load (Hernández-Pando et al., 2006). In vitro and in vivo studies using PGES−/− mice, which cannot produce PGE2, showed a significantly higher mycobacterial burden in these mice than control mice. These findings suggested that PGE2 plays a critical role in the inhibition of M. tuberculosis replication (Chen et al., 2008). In tuberculosis patients, the production of PGE was increased in both lungs, but the hyperproduction of PGF2α occurred in the impaired lung (Kaminskaia et al., 1991). The spontaneous development of tuberculosis in guinea pigs was accompanied by phasic changes in PG. There was an increase in PGs during a swift progression of inflammatory and necrotic changes, with an abundance of PGF being noted; however, the PG content in the lung tissue normalized after tuberculosis treatment (Kaminskaia et al., 1990). On the other hand, calcitriol has been reported to regulate the expression of several key genes involved in the PG pathway, causing a decrease in PG synthesis (Moreno et al., 2005). Calcitriol and its analogs have been shown to selectively inhibit the activity of cyclooxygenase-2 (Apama et al., 2008). These findings suggested that vitamin D plays a role in modulating the inflammatory process in tuberculosis.

ROS are produced by activated phagocytes as a part of their microbicidal activities. ROS are capable of damaging the host tissue through lipid peroxidation. The end product of lipid peroxidation, MDA, serves as a marker of cellular damage. The serum ROS levels in active lung tuberculosis and sequel lung tuberculosis cases were significantly higher than those of the control group (Ceylan et al., 2005). The levels of lipid peroxidation end products, 9,11-linoleic acid, and free radical activity were higher in the active pulmonary tuberculosis cases than the inactive pulmonary tuberculosis cases (Jack et al., 1994). The high levels of free radicals in active pulmonary cases decreased to normal at the end of treatment (Makinskii et al., 2002). In another study, MDA and lipid peroxidation products in sputum smear-positive patients with advanced pulmonary tuberculosis and sputum smear-negative patients with small radiographic changes were significantly higher than those of a healthy control groups (Kwiatkowska et al., 1999). The elevated exhalation of hydrogen peroxide was also reported in patients with pulmonary
tuberculosis (Kwiatkowska et al., 2007). Decreased SOD activity was noted in the disseminated stage of military and infiltrative tuberculosis. SOD activity is known to increase when there is no dissemination (Safarian and Karapetian, 1990). Similarly, calcitriol has been reported to exert a receptor-mediated effect on the secretion of hydrogen peroxide by human monocytes (Cohen et al., 1986). Human monocytes in culture gradually lose their capacity to produce superoxide when stimulated. The addition of calcitriol, lipopolysaccharide or lipoteichoic acid restored the ability of stimulated monocytes to produce superoxide and increased their oxidative capacity compared with unstimulated monocytes (Levy and Malech, 1991). Calcitriol can also protect nonmalignant prostate cells from oxidative stress-induced cell death by eliminating ROS-induced cellular injuries (Bao et al., 2008). Vitamin D metabolites and vitamin D analogs were reported to induce lipoxygenase mRNA expression, lipoxygenase activity and ROS in a human bone cell line (Somjen et al., 2011). Vitamin D can also reduce the extent of lipid peroxidation and induce SOD activity in the hepatic anti-oxidant system in rats (Sardar et al., 1996). These findings suggested that vitamin D modulates oxidative stress in tuberculosis.

Cytokine-activated macrophages produce RNI to combat pathogens. In vitro, the expression of RNI at physiological concentrations may prevent M. tuberculosis from proliferating, but the removal of RNI allows mycobacteria to proliferate (Firmani and Riley, 2002). In most murine models, iNOS is essential for the killing of intracellular mycobacteria (Chan et al., 1992), iNOS−/− mice proved highly susceptible to M. tuberculosis, which exhibited faster replication in the lungs of these mice than in the lungs of hosts deficient in other genes (MacMicking et al., 1997), and iNOS inhibitors also render similar deleterious effects on tuberculosis infection in mice, as assessed by mortality, bacterial burden, and pathological tissue damage (Chan et al., 1995). These findings confirmed the importance of RNI in resistance against M. tuberculosis. In granulomatous diseases, such as tuberculosis, the activation of macrophage 1α-hydroxylase results in an increase in 1,25(OH)2D3, which inhibits iNOS expression and reduces NO production by LPS-stimulated macrophages (Chang et al., 2004). This calcitriol production by macrophages may provide protection against the oxidative injuries caused by the NO burst. Calcitriol is known to inhibit LPS-induced immune activation in human endothelial cells (Equils et al., 2005). Calcitriol enhances the intracellular glutathione pools and significantly reduces the nitrite production that is induced the by LPS (Garcion et al., 1999). In human macroage-like cells, calcitriol induces iNOS and suppresses the growth of M. tuberculosis (Rockett et al., 1998). Finally, vitamin D affects the RNI to combat tuberculosis.

PZA is an important medication for tuberculosis, and plays a key role in shortening tuberculosis treatment from 9-12 months to 6 months. UV light enhances the activity of PZA against M. tuberculosis in vitro (Wade and Zhang, 2006). These authors suggested that UV light can generate free radicals that cause damage to macromolecules – such as DNA – and can also affect membrane integrity. UVB radiation, however, was reported to induce calcitriol from 7-dehydrocholesterol in human keratocytes (Lehmann et al., 2000; Vantieghem et al., 2006). Calcitriol synergizes with PZA to kill tuberculous bacilli in cultured human macrophages (Crowle et al., 1989). Chloroquine has been used to control hypercalcemia in granulomatous patients (O’Leary et al., 1986), and chloroquine therapy has been associated with a significant reduction in the
18. Vitamin D and tuberculosis

serum levels of 1,25(OH)$_2$D3 and urinary calcium, perhaps via inhibition of the conversion of 25(OH)D to 1,25(OH)$_2$D3. A combination of three drugs (chloroquine, 1,25(OH)$_2$D3, and PZA) kill tuberculous bacilli faster than the combination of 1,25(OH)D and PZA in cultured human macrophages (Crowle and May, 1990). Ketoconazole has been reported to decrease the serum levels of ionized calcium and 1,25(OH)$_2$D3 in tuberculosis-associated hypercalcemia (Saggese et al., 1993) and has also been shown to act against *M. tuberculosis* both in vitro and in a mouse model (Byrne et al., 2007). Ketoconazol, however, was reported to synergize with calcitriol by inhibiting 24-hydroxylase and increasing the activity of calcitriol in human skin and prostate cancer cells (Kang et al., 1997; Peehl et al., 2002).

18.4 Conclusion

The relationship between vitamin D and tuberculosis has been discussed. Vitamin D clearly has a beneficial role in some patients with tuberculosis. Genetic studies have provided the opportunity to determine which proteins link vitamin D to tuberculosis pathology. Vitamin D also exerts its effect on tuberculosis through non-genomic mechanisms. It is necessary to check the vitamin D status of patients with tuberculosis. Calcitriol is best used for tuberculosis because it is the active form of vitamin the D3 metabolite, has receptors in the lung, and inhibits inflammatory cytokine expression.

References


Ametaj, B., Reihard, T. and Nonnecke, B., 1996. 1,25-dihydroxyvitamin D3 inhibits secretion of interferon-
gamma by mitogen- and antigen-stimulated bovine mononuclear leukocytes. Vet Immunol Immunopathol. 52, 
77-90.

Amirzargar, A., Yalda, A., Hajabolbaghi, M., Khosravi, F., Jabbari, H., Rezaei, N., Niknam, M., Ansari, B., Moradi, B. 
and Nikbin, B., 2004. The association of HLA-DRB, DQB1 alleles and haplotype frequency in Iranian patients 
with pulmonary tuberculosis. Int J Tuberc Lung Dis. 8, 1017-1021.

and the inhibitor TIMP-1 in pulmonary tuberculosis. 133, 126-131.


Ane-Anyangwe, I., Akenji, T., Mbacham, W., Penlap, V. and Titanji, V., 2006. Seasonal variation and prevalence of 

Andraos, C., Koorsen, G., Knight, J. and Bornman, L., 2011. Vitamin D receptor gene methylation is associated 

Aparna, R., Subhashini, J., Roy, K., Reddy, G., Robinson, M., Uskokovic, M., Venkateswara Reddy, G. and Reddanna, 

Ates, O., Dolek, B., Dalyan, L., Musellim, B., Ongen, G. and Topal-Sarikaya, A., 2011. The association between BsmI 

Atun, R., Samyshkin, Y., Drobniewski, F., Kuznetsov, S., Fedorin, I. and Coker, R., 2005. Seasonal variation and 

Austin, C., Ma, X. and Graviss, F., 2008. Common nonsynonymous polymorphisms in the NOD2 gene are associated 
with resistance or susceptibility to tuberculosis disease in African Americans. Infect Defs. 197, 1713-1716.

Awaisu, A., Haniki Nik Mohamed, M., Noordin, N., Muttalif, A., Aziz, N., Syed Sulaiman, S. and Mahayiddin, A., 
2011. The SCIDOTS project: evidence of benefits of an integrated tobacco cessation intervention in tuberculosis 
care on treatment outcomes. Substance Abuse Treatment, Prevention, and Policy. 6, 26.

Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. 
Tuberculosis (Edinb). 87, 295-302.

the human NRAMP1 gene and susceptibility to tuberculosis in Morocco. Int J Tuberc Lung Dis. 7, 599-602.

and its attenuation by vitamin D. J Cell Physiol. 222, 729-737.

Bao, B., Ting, H., Hsu, J. and Lee, Y., 2008. Protective role of 1α-dihydroxyvitamin D3 against oxidative stress in 

D and calcium absorption. J Clin Endocrinol Metab. 87, 4952-4956.

Barthel, R., Piedrahita, J., McMurray, D., Payeur, J., Baca, D., Súarez Güemes, F., Perumailla, V., Ficht, T., Templeton, 
J. and Adams, L., 2000. Pathologic findings and association of Mycobacterium bovis infection with the bovine 
NRAMP1 gene in cattle from herds with naturally occurring tuberculosis. Am J Vet Res. 61, 1140-1144.

control: mathematical modeling analysis. BMJ. 343, d5506.
18. Vitamin D and tuberculosis


18. Vitamin D and tuberculosis


18. Vitamin D and tuberculosis


18. Vitamin D and tuberculosis


Lehmann, B., Knuschke, P. and Meurer, M., 2000. UVB-induced conversion of 7-dehydrocholesterol to 1α,25-dihydroxyvitamin D3 (Calcitriol) in the human keratinocyte line HaCaT. Photochem Photobiol. 72, 803-809.


18. Vitamin D and tuberculosis


Handbook of *vitamin D in human health* 347


18. Vitamin D and tuberculosis


K. vinh quôc Luong and L.T.H. Nguyên


Key facts

- Periodontal disease is common and includes gingivitis (inflammation of the soft tissues surrounding teeth) and periodontitis (inflammation and destruction of soft tissue and bone surrounding teeth).
- According to the 2000 US Surgeon General report, dental caries (infection and destruction of tooth structure) is the single most common chronic childhood disease.
- Inadequate levels of vitamin D may increase the risk for developing dental caries and periodontal disease.
- Diseases of the oral cavity have multifactorial causes.
- Vitamin D is necessary for the mineralization of tissues in the oral cavity including bone and teeth.

Summary points

- Gingivitis is an inflammatory condition confined to the soft tissues surrounding teeth that does not result in destruction of bone or the attachment apparatus.
- Vitamin D receptors are found in cells involved with calcium and bone metabolism, and immune regulation, and these receptors are present in cells of the oral cavity.
- Periodontitis is an inflammatory infectious disease of the supporting tissues of the teeth and results in destruction of bone and connective tissue attachment around the tooth.
- There is a growing body of evidence to suggest that vitamin D contributes directly to bone strength and plays a role in host immunity, both of which can alter the pathogenesis of gingivitis and periodontitis.
- Vitamin D levels can affect the calcification of tooth enamel of permanent teeth, the rate of tooth eruption, and the position of teeth.
- Caries incidence may be related to indirect vitamin D levels such as diet and sunlight exposure.
- High levels of vitamin D may have protective effects on bone and teeth in the oral cavity.
- Vitamin D toxicity may adversely affect tooth morphology and oral bone development.
19. Vitamin D and dental health

E. Jordan, S. Davis, P. Rakes, L.K. McCauley and J. Bashutski
Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 North University Avenue, Ann Arbor, MI 48109-1078, USA; jillbash@umich.edu

Abstract

Common oral diseases include gingivitis (inflammation of soft ‘gum’ tissue), periodontitis (infection of hard and soft tissues around teeth) and caries (infection of teeth). These conditions are influenced by the body’s immune function as well as its ability to promote mineralization of hard tissues in the area. As a mediator of mineralization and immunity, vitamin D levels may influence these conditions. Vitamin D has anti-inflammatory and anti-bacterial roles in the oral cavity. Its anti-inflammatory effects are a result of many different interactions including activation of both acquired and innate immunity. The presence of vitamin D also provides indirect antimicrobial effects through increasing cathelicidin levels, which destroy both gram negative and gram positive bacteria. As a result, vitamin D deficiency may compromise immunity and allow bacterial growth within the oral cavity, ultimately increasing local inflammation and the development of periodontal disease. Vitamin D is also necessary for mineralization of dental tissues including alveolar bone, enamel, and dentin. Inadequate mineralization of these tissues as a result of vitamin D deficiency can contribute to decreased integrity of these structures, placing them at higher risk for disease. In the presence of inflammation and pathogenic bacteria, which is also exacerbated by vitamin D deficiency, oral conditions such as periodontitis and caries may be at higher risk of development. While most evidence shows an association between vitamin D deficiency and conditions of the oral cavity, it is important to remember that oral diseases are often multifactorial. Evidence linking vitamin D deficiency with increased risk for oral disease requires more investigation to show causality and determine the magnitude of the contribution. Vitamin D is involved in many vital aspects of mineralization, immunity, and tissue integrity that suggest a key role in preventing oral disease.

Keywords: caries, gingivitis, periodontitis, vitamin D toxicity
19.1. Introduction

Establishing and maintaining health of the oral cavity requires harmony of various factors related to one's habits, genetics and systemic health. For the majority of people, oral and dental health can be maintained through brushing and flossing in addition to regular prophylactic cleanings and exams with an oral health care provider. However, often there are additional factors that impact health of the dentition. The oral cavity is composed of many tissue types including epithelium, connective tissue, blood vessels and lymphatic vessels. Uniquely, teeth are composed of pulp (blood vessels, nerves and collagen fibers), dentin, cementum and enamel, with the apical portions surrounded by bone. These tissues rely on both local and systemic factors for repair, regeneration and maintenance of health. For example, studies have shown the strong association between the use of fluoride and preventing dental caries where fluoride strengthens enamel through the formation of fluorohydroxyapatite (Buzalaf et al., 2011). Conversely, mutations in certain genes can cause defects in enamel formation (Simmer and Hu, 2001), potentially increasing susceptibility to dental caries.

Vitamin D plays a pivotal role in calcium homeostasis and immune regulation. Calcium and vitamin D have a documented role in preventing demineralization, encouraging remineralization and establishing the strength of mineralized tissues (i.e. bone and teeth). Therefore, a plausible relationship exists between the health of the periodontium, which is directly influenced by the health of the surrounding soft and mineralized tissues, and the presence of vitamin D and calcium. Furthermore, vitamin D has been associated with the arrest and prevention of dental caries through its effects on enamel mineralization.

In this chapter, we will explore the link between vitamin D and key conditions that affect dental health. Specifically, the relationship between vitamin D and gingivitis, periodontitis and dental caries will be discussed. As vitamin D is a fat-soluble vitamin, there are potential negative effects in the oral cavity when vitamin D levels remain elevated. Accordingly, information regarding the toxicity of vitamin D in the oral cavity will also be discussed.
19. Vitamin D and dental health

19.2 Vitamin D and gingivitis

19.2.1 Definition, nomenclature, and epidemiology

Diseases affecting the oral cavity typically impact the periodontium. The periodontium is defined as the surrounding and supporting structures of the tooth, and is broken down into two main categories, the gingiva and the attachment apparatus. The attachment apparatus consists of the periodontal ligament, cementum and alveolar bone. Cementum is an avascular calcified tissue, which comprises the outer portion of the root. The periodontal ligament is responsible for connecting the cementum (and therefore the root) to the surrounding alveolar bone (that portion of the maxilla and mandible forming the tooth socket). The gingiva is the soft tissue surrounding the tooth comprised of connective tissue covered by a layer of epithelium and its primary purpose is to protect the underlying tissues and referred to as the ‘gums’ in lay terminology. It aids in forming the gingival sulcus, which is a shallow ‘V’ shaped space bound by tooth structure on one side and epithelium on the other.

Gingivitis is an inflammatory condition confined to the soft tissues surrounding teeth that does not result in destruction of bone or the attachment apparatus (Armitage, 1999). The characteristic signs are redness and swelling of the gingiva, or gingiva that bleeds easily. Although gingival diseases can be related to a number of factors, the most common form is plaque-induced gingivitis. This gingivitis results from the presence of thin layer of bacteria and bacterial byproducts in a polysaccharide matrix, which is known as a dental biofilm (Page, 1986). Dental plaque is a biofilm that forms a soft deposit around teeth and other hard surfaces of the oral cavity. Plaque contains both organic and inorganic components but is primarily made up of microorganisms. These microorganisms are predominantly bacterial in origin and are roughly equal parts gram-positive and gram-negative. Some of the bacteria in the biofilm produce enzymes and tissue-destructive factors that can contribute to the breakdown of gingival tissues. However, the redness and swelling of the tissues seen in gingivitis is actually a manifestation of the body’s immune response to the presence of these microbes. In the initial stages of gingivitis, increased vascular permeability and subsequent infiltration of neutrophils and lymphocytes occurs (Page et al., 1997). Once permeability increases, microbes capable of producing products such as collagenase and endotoxin, may cause epithelial and connective tissue damage.

The etiology of gingivitis is multi-factorial but is most commonly due to the interaction of microorganisms within dental plaque and inflammatory cells of the gingival tissues. As with most inflammatory diseases, gingivitis can be modified by both local and systemic factors. Local factors that exacerbate gingival inflammation include the formation of calculus, a hard deposit that results from the mineralization of plaque. Systemic factors such as hormonal changes (puberty and pregnancy), medications (anticonvulsants, immunosuppressants, calcium channel blockers) and severe malnutrition may also exacerbate gingivitis.

Clinically gingivitis can be categorized as acute or chronic and localized or generalized. Diagnosis of gingivitis is typically made by elicitation of bleeding upon gentle probing of the gingival sulcus.
Gingivitis is also demarcated by changes in color, texture and position of the gingiva. According to a national survey of the US population, approximately 54% of individuals age 13 years or older have gingivitis in at least one site (Surgeon Generals’ Report, 2000). The same study found that gingivitis was most prevalent among younger and older age groups as compared to middle age groups. Gingivitis can be effectively treated by proper oral hygiene as well as regular dental prophylaxis.

19.2.2 Biological mechanisms of action of vitamin D in gingival tissue

Since bacteria and the immune response to these bacteria are the primary cause for diseases of the periodontium, this creates a unique environment for the role of the immune system in mediating conditions of the oral cavity. Evidence of the role of vitamin D in immunity is growing. VDR is a part of the superfamily of nuclear hormone receptors. VDRs are found in cells involved with calcium and bone metabolism, and immune regulation, and these receptors are present in cells of the oral cavity. When bound to the active form of vitamin D, 1,25(OH)₂D₃, VDRs bind to certain sequences in vitamin D-responsive genes and influence the rate of RNA polymerase II-mediated transcription (Mathieu and Adorini, 2002). 1,25(OH)₂D₃ binds to VDRs on T cells and antigen-presenting cells, thereby modulating the immune response. Because VDRs function based on binding to the active form of vitamin D, the enzyme responsible for the conversion of 25(OH)D into 1,25(OH)₂D₃ is crucial. In an animal study, the 25(OH)D-[1(OH)ase] enzyme was ablated in mice, resulting in hypocalcemia, secondary hyperparathyroidism and a reduction in CD4⁺ and CD8⁺ T cell populations (Panda et al., 2001).

The anti-inflammatory effects of vitamin D are well-supported through invitro human studies. The biologically active form, 1,25(OH)₂D₃ has been shown to decrease antigen-induced T cell proliferation through the inhibition of IL-2 (Bhalla et al., 1984), as well as inhibit the production of IFN-γ by T-lymphocytes (Rigby et al., 1987). Furthermore, several studies have documented the role of 1,25(OH)₂D₃ in inhibiting the differentiation, maturation and proliferation of antigen presenting cells (Berer et al., 2000; Penna and Adorini, 2000; Piemonti et al., 2000). 1,25-dihydroxyvitamin D has also been shown to impact innate immunity, leading to the destruction of infectious agents (i.e. Mycobacterium tuberculosis). When macrophages or monocytes are stimulated by an LPS or infectious agent, the expression of the VDR and the 25(OH)D-[1(OH)ase] enzyme are up-regulated; this in turn increases the production of 1,25-dihydroxyvitamin D, which increases the expression of cathelicidin (Holick, 2007). Cathelicidins are peptides capable of promoting innate immunity and promoting the destruction of infectious agents. They have documented antimicrobial activity, effective against both gram positive and gram negative bacteria (Zanetti et al., 2004). They have also been shown to impact the activity of antigen presenting cells (Bowdish et al., 2006). Thus, vitamin D may play a critical role in influencing the effect of bacteria on periodontal destruction through mediating the immune response to bacteria as well as antimicrobial effects.
19.2.3 Review of studies correlating vitamin D and gingivitis

In a recent study, McMahon et al. (2011) described the impact of vitamin D on gingival epithelial cells and their defense against the well-established periodontal pathogen *Aggregatibacter actinomycetemcomitans* (McMahon et al., 2011). Exposure of epithelial cells to the active form of vitamin D, 1,25(OH)₂D₃, induced the expression of the human cathelicidin LL-37 and also the innate immune regulator triggering receptor on myeloid cells (triggering receptor on myeloid cells), which has also been shown to impact the regulation of innate immune response. These gingival epithelial cells were shown to have increased antibacterial activity, measured through their increased ability to destroy *A. actinomycetemcomitans*. These findings support the notion of the effect of vitamin D on immune regulation and innate immunity in the oral cavity.

Even with the evidence of the role of vitamin D and its impact on modulators of inflammation and immunity, information regarding the role of the anti-inflammatory effects of vitamin D in humans is limited. A study by Dietrich et al. (2005) examined data from the 3rd NHANES data set and made associations between patients’ serum vitamin D levels and chronic gingivitis (Dietrich et al., 2005). They found a strong negative association between the serum level of vitamin D and chronic gingivitis, as indicated by BOP. Subjects in the top quintile of serum vitamin D levels had 20% lower odds of BOP; they also found that increasing the vitamin D levels by 30 nmol/l was associated with a 10% lower odds for BOP. Because of the known function of the immune response in the formation of gingivitis and the developing relationship between vitamin D and the immune response, there is reason to assume a possible association between vitamin D and gingivitis. Findings such as those made by Dietrich substantiate further investigation into this relationship.

19.3 Vitamin D and periodontitis

19.3.1 Definition, nomenclature, and epidemiology

Periodontitis is defined as ‘an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in the progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both’. Unlike gingivitis where the inflammatory process affects the gingival tissues only, periodontitis results in destruction of bone and connective tissue attachment around the tooth (Page, 1986). The result of attachment loss is typically the formation of a periodontal pocket, or pathological deepening of the gingival sulcus, which serves as a reservoir for pathogenic bacteria. Periodontitis is classified into three main categories based on its etiology, progression and response to therapy and includes: chronic periodontitis, aggressive periodontitis, and periodontitis as a manifestation of systemic disease (Armitage, 1999). Diagnosis of periodontal disease is based on an evaluation of dental radiographs demonstrating bone loss, probing of the periodontal pocket to measure attachment loss as well as the presence of bleeding and numerous other clinical parameters (Page, 1986).
Chronic periodontitis is the most common form of periodontal disease and is typically seen in adults older than 35 years of age (Papapanou, 1996). The primary etiology of this condition is the host immune response to the presence of bacterial plaque. Characteristically, chronic periodontitis causes a slow to moderate rate of destruction but may have rapid bursts of activity in the presence of local, systemic or environmental factors. Disease severity is generally based on the length of time the periodontal tissues have been challenged by inflammation. Because dental plaque is the main cause of chronic periodontitis, any plaque retentive factors such as calculus (calcified plaque), will aid in the progression of the disease. Calculus, which firmly attaches to the root surface, gives bacterial plaque an ideal place to harbor and grow. Therapeutic measures to control this condition are aimed at removal of calculus and control of periodontal inflammation.

Aggressive periodontitis differs from chronic periodontitis in that the rate of bone breakdown and attachment loss is typically rapid. Furthermore, the amount of plaque and calculus is minor when compared to the amount of destruction present. Despite the presence of deep periodontal pockets, the amount of inflammation present is generally minimal. Interestingly, aggressive periodontal disease is typically found in individuals under the age of 30 and may even show a pubertal onset but only affects a very small segment of the population (Papapanou, 1996). A genetic component appears to be present in aggressive periodontal disease based on a familial aggregation of the disease. Some, but not all individuals with this condition may show abnormalities in phagocyte function or a hyper responsiveness of macrophages pointing to an abnormal immune response.

Periodontitis as a manifestation of systemic disease can be found in individuals with certain hematologic and genetic conditions. Leukemias, Down syndrome and leukocyte adhesion deficiencies are among some of the conditions that lead to periodontal destruction. In general, it is believed that a disruption in the host defense mechanisms is responsible for the disease progression observed.

19.3.2 Review of studies correlating vitamin D and periodontitis

Because of the well-established link of vitamin D and its relationship to calcium homeostasis and regulation in bones, as well as its role in host immunity, there is biological plausibility to assume a relationship between vitamin D and periodontal health. Table 19.1 summarizes pertinent studies evaluating this relationship.

In animal models, vitamin D deficiency has been shown to increase osteoclast activity and bone resorption. Anderson et al. (2011) showed that in rats, serum vitamin D levels directly impacted bone mineralization. The rats were given a diet of 0.4% calcium and different levels of vitamin D3, which produced serum levels ranging between 10 and 115 nmol/l. There was a positive correlation between the trabecular bone mineral volume (measured in the distal femoral metaphysis) and the circulating 25(OH)D levels ($r^2=0.42$, $P<0.01$). There was an observed increase in the ratio of receptor activator of NFκB ligand:OPG mRNA for animals with low serum vitamin D levels. The receptor activator of NFκB ligand is a mediator of osteoclastogenesis and OPG is a receptor decoy antagonist. In an unrelated study, periodontal findings were evaluated in sheep that had
Table 19.1. Studies relating vitamin D with gingivitis and periodontitis.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subject demographic</th>
<th>Dental outcome measurement</th>
<th>Systemic outcome measurement</th>
<th>Type of study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietrich et al., 2005</td>
<td>6,700 adults aged 13 to &gt;90 years</td>
<td>Gingivitis measured by bleeding on probing</td>
<td>Serum concentration 25(OH)D</td>
<td>Cross-sectional</td>
<td>Subjects in the highest 25(OH)D quintile were 20% less likely to have bleeding on probing than the lowest quintile.</td>
</tr>
<tr>
<td>Krall et al., 2001</td>
<td>145 healthy adults aged 65 yrs and over</td>
<td>Tooth loss</td>
<td>Supplementation with calcium (500 mg/day) and vitamin D (700 IU/day)</td>
<td>Randomized controlled trial</td>
<td>Fewer subjects (13% vs. 27%) taking supplements lost teeth during the trial period and during the two year follow up period (40% vs. 59%) than those not taking supplements.</td>
</tr>
<tr>
<td>Dietrich et al., 2004</td>
<td>11,202 adults ≥20 yrs</td>
<td>Clinical attachment loss</td>
<td>Serum concentration 25(OH)D</td>
<td>Cross-sectional</td>
<td>Inverse association between serum 25(OH)D levels and attachment loss.</td>
</tr>
<tr>
<td>Miley et al., 2009; Garcia et al., 2010</td>
<td>51 postmenopausal women and men aged 50-80</td>
<td>Probing depths, sites with bleeding on probing, clinical attachment loss, alveolar bone crest height</td>
<td>Supplementation with calcium (≥1000 mg/day) and vitamin D (≥400 IU/day)</td>
<td>Cross-sectional/longitudinal</td>
<td>Subjects in the supplementation group had shallower probing depths, fewer sites with bleeding on probing, less attachment loss and less alveolar bone crest height loss, compared with those not taking supplementation.</td>
</tr>
<tr>
<td>Bashutski et al., 2011</td>
<td>40 adults (aged 30-65)</td>
<td>Probing depths and clinical attachment loss</td>
<td>Serum 25(OH)D</td>
<td>Randomized controlled trial</td>
<td>Vitamin D sufficient subjects (≥20 ng/ml) showed more CAL gain (0.92 vs. -0.43 mm) and greater pocket depth reduction (1.83 mm vs. 0.43 mm) than vitamin D deficient patients (16-19 ng/ml)</td>
</tr>
<tr>
<td>Chen et al., 2011</td>
<td>Asian and Caucasian adults</td>
<td>CP or AP</td>
<td>VDR polymorphisms: TaqI, Bsml, FokI, and Apal</td>
<td>Meta-analysis</td>
<td>Significant association between the VDR TaqI variants and CP in Asians. FokI allele is a risk factor for AP.</td>
</tr>
<tr>
<td>Deng et al., 2011</td>
<td>Asian and Caucasian adults</td>
<td>CP or AP</td>
<td>VDR polymorphisms: TaqI, Bsml, FokI, and Apal</td>
<td>Meta-analysis</td>
<td>Significant association between TaqI variant and CP. Among Asian CP patients, lower frequencies of the Bsml variant and higher frequencies of the Apal variants</td>
</tr>
</tbody>
</table>
been given a calcium and vitamin D-restricted diet, ovariectomy, and glucocorticoid injections, and compared with sheep without these alterations (Dvorak et al., 2009). The authors reported that sheep in the test group had more gingival recession than did those of the control group. Of note, however, is the age of the test group (mean 3.8±0.9 years) was different than the control group (mean 7.5±1.0 years), as age can influence degenerative changes of the periodontium. Furthermore, the authors admit that they could not implicate which single parameter influenced changes in the periodontium.

Although limited evidence of vitamin D and periodontal disease exists in animal studies, there has been growing work examining this relationship in humans. Patients with vitamin D-dependent rickets, who have a hereditary defect in vitamin D metabolism, frequently present with chronic periodontal disease (Zambrano et al., 2003). In a 2001 study, Krall reported a decreased incidence of tooth loss in elderly patients taking calcium and vitamin D supplementation versus those who did not (13% vs. 27%) (Krall et al., 2001). These effects lasted even after the end of supplementation for up to two years, as only 40% of subjects supplementing 1000 mg calcium lost one or more teeth, compared to nearly 60% of subjects who took less. Although the results of this study pointed to the impact of vitamin D and calcium supplementation on tooth loss, specific parameters of periodontitis were not measured and the tooth loss cannot be attributed to periodontal disease alone. However, in a 2004 report, data from the third National Health and Nutrition Examination Survey were used to evaluate the association between serum 25(OH)D levels and indicators of periodontal disease (Dietrich et al., 2004). It was shown that for persons >50 years old, there was a statistically significant, inverse association with attachment loss and serum vitamin D levels. When comparing persons from the lowest quintile of serum vitamin D with those from the highest quintile, there was 0.39 mm greater attachment loss for men and 0.26 mm greater attachment loss for women. Similarly, among patients receiving periodontal therapy, those with the highest serum vitamin D level show significantly less bleeding sites and lower mean pocket depth and clinical attachment (Teles et al., 2011). In a cohort of patients undergoing periodontal maintenance therapy, although marginally significant, patients who took ≥400 IU/day of vitamin D and ≥1000 mg/day of calcium had shallower probing depths, less attachment loss, fewer bleeding sites and less alveolar crest height loss for up to one year (Garcia et al., 2010; Miley et al., 2009). Though these studies did not directly measure serum 25(OH)D levels, the effects of supplementation are noteworthy and revealed the need for further investigation into this area.

Only one study has provided insight into the impact of serum vitamin D levels and response to periodontal therapy in patients with advanced periodontal disease. A post-hoc analysis of a study involving the anabolic peptide teraparatide showed a positive correlation between serum vitamin D levels and periodontal parameters (Bashutski et al., 2011). Forty subjects with severe chronic periodontitis planned for periodontal surgery were given teriparatide or placebo in conjunction with daily vitamin D and calcium supplements. Both clinical and radiographic parameters were measured over the course of 1 year and serum vitamin D levels were measured at baseline, 6 weeks and 6 months post surgery. The authors reported that placebo patients with vitamin D deficiency (defined as 16-19 ng/ml serum 25(OH)D) at the time of surgery had significantly less
clinical attachment level gain and pocket depth reduction than vitamin D sufficient patients at 12 months. Interestingly, even with growing evidence of the potential benefits of vitamin D and periodontal health, only 7% of adults enrolled in a periodontal maintenance program report taking the US Food and Nutrition Board’s recommended amount of supplementation (Dixon et al., 2009). This suggests that patients at high risk for periodontitis are also receiving inadequate amounts of vitamin D.

The role of vitamin D in periodontal health is gaining popularity, with increased interest into the precise nature of the association. This relationship, however, may not be limited solely to supplementation and circulating levels of serum 25(OH)D. There is evidence to suggest a prominent role of the VDR and its impact on periodontitis. Mutations in the VDR gene have been linked to diseases such as cancer, primary hypoparathyroidism, and renal failure (Valdivielso and Fernandez, 2006). Accordingly, VDR polymorphisms have been associated with both chronic and aggressive periodontitis (Meng et al., 2007; Yoshie et al., 2007). Four VDR polymorphisms have been identified in association with periodontal diseases: VDR TaqI, VDR BsmI, VDR FokI, and VDR ApaI. A meta-analysis by Chen et al. (2011) found a significant association between the TaqI variants and chronic periodontitis in Asians and found that the FokI allele is a risk factor for aggressive periodontitis. Deng et al. (2011) also conducted a meta-analysis examining the same four polymorphisms. Like the previous study, these authors found a similar association between the TaqI variants and chronic periodontitis; however, they also reported that among Asian chronic periodontitis patients, lower frequencies of the BsmI variant and higher frequencies of the ApaI variants were found.

Such findings highlight the interaction between vitamin D, its receptor, and maintenance of periodontal health. Vitamin D contributes directly to bone strength and plays a role in host immunity, both of which can alter the pathogenesis of periodontitis.

19.4 Vitamin D and dental caries

19.4.1 Definition, nomenclature, and epidemiology

As reported by the 2000 US Surgeon General report, dental caries is the single most common chronic childhood disease. Dental caries is five times more common than asthma and seven times more common than hay fever. Reports from the NHANES from 1988-1994 and from 1999-2004 suggest that the prevalence of dental caries is increasing, despite improvements in a number of other areas and factors such as sealants, tooth retention, periodontal health, and edentulism (Dye et al., 2007).

Early studies looking at the properties of vitamin D supplementation in children were conducted as early as 1924 where vitamin D was proposed to play a critical role in tooth development along with the theory that properly developed teeth are less susceptible to caries, which was a point of contention at the time with some dental authorities (Mellanby, 1924). Mellanby and Pattison
(1928) evaluated the use of dietary supplements, and determined that the addition of vitamin D prevented the initiation of new carious foci, limited the spread of caries and arrested active caries. McBeath (1934) evaluated 425 institutionalized males and females, ages eight to fourteen, and found that those receiving increased levels of vitamin D in evaporated milk and dairy milk, compared to positive and negative controls, had fewer dental caries. This group concluded that vitamin D is an important factor in the nutritional control of dental caries.

Studies have analyzed the regional geographic differences between the amount of average hours of sunlight and prevalence of caries (McBeath, 1934). In a study of 94,337 Caucasian boys ages 12-14, East (1939) found an inverse relationship between the hours of sunlight exposure in areas of residence and caries rate.

19.4.2 Impact on vitamin D on teeth

The ability of vitamin D to affect the calcification of tooth enamel of permanent teeth, the rate of tooth eruption, and the position of teeth was suggested as early as 1918 (Mellanby, 1918). Physical effects of vitamin D deficiency in teeth include dysplastic enamel in the form of enamel hypoplasia (Liu et al., 2009; Papagerakis et al., 1999) and is commonly associated with vitamin D-dependent rickets type I, hereditary vitamin D-dependency rickets, hypoparathyroidism, and other developmental disorders (Nikiforuk and Fraser, 1981).

Structurally, tooth-specific matrix proteins are differentially regulated by vitamin D3 during tooth development. Papagerakis et al. (1999) found that vitamin D3 up-regulates the transcription of amelogenin, and presumably plays a role in the genetic and hormonal control of enamel formation as seen in rats (Liu et al., 2009). Findings in vitamin D deficient animals include enamel defects found in the main enamel prism proteins as well as alterations in dentin formation with respect to shape, thickness, and composition (Papagerakis et al., 2002).

The effects of vitamin D metabolism are directly linked to the physiologic levels of calcium and phosphorus available extracellularly (Christakos et al., 2010; Liu et al., 2009). Lui et al. (2009) suggest that defects in teeth and mandibles during mineralization induced by vitamin D3 deficiency are similar to long bone defects as seen in 1α-hydroxylase knockout mice, which represents the vitamin D-dependent rickets type I loss of function mutation. Similarly, Krall et al. (2001) found that supplements of calcium and vitamin D in an elderly population of men and women contribute to a reduced the risk of tooth loss, a reduction of moderate bone loss, and nonvertebral fracture risk.

19.4.3 Review of studies correlating vitamin D and caries

Vitamin D and its relationship to dental caries was initially presented in the 1920s and 1930s (East, 1939; McBeath, 1934; Mellanby and Pattison, 1928) and has been continually studied to the present day. Table 19.2 summarizes several of the studies between vitamin D and dental caries, many of which support the benefits of nutritional supplementation with vitamin D to improve oral
19. Vitamin D and dental health

Mellanby and Pattison (Mellanby and Pattison, 1928) stated in multiple publications that diets high in vitamin D-containing foods caused a "hardening" effect on existing carious lesions and acted by suppressing the carious process. While there are limitations of their study in regards to how effectively the amount of available vitamin D from the dietary sources could be measured, these studies support the ability of carious lesions to be reversed through remineralization in the presence of adequate vitamin D. In a similar study, McBeath (1934) also applied dietary changes by increasing vitamin D intake through increased milk consumption. In addition, he added a group who received ultra-violet lamp treatments to further increase the amount of vitamin D the patients received. He then examined each individual and recorded the total number of carious surfaces as a percentage of the number of surfaces in the mouth over the course of a year and found an inverse correlation between vitamin D intake and the presence of caries. Pacey et al. (2010), through a cross-sectional health survey found a protective effect associated with milk intake, while sugar intake had a deleterious association in respect to reported caries experience. Schroth et al. (2005) considered maternal vitamin D supplementation with respect to primary tooth eruption, mean number of teeth with enamel hypoplasia and decayed, extracted, and filled teeth. No association was found with vitamin D supplementation and fewer decayed teeth; however, the eruption of primary teeth was found to be earlier in mothers who received vitamin D supplementation. Krall et al. (2001) evaluated tooth loss in the elderly using a longitudinal study and found that calcium and vitamin D supplementation prevented tooth loss. Possibly one of the longest longitudinal studies on the effects of vitamin D on caries was conducted over a 25-year period and evaluated calcium and phosphate levels in individuals with vitamin D deficiency over time (Nikiforuk and Fraser, 1981). Disorders that were monitored in this study included hereditary vitamin D-dependent rickets, X-linked hypophosphatemic rickets, permanent hypoparathyroidism, transient neonatal hypoparathyroidism and pseudohypoparathyroidism. This study observed the prevalence of enamel hypoplasia in both the primary and permanent dentition as well as the presence of dental lesions and found a positive association between hypocalcemia and enamel hypoplasia. No relationship was observed between hypophosphatemia and enamel lesions. Environmental factors such as sunlight exposure and regional location have also been evaluated for their role in dental caries, through indirect changes in vitamin D levels. Many studies have found that caries incidence is inversely related with annual sunlight exposure (East, 1939; Fraser and Nikiforuk, 1982; Krall et al., 2001).

19.5 Vitamin D toxicity in the oral cavity

While a deficiency in vitamin D may prevent calcification and compromise immunity, high levels of vitamin D may also be detrimental to oral tissues through promoting extensive bone resorption, ectopic calcifications and alterations in dental tooth morphology. A case report of a young girl affected by hypervitaminosis D showed enamel hypoplasia and focal pulp calcifications in the permanent teeth (Giunta, 1998). The effects of hypervitaminosis D on tooth structure may also include enamel hypoplasia, hypermineralization of enamel matrix, defective dentine formation, excessive hypercementosis and calcification of pulpal and periodontal tissues (Cagnone, 1970). However, this hypermineralization may have protective effects against caries as well. One study
Table 19.2. Studies relating vitamin D with caries.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subject demographic</th>
<th>Dental outcome measurement</th>
<th>Systemic outcome measurement</th>
<th>Type of study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mellanby and Pattison, 1928</td>
<td>21 children</td>
<td>Caries</td>
<td>N/A</td>
<td>Cross-sectional</td>
<td>Carious process could be inhibited by increasing the intake of fat-soluble vitamins</td>
</tr>
<tr>
<td>Mellanby and Pattison, 1932</td>
<td>22 children</td>
<td>Caries</td>
<td>N/A</td>
<td>Cross-sectional</td>
<td>Vitamin D and calcium rich diets that lack cereals has a greater inhibitory and curative effects on dental caries</td>
</tr>
<tr>
<td>McBeath, 1934</td>
<td>425 children</td>
<td>Total number of carious tooth surfaces</td>
<td>N/A</td>
<td>Cross-sectional</td>
<td>Correlation of degree of control of caries with a quantitative dose of vitamin D</td>
</tr>
<tr>
<td>East, 1939</td>
<td>94,337 boys, aged 12-14</td>
<td>Mean caries incidence per 100 children</td>
<td>Mean annual amounts of sunshine in hours</td>
<td>Cross-sectional</td>
<td>Mean caries incidence varies inversely with the hours of sunshine present in the area of residence</td>
</tr>
<tr>
<td>Nikiforuk and Fraser, 1981</td>
<td>56 children with vitamin D abnormalities</td>
<td>Enamel hypoplasia</td>
<td>Hypocalcemia</td>
<td>Longitudinal</td>
<td>Enamel hypoplasia occurred only in children who had hypocalcemia</td>
</tr>
<tr>
<td>Krall et al., 2001</td>
<td>145 healthy adults (aged 65 or older)</td>
<td>Tooth loss</td>
<td>N/A</td>
<td>Longitudinal</td>
<td>Intake levels of calcium and vitamin D used for preventing osteoporosis are beneficial to tooth retention</td>
</tr>
<tr>
<td>Schroth et al., 2005</td>
<td>98 children</td>
<td>DEFT</td>
<td>N/A</td>
<td>Cross-sectional</td>
<td>Mean DEFT for study group was high and 50% had enamel defects</td>
</tr>
<tr>
<td>Pacey et al., 2010</td>
<td>388 Nunavut Inuit children</td>
<td>RCE</td>
<td>Nutritional supplements, food questionnaire, dietary recalls</td>
<td>Cross-sectional</td>
<td>High prevalence of RCE. Milk intake showed protective associations. Sugar intake showed deleterious associations with RCE</td>
</tr>
</tbody>
</table>
reported that indirect effects of high levels of vitamin D, as assessed through ultraviolet radiation exposure, reduced caries rates. Interestingly, there was no adverse effect on teeth at higher levels of exposure, although this would not necessarily relate to toxic levels of vitamin D (Grant, 2011).

Hypervitaminosis D also affects the tissues surrounding teeth including alveolar bone, connective tissue, and epithelium. The effects of high levels of vitamin D on several tissue types were compared to a control group who received normal levels using an animal model (Haschek et al., 1978). In this porcine study, 825,000 IU/kg of vitamin D had detrimental effects to both bone and soft tissue within 2 weeks of administration. Notably, osteonecrosis, osteocytic osteolysis, and epithelial cell degeneration occurred. Although this study did not specifically evaluate oral tissues, both bone and epithelial integrity is critical to prevent many types of oral disease. An animal study evaluated the effects of 5,000 IU vitamin D/10 g body weight on the developing bones and teeth of rats (Hammarstrom et al., 1973). Within days, acid phosphatase activity increased in the alveolar bone regions, but did not increase in tooth enamel. In addition, ectopic calcification occurred near erupting teeth and in the connective tissues surrounding teeth, which ultimately altered the developing tooth morphology. Consequently, the effects of excessive levels of vitamin D in the oral cavity include increased alveolar bone resorption, loss of epithelial and connective tissue integrity, increased enamel mineralization, and tooth anomalies. While an insufficient number of studies have been conducted in humans, this information suggests that patients with hypervitaminosis D may be at increased risk for chronic periodontitis, altered tooth eruption, and decreased caries risk.

**19.6 Summary and conclusions**

Vitamin D is necessary for mineralization and immune support, both of which affect conditions of the oral cavity including gingivitis, periodontitis, and caries. Figure 19.1 summarizes the proposed effects of vitamin D deficiency on teeth and the periodontium. Vitamin D has anti-inflammatory and anti-bacterial roles in the oral cavity. Its anti-inflammatory effects are a result of many different interactions including activation of both acquired and innate immunity through upregulation of T-cells and antigen-presenting cells, as well as a feedback loop of increased VDR activation when monocytes and macrophages are infected by bacteria. The presence of vitamin D also provides indirect antimicrobial effects through increasing cathelicidin levels, which destroy both gram negative and gram positive bacteria. As a result, vitamin D deficiency may compromise immunity and allow bacterial growth within the oral cavity, ultimately increasing local inflammation and the development of gingivitis.

Vitamin D is also necessary for mineralization of dental tissues including alveolar bone, enamel, and dentin. Inadequate mineralization of these tissues as a result of vitamin D deficiency can contribute to decreased integrity of these structures, placing them at higher risk for disease. In the presence of inflammation and pathogenic bacteria, which is also exacerbated by vitamin D deficiency, oral conditions such as periodontitis and caries may be at higher risk of development.
While most evidence shows an association between vitamin D deficiency and conditions of the oral cavity, it is important to remember that oral diseases are often multifactorial. Evidence linking vitamin D deficiency with increased risk for oral disease requires more investigation to show causality and determine the magnitude of the contribution. Vitamin D is involved in many vital aspects of mineralization, immunity, and tissue integrity that suggest a key role in preventing oral disease.

References


19. Vitamin D and dental health


E. Jordan, S. Davis, P. Rakes, L.K. McCauley and J. Bashutski


Penna, G. and Adorini, L., 2000. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. Journal of Immunology 164, 2405-2411.


Key facts

- Vitamin D is mainly produced by ultraviolet B radiation in the skin, and has to be hydroxylated in liver and kidney to its active form 1,25-dihydroxyvitamin D. After ligation to vitamin D receptor, it modulates gene transcription in a wide range of cells.
- These effects have been shown in the immune system, inducing a shift from a pro-inflammatory Th1/Th17 response to an anti-inflammatory Th2/Treg profile.
- Vitamin D deficiency could be involved in such pathologies as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes, multiple sclerosis, cancer, cardiovascular and infectious diseases.
- Local modulation of the immune microenvironment requires high vitamin D doses. New pharmacologic forms are needed to avoid general toxicity, especially hypercalcemia.
- Randomized controlled trials will help to define new therapeutic modalities.

Summary points

- Vitamin D, beyond its role in bone metabolism, could be involved in many inflammatory diseases.
- Vitamin D receptor and the enzymes required for vitamin D metabolism are widely expressed in the immune system, and seem to induce an overall anti-inflammatory effect.
- Vitamin D deficiency is a frequent condition, with an increased prevalence in inflammatory diseases as shown in observational studies.
- Vitamin D supplementation has therapeutic effects in dysimmune disease models.
- The supplementation modalities remain unclear.
20. Inflammation and vitamin D

X. Guillot¹, C. Prati¹, N. Saidenberg-Kermanac’h², L. Semerano², G. Falgarone², M.C. Boissier² and D. Wendling¹
¹University Hospital Jean Minjoz, Rheumatology Department, Boulevard Fleming, 25030 Besançon, France; ²University Hospital Avicenne, AP-HP, Rheumatology Department, 125 rue de Stalingrad, 93009 Bobigny cedex, France; xguillot@chu-besancon.fr

Abstract

Calcitriol, or 1,25-dihydroxyvitamin D₃ is a well-known endocrine regulator of calcium homeostasis. More recently, local calcitriol production by immune cells was shown to exert autocrine or paracrine immunomodulating effects. Immune cells that produce calcitriol also express the vitamin D receptor and the enzymes required to metabolize vitamin D₃ (1α-, 25- and 24-hydroxylases). These immunomodulating effects, shown in animal models and cell culture experiments, are both direct and indirect and involve T cells, B cells, and antigen presenting cells (dendritic cells and macrophages), affecting both innate and adaptative immunity. The overall effect is a switch from a Th1/Th17 to a Th2/Treg profile. These immunomodulating properties could explain the epidemiological associations, suggested by observational studies, between vitamin D serum levels and many autoimmune and inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel diseases, type 1 diabetes, but also infections, cancer, transplant rejection and cardiovascular diseases. Furthermore, vitamin D supplementation showed therapeutic effects in animal models for these diseases. Thus, vitamin D is a key focus for public health efforts and may hold promise for the treatment of dysimmune diseases.

Keywords: 1,25-dihydroxyvitamin D₃, vitamin D receptor, 1α-hydroxylase, 25-hydroxylase, dendritic cell, macrophage, T cell, B cell
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)₂D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40-ligand</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclo-oxygenase 2</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DCs</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>KO</td>
<td>Knockout</td>
</tr>
<tr>
<td>LT</td>
<td>T lymphocyte</td>
</tr>
<tr>
<td>mDC</td>
<td>Myeloid dendritic cells</td>
</tr>
<tr>
<td>MHC2</td>
<td>Class 2 major histocompatibility complex</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>NFκB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NKT</td>
<td>Natural killer T cells</td>
</tr>
<tr>
<td>NOD</td>
<td>Nonobese diabetic</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Th</td>
<td>T helper</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>Tr1</td>
<td>Type 1 regulatory T cells</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T cells</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
</tbody>
</table>

## 20.1 Introduction

Vitamin D is a secosteroid hormone mainly produced in the skin following exposure to ultraviolet B. It can also be provided by the diet or by supplements. The crucial role for vitamin D in calcium-phosphate homeostasis and bone metabolism is well established, with optimal serum levels considered to be at least of 30 ng/ml (75 nmol/l) in order to limit the release of parathyroid hormone and promote the intestinal absorption of calcium (Holick, 2007).

In order to become biologically active, cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) must be hydroxylated at position 25- in the liver and then at position 1- in the proximal tubule of the kidney, resulting in the production of the active form calcitriol (1,25(OH)₂D). This liposoluble hormone binds to VDR, which is widely expressed in targets relevant to calcium.
Inflammation and Vitamin D

20. Inflammation and vitamin D homeostasis such as small bowel, osteoblasts. Vitamin D interacts with its target cells as a steroid hormone, by binding to its nuclear receptor VDR. VDR then undergoes heterodimerization, usually with the retinoid X receptor, and binds to specific DNA sequences (VDR elements) located in promoter regions, thereby controlling the transcription of target genes involved in calcium metabolism (e.g. binding proteins) and the immune response (Figure 20.1).

Recent observational studies suggest extra osseous effects of vitamin D. Indeed, vitamin D deficiency was associated with the risk and/or severity of many diseases including cancer, cardiovascular disease, sarcopenia, osteoarthritis, infections and transplant rejection, and notably autoimmune diseases such as diabetes, MS, and more recently IBD, and connective tissue diseases (mainly RA and SLE). These extra osseous effects of vitamin D, suggested by observational studies, are linked with vitamin D's immunomodulating properties, whose mechanisms have been shown in animal studies and cell culture experiments. Vitamin D influences the regulation of immune cells' proliferation, differentiation and function both in direct and indirect ways (Guillot et al., 2010; Meyer et al., 2011).

20.2 Immunomodulating effects of vitamin D

20.2.1 Enzyme metabolism

In addition to regulating calcium homeostasis, vitamin D has many other metabolic effects which were discovered more recently, notably via its immunomodulating properties first described about 20 years ago (Arnson et al., 2007; Lemire et al., 1984; Maruotti and Cantatore, 2010; Mora et al., 2008; Rigby et al., 1984; Sigmundsdottir et al., 2007) (Table 20.1). Vitamin D can accumulate in the microenvironment of lymphoid organs, where it exerts specific autocrine and/or paracrine effects without unwanted systemic effects (such as hypercalcemia and increased bone resorption). Activated T (and probably B) cells can only hydroxylate 25(OH)D3 to 1,25(OH)2D3, while macrophages and some DCs have both of the enzymes needed to convert vitamin D to

Figure 20.1. Mechanism of action of vitamin D within the nucleus of target cells.
1,25(OH)\textsubscript{2}D\textsubscript{3} (Fritsche et al., 2003; Sigmundsdottir et al., 2007) allowing 1,25(OH)\textsubscript{2}D\textsubscript{3} to be produced locally and to exert paracrine effects. In contrast to renal cells, antigen-presenting cells are not subjected to negative regulation of 1-alpha-hydroxylase by parathyroid hormone nor by 1,25(OH)\textsubscript{2}D\textsubscript{3}. In antigen-presenting cells, 1-alpha-hydroxylase is induced by many factors, including IFN\gamma, and undergoes downregulation as the DC matures (Hewison et al., 2003).

### 20.2.2 Role and expression of the vitamin D receptor

When activated by 1,25(OH)\textsubscript{2}D\textsubscript{3}, the VDR regulates gene expression in numerous tissues targeted by vitamin D. The VDR is expressed by many types of immune cells including circulating monocytes, macrophages, DCs, and activated T cells (Adams et al., 1983; Proveddini et al., 1983).
In VDR-KO mice, 1,25(OH)_2D3 can no longer induce the differentiation of haematopoietic bone marrow progenitors to monocytes/macrophages, and plays a key-role in spleen cell Th1 response (O’Kelly et al., 2002). However, VDR-KO mice have normal immune-cell populations and reject allogeneic and xenogeneic grafts as frequently as wild-type mice (Mathieu et al., 2001). VDR-KO mice with induced asthma exhibit no airway inflammation, hypereosinophilia, nor bronchial hyperactivity despite high levels of immunoglobulin E and Th2 cytokines, suggesting a possible key-role of vitamin D in allergic response (Wittke et al., 2004).

20.2.3 Effects on macrophages

Macrophages and DCs are regulated by 1,25(OH)_2D3, which thereby plays an important role in innate immune responses. 1,25(OH)_2D3 promotes monocyte-to-macrophage differentiation, stimulates macrophages to produce the immunosuppressant cytokine PGE_2 and inhibits granulocyte-macrophage colony stimulating factor expression (Towers et al., 1999). It also down-regulates macrophage production of pro-inflammatory cytokines and chemokines (Zhang et al., 2012), and enzymatic activities (COX-2, inducible NO-synthase) (Helming et al., 2005). Vitamin D deficiency impairs macrophage maturation and the production of macrophage-specific membrane antigens, lysosomal acid phosphatase, and hydrogen peroxyd required for antimicrobial activity (Abu-Amer et al., 1994). The addition of 1,25(OH)_2D3 increases the expression of these membrane markers, enzymes and free oxygen radicals and up-regulates chemotaxis and phagocytosis (Goldman, 1984; Koeffler et al., 1984). Activated macrophages produce 1,25(OH)_2D3 in response to IFNγ and activation of the TLRs. TLR2 stimulation has been shown to induce the expression of CY27B1 gene, coding for 1α-hydroxylase (Schauber et al., 2007). In human monocyte cultures, the addition of 100 nM of 1,25(OH)_2D3 inhibits the expression of the innate-immunity receptors TLR2, TLR4 and TLR9 and impairs the TLR9-dependant production of IL-6 (Dickie et al., 2010). Other effects of 1,25(OH)_2D3 may in contrast stimulate innate immunity: indeed, it downregulates cell membrane expression of MHC2 antigens and induces the production of cathelicidin, a peptide involved in antimicrobial response (Cantorna and Mahon, 2004; Gombaert et al., 2005; Liu et al., 2006; Wang et al., 2004). Furthermore, 1,25(OH)_2D3-induced downregulation of the membrane expression of MHC2 can also impair the antigen-presenting function of macrophages.

20.2.4 Effects on myeloid dendritic cells

1,25(OH)_2D3 inhibits mDC proliferation, maturation, differentiation and survival, monocyte-to-DC differentiation, therefore decreasing mDC stimulating effect on LT (Griffin et al., 2001; Penna et al., 2005; Piemonti et al., 2000). The NFκB transduction pathways is involved via vitamin D-induced inhibition of p65 subunit phosphorylation. 1,25(OH)_2D3 also inhibits IL-12 production by mDC, subsequently indirectly inhibiting the Th1 response – the direct interaction between 1,25(OH)_2D3, VDR and NFκB affecting IL-12 gene transcription (D’Ambrosio et al., 1998). In addition to IL-12 inhibition, 1,25(OH)_2D3 also increases IL-10 production by mDC. The overall result is a decrease in the Th1 response and probably an induction of IL-10-producing Tr1 cells. 1,25(OH)_2D3 also modulates LT-DC interactions by acting on co-stimulatory molecules.
It down-regulates CD40L-induced pro-inflammatory cytokine production (notably IL-1 and TNF-α) and membrane MHC2, CD40, CD80, CD86 expression, causing a decreased production of IL-12 and IFNγ and an increased production of IL-10 and transforming growth factor-β (Adorini et al., 2005; Almerighi et al., 2009; Gauzzi et al., 2005; Hardin, 2005; Lyach et al., 2005). CD-40, CD-80 and CD-86 down-regulation in antigen presenting cells (notably in mDCs) induces a decreased activation of LT (Van Etten et al., 2005). 1,25(OH)2D3 can also induce autoreactive T cell apoptosis via PD-L receptor up-regulation and can promote CD4+CD25+ Treg induction by immature mDCs (Boissier et al., 2009). In a murine colitis model, 1,25(OH)2D3 increased the expression of Fox-P3 transcription factor and IL-10, two critical factors for Treg induction (Daniel et al., 2008). This tolerogenic DC and Treg induction decreased the risk of rejection in a murine organ transplantation model (Gregori et al., 2001).

20.2.5 Effects on T cells

Vitamin D exerts direct effects on T and B cells and alters their response to activation, thereby playing a key role in adaptive immune response. Quiescent CD4+ T cells express VDR at a low rate, which shows a five-fold increase after cell activation (Mahon et al., 2003). To date, 102 target genes for 1,25(OH)2D3 have been identified in CD4+ T cells (Mahon et al., 2003). The effect of 1,25(OH)2D3 on T cells is both direct via inhibition of T-cell proliferation, notably IFNγ and IL-2 producing Th1 cells (Daniel et al., 2008; Van Etten et al., 2005) and indirect: 1,25(OH)2D3 inhibits the Th1 response by inhibiting DCs’ IL-12 production (Penna and Adorini, 2000). Furthermore, 1,25(OH)2D3 affects T-cell differentiation, inhibiting the Th1 response (cell proliferation, IFNγ and IL-2 gene transcription and protein expression) and stimulating the Th2 response (IL-4, IL-5 and IL-10) both indirectly by decreasing the production of IFNγ (Boonstra et al., 2001, Van Etten et al., 2005) and directly by increasing the production of IL-5 and IL-10 via an up-regulation of the Th2-related transcription factor GATA-3. The role of 1,25(OH)2D3 in IL-4 regulation is more controversial (Cantorna et al., 1998a; Staeva-Vieira and Freeman, 2002). In association with corticosteroids, 1,25(OH)2D3 induces IL-10 producing-Tr1 cells, which repress the immune response and could in part explain vitamin D’s therapeutic effects observed in animal models of autoimmune diseases (Barrat et al., 2002). CD4+ T cells from VDR-KO mice produce more IFNγ and less IL-2, IL-4 and IL-5 compared to those from wild-type mice (Froicu et al., 2003). In addition, 1,25(OH)2D3 inhibits Th17 response both directly by inhibiting CD4+ differentiation into a Th17 phenotype and IL-17 synthesis (Liu et al., 2006), and indirectly by repressing IL-6 and IL-23 synthesis (Daniel et al., 2008). This decreased IL-23 production is linked with a vitamin D-induced down-regulation of the Th-17 related transcription factor RORyt (Mus et al., 2010). In cell culture experiments, 1,25(OH)2D3 induces Treg in the presence of IL-2, by up-regulating the transcription of Fox-P3 and CTLA4 genes (Jeffery et al., 2009; Penna et al., 2005). T cell homing is also affected by 1,25(OH)2D3, which can act synergically with IL-12 to induce skin-homing marker CCR10 expression on T cell membrane surface, while keratinocytes express its ligand CCL27, which could promote T cell circulation and/or retention within epidermal tissues (Sigmundsdottir et al., 2007). Conversely, 1,25(OH)2D3 can also downregulate the expression of chemokines (CCL2, CXCL10) involved in pancreatic Th1 cell homing and infiltration in NOD mice, a model of type 1 diabetes (Giarrantana et al., 2004) or in monocyte infiltration in EAE,
20. Inflammation and vitamin D

A murine model of MS (Pedersen et al., 2007). In addition, 1,25(OH)$_2$D3 inhibits CD8+ T cell-mediated cytotoxic response (Meehan et al., 1992) and increases NKT cell function. In summary, 1,25(OH)$_2$D3 inhibits the Th1 and Th17 proinflammatory responses and promotes the Th2, Treg and Tr1 immunomodulating responses, resulting in an overall downregulation of the effector T-cell immune response (Figure 20.2).

20.2.6 Effects on B cells

As concerns B cells, 1,25(OH)$_2$D3 inhibits their proliferation, their differentiation into memory B cells, plasma cells, and immunoglobulin production, especially in SLE patients (Chen et al., 2007; Lemire et al., 1984; Linker-Israeli et al., 2001).

20.3 Vitamin D and inflammatory diseases

Many studies have shown significantly lower vitamin D serum levels in patients with autoimmune diseases compared to healthy controls. Furthermore, vitamin D3 supplementation has been reported to prevent the onset or decrease severity of autoimmune diseases (Table 20.2), and relationships have been suggested between vitamin D deficiency and many autoimmune diseases (Holick, 2007).

Figure 20.2. Vitamin D effects on immune cells.
Vitamin D deficiency is more prevalent in RA patients compared to the general population, but it is difficult to determine if this deficiency is a cause or a consequence of the disease (Aguado et al., 2000; Als et al., 1987; Ralston et al., 1988). In the Iowa Women's Health Study, a higher vitamin D intake, assessed using a dietetic questionnaire, correlated with a lower RA frequency (Merlino et al., 2004). However, a serum-bank case-control study from the Netherlands found no correlation between serum 25(OH)D3 levels and the onset of RA (Nielen et al., 2006). In an evaluation of the Nurse's Health Study cohort including 190 SLE patients and 722 RA patients, there was no correlation between vitamin D intake estimated from semi-quantitative food frequency questionnaires and the risk of SLE or RA (Costenbader et al., 2008). In RA patients, serum
20. Inflammation and vitamin D

25(OH)D3 levels correlated negatively with disease activity (Cutolo et al., 2007; Patel et al., 2007). In interventional trials, supplementation with 1 μg 1,25(OH)2D3 had no significant effect (Hein et al., 2000), while higher doses were associated with reduced pain and significant CRP decrease (Andjelkovic et al., 1999). In collagen-induced arthritis, a murine model of RA, supplementation with 1,25(OH)2D3 prevents arthritis development and blocks its progression (Boissier et al., 1992; Cantorna et al., 1998b). In cultured peripheral blood mononuclear cells from early RA patients, the supplementation with 1,25(OH)2D3 at the physiological concentration of 100 nM/l decreased IL-17, IFNγ and increased IL-4 production (Colin et al., 2010). Vitamin D also decreased Th17-driven synovial fibroblast activation in early RA patients (Van Hamburg et al., 2010), and acted synergically with TNF blockers to inhibit synovial inflammation (van Hamburg et al., 2012). In human chondrocyte culture experiments, vitamin D metabolites act via the VDR to regulate the transcription of numerous genes involved in chondrocyte metabolism, resulting for instance in a modulation of proteoglycan and collagen synthesis, and the expression of specific matrix metalloproteinases (Dean et al., 1997). In TNF transgenic mice, a murine model of arthritis, a KO for VDR gene aggravated clinical arthritides and synovial inflammation, with increased joint erosions, cartilage damages and an increased macrophage joint infiltration. VDR-KO macrophages showed a TNF-hyper responsive phenotype with prolonged MAP-kinase activation and cytokine secretion. VDR-KO monocytes also had an increased potential to differentiate into bone reabsorbing osteoclasts in vitro (Zwerina et al., 2011). This suggests a key-role of vitamin D deficiency in RA activity and severity and potential therapeutic implications (Buckland, 2012).

20.3.2 Inflammatory bowel disease

The prevalence of IBD is higher in areas less exposed to sunlight, such as the northern parts of Europe and America (Cantorna, 2006; Podolsky, 1991; Sonnenberg and Wasserman, 1991). Patients with IBD have low 25(OH)D3 serum levels compared to controls (Jahnsen et al., 2002). This result could be related to a combination of confounding factors including decreased intestinal absorption, lower dietary intakes, and limited sunlight exposure. Patients with newly diagnosed IBD also have lower serum 25(OH)D3 levels compared to controls (Lamb et al., 2002). In a recent double-blind randomized controlled trial including 108 patients with Crohn’s disease, daily oral supplementation with 1,200 UI of 25(OH)D3 increased the mean serum 25(OH)D3 levels from 69 to 96 nmol/l and reduced the relapse rates over the 1-year follow-up (13% vs. 29%; P=0.06) compared to the placebo (Jorgensen et al., 2010). In IL-10 KO mice, vitamin D deficiency was associated with accelerated bowel inflammation, while vitamin D3 supplementation had therapeutic effects in this murine model of IBD (Cantorna, 2000; Cantorna et al., 2000; Froicu et al., 2003). In a murine model of Th1-mediated experimental colitis, a low calcemic vitamin D analog exerted both preventive and therapeutic effects (Daniel et al., 2006).

20.3.3 Systemic lupus erythematosus

In the US, African Americans have a three-fold higher incidence of SLE with earlier disease onset and higher mortality and morbidity rates compared to Caucasians (Alarcon et al., 1999). A genetic factor is unlikely involved, as SLE is rare in Western Africa. This result is more probably related to
a reduced sunlight exposure and a decreased cutaneous penetration of ultraviolet B radiation due to high melanine levels. Vitamin D deficiency is common in SLE patients, whatever the disease duration, but it might be a consequence of the disease, related to an increased photoprotection (Bultink et al., 2005; Kamen et al., 2006; Muller et al., 1995). Conversely, a cross-sectional study found no significant difference in serum 25(OH)D3 between 25 women with SLE and 25 women with fibromyalgia (Huisman et al., 2001). In one study, serum 25(OH)D3 levels were lower in patients with undifferentiated connective tissue diseases compared to controls and vitamin D deficiency seemed to be involved in the subsequent development of differentiated connective tissue diseases in these patients such as RA, Sjögren’s syndrome, SLE, overlap syndromes, systemic vasculitides, and antiphospholipid syndrome. In addition, skin and pleural involvement correlated negatively with serum 25(OH)D3 levels in this study (Zold et al., 2008). In murine models of SLE, vitamin D supplementation had therapeutic effects, decreasing proteinuria and increasing life span (Lemire et al., 1992; Vaisberg et al., 2000).

20.3.4 Diabetes and multiple sclerosis

In NOD mice, a model of diabetes, administration of a 1,25(OH)2D3 analog diminished the expression of IL-12 and IL-12 and IFNγ, prevented DC maturation and pancreatic islet infiltration by Th1 cells, and halted disease progression (Mathieu et al., 1992). Treg cells were probably involved in this effect, as they were found in increased numbers in the pancreas-draining lymph-nodes (Gregori et al., 2002).

Vitamin D supplementation also showed a therapeutic effect in an animal model of autoimmune thyroiditis (Fournier et al., 1990). Vitamin D supplementation showed preventive and therapeutic effects in EAE, a murine model of MS (Lemire and Archer, 1991; Van Etten et al., 2003). These effects were mediated by IL-10 (Spach et al., 2006).

20.3.5 Transplantation, cardiovascular and infectious diseases

Vitamin D could be used as an adjuvant therapy in association with immunomodulating drugs in organ transplantation. In a murine model of heart allograft, 1,25(OH)2D3 and one of its analogs prolonged allograft survival (Amuchastegui et al., 2005). Furthermore, even if 1,25(OH)2D3 contributes to prevent graft rejection, it does not seem to interfere significantly with the protective anti-infectious immune response (Cantorna et al., 1998c). Vitamin D deficiency seems to affect blood pressure and myocardial function in a possibly TNF-α-dependant fashion (Zittermann et al., 2003). 1,25(OH)2D3 is also able to induce antimicrobial peptide gene expression (Wang et al., 2004).

20.4 Therapeutic implications

The optimal serum 25(OH)D3 concentration for bone health should be at least 30 ng/ml (75 nmol/l), with the ideal range being estimated at 30-60 ng/ml. Higher serum levels could
be required for vitamin D to exert its beneficial extra osseous effects (Meyer et al., 2011). Vitamin D poisoning occurs at levels greater than 150 ng/ml (Holick, 2007). Only fragmentary information is available on the serum 25(OH)D3 concentration required for vitamin D to exert immunomodulating effects. In one observational study, the risk of multiple sclerosis decreased by 41% for each 20 ng/ml increase in serum 25(OH)D3, starting at a threshold level of about 24 ng/ml (60 nmol/l) (Munger et al., 2004). When serum 25(OH)D levels fall below 20 ng/ml (50 nmol/l), human monocytes and macrophages are unable to initiate some of the innate immune responses in vitro, which could explain the increased risk of tuberculosis in populations with prevalent vitamin D deficiency such as African-Americans (Liu et al., 2006). No serum 25(OH)D threshold has been documented for RA. However, in one observational study, each 10 ng/ml increase in serum 25(OH)D was associated with a 0.3-point decrease in mean DAS28 and a 25% decrease in serum CRP (Patel et al., 2007). The pleiotropic roles of vitamin D in the immune response suggest it could be used as a therapeutic agent in many autoimmune diseases. The incidence and severity of such pathologies as RA, type 1 diabetes, IBD and MS could be reduced by increasing vitamin D intake (Cantorna and Mahon, 2004). In active RA patients, supplementation with α-calcidiol (2 μg/day) improved inflammation parameters (Andjelkovic et al., 1999). Obtaining immunomodulating effects in vitro requires local concentrations of 1,25(OH)2D3 of about 10−10 M in the immune microenvironment. The obtention of these concentrations requires supraphysiological doses of vitamin D3, associated with a non-acceptable risk of hypercalcemia. Therefore, vitamin D analogs inducing no hypercalcemia have been developed (Baeke et al., 2011; Brown and Slatopolsky, 2008; Plum and DeLuca, 2010). Vitamin D can also be used synergistically with immunomodulating drugs such as ciclosporine or with bisphosphonates (Daniel et al., 2006; Van Etten et al., 2005). In vivo studies of murine models suggest that not only vitamin D but also a sufficient calcium intake contribute to modulate the immune response in the gastrointestinal tract and in the central nervous system. Concomitant calcium supplementation enhances the ability of vitamin D to prevent IBD and MS (Cantorna et al., 1999, 2000).

20.5 Conclusion

Vitamin D’s immunomodulating properties may hold therapeutic promise in a wide range of inflammatory diseases, including autoimmune diseases, infections, malignancies and cardiovascular diseases. These immunomodulating effects could be related to anti-Th1 and anti-Th17, pro-Th2 and Treg/Tr1-inducing effects (Boissier et al., 2008). Treatment modalities (doses, target levels, pharmacological forms, association with calcium) remain to be established in randomized controlled trials.

References


20. Inflammation and vitamin D


20. Inflammation and vitamin D


X. Guillot et al.

Vitamin D and cancer
Key facts

- Pancreatic cancer is one of the most lethal cancers.
- Vitamin D has functions other than maintaining good bones.
- Vitamin D deficiency is a risk factor for pancreatic cancer and other cancers.
- Vitamin D analogs have potential to treat advanced pancreatic cancer.

Summary points

- Risk factors for pancreatic cancer are smoking, heavy alcohol consumption, obesity and diabetes mellitus, among others.
- The anti-tumor actions of 1α,25(OH)₂D and its analogs may include pro-apoptosis, anti-angiogenesis, anti-inflammatory in addition to anti-proliferation and pro-differentiation.
- The vitamin D analog, MART-10 is about 1000-fold more active than 1α,25(OH)₂D3 in inhibiting the proliferation of LNCaP and PC-3 prostate cancer cell lines and HepG2 liver cancer cell line in vitro.
21. Vitamin D in the prevention and treatment of pancreatic cancer

T.C. Chen1 and K.C. Chiang2
1Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA; 2Department of Surgery, Chang Gung Memorial Hospital and Chang Gung University, Keelung, Taiwan; taichen@bu.edu

Abstract

Pancreatic cancer is one of the most lethal cancers with a 5-year survival rate of less than 5%. The more aggressive nature of this disease contributes to its usual late stage diagnosis that makes most patients unsuitable for receiving curative surgery. In addition, traditional chemotherapy and radiotherapy are not effective in this group of patients, leaving little therapeutic choices for them. 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3), the biologically active form of vitamin D3, is now recognized to regulate more than 200 genes and to exert a variety of biological effects in almost every tissue in the body, including antiproliferative, pro-apoptotic, pro-differentiation and anti-angiogenesis effects in cancer cells in vivo and in vitro. The clinical use of 1α,25(OH)2D3 is impeded by the lethal side effects of hypercalcemia and hypercalciuria. Therefore, 1α,25(OH)2D3 analogs, which are either equipotent or more potent than 1α,25(OH)2D3 in inhibiting tumor cell growth but with less hypercalcemic and hypercalciuric side effects, have been developed for the treatment of different cancers, and either used alone or in combination with other anticancer agents. The inversed association between vitamin D status and incidence of many forms of cancer has suggested that vitamin D could play a role in the prevention of these types of cancer. Although it is still controversial whether this association exists for pancreatic cancer, biochemical evidence clearly indicates that pancreatic cancer cells are responsive to the inhibitory effect of 1α,25(OH)2D3 and its analogs in vitro and in vivo. In this chapter, we summarize the recent progress in the epidemiological studies of sunlight, and vitamin D nutritional status, and discuss the biochemical studies using 1α,25(OH)2D3 and its analogs in pancreatic cancer cells with particular emphasis on the molecular mechanism of anti-cancer actions of 1α,25(OH)2D3.

Keywords: analogs, metabolism, apoptosis, differentiation, antiproliferation
21.1 Introduction

PCA is one of the most lethal human malignancies. PCA ranks fourth in the Western countries and fifth worldwide among the most common causes of cancer-related mortality (American Cancer Society, 2008). In the US, an estimate of 43,920 new cases of PCA will be identified and 37,390 will die from this disease in 2012 (Siegel et al., 2012). The almost 1:1 ratio of incidence to mortality clearly indicates a poor prognosis and the lethal nature of PCA, due to difficulty of early diagnosis, early local spread, distant metastasis and resistance to traditional chemotherapy and radiotherapy. The overall five-year survival rate is in the range of 1-4%, which is much lower than the other types of cancers, such as colon, breast and prostate cancers (American Cancer Society, 2008). Until the present time, surgical extirpation has been the choice of treatment. However, the overall five-year survival rate after resection is only about 10-29% (Nitecki et al., 1995; Trede et al., 1990; Yeo et al., 1997). Moreover, at the time of presentation, 40% patients already had distant metastasis and another 40% were diagnosed with locally advanced cancer (Haller, 2003), which excluded them from being good candidates for resection.

Although the exact causes of pancreatic cancer remain poorly understood, certain environmental factors have been implicated. The best established and single most important risk factor for pancreatic cancer and the one to clearly avoid is tobacco smoke. Abundant epidemiological studies conducted since 1966 have linked tobacco smoking to an increase in the incidence of pancreatic cancer (Raimondi et al., 2009) and the risk increases with increasing frequency and length of tobacco exposure (Hassan et al., 2007). After cessation of cigarette smoking for 10 years or longer, the risk of pancreatic cancer dwindles (Iodice et al., 2008). Although the association between alcohol and pancreatic cancer is controversial (Jiao et al., 2009; Li Y et al., 2011; Michaud et al., 2010; Rohrmann et al., 2009; Schatzkin and Stolzenberg-Solomon, 2009;
Tramacere et al., 2010), recent evidence has pointed to an association between heavy drinking and pancreatic cancer mortality (Gapstur et al., 2011; Genkinger et al., 2009; Gupta et al., 2010). A pooled analysis of 14 cohort studies reported that in women who consumed more than 30 g of alcohol per day had a positive relationship between pancreatic cancer and alcohol consumption (Genkinger et al., 2009). A prospective study including 1,030,467 US adults 30 years and older reported that consumption of 3 or more drinks per day was associated with pancreatic cancer mortality in never smokers and in ever smokers (Gapstur et al., 2011). Similarly, a population-based case-control study including 532 cases and 1,701 controls in San Francisco Bay Area provides support for heavy alcohol consumption as a risk factor for PCA in men (Gupta et al., 2010). When heavy drinking was combined with heavy smoking, PCA risk increased to 4.3-fold (Talamini et al., 2010). Interestingly, consuming beer or wine had no association with PCA (Gapstur et al., 2011). High caloric intake and obesity were also found to be risk factors for pancreatic cancer (Berrington de Gonzalez et al., 2003; Fryzek et al., 2005; Patel et al., 2005; Reeves et al., 2007), except in Japan (Nitsche et al., 2011). Diets high in processed or red meat, diets low in phytochemicals such as lycopene and flavonols, have been proposed and refuted as risk or protective factors in different trials. Whether fruits and vegetables offer any protective benefit for pancreatic cancer is also conflicting. An earlier large-scaled cohort study failed to show positive benefit of fruits and vegetables (Vrieling et al., 2009). However, a recent case-control study strongly suggests that lower consumption of fruits, vegetables, whole grains, and fiber is associated with a higher pancreatic cancer risk (Jansen et al., 2011). Others, like intake of coffee, use of aspirin and previous cholecystectomy may be contributing factors for pancreatic cancer as well, although less conclusive (Batty et al., 2009; Landi, 2009; Lowenfels and Maisonneuve, 2006). Occupational exposures to chlorinated hydrocarbon solvents, synthetic polymer dust, ionizing radiation, pesticides, diesel and gasoline engine exhaust may also link to pancreatic cancer (Santibanez et al., 2010). For the development of pancreatic cancer, chronic pancreatitis, especially hereditary pancreatitis, is a risk factor (Maisonneuve et al., 2010; Nitsche et al., 2011). DM is also widely considered to be a risk factor for pancreatic adenocarcinoma independent of smoking (Li D et al., 2011). Furthermore, several case-control studies suggest that DM could be both an early manifestation and an etiologic factor of pancreatic cancer in males and females (Ben et al., 2011a,b; Maisonneuve et al., 2010).

To search for a potential geographical variation in the incidence of pancreatic cancer, epidemiologists found that the incidence of pancreatic cancer was 3- to 4-times higher in northern latitudes than in areas closer to equator (Curado et al., 2007). Similar variation has been observed previously with prostate, colon and breast cancers (Garland and Garland, 1980; Gorham et al., 1990; Schwartz and Hulka, 1990). This north/south variation has been attributed to sunlight or UV B radiation, which is proportional to vitamin D synthesis in humans, and further suggest that vitamin D deficiency may be a risk factor for pancreatic cancer.

Since pancreatic cancer has poor prognosis due to late diagnosis, and currently available chemotherapy and radiotherapy for pancreatic cancer are relatively ineffective, there is an urgent need to explore new regimens or strategies to prevent and/or treat this disease. In this chapter, we describe an overview of the current advances in the understanding of pancreatic cancer.
etiology and development, the pancreatic cancer/vitamin D connection, and the rationales for the potential use of vitamin D and its analogs for the prevention and treatment of pancreatic cancer.

21.2 Pancreatic cancer

21.2.1 Overview

The pancreas, a glandular organ located deep in the abdomen and lay partially behind the stomach and within the concavity of duodenum, is an integral part of the digestive system. The organ has two different functional components, the endocrine and the exocrine parts. The Islets of Langerhans which are the endocrine cells of the pancreas produce and secrete hormones, such as insulin and glucagon, into the bloodstream to regulate the blood glucose levels. The exocrine cells or the Acinar cells produce and excrete digestive enzymes into the duodenum where they assist in the digestion of food. Since the two parts have different normal functions, different kinds of symptoms will occur when tumors arise in either part. However, the vast majority of tumors arise in the exocrine part (Hernández-Muñoz et al., 2008). Cancer of the pancreas is not a single disease (Hruban and Adsay, 2009). In fact, there are as many as twenty different tumors classified under the term ‘cancer of the pancreas.’ Each of these tumors has a different appearance when examined under a microscope, some require different treatments, and each carries its own unique prognosis. An understanding of the different types of pancreatic tumors is required for rational treatment of this deadly disease (Mahalingam et al., 2009).

21.2.2 The origin and progression of pancreatic adenocarcinoma

PCA originates mainly from the pancreatic ductal epithelium. The disease is believed to evolve from premalignant lesions to invasive cancer, combined with successive accumulation of various gene mutations during the progressive process (Vogelstein and Kinzler, 2004). The premalignant lesions include PanINs, IPMN and MCN, with PanINs being the most common and best characterized histological precursor of pancreatic cancer (Hruban et al., 2008; Takaori, 2007). PanINs are microscopic ductal lesions (less than 5 mm) located in the head of the pancreas, consisting of columnar to mucins-containing cuboidal cells (Hruban et al., 2004). Fourtier classifications are used to describe PanINs, which are PanINs-1A, PanINs-1B (low grade PanINs and refer to as flat and papillary type, respectively), PanINs-2 (intermediate grade PanINs), and PanINs-3 (high grade PanINs), and to reflect its sequentially evolutionary process to PCA. IPMN and MCN are both macroscopic lesions. Current literatures indicate that majority of IPMN may progress into invasive cancer (Delpu et al., 2011). MCNs are large mucin secreting neoplasms and rare. About 20% of MCNs are associated with PCA. Other than originating from premalignant lesions, PCA may derive from a subgroup of about 1-5% of cells with stem cell properties through unlimited self-renewal and asymmetric division (Herrmann et al., 2007; Li et al., 2007). During the progression from low to high grade PanINs, and from precursor lesions to carcinoma, accumulation of gene mutations has been observed, which includes chromosome 7 alterations leading to enhanced expression of EGFR (Korc et al., 1986), up-regulation of the
21. Vitamin D in the prevention and treatment of pancreatic cancer

Oncogene KRAS2 (Almoguera et al., 1988) and down-regulation of tumor-suppressor genes, such as CDKN2A (Caldas et al., 1994), DKN2A (Yonezawa et al., 2008), TP53 (Redston et al., 1994), and DPC 4 (Hahn et al., 1996). Among the tumor-suppressor genes, TP53 mutation is the most common single point mutation in human cancer, accounting for up to 75% of PCA (Yonezawa et al., 2008), and is a late event during PanIN multistep progression to adenocarcinoma (Yachida and Iacobuzio-Donahue, 2009).

In addition to various gene mutations, epigenetic alterations have been linked to the etiology of PCA. This kind of alteration is the result of DNA methyltransferase-dependent methylation in CpG islands, histone modification and miRNA expression (Maitra and Hruban, 2008). Sato et al. (2003) reported that gene hypomethylation was a frequent epigenetic event in pancreatic cancer and was commonly associated with the overexpression of affected genes which were not expressed in normal pancreatic tissues. The discovery of miRNA also leads to the finding that numerous miRNAs were aberrantly and differentially expressed in PanINs (Li et al., 2010) and in IPMN lesions (Habbe et al., 2009). These miRNAs have been implicated in the abnormal regulation of cellular differentiation, proliferation, and apoptosis (Maitra and Hruban, 2008). Therefore, it has been suggested that miRNA alterations may contribute to the development of pancreatic cancer and may serve as markers for the early detection of pancreatic neoplasia (Yu et al., 2011).

21.2.3 Therapies for pancreatic cancer

Currently, the standard treatment for resectable pancreatic cancer remains surgery. However, less than 20% of PCA patients are surgically operable (Haller, 2003; http://www.seer.cancer). After operation, adjuvant chemotherapy with either gemcitabine or a combination of 5-fluorouracil and leucovorin is able to increase progression-free period and overall survival (Neoptolemos et al., 2004; Oettle et al., 2007; Regine et al., 2008). Combination of adjuvant chemotherapy and radiation therapy seems to improve overall survival; however, the results are not significant (Herman et al., 2008). For unresectable pancreatic cancer, the principle of treatment is mainly palliative. Historically, 5-fluorouracil was used in spite of poor overall response. Currently, the standard chemotherapy for this group of patients is gemcitamine (Renouf and Moore, 2010). Recently, there have been clinical studies using two new regimens, namely the combination of gemcitabine and erlotinib, or gemcitabine and leucovorin, that demonstrated improvement over gemcitabine alone (Conroy et al., 2011; Moore et al., 2007). Unfortunately, there was a significant increase in toxicity with leucovorin (Oberstein and Wasif Saif, 2011). There are clinical trials underway studying the combination therapy with erlotinib and capecitabine, and the combination of panitumumab with gemcitabine plus erlotinib for dual EGFR inhibition (Oberstein and Wasif Saif, 2011).
21.3 Roles of vitamin D in cancer

21.3.1 History of vitamin D

The discovery of vitamin D is closely associated with the childhood bone disease, rickets. In 1822, Sniadecki, a Polish physician, noted that children living on farms had a lower prevalence of rickets compared to those who lived in the city of Warsaw, Poland. In late 19 century, Theodore Palm, a medical missionary, observed that children living closer to equatorial areas did not have rickets and suggested sunbathing as a possible cure and strategy for rickets prevention. They both attributed their finding of the geographic differences in rickets incidence to varied exposures to sunlight (Mozolowski, 1939; Palm 1890). In 1918, Sir Edward Mellanby successfully raised dogs rachitic by feeding the animals with oats exclusively and keeping them indoors without exposing to sunlight, and was able to cure the rachitic dogs with cod liver oil. During that period, cod liver oil was known to treat night blindness and fracture. Mellanby did not know whether the cure of rickets was due to the newly discovered vitamin A present in cod liver oil (McCollum et al., 1916) or a new substance. It was not until 1922 McCollum clearly demonstrated that the anti-rachitic principle present in cod liver oil was a new substance and named it 'vitamin D' (McCollum et al., 1922). About the same period, Huldshinsky discovered that children with rickets could be cured by exposing to sunlight (Huldshinsky, 1919). Steenbock and Black (1924) then noted that UV-irradiated food could cure rickets, which led to a great discovery later that UV light was capable of transforming one substance stored in food and skin to another form. The findings suggested a close relationship between sunlight exposure and vitamin D. In other words, UV light could produce vitamin D, which possesses anti-rachitic activity.

21.3.2 Sources and metabolism of vitamin D

Vitamin D is rare in food. Therefore, the primary source of vitamin D for humans is the exposure to sunlight in the absence of vitamin D-fortified food and vitamin D supplement. Today, we increasingly depend on vitamin D supplementation to meet our vitamin D requirement due to the concern of UV-induced skin cancers. Vitamin D has two major forms: vitamin D2 and vitamin D3. Vitamin D2 is synthesized from ergosterol found in plants and fungi, whereas vitamin D3 is produced from 7-dehydrocholesterol in animal. When human skin is exposed to UV irradiation (wavelength 290-315 nm), 7-dehydrocholesterol, stored in the basal and suprabasal layers of skin, is photolyzed to form previtamin D3, which is then thermoisomerized to vitamin D3 (Holick et al., 1980). Either vitamin D3 or ingested vitamin D2 enters the blood circulation and is carried by DBP to other organs, including the liver which is the major site of 25-hydroxylation catalyzed by vitamin D-25-hydroxylase (CYP2R1) to form 25(OH)D (Ponchon and DeLuca, 1969; Cheng et al., 2004). Serum 25(OH)D, the index of vitamin D status in humans, has the highest affinity for DBP (Bouillon et al., 1980; Haddad and Walgate, 1976), and naturally is bound to DBP in circulation. 25(OH)D is then delivered to the kidneys to be hydroxylated by CYP27B1 to form 1α,25(OH)2D, the biologically active form of vitamin D. Both 25(OH)D and 1α,25(OH)2D can be hydroxylated by renal CYP24A1 to form their corresponding 24-hydroxylated metabolites. Hydroxylation at carbon 24 of the vitamin D molecule by CYP24A1 is the first step of inactivation...
process for 25(OH)D and 1α,25(OH)₂D (Schuster, 2011). However, it is now established that CYP27B1 and CYP24A1 are expressed not only in the kidneys but also in many tissues and cells (Schuster, 2011; Zehnder et al., 2001), including the expression of CYP27B1 in the pancreas (Schwartz et al., 2004). Thus, the pancreas has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion (Chiang and Chen, 2009).

### 21.3.3 Functions of vitamin D

The action of 1α,25(OH)₂D, the active form of vitamin D, is mediated through its binding to VDR, an endocrine member of the nuclear receptor superfamily (Haussler et al., 1997) to regulate its target genes. The first evidence indicating that vitamin D might have actions beyond regulating calcium and bone metabolism was obtained by an autoradiographic study injecting high specific activity of tritium-labelled 1α,25(OH)₂D₃ to vitamin D-deficient rats (Stumpf et al., 1979). The authors found that the radioactivity was concentrated not only in the nuclei of small intestine, renal tubules and glomeruli, but also in the nuclei of the epidermis, stomach, pituitary and parathyroid, suggesting that the target cells for 1α,25(OH)₂D₃ might include those not known for calcium and bone metabolism (Colston et al., 1980). Using a Chip-sequencing assay to define genome-wide mapping of VDR binding, Ramagopalan et al. recently reported that VDR was bound to 2,776 genomic sites in 229 vitamin D-regulated genes (Ramagopalan et al., 2010). It is now well-established that 1α,25(OH)₂D exhibits anti-proliferative, pro-differentiating, anti-inflammatory, pro-apoptotic activities, immune regulation and many other functions in a tissue- and cell-specific manner (Adams and Hewison, 2010; Bikle, 2009; Feldman et al., 2008) and, so far, it has been shown to have growth inhibitory effect on prostate, colon, breast, lung, liver and pancreatic cancer cells, which express VDR (Chiang et al., 2011; Colston et al., 1980; Cross et al., 1991; Kawa et al., 1997; Skowronska et al., 1993).

### 21.3.4 The mechanisms of anti-cancer effects of vitamin D

The mechanism involved in VDR-mediated anti-cancer effects includes (1) cell growth arrest; (2) apoptosis; (3) anti-angiogenesis; (4) pro-differentiation; and (5) anti-inflammation (Adams and Hewison, 2010; Bikle, 2009; Chen and Holick, 2003; Feldman et al., 2008; Welsh, 2011).

#### Cell growth arrest

Evidence that VDR is required for the antiproliferative effect of 1α,25(OH)₂D₃ in cancer cell lines has been obtained using stable transfection of cDNA that encodes the VDR into JCA-1 prostate carcinoma cells to increase the VDR concentration (Miller, 1998). This caused proportional increases in antiproliferative effects and CYP24A1 activity in the presence of 1α,25(OH)₂D₃. Conversely, stable transfection of antisense VDR cDNA to ALVA-31 prostate cancer cells to knockdown VDR (Miller, 1998) attenuated the ability of 1α,25(OH)₂D to inhibit cell growth and induce CYP24A1 expression by 1α,25(OH)₂D₃. Using a different approach, Zinser et al. (2003) studied cancer cells derived from VDR null animals and showed that the cells were completely resistant to 1α,25(OH)₂D₃-mediated growth arrest and apoptosis over the range of 0.01-100
nM 1α,25(OH)₂D₃. Overall, these data demonstrate that the 1α,25(OH)₂D-dependent induction of cell cycle arrest, CYP24A1 upregulation, and apoptosis in cancer cells is dependent on the nuclear VDR. In pancreatic cancer cells, 1α,25(OH)₂D₃ inhibited their proliferation through cell cycle arrest at the G₀/G₁ phase (Kawa et al., 1996), which in turn is mediated through the up-regulation of p21 and p27, followed by the down-regulation of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors (Kawa et al., 1997). During the cell cycle progression, E2F-1 transcription factor binds specifically to hypophosphorylated retinoblastoma protein (Powers et al., 2004). In mid to late G₁ phase, cyclin-dependent kinases phosphorylate retinoblastoma protein and displace the E2F-1 transcriptional factor, which further activates the expression of S phase genes required for DNA replication (Krtolica et al., 1998). The activity of cyclin-dependent kinases required for G₁/S transition is regulated by endogenous CDK inhibitors (i.e. p21 and p27). Interestingly, studies using LNCaP prostate cancer cells and HepG2 liver cancer cells have suggested that the up-regulation of p27 proteins induced by 1α,25(OH)₂D₃ or its analogs may not involve new p27 mRNA synthesis (Luo et al., 2009; Yang and Burnstein, 2003; Yang et al., 2002). In fact, the up-regulation of p27 protein expression is likely the consequence rather than the cause of 1α,25(OH)₂D₃-induced growth inhibition (Flores et al., 2010). Flores et al. (2010) infected LNCaP cells with a retrovirus containing a sh-p27 or sh-Luc (served as control) and found that while p27 was depleted by 74% in sh-p27 infected cells, compared to the cells infected with sh-Luc, the knockdown of p27 had no effect on 1α,25(OH)₂D₃-induced cell growth inhibition. Thus, it was concluded that p27 up-regulation in LNCaP prostate cancer cells was not essential for 1α,25(OH)₂D₃-mediated growth inhibition. Taken together, further investigation using pancreatic cancer cells infected with sh-p27 is needed to establish the role of p27 in the antiproliferative effect of 1α,25(OH)₂D₃.

In addition to the direct effects of 1α,25(OH)₂D₃ on cell cycle-related genes, the hormone may act indirectly through other mechanisms on these genes. For example, 1α,25(OH)₂D₃ has been shown to up-regulate the insulin-like growth factor binding protein-3 and transforming growth factor-β signaling pathways and down-regulate the EGFR signaling cascade (Huynh et al., 1998; Tong et al., 1999; Yanagisawa et al., 1999). In prostate cells, it has been proposed that the modulation of prostaglandin concentration by 1α,25(OH)₂D₃ may be one mechanism responsible for the growth inhibitory action of 1α,25(OH)₂D₃ (Moreno et al., 2005).

**Apoptosis**

1α,25(OH)₂D₃ induces apoptosis in a variety of cancer cells to exert anti-tumor effects by repressing the expression of the anti-apoptotic proteins BCL2 and BCL-Xₐ, or inducing the expression of pro-apoptotic proteins, such as BAX, BAK and BAD (Chen and Holick, 2003). In addition, 1α,25(OH)₂D₃ may directly activate caspase effector molecules to induce apoptosis. Activation of the caspase cascades, particularly caspase-3, -6, -7 and -9, is mainly induced by the release of cytochrome c from the mitochondrial membrane, into the cytosol. Among them, caspase-3 is the prime inducer of apoptosis (Duan et al., 2003; Gown and Willingham, 2002; Resendes et al., 2004). In our studies with HepG2 cells, no expression of active caspase 3 protein was found either in the control or the group treated with 1α,25(OH)₂D₃ as measured by western
21. Vitamin D in the prevention and treatment of pancreatic cancer

Blot analysis (Chiang et al., 2011). Flow cytometry study with Annexin V-FITC and PI staining to analyze apoptotic and necrotic cell populations of HepG2 cells after 1α,25(OH)_{2}D3 treatment also showed similar apoptotic and necrotic cell populations between the control and the treated groups, suggesting that the decrease in cell number after 1α,25(OH)_{2}D3 treatment in HepG2 cells is apoptosis-independent. The 1α,25(OH)_{2}D3-induced apoptotic response may also be mediated by the destabilization of telomerase reverse transcriptase mRNA, that will lead to the down-regulation of telomerase activity (Jiang et al., 2004). Therefore, 1α,25(OH)_{2}D3 induces apoptosis in a cell-specific manner (Chaudhry et al., 2001; Ylikomi et al., 2002) and may involve different mechanisms in different cells.

**Anti-angiogenesis**

The evidence that vitamin D might have effects on angiogenesis was first suggested by Merke et al. (1989), who demonstrated the expression of VDR by immunohistochemical technique using a specific monoclonal antibody against VDR in venular and capillary endothelial cells of human skin biopsies. In their study, they were able to demonstrate CYP27B1 activity and the growth inhibition in the presence of 25(OH)D3 and 1α,25(OH)_{2}D3 in the bovine aortic endothelial cells. Subsequently, various studies showed 1α,25(OH)_{2}D3 inhibited the proliferation of cultured endothelial cells and reduced angiogenesis in animal models (Chung et al., 2006; Iseki et al., 1999; Mantell et al., 2000). 1α,25(OH)_{2}D3 can also up-regulate mRNA levels of the potent anti-angiogenic factor thrombospondin 1 in human colon tumor cells (Fernandez-Garcia et al., 2005). The hormone can also interrupt the angiogenic factor interleukin 8 signaling, leading to the inhibition of endothelial cell migration and tube formation (Bao et al., 2006). It has been proposed that the inhibitory effect of 1α,25(OH)_{2}D3 on metastasis observed in prostate and lung murine models may partially depend on its anti-angiogenic property (Getzenberg et al., 1997; Nakagawa et al., 2005). It should be noted that in certain cells, 1α,25(OH)_{2}D3 may be angiogenic instead of anti-angiogenic, e.g. it induces the angiogenic factor interleukin 8 in SCC cells (Lin et al., 2002) and upregulates VEGF mRNA in vascular smooth muscle cells (Cardus et al., 2006).

**Pro-differentiation**

The seminal observation by Abe et al. (1981) that 1α,25(OH)_{2}D3 was capable of inducing mouse myeloid leukemia cells to differentiate into multinucleated macrophages provides the first evidence that 1α,25(OH)_{2}D3 has functions other than regulating calcium and phosphate homeostasis. Later, it was shown that 1α,25(OH)_{2}D3 can inhibit proliferation and increase the expression of differentiation markers, including involucrin, transglutaminase, loricrin and filaggrin, and enhance cornified envelope formation in cultured keratinocytes (Bikle, 2009). By maintaining the ordered cellular proliferation and differentiated epithelium, 1α,25(OH)_{2}D3 is able to contribute to skin cancer prevention (Bikle, 2009). Now, we know that in cultured cells, administration of 1α,25(OH)_{2}D3 or its analogs can regulate the expression of a variety of genes that are associated with the differentiated cell of origin (Akutsu et al., 2001; Guzey et al., 2004; Palmer, 2003), and thus modulate processes critical for tumor growth and metastasis (Fernandez-Garcia et al., 2005). In SW 480-ADH human colon carcinoma cell line, 1α,25(OH)_{2}D3 induces
differentiation by promoting the expression of proteins implicated in adherent junction formation, including differentiation marker E-cadherin (Palmer et al., 2001). This process is mediated by the up-regulation of Id1gene and down-regulation of Id2 gene in response to 1α,25(OH)₂D₃ (Fernandez-Garcia et al., 2005). In breast cancer cell lines, the induction of differentiation markers, such as E-cadherin, was also observed following 1α,25(OH)₂D₃-induced growth arrest (Kemmis et al., 2006; Lazzaro et al., 2000; Pendas-Franco et al., 2008). Thus, 1α,25(OH)₂D₃-induced differentiation may be one mechanism responsible for inhibiting tumor growth and metastases.

**Anti-inflammation**

Animal studies have linked the anti-cancer effects of 1α,25(OH)₂D₃ to its ability to regulate inflammation (Welsh, 2011). In colon carcinoma cells, 1α,25(OH)₂D₃ is capable of interrupting the wnt-mediated crosstalk between tumor epithelial cells and macrophages in the tumor microenvironment by blocking the production of IL-1β, an inflammatory cytokine produced by tumor-associated macrophages (Kaler et al., 2009). Conversely, any disruption of the 1α,25(OH)₂D₃/VDR signaling pathway, such as up-regulation of the transcriptional repressor SNAIL, may enhance epithelial cell inflammation and exacerbate colon cancer progression. Feldman and colleagues performed cDNA-microarray analyses of normal and cancer-derived primary prostate epithelial cells (Peehl et al., 2004) and LNCaP cells (Krishnan et al., 2004) and found that 1α,25(OH)₂D₃ regulated a wide array of genes, including those involved in the synthesis and catabolism of prostaglandins, well-established inflammatory compounds. They showed that 1α,25(OH)₂D₃ up-regulated the expression of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase gene and down-regulated cyclooxygenase-2 (COX-2) expression. Since prostaglandins are known to play a role in the development and progression of many cancers (Mantovani et al., 2008), the ability of 1α,25(OH)₂D₃ to decrease prostaglandin concentration strongly suggests that one mechanism of anti-cancer effect of vitamin D may be mediated through its anti-inflammatory action.

### 21.4 Vitamin D and pancreatic cancer

#### 21.4.1 Overview

Vitamin D deficiency has been implicated in many chronic diseases and cancers (Garland et al., 2009; Holick, 2007). Regarding pancreatic cancer, laboratory studies have firmly established the anti-tumor effects of 1α,25(OH)₂D₃ in cultured pancreatic cancer cells and in animal models. However, the epidemiological data are conflicting. This could be due to the aggressive nature of this disease, and the lack of a ‘zero’ or ‘near zero’ lag time blood drawing between diagnosis and serum 25(OH)D measurement that is critical to demonstrate the inverse relationship between the incidence of pancreatic disease and vitamin D status (Mohr et al., 2010).
21. Vitamin D in the prevention and treatment of pancreatic cancer

21.4.2 Biochemical evidence

As mentioned in Sections 21.3.3 and 21.3.4, 1α,25(OH)₂D₃ possesses anti-tumor activity through anti-proliferative, pro-apoptotic, and pro-differentiation actions in a cell- and tissue-specific manner (Adams and Hewison, 2010; Bikle, 2009; Chen and Holick, 2003; Feldman et al., 2008; Plum and DeLuca, 2010). Regarding pancreatic cancer, 1α,25(OH)₂D₃ has been shown to up-regulate the expression of p21 and p27 and down-regulate the expression of cyclins A, D1, and E, leading to cell cycle arrest at G₀/G₁ phase (Kawa et al., 1997). However, 1α,25(OH)₂D₃ is known to cause hypercalcemia and hypercalciuria side effects. To overcome these lethal side effects caused by systemic administration of 1α,25(OH)₂D₃, numerous less calcemic or noncalcemic analogs of 1α,25(OH)₂D₃ have been synthesized and studied in vitro and in vivo animal models. Some of them have been found to have more potent anti-tumor activities mediated by promoting cell-cycle arrest, cellular differentiation, and/or apoptosis in pancreatic cancer cells in vitro and in the xenograft animal model (Colston et al., 1997; Kawa et al., 1996, 1997; Pettersson et al., 2000; Schwartz et al., 2008; Zugmaier et al., 1996). One of these analogs, EB-1089, has been investigated in a phase II clinical trial to treat advanced pancreatic cancer. However, the trial showed that the analog failed to significantly prolong the survival of patients (Evans et al., 2002). In a more recent published phase II clinical trial enrolling 25 advanced pancreatic cancer patients, a combination of oral 1α,25(OH)₂D₃ (0.5 μg/kg) and docetaxel significantly increased the period of time-to-progression of pancreatic cancer as compared to treatment with docetaxel alone (Blanke et al., 2009). A VDR-alkylating derivative of 1α,25(OH)₂D₃, 1α,25-dihydroxyvitamin D₃-3-bromoacetate, has been studied in vitro and was shown to inhibit the growth of several pancreatic cancer cell lines to a greater extent than 1α,25(OH)₂D₃ (Persons et al., 2010). The in vitro activity was further enhanced by combining with 5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside (Persons et al., 2010). In our laboratory, a new vitamin D analog, MART-10 has been shown to be about 1000-fold more active than 1α,25(OH)₂D₃ in inhibiting the proliferation of LNCaP and PC-3 prostate cancer cell lines and HepG2 liver cancer cell line in vitro (Chen et al., 2007; Chiang et al., 2011; Flanagan et al., 2009; Iglesias-Gato et al., 2011). Most importantly, MART-10 does not increase serum calcium in animals (Iglesias-Gato et al., 2011). Furthermore, MART-10 is more resistant to CYP24A1-mediated degradation pathway and has a lower binding affinity for DBP compared to 1α,25(OH)₂D₃, suggesting that this analog would be more bioavailable than 1α,25(OH)₂D₃ in circulation (Flanagan et al., 2009; Iglesias-Gato et al., 2011). Given the poor prognosis and little effective therapeutic options against pancreatic cancer, these two new analogs are promising candidates for further pre-clinical studies, and subsequent clinical trials for pancreatic cancer patients.

21.4.3 Ecological and epidemiological studies of vitamin D and pancreatic cancer

Vitamin D status has been shown to possess positive impacts on the incidence of prostate, colon and breast cancers in a number of ecological and epidemiological studies (Garland and Garland, 1980; Gorham et al., 1990; Schwartz and Hulka, 1990). For pancreatic cancer, two earlier epidemiologic studies reported inconsistent relationship between pancreatic cancer incidence and vitamin D status (Skinner et al., 2006; Stolzenberg-Solomon et al., 2006). A later nested case-
control study conducted by Stolzenberg-Solomon et al. (2009) again failed to support the inverse association between the circulating concentration of 25(OH)D and the risk of pancreatic cancer. The same group further reported that a high 25(OH)D level exceeding 100 nmol/l (40 ng/ml) would have a 2-fold increase in pancreatic cancer incidence (odds ratio = 2.12, 95% confidence interval: 1.23, 3.64) (Stolzenberg-Solomon et al., 2010). On the contrary, Mohr et al. (2010) reported that pancreatic cancer incidence rates were half as high in countries with estimated serum 25(OH)D >30 ng/ml than in those with ≤30 ng/ml. A recent report by Wolpin et al. (2012) demonstrated that among participants in a pooled analysis of nested case-control studies with 451 cases and 1,167 controls from five large prospective cohorts with a follow-up ranging from 12.2 yr in Women's Health Initiative-Observational Study to 25.3 yr in Physician's Health Study, higher plasma levels of 25(OH)D were associated with a lower risk of pancreatic cancer. The study further implies that low circulating levels of 25(OH)D may predispose individuals to the development of pancreatic cancer. No increased risk was found in subjects with 25(OH)D ≥100 nmol/l as reported in a previous study by Stolzenberg-Solomon et al. (2010). The lack of association between serum 25(OH)D levels and pancreatic cancer risk in some studies could be due to that the studies utilized a single serum sample obtained years prior to diagnosis for 25(OH)D measurement. It may well be that serum 25(OH)D changed over the years after the measurement. For example, Yin et al. (2010) conducted case-control studies with zero lag time between diagnosis and serum 25(OH)D measurement, not nest studies, and found an inverse correlation between serum 25(OH)D level and breast cancer.

 Interestingly, the inverse relation between sunlight exposure and pancreatic cancer has been more consistent. For example, the death rate of pancreatic cancer has been found to be inversely associated with sun exposure (Boscoe and Schymura, 2006; Grant, 2007; Stolzenberg-Solomon et al., 2006; Tuohimaa et al., 2007). Stolzenberg-Solomon et al. (2009) did confirm a positive association among subjects with low estimated annual residential solar UVB exposure and pancreatic cancer risk in the same study which did not show an inverse relation between serum 25(OH)D levels and pancreatic cancer risk. Mohr et al. (2010) as well as many other studies (Giovannucci et al., 2006; Kato et al., 1985; Neale et al., 2009) also found an inverse association between UVB irradiation and incidence rates of pancreatic cancer worldwide.

Results from investigating association of insulin and glucose levels and the development of pancreatic cancer have also indicated a positive association between high insulin and glucose levels and pancreatic cancer (Henning et al., 2004; Huxley et al., 2005; Michaud et al., 2007; Stolzenberg-Solomon et al., 2005). Since vitamin D is capable of regulating the synthesis, binding and actions of insulin (Maestro et al., 2000, 2003; Mathieu et al., 2005), the finding may imply that an inverse relationship between pancreatic cancer incidence and vitamin D status could very well exist.

To resolve the question whether vitamin D status has a preventive benefit against the development of pancreatic cancer, more careful selection of study population according to VDR genotypes and possibly the genotypes of DBP and CYP enzymes involved in vitamin D metabolism, such as CYP24A1, CYP27B1 and CYP2R1, may be essential. Several recent genome-wide association
studies have suggested that variation in vitamin D pathway genes may affect serum concentrations of 25(OH)D (Ahn et al., 2010; McGrath et al., 2010; Signorello et al., 2011; Wang et al., 2010).

21.5 Conclusions

Pancreatic adenocarcinoma remains to be a lethal human malignancy without effective treatments at the present time. During the past few years major advances have been made in the understanding of the molecular mechanisms underlying the initiation and progression of pancreatic cancer, mainly through the efforts of genome- and epigenome-wide screening techniques. By knowing the earliest molecular signatures of carcinogenesis, we may be able to either prevent its initiation, detect the precursor lesions at the early stages or before the tumor cells metastasize to other organs. The active form of vitamin D3, 1α,25(OH)2D3, has been recognized as one of the most potent compounds in inhibiting cell proliferation and inducing cellular differentiation of normal and cancer cells for more than 30 years, including pancreatic cells. Recently, a genome-wide mapping of VDR binding study using a Chip-sequencing assay indicates that there are 2,776 genomic binding sites for VDR in 229 vitamin D-regulated genes. Coupled with this information, genomic profiling has been used to gain insight into the anti-tumor mechanisms of vitamin D. We now know that the anti-tumor actions of 1α,25(OH)2D and its analogs may include pro-apoptosis, anti-angiogenesis, anti-inflammatory in addition to anti-proliferation and pro-differentiation. However, at the present time we still don’t know whether some of the vitamin D-affected genes can prevent the normal or precancerous cells from becoming cancerous. As vitamin D deficiency is implicated with the increased incidence of many cancers and other chronic diseases, the likelihood that this kind of ‘prevention’ genes is present is very high. Regarding the treatment of advanced pancreatic cancer, a phase II trial using a combination of 1α,25(OH)2D and docetaxel showed a significant increase in the period of time-to-progress of pancreatic cancer as compared to docetaxel alone. In our laboratory, we have studied an analog of 1α,25(OH)2D3, MART-10, and demonstrate that this analog is non-calcemic, and is much more potent and more bioavailable than 1α,25(OH)2D3 in prostate and liver cancer cells. Knowing the properties of this analog, we may be able to use this analog in combination with docetaxel or other chemotherapeutic agents to treat advanced pancreatic adenocarcinoma patients. In conclusion, from the current biochemical, epidemiological and ecological evidence accumulated for the past several decades, sufficient vitamin D nutrition may be beneficial for the prevention of cancers, including pancreatic adenocarcinoma, and the non-calcemic and highly potent 1α,25(OH)2D, such as MART-10, in combination with other chemotherapeutic agents could be a promising regimen for treating advanced pancreatic cancer.

References


21. Vitamin D in the prevention and treatment of pancreatic cancer


T.C. Chen and K.C. Chiang


21. Vitamin D in the prevention and treatment of pancreatic cancer


Herrmann, C., Abel, U., Stremmel, W., Jaeger, D. and Herrmann, T., 2007. Short time to progression under first-line chemotherapy is a negative prognostic factor for time to progression and residual survival under second-line chemotherapy in advanced pancreatic cancer. Oncology 73(5-6), 335-339.


21. Vitamin D in the prevention and treatment of pancreatic cancer


McCollum, E.V., Simmonds,N. and Pitz,W., 1916. The relatian of the unidentified dietary factors, the fat-soluble A and watersoluble B of the it to the growth promoting properties of milk. J Biol Chem 27, 33-38.


Palm, T., 1890. The geographical distribution and etiology of rickets. Practitioniner 45, 270-279.
21. Vitamin D in the prevention and treatment of pancreatic cancer


21. Vitamin D in the prevention and treatment of pancreatic cancer


Key facts

- Melanoma is the most commonly lethal form of skin cancer, and although it is known to be caused by sun exposure, the relationship between patterns of sun exposure and risk is very complex.
- Sun exposure is critical to human health, however, in terms of synthesis of vitamin D, low vitamin D is associated with increased risk of many diseases.
- The balance between sun exposure and protection is therefore the key issue for melanoma and that will depend on where people live, their skin type (how sensitive they are to sunburn), their lifestyle (especially behaviour in the sun) and cultural clothing requirements.
- Although epidemiological studies consistently implicate sunburn as a major risk factor for melanoma, there is some evidence that regular moderate sun exposure might be protective for melanoma.
- Vitamin D levels may also be important to survival for melanoma patients but we don’t yet know the optimal blood levels.

Summary points

- Excessive sun exposure increases the risk of melanoma of the skin in pale skinned peoples.
- The role of vitamin D in protection from melanoma is not known. There is some evidence that pale-skinned people who spend a lot of time in the sun in temperate climates may be at a reduced risk compared with those who spend most of their time indoors.
- There appear to be differences in the relationship between sustained sun exposure and melanoma risk at different latitudes for pale-skinned people.
- There are some genetic data looking at inheritance of the gene coding for the vitamin D receptor, for a role for vitamin D in the cause of melanoma.
- Melanoma patients in the Leeds Melanoma Cohort who have lower levels of vitamin D at diagnosis have thicker, poorer prognosis tumours and are more likely to die of their melanoma.
- Blood levels of vitamin D are affected by obesity, sun exposure, dietary supplementation and genetics. Variation in the gene coding for vitamin D binding protein plays a significant role in determining blood levels.
- Vitamin D has an anti-proliferative effect on melanoma cells in vitro and therefore higher levels may be beneficial in terms of survival for melanoma patients, however we have concerns to avoid excessively high levels of vitamin D fearing an adverse effect on immune-surveillance at high doses. A conservative level to aim for might be 60 to 85 nmol/l throughout the year.
22. Vitamin D and melanoma

J.A. Newton-Bishop and J.A. Randerson-Moor
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds LS9 7TF, United Kingdom; j.a.newton-bishop@leeds.ac.uk

Abstract

Melanoma is predominantly a cancer of pale-skinned peoples and there is no doubt that sunburn is the best short-term measure known of the adverse effects of intense UV exposure. The genetics of susceptibility reinforce the role of sunburn as the commonest susceptibility genes are associated with sun-sensitivity (pigment genes). Other common genes are also naevus genes and there are newly identified susceptibility genes of unknown role which when further studied will tell us more about the biological basis of susceptibility. While observational studies, proving a role for vitamin D in susceptibility and survival from melanoma, are not achievable, there are some lines of circumstantial evidence, which can be interpreted this way. A case-control study suggests that moderate regular sun exposure may be protective for melanoma and this could be explained by photoadaption after sun exposure having the effect of reducing the likelihood of sunburn, or a protective role for vitamin D. Genetic association studies of inheritance of polymorphisms in the gene encoding the vitamin D receptor are suggestive of a role for vitamin D in protection from melanoma but this is as yet unproven. Melanoma patients in the Leeds Melanoma Cohort who have lower levels of vitamin D at diagnosis have thicker tumours and poorer prognosis even when the data are stratified for the thickness of the tumours, but these data have not as yet been confirmed in other studies. There are in vitro data to suggest that vitamin D is anti-proliferative for melanoma cells and the putative effect of vitamin D on melanoma survival might be attributed to those anti-proliferative effects. Vitamin D however has complex effects on the immune system, some of which might be beneficial for melanoma patients and some theoretically harmful. Consequently the optimal approach for managing vitamin D levels after diagnosis of melanoma (especially when melanoma patients tend to be sun-avoidant after diagnosis) is not known. A sensible approach might be to aim for levels at the lower end of the normal range (e.g. 60 to 85 nmol/l), whilst this difficult issue is being resolved.

Keywords: optimal levels, prevention, survival, immune-surveillance, sunburn
22.1 Introduction

Melanoma of the skin is the most potentially lethal form of skin cancer. It arises either from a benign melanocytic naevus or de novo. Most cases arise on sun-exposed skin in pale skinned peoples, although rare subtypes occur on sun-protected body sites at approximately the same incidence in all ethnic groups. The commonest type is the superficial spreading type, which is most common on the back of men and the legs of women. The nodular subtype is a less common, more rapidly growing sub-type and finally, melanomas also arise from precursor lesions called lentigo maligna, on the chronically sun-exposed skin of the face. The incidence of melanoma on sun-exposed skin has increased considerably in most white skinned populations and this is attributed to greater exposure to sunny holidays or leisure time sun exposure. Although most melanomas are cured by excision, the treatment of metastatic melanoma remains very difficult because of resistance to chemotherapy.

22.2 Melanoma susceptibility genes

The majority of cases of melanoma are of the superficial spreading or nodular varieties and these occur rarely in individuals who do not have white skin, so genes are clearly important in susceptibility. Within the white-skinned populations, those with pale skin which has a tendency to burn are especially at risk (Gandini et al., 2005c). A tendency to burn in the sun is related to inherited pigmentary genotypes such as variants in the gene coding for the MC1R and pigment genes, such as MC1R, are also known to be associated with melanoma risk (Valverde et al., 1996). Genome wide association studies have recently identified SNPs in a number of additional pigment genes such as tyrosinase and the agouti locus (an MC1R agonist) (Gudbjartsson et al., 2008) as melanoma risk alleles. Thus melanoma is actually a strongly genetically determined type of cancer and a number of the susceptibility genes are pigment genes related to how skin reacts to sunlight.

There are other groups of susceptibility genes, which are not pigment genes. The strongest phenotypic risk factor for melanoma is the presence of multiple melanocytic naevi (moles) (Gandini et al., 2005a). Individuals with multiple moles also commonly have clinically atypical...
22. Vitamin D and melanoma

moles, which are larger than the norm (>5 mm in diameter), with an irregular shape and colour. This phenotype is commonly known as the atypical mole syndrome phenotype. Twin studies showed that this phenotype is predominantly genetically determined (Easton et al., 1991; Wachsmuth et al., 2001) and therefore, not surprisingly, the second group of identified susceptibility genes are genes also associated with naevus number, such as a locus on chromosome 9, PLA2G6 (Falchi et al., 2009) and a gene called IRF4 (Duffy et al., 2010). Although this ‘at risk’ phenotype is primarily genetically determined, there is a measurable effect of sun exposure seen both in twin studies (Wachsmuth et al., 2005) and in melanoma case-control studies such as one recently reported by our group in which increased number of naevi was related especially to sunny holidays (Newton-Bishop et al., 2010). Some have considered the development of melanoma in people with many naevi as occurring by a different ‘route’ than those developing as a result of inherited pigment genes (Whiteman et al., 2003), but it can be seen that sun exposure is likely critical for both.

Very recent genome wide association studies have identified new groups of susceptibility genes, in the vicinity of genes such as MX2 (Barrett et al., 2011) which have not previously been associated with melanoma biology and are not related to the known melanoma-associated risk factors of pigmentation and naevus count, and hence likely indicate different pathogenetic ‘routes’ to melanoma, and exploration of these genes is likely to cast new light on pathogenesis in the near future.

In terms of genetic predisposition to melanoma then, pigment genes are important as are genes associated with increased numbers of naevi, and there are emerging data suggestive of genes not associated either with fair skin or increased numbers of naevi, and these remain to be understood.

22.3 Sun exposure and risk

That genes associated with a tendency to burn in the sun are melanoma susceptibility genes, and that the ‘at risk’ naevus phenotype is related to sunny holidays is good evidence for a role for sun exposure in the cause of melanoma. Epidemiological evidence is also strong. The world variation in occurrence shows that melanoma incidence is highest where pale-skinned people live closest to the equator. The highest incidence is in Australasia where the melanoma population is primarily genetically derived from the UK (Bishop et al., 2009) and is therefore pale-skinned. The high incidence in Australasia has resulted from a combination of inherited fair skin genes and very high levels of sun exposure, for a population, which, it might be said, had evolved to thrive in northern Europe.

Case-control studies have provided very strong evidence for a role for sunburn in the pathogenesis of melanoma, reported as a meta-analysis (Gandini et al., 2005b) and more recently as a pooled data analysis (Chang et al., 2009) of multiple such studies. In both a very consistently elevated risk associated with sunburn was seen for melanoma at all latitudes. Many studies have reported a particularly strong effect for sunburn in childhood. These epidemiological data identifying sunburn as a risk factor are consistent with laboratory data developed in a fish model in which
intense ultraviolet B exposure in adults was associated with the development of melanoma (Fernandez et al., 2012). The relative importance of early sunburn is supported by data summarized by Noonan (Noonan et al., 2003) in which early exposure to ultraviolet light was necessary to initiate melanoma in hepatocytes growth factor/ scatter factor transgenic mice (Zaidi et al., 2011).

Although there is no doubt that sunburn is associated with melanoma risk, there is a much less clear relationship between chronic sun exposure and risk. There is (at least in temperate climates) no dose response relationship between cumulative sun exposure and risk (Gandini et al., 2005b) so that there appears to be something particular about sunburn which causes melanoma even if the total cumulative sun exposure through life is not high.

The mechanisms via which intense sun exposure, and consequent sunburn, increase melanoma risk were first extensively explored by Margaret Kripke and her colleagues. Using experiments in mice, they showed that ultraviolet B exposure may result both in local immunosuppression in the skin and systemic immunosuppression, and that these effects are related to growth of melanomas in the skin of those mice (Donawho and Kripke, 1991; Fisher and Kripke, 1977; 2002). The cellular and molecular basis of this UV-induced immunosuppression in man is now fairly well understood. The chromophores which mediate UV-induced immunosuppression in animals, including man, include products of damaged DNA (pyrimidine dimers), urocanic acid and membrane components which generate free radicals and oxidative stress (see review by Norval, 2011). UV-induced changes in the chromophores result in the release of inflammatory mediators, and migration of Langerhans and dermal dendritic cells from the skin to the draining lymph nodes. Immunosuppression involves production of T regulatory cells, which secrete the immunosuppressive cytokine IL-10. The accumulation of macrophages and mast cells in the skin also occurs. The theory developed by Kripke and her colleagues and reviewed eloquently by Norval (McGill et al., 2002; Norval, 2011) is that UV exposure, if intense, may induce genetic mutations in the skin as well as local and systemic immunosuppression so that those mutant cells can proliferate unchecked. In this way they have proposed an explanation for the peculiar relationship between sunburn and melanoma risk. The propensity to melanoma as a result of sunburn is postulated also to result from the fact that melanocytes are highly differentiated cells, which are protected from apoptosis due to high expression of molecular inhibitors of apoptosis such as Bcl-2 (McGill et al., 2002). It is postulated therefore, that mutated melanocytes are likely to persist in sunburn damaged cells because the mutated cells are less likely to apoptose than keratinocytes and less likely to be killed by immune-defence systems.

There is therefore no doubt that sun exposure inducing sunburn is strongly related to melanoma risk, and the concern is that the incidence of melanoma continues to rise in many countries predominantly populated by pale-skinned peoples who have access to sunny holidays (Downing et al., 2006), given that holiday sun exposure is the behaviour most strongly related to sunburn in epidemiological studies (Newton-Bishop et al., 2011). The difficulty however in converting the epidemiological data to public health advice, is the lack of a simple relationship between cumulative sun exposure and risk. If melanoma aetiology was related to sun exposure (some have argued) then there would be a linear relationship between cumulative sun exposure and
22. Vitamin D and melanoma

risk as there is, for example, for squamous cell carcinoma. There is in fact, for melanoma, little epidemiological evidence of such a relationship except in countries near to the equator such as Australia and Hawaii (Chang et al., 2009). In temperate climates there are data which suggest the reverse, in that outdoor workers in some case-control studies appeared to be at somewhat reduced risk (Gandini et al., 2005b). Sceptics have therefore doubted the relationship between sun exposure and risk.

We have recently reported a very detailed exploration of different patterns of sun exposure and melanoma risk in the UK (Newton-Bishop et al., 2011) in 960 melanoma cases and 500 controls. In this study the clear increased risk associated with sunburn was seen, but 5 or more hours of regular sun exposure per day at weekends was actually associated with a reduced melanoma risk (OR 0.67, 95% CI 0.50-0.89 for highest versus lowest tertile of weekend exposure). This pattern of sun exposure was associated with higher blood levels of 25-hydroxyvitamin D3 (henceforth referred to as vitamin D when referring to serum levels) and this might suggest that vitamin D was protective for melanoma. Certainly vitamin D appears to be important in the skin's responses to the sun (see below) but there are a number of additional explanations for this finding. Regular weekend sun exposure is likely to result in 'photoadaption'; a process by which regular sun exposure results in a number of changes in the skin including tanning and epidermal thickening, which make sunburn less likely on sunny days. Thus this type of exposure might, in the long term, be protective for melanoma by reducing sunburn; although there are data to suggest that UV-induced immunosuppression will not necessarily be reduced as a result of photoadaption (Norval and Wulf, 2009). There may be alternative explanations too, such that higher vitamin D levels might be a marker of another (as yet unidentified) protective factor. However, vitamin D does remain a possibility in terms of melanoma prevention (not least because it is produced within the skin) and thus we will explore the significance of the published data.

There are very few data on vitamin D intake and risk. One small study from Italy showed some evidence of risk associated with lower reported levels of intake of vitamin D (Vinceti et al., 2011), the adverse effect of low intake on risk being stronger in males.

In the Leeds case-control study there was some suggestion of a case-control difference in vitamin D levels in that cases had non-statistically significant lower levels than controls (Newton-Bishop et al., 2011), but such studies are subject to considerable difficulties in interpretation because of effects consequent upon the diagnosis and case-control bias. Cohort studies of healthy individuals and risk are therefore much more likely to yield meaningful results. In the Women’s Health Initiative Randomized Controlled Trial 36,282 post menopausal women were randomized to receive calcium and 400 IU vitamin D3 per day or placebo, with a mean follow up of 7 years (Tang et al., 2011). Although there was no effect overall of vitamin D3 and melanoma risk, there was a reduction in risk for women who had previously had non-melanoma skin cancer (hazard ratio 0.43, 95% CI 0.21-0.90). Moreover, examination of the published Kaplan-Meier estimates of the cumulative hazard ratio for melanoma is suggestive of the emergence of reduced melanoma risk just beginning, after what is actually a small period of time (7 years) for a cancer which is thought
Vitamin D synthesis starts in the skin after irradiation with UVB, when UVB-mediated photolysis of 7-dehydrocholesterol occurs to form pre-vitamin D3. Subsequent enzymic conversion occurs outside the skin predominantly, although both the necessary enzymes, 25-hydroxylase (CYP27A1) and 1α-hydroxylase (CYP27B1) are present in skin, in keratinocytes. Thus the active form of vitamin D, 1,25-dihydroxyvitamin D3 may be present in the skin and what is known about its function there suggests that it might be photoprotective as it enhances repair of pyridimine dimers and, in some circumstances, protects against UV-induced immunomodulation. It is clear that injury to the skin moreover results in increased synthesis of vitamin D in the skin and there is evidence that the vitamin D then increases the expression of pattern recognition receptors important in innate immunity, such as toll-like receptors and CD14 (Miller and Gallo, 2010). Innate immunity is classically described as important in early defence systems. So, cutaneous vitamin D might protect against malignancy by increasing DNA repair or via a role in innate immunity. Certainly vitamin D deficiency is thought to be related to an increased tendency to infection, as was seen in terms of chest infections in children with rickets in the past. Dermatologists are also familiar with the use of ultraviolet light to treat cutaneous tuberculosis (Norval, 2011) but it’s not clear whether the beneficial effects could be attributed to the UV exposure itself or consequent vitamin D synthesis.

Although cutaneous vitamin D may have a protective function by playing a role in innate immunity, it is known to suppress Th1 and Th17 cytokine responses thus potentially leading to suppression of adaptive immune responses (Norval, 2011), and there is clinical evidence for suppression of immunity as sun exposure commonly results in reactivation of latent viral infections with herpes simplex. Exposure to intense ultraviolet light can moreover also suppress immunity both in the skin and systematically as described above, so that the net effect of sun exposure on cutaneous immunity is likely to depend upon many factors such as UV dosage and possibly the genetic characteristics of the host.

In summary then, it is possible that our observation that regular weekend sun exposure in the summer in the UK was protective for melanoma and associated with higher serum vitamin D levels whilst sunburn was associated with risk, might reflect this complex interaction between sun exposure, vitamin D synthesis and immunity. One interpretation might be that moderate but persistent sun exposure results in increased levels of cutaneous vitamin D, thereby controlling innate immunity in the skin and providing protection from the profound negative effects on the immune system resulting from sudden intense sun exposure as suggested by Dixon et al (2010). The ability of an individual to respond to regular sun exposure in terms of protection from melanoma would however depend upon the individual’s genetically determined ability to...
synthesize vitamin D in the skin, their pigmentation, genetic variation in DNA repair capabilities, or innate immunity, use of clothing, etc.

22.5 Genetic data

Sun exposure, if intense and especially if associated with sunburn, is clearly causal for melanoma. However if there is a complex relationship between doses of sun exposure and risk, so that moderate but sustained exposures might protect against melanoma by resulting in higher levels of vitamin D, then elucidating the relationships involved in the effects of sunshine on the skin is understandably difficult. A number of groups have therefore sought to use genetic association studies to study this relationship. The genomic effects of vitamin D on the cell are mediated by binding of vitamin D to a heterodimer made up of the nuclear VDR and the retinoid receptor, RXR. The binding results in transcription of a large number of genes explored recently using ChIP sequencing (Ramagopalan et al., 2010) in which 229 genes were identified with significant changes in response to vitamin D. It is thought that vitamin D may also have transcription-independent effects mediated by binding elsewhere possibly even to cell surface molecules such as the epidermal growth factor, summarized by Deeb et al. (2007), but these effects are less well studied. The changes consequent upon these non-genomic effects of vitamin D however are much more rapid then the genomic effects (Dixon et al., 2010) taking seconds or minutes rather than hours or days.

The gene coding for the VDR is polymorphic and a number of SNPs in the gene are thought to modify the expression and/or activity of the receptor. The functional implications of six variants in particular have been extensively investigated: the coding non-synonymous variant FokI (p.Met1Thr, rs2228570); the promoter SNPs Cdx-2 (rs11568820) and GATA (A-1012G, rs4516035); and the 3’ gene SNPs BsmI (rs1544410), ApaI (rs7975232) and TaqI (rs731236). The FokI ‘T’ variant located in exon 2 of the \textit{VDR} gene, the only non-synonymous \textit{VDR} SNP so far identified, leads to the creation of an alternative start site and results in the expression of an extended VDR protein (427aa) which has been shown to both have diminished transcriptional activity compared to the shorter ‘C’ variant protein (424aa) (Colin et al., 2000; Jurutka et al., 2000; Whitfield et al., 2001) as well as less efficient interaction with the TFIIB transcription factor (Jurutka et al., 2000) suggesting that the ‘C’ variant protein represents a more transcriptionally potent VDR protein. Additionally two promoter variants which affect a Cdx-2 and GATA-3 transcription binding site respectively have also been shown to alter \textit{VDR} expression. The Cdx-2 ‘A’ variant has been shown to bind the intestinal specific transcription factor Cdx-2 more strongly than the wild type ‘G’ variant with a concurrent increase in transcriptional activity (Arai et al., 2001). It is proposed that the ‘A’ allele leads to increased intestinal VDR expression and thus better absorption of calcium (reviewed by Uitterlinden et al., 2004). The GATA ‘G’ variant results in destruction of a strong GATA-3 core binding site (Halsall et al., 2004). The GATA-3 transcription factor is reported to be important in directing the polarization of naïve T-cells to Th2 (T-helper Type 2) lymphocytes (Rengarajan et al., 2000) and has been shown to be upregulated by 1,25(OH)$_2$D3 (Boonstra et al., 2001). Consequently the VDR GATA polymorphism is believed to
have a role in the anticancer immune response (Halsall et al., 2004). The functional consequences of the BsmI, ApaI (both located in intron 8) and the TaqI (p.Ile352Ile) variants, which are known to be in strong linkage disequilibrium, are less clear, with conflicting reports of the effects of the most commonly found haplotypes on VDR expression, mRNA stability and protein activity published by a number of investigators (summarized in Uitterlinden et al., 2004). However individually carriers of the homozygous wild type TaqI variant display lower levels of circulating active vitamin D (Hustmyer et al., 1993; Ma et al., 1998; Morrison et al., 1994), and the BsmI variant may affect gene expression via regulation of mRNA stability (Jurutka et al., 2001).

Association of these SNPs with melanoma risk has been investigated by a number of groups with somewhat variable results, as is the norm for relatively small candidate gene association studies. To date four meta-analyses of VDR polymorphisms in skin cancer have been reported (Gandini et al., 2009b; Mocellin and Nitti, 2008; Orlow et al., 2012; Randerson-Moor et al., 2009) focusing primarily on limited subsets of the functional gene variants. In 2009 we reported a study of the six VDR SNPs detailed above (Randerson-Moor et al., 2009) in which there was no significant evidence of an association between any of the SNPs and risk in 1028 population-ascertained cases and 402 controls from Leeds, UK. However in a second Leeds case-control study (299 cases and 560 controls) the FokI T allele was associated with increased melanoma risk (OR 1.42, 95% CI 1.06-1.91, \(P=0.02\)). In the accompanying meta-analysis performed in conjunction with published data from other smaller data sets (total 3769 cases and 3636 controls), the FokI T allele was associated with increased melanoma risk (OR 1.19, 95% CI 1.05-1.35), whilst the BsmI A allele was associated with a reduced risk (OR 0.81, 95% CI 0.72-0.92), in each instance under a parsimonious dominant model. A second meta-analysis of BsmI and FokI was reported concurrently in the same year (Gandini et al., 2009b), which reported similar findings. Most recently a meta-analysis of GATA, BsmI and TaqI, which included data from an additional Polish case control series (Gapska et al., 2009), showed a similar protective effect for BsmI to that observed in previous meta-analyses (Orlow et al., 2012).

Genome wide association studies have identified a number of SNPs associated with serum levels of vitamin D located near genes involved in vitamin D synthesis, activation or transport; the strongest being an intronic SNP (rs2282679) in the gene coding for the vitamin D binding protein (Ahn et al., 2010; Wang et al., 2010) confirmed in replication cohorts. Large-scale studies of this SNP in a series of case-control studies are indicated to investigate whether this gene is likely to be implicated in melanoma risk.

These data would appear therefore to provide modest support for the hypothesis that vitamin D might be protective for melanoma if we accept the view that an association between genetic variation in the receptor to which it binds has a small (but potentially biologically significant) association with risk. The most recent data from the GEM study do however provide somewhat conflicting information, in that in this study in which individuals with a single melanoma primary were compared with those with multiple primaries, inheritance of the BsmI variant was associated with an increased risk of multiple primary tumours (Mandelcorn-Monson et al., 2011). The comparison reported by the GEM group was however between single case and
multiple case individuals, so it is difficult to compare with the other comparisons of cases and controls. SNPs in genes in the vitamin D pathway have furthermore not so far been identified in genome wide association studies reported by a number of groups (Amos et al., 2011; Barrett et al., 2011; Bishop et al., 2009; Gudbjartsson et al., 2008; Macgregor et al., 2011) as melanoma risk alleles, so although this does not exclude an association it suggests that any such association would be weak in magnitude.

22.6 Summary, vitamin D and melanoma risk

In summary then, there is no doubt that intermittent sun exposure such as that which pale-skinned people get on holiday is associated with melanoma risk, if that sun exposure causes sunburn. That outdoor workers in temperate climates are not at increased risk of melanoma and may even have a lower risk (Gandini et al., 2005b), suggests a complex relationship between sun exposure and the aetiology of melanoma. Allied with recent findings of a protective effect of regular weekend sun exposure of 5 or so hours each day at the weekend in our case-control study in the UK, these observations suggest that regular moderate sun exposure might reduce risk and this could be related to higher levels of vitamin D (although photoadaption is another possible explanation). There are biological reasons to suppose that vitamin D production in the skin might control innate immunity and potentially mediate immunological responses to UV-induced damage and therefore that it might be protective for skin cancer. Furthermore vitamin D has demonstrable anti-proliferative effects on melanoma cells in vitro (Reichrath et al., 2007b) so that vitamin D might be protective in terms of limiting growth of precursor lesions such as naevi. The immunological effects of vitamin D are however very complex and one could cite cogent arguments why its effects might actually have the reverse effect. The genetic association studies between polymorphisms in the gene coding for the vitamin D receptor and risk provide some support for a relationship but these data remain relatively weak currently.

22.7 Advice to the general public on prevention of melanoma and vitamin D

The data on vitamin D and risk of melanoma is interesting but immature and certainly too weak to support public health programmes suggesting that vitamin D supplementation should be considered to prevent melanoma.

The ‘at risk’ population for melanoma are however fair-skinned, and the majority easily burn in the sun. There is no doubt that sunburn causes melanoma, therefore in these people it is sensible to give the advice that they should limit their sun exposure to avoid burning. In the Leeds Melanoma Cohort we saw that those people (cases and controls) with sun-sensitive phenotypes actually spend less time in the sun than the non-sensitive (Newton-Bishop et al., 2011) (presumably because of their tendency to burn), yet those phenotypes are associated with risk. Furthermore the vitamin D levels were lower in these people (Davies et al., 2011) and this observation suggests that in practice it is difficult for the fair-skinned to avoid sunburn without becoming vitamin
D deficient. As low levels of vitamin D are associated with reduced bone health and there are some data suggestive of lower overall mortality with supplementation (Autier and Gandini, 2007) one can argue that the fair-skinned should avoid the sun and take supplementation to avoid sub-optimal vitamin D levels. The level of supplementation remains controversial and will be discussed below.

It will be important in public health campaigns to find a way of allowing individuals to understand the relative risk benefit for them of sun exposure related to their skin type; how their skin behaves in the sun. The balance of risk will also depend upon the intensity of sun exposure so that advice will depend upon latitude of residence. What is clear is that skin colour is the main determinant of that balance and we will have to learn how best to convey this effectively. Such issues have been explored in relation to New Zealand where the melanoma rates are the highest in the world and the population comprised of Maori and Pacific peoples, as well as those of European origin (Callister et al., 2011).

22.8 Melanoma progression/prognosis and vitamin D

In 2005 Berwick et al. reported a better outcome for melanoma patients recruited to a case-control study in the US, in the presence of solar elastosis in the skin (Berwick et al., 2005). Solar elastosis is histologically detected damage to the connective tissues in the dermis, which is seen in elderly skin on sun-exposed sites. The inference made was that sun exposure was associated with better survival in melanoma but that the observation might also be explained if biological differences between melanomas arising in chronically sun-exposed skin and other tumours might ‘explain’ different survival. This first finding however was of interest.

We subsequently reported data from the Leeds Melanoma Cohort which suggested that melanoma patients bled after diagnosis (we attempted to sample 3 to 6 months after diagnosis) who had higher levels of vitamin D had thinner tumours, and survival data corrected for thickness and other factors known to predict outcome, such as age and sex, showed an independent survival benefit for the higher vitamin D levels (Newton-Bishop et al., 2009). There has been a small subsequent study which showed a protective effect in stage IV melanoma (Nurnberg et al., 2009), but the lack of sufficiently large cohorts with stored serum has meant that there has, to date, been limited opportunity to look at this in other cohorts. The data must therefore be taken as un-validated. The literature furthermore contains several instances of failure of randomised clinical trials to validate strong observational evidence linking the use of vitamins to lower risk outcome in cancer. This was shown for vitamin E and beta-carotene and in meta-analysis there even appeared to be an increased mortality associated with supplementation (Fernandez et al., 2012) thereby raising the possibility that vitamin levels might simply be markers of other determinants of risk, or even that they might have different effects in terms of primary prevention than secondary prevention. It does not seem inconceivable, for example, that antioxidants might be important in primary prevention but could impair survival for cancer patients if host defence mechanisms utilised oxidative stress.
Although there are *in vitro* data to suggest that vitamin D is antiproliferative for melanoma (Reichrath *et al*., 2007a), and consequently there might be a valuable biological effect of vitamin D on melanoma, there are considerable difficulties in exploring this further. Vitamin D levels are known to be higher in people with lower body mass index (Brock *et al*., 2010), and to be associated with more exercise and generally better health and therefore some have argued that the association between higher vitamin D levels and lower risk of many diseases might reflect a causal relationship, not between vitamin D and those diseases, but between other medical conditions associated with poor outcomes such as the metabolic syndrome. There seems to be a clear relationship between lower serum levels and increased mortality (discussed in Chapter 10 of this book) but the suggestion is that serum vitamin D levels could be associated here with outcome for melanoma just as markers of other causal aspects of health. The argument is rather circular, as others have reported evidence that low vitamin D levels might be a cause of the metabolic syndrome (Pacifico *et al*., 2011), and a modest reversal of the metabolic syndrome phenotype has been demonstrated after supplementation (Al-Daghri *et al*., 2011).

We have therefore explored Mendelian Randomization as a method of using measured variation in genes of known function (in this case the vitamin D binding protein) to examine the causal effect of a modifiable exposure, (that is serum vitamin D level). This was first described by Gray and Wheatley in 1991 as a method for obtaining unbiased estimates of the effects of a putative casual variable which they suggested might be achieved without conducting a traditional randomized trial (Gray and Wheatley, 1991). If the reported association between serum vitamin D levels and survival is actually a marker for some other more potent determinant, such as the metabolic syndrome, then the association between the genetic determinants of serum levels would show a different relationship to melanoma survival than do the serum levels. The genetic determinants of vitamin D level in the serum have been published from genome wide association studies, which showed the strongest effect for a SNP rs2282679 in the gene coding for the vitamin D binding protein (Ahn *et al*., 2010; Wang *et al*., 2010). We have subsequently reported a strong association between this SNP and lower levels of vitamin D (Davies *et al*., 2011); dietary supplementation, holiday and regular weekend sun exposure, body mass index and skin type were also major determinants of serum levels (Davies *et al*., 2011). We postulated that if inheritance of the SNP associated with lower levels of vitamin D was associated with poorer survival from melanoma then, using the principle of Mendelian Randomization, this would provide support for the hypothesis that vitamin D has a causal relationship with survival. These data are currently being generated.

There is an enormous literature on the association between serum vitamin D levels and cancer risk and an equally large literature of the biological effects of vitamin D on cancer cells. Certainly there are strong *in vitro* data to suggest that vitamin D is anti-proliferative for some but not all melanoma lines (Seifert *et al*., 2004) which we have confirmed in our own laboratory using pharmacological doses of vitamin D. It is supposed that these anti-proliferative effects likely 'explain' the potential beneficial effects of vitamin D in melanoma, and that these effects would be mediated by the VDR. Expression of VDR mRNA corresponds to responsiveness to added vitamin D *in vitro* (Essa *et al*., 2010) suggesting that the effects of vitamin D are mediated by
transcriptional changes in the cells. In vivo there is evidence that VDR expression diminishes with melanoma progression (Brozyna et al., 2011) and that expression is particularly reduced in pigmented tumours. So that, in life at least, any putative protective effects of vitamin D in melanoma patients are likely to vary according to the genomic profile of the tumour.

There are further potential effects of vitamin D for cancer patients as the hormone has been reported to have anti-angiogenic effects (Iseki et al., 1999; Shokravi et al., 1995). Vitamin D is also thought to have a role in controlling excessive immune mediated inflammation, evidence coming from the susceptibility of VDR knockout mice to a type of inflammatory bowel disease (Soudja et al., 2010) and evidence of inhibition of TH17 cells in a transgenic mouse, mediated by the VDR (Joshi et al., 2011). Chronic inflammation is thought to be deleterious in melanomas, driven by cytokines produced by tumour cells and macrophages, and leading to angiogenesis and suppression of adaptive immunity, so that in an individual in which this was the dominant process one could hypothesise that increasing vitamin D levels might be beneficial. There are also data to suggest that vitamin D enhances natural killer cell killing of melanoma cells (Lee et al., 2011) which might be beneficial too.

Figure 22.1. Kaplan Meier curve showing survival for 1,204 patients in the Leeds Melanoma Cohort by serum vitamin D level at recruitment to the study (levels were measured where possible 3 to 6 months after diagnosis). The levels are split into tertiles showing better survival for participants with serum vitamin D levels in excess of 65.6 nmol/l.

---
In the period since we reported the relationship between serum vitamin D levels and outcome in the Leeds Melanoma Cohort, the effects of vitamin D level around diagnosis on survival have persisted (Figure 22.1).

The role of vitamin D in survival for melanoma patients however remains unclear. There is clear evidence that the prevalence of suboptimal (even deficient) levels of serum vitamin D in the UK melanoma population is high at diagnosis. There is also evidence that sun avoidance is common after diagnosis (indeed dermatologists usually advise this) (Idorn et al., 2011) so that the proportion of melanoma patients who are deficient is likely to increase after diagnosis.

We therefore took the view that it would be prudent to advise patients to be aware of this risk and to avoid further deficiency by compensatory ingestion of fortified foods or modest vitamin D supplements. However it is clear that there are potential ill effects for melanoma patients of higher vitamin D levels. There are data from breast cancer studies (Goodwin et al., 2009) and cardiovascular disease (Wang et al., 2008) of a U-shaped effect so that both low and high levels of vitamin D might be harmful. More recently too, data have been published in which CRP levels in a large sample set were used as a marker of a pro-inflammatory state and therefore increased risk of cardiovascular disease in otherwise healthy people, and was correlated with serum vitamin D levels (Amer and Qayyum, 2012). The lowest levels of CRP were actually associated with vitamin D levels around 52.5 nmol/l. There was a fall in CRP by a geometric mean of 0.285 mg/dl for every 25 nmol/l increase in serum vitamin D level up to 52.5 nmol/l but then a rise of 0.05 mg/dl for every 25 nmol/l of vitamin D above 52.2 nmol/l, suggesting again that optimal levels for some measures of health (other than for bone health) might be at the lower end of ‘normal’ levels previously reported. The observed CRP changes, used as a measure of chronic inflammation, suggest that there would be no benefit (and may even be a small adverse effect) of increasing serum levels of vitamin D above 52.5 nmol/l.

Of particular concern for melanoma patients is the complex relationship between vitamin D and immunity. Melanoma is an immunogenic tumour. Spontaneous regression of advanced disease has been described (albeit rarely). Tumour infiltrating lymphocytes are common in primary tumours and brisk infiltration carries a better prognosis (Elder and Murphy, 1991), the development of vitiligo (auto-immune mediated damage to melanocytes in the skin) during chemotherapy is a good prognostic sign. Most recently immunotherapy with antibodies to the immunosuppressive molecule CTLA4 have shown survival benefit from treated patients, even those patients with pre-treated stage IV melanoma (Robert et al., 2011). It is clear that vitamin D has potent effects on the immune system but they are complex and there are concerns that for cancer patients some of those effects might be harmful if the net effect of increasing vitamin D levels is immunosuppression.

We could hypothesise that the effects of increasing vitamin D levels for melanoma patients would depend upon the relative activity of the innate versus adaptive immune systems. As discussed above the absence of the VDR in mice provides a very interesting model of inflammatory bowel disease (Froicu and Cantorna, 2007) in which the mice develop excessive uncontrolled
inflammation (mediated by innate immune systems) after minor bowel injury with high expression of inflammatory cytokines. Thus vitamin D may be a regulator of innate immunity.

Although the development of adaptive immunity appears to be beneficial for melanoma patients, in mice models tumour-initiated inflammation overrides the protective effects of adaptive immunity (Soudja et al., 2010). Thus, whilst induction of innate immune-like responses using toll-like receptor agonists such as Aldara has been shown to be effective treatment of melanoma in the skin (Schon and Schon, 2007), protracted inflammation may be deleterious so that moderation of that chronic inflammation would likely be beneficial if indeed increasing vitamin D levels had such an effect.

Vitamin D however also appears to suppress adaptive immunity and it is thought that supplementation with vitamin D might be useful in autoimmunity by having such an effect (Zold et al., 2008). Suppression of adaptive immunity in melanoma patients by whatever means would be inadvisable one would suppose, and this is the theoretical concern around supplementation in cancer patients.

The avoidance of ‘high’ serum levels of vitamin D would seem to be necessary. One of the fundamental difficulties however is that there is a lack of clarity as to what constitutes a ‘high’ level. Some argue that it’s difficult to generate toxicity from vitamin D but hypercalcaemia may occur; indeed prevention trials have reported an increased risk of kidney stones in vitamin D treated patients, for example in the Women’s Health Initiative Trial (Glendenning et al., 2012). The usual lower end of the quoted ‘optimal’ blood range of serum vitamin D is 60 to 78 nmol/l summarised in a recent review (Field and Newton-Bishop, 2011). Toxicity is described as unlikely at under 325 nmol/l and although the upper range in many laboratories is around 125 nmol/l life-guards often have levels higher than this (Holick, 2004)

Parathyroid levels are high in the presence of low vitamin D levels but they plateau at serum levels of vitamin D of around 60 to 78 nmol/l although it is clear from a meta-analysis by Bjorkman that even this is complex (Bjorkman et al., 2009). In our studies in which we reported the associations between serum levels, sun exposure, supplementation and inherited variation in genes reported to be associated with serum levels (reviewed by McGrath et al., 2010), the median level of vitamin D also plateaued at 60 nmol/l (Davies et al., 2011) with increased sun exposure (Figure 22.2). The observations made by Amer and Qayyum (2012), discussed above relating to CRP levels and serum vitamin D, and the suggestion of a U-shaped curve for protection from breast cancer deaths (Goodwin et al., 2009) or cardiovascular disease (Wang et al., 2008), to us at least suggest that for cancer and cardiovascular disease it may be that minimal serum levels of vitamin D of around 60 nmol/l might be preferable. It must be said that in our studies we have not seen any evidence at all of a deleterious effect of higher vitamin D levels in the Leeds Melanoma Cohort but it is also true that levels above 100 nmol/l were rare. There is of course marked seasonal variation in levels and peak to trough seasonal variation has been reported to be around 25 nmol/l (Shoben et al., 2011). We suggest therefore that we should consider aiming for levels in the range 60 to
22. Vitamin D and melanoma

Figure 22.2. The median serum vitamin D levels achieved by participants in the Leeds Melanoma Cohort as a result of increasing numbers of hours spent outside at weekends in the warmer months (Davies et al., 2011). The data are divided into those for individuals defined as sun sensitive and non-sun sensitive (on the basis of responses to questions about skin pigmentation and the effects of UV exposure). It can be seen that the sun-sensitive have median levels of vitamin D which are lower than those for the not sun-sensitive (hypothesised to be because the sun-sensitive find it difficult to stay outside in the sun for long enough without burning). It can also be seen that the median levels reach a plateau at around 60 nmol/l after 4 hours of daily weekend sun exposure for the not sun-sensitive and 6 hours for the sun-sensitive. Median levels of 60 nmol/l were reached in those taking supplements irrespective of sun exposure.

85 nmol/l throughout the year in cancer patients in the period before randomized clinical trials have been performed.

The literature suggested however that achieving this is likely to be difficult. Variation in sun exposure and behaviour in the sun will have a marked effect. There are also marked differences in how individuals respond to sunlight exposure and oral intake of vitamin D. Skin colour is a strong determinant. Melanoma patients tend to be white and the biggest differences are between black and white-skinned people but even in the white-skinned peoples there is variation. Three
studies have shown that the palest people have lower levels (Davies et al., 2011; Glass et al., 2009), presumably because in real life individuals who are sensitive to the sun find it difficult to be outside uncovered for long enough to produce sufficient vitamin D without burning (Figure 22.2). Levels tend to be lower in the obese and our own studies have suggested that inherited variation in the gene coding for the vitamin D binding protein determines serum levels of vitamin D achieved by increased sun exposure and supplementation (Figure 22.3). A review by Balk (2011) summarised the findings of studies designed to evaluate the supplementation required to maintain levels at 62.5 nmol/l in adults which ranged from 400 to around 1,500 IU per day.

We have measured vitamin D levels in melanoma patients in follow up in the UK and have experienced this variation. Some patients appear to increase their serum levels from less than 20 nmol/l to 70 nmol/l, for example, in 3 months with 1 cod liver oil capsule whereas others struggle to reach these levels using oral supplements in over 12 months. This has led many to give high loading doses to deficient patients and this might be preferable for cancer patients if we take the view, as some have done, that it makes sense to increase the levels as quickly as possible after cancer diagnoses. The published evidence of suppression of adaptive immunity by vitamin D however causes us concern about rapid changes of level.

Figure 22.3. The median serum vitamin D levels achieved by participants in the Leeds Melanoma Cohort as a result of increasing numbers of hours spent outside at weekends in the warmer months (Davies et al., 2011). The three curves indicate levels reached according to inheritance of the SNP rs2282699 in the gene coding for the vitamin D binding protein. Individuals homozygous for the SNP associated with lower levels of the protein run at significantly lower levels of vitamin D for similar periods of sun exposure.
Our personal view is that we should aim for levels of serum vitamin D in the range 60 to 85 nmol/l achieved by daily supplementation with vitamin D3 in the range 400 to 1000 IU per day depending on baseline levels of vitamin D at diagnosis. This advice is based upon a cautious view of the literature but fully cognisant that the necessity is to perform a randomised clinical trial.

The Italians have published a plan for such an adjuvant trial in stage 1 to 2 melanoma patients (Gandini et al., 2009a) but we are not aware if it has started as yet. Whilst a randomized clinical trial is very desirable it would be a very difficult trial indeed to design. It is not clear what blood level we would aim for or how to achieve that level. It seems likely to us that responses to supplementation might depend furthermore on the status of host/tumour interaction at the time.

We have elected to continue to research the relationship between vitamin D and melanoma in order to inform the development of such a randomized trial, using genomics and by looking at serum samples in stored sample sets from clinical trials. In the meantime, in clinic we do not wish our melanoma patients to become even more vitamin D deficient after diagnosis than they were at presentation. We therefore gave general advice about avoiding supplementation but when we checked blood levels we found that the majority of our patients remained at low levels. We therefore now counsel:

- measurement of vitamin D levels in melanoma patients soon after diagnosis in the winter and summer;
- modest supplementation if levels are lower than 50 nmol/l depending on season;
- aiming for levels between 60 nmol/l and 85 nmol/l;
- that a patient who has had a poor prognosis tumour in the past but suffered no relapse has shown good evidence of effective host/tumour interaction and it might be prudent for them to avoid supplementation.

References


22. Vitamin D and melanoma


Callister, P., Galtry, J. and Didham, R., 2011. The risks and benefits of sun exposure: should skin colour or ethnicity be the main variable for communicating health promotion messages in New Zealand? Ethnicity & Health 16, 57-71.


Froicu, M. and Cantorna, M.T., 2007. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. BMC Immunology 8, 5.


22. Vitamin D and melanoma


22. Vitamin D and melanoma


Handbook of vitamin D in human health

J.A. Newton-Bishop and J.A. Randerson-Moor


**Key facts**

- Prostate cancer is the most diagnosed cancer in men.
- Low sunlight exposure is a risk for prostate cancer.
- Vitamin D has functions other than maintaining good bones.
- Prostate has the ability to produce its own active form of vitamin D.
- Inhibitors of the enzyme which destroys the active form of vitamin D have potential to treat prostate cancer.

**Summary points**

- There is biochemical, ecological and epidemiological evidence for the connection between vitamin D and prostate cancer.
- Six cytochrome P450 enzymes have been implicated in the hydroxylation of vitamin D to 25(OH)D: CYP2C11, CYP27A1, CYP3A4, CYP2J3, CYP2D25 and CYP2R1. The prostate expresses CYP2R1, CYP27B1 and CYP24A1.
- CYP2R1 is expressed more in the prostate than in the liver, and can be down-regulated by 1α,25(OH)2D3.
- The epidermal growth factor may play an important role in the development of prostate cancer and CYP27B1 is likely a tumor suppressor in the prostate.
- CYP24A1 is responsible for the hydroxylation at C-24 of 25(OH)D and 1α,25(OH)2D, serving as the initial step to inactivate 1α,25(OH)2D.
- MART-10 has shown to be more active in inhibiting prostate cell proliferation and is less susceptible to CYP24A1 degradation.
- Inhibitors of CYP24A1, such as VIMI and CPA1, decrease the degradation of the active hormone. CPA1 shows an 80-1 selective inhibition of CYP24A1 over CYP27B1.
23. Vitamin D autocrine system and prostate cancer prevention and treatment

T.C. Chen
Boston University School of Medicine, 715 Albany Street, Boston MA, 02118 USA; taichen@bu.edu

Abstract

Cytochrome P450 (CYP) enzymes play crucial roles in vitamin D metabolism and actions, including hepatic vitamin D-25-hydroxylase and renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) for the synthesis of the active form 1α,25-dihydroxyvitamin D3 (1α,25(OH)₂D₃), and 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) also in the kidneys to inactive 1α,25(OH)₂D₃. The active hormone has high affinity for and interacts with vitamin D receptor (VDR), a transcription factor, to induce anti-proliferative, anti-invasive, anti-angiogenic, anti-inflammatory, pro-apoptotic and pro-differentiation actions in prostate cancer cells. However, it is now recognized that CYP27B1 and CYP24A1 are also expressed in many tissues and cells, including the prostate. Although six CYP enzymes have been identified with 25-hydroxylase activity, the two major ones are CYP27A1 and CYP2R1, and both are expressed in the prostate with CYP2R1 as the main 25-hydroxylase. The finding demonstrates that prostate tissue has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion. Recently 25-hydroxyvitamin D3 (25(OH)D3) and its analogs, without converting to their 1α-hydroxylated counter parts, have been shown to bind to VDR as agonists to modulate various gene expressions that ultimately lead to cell growth arrest and other anti-tumor activities. The finding suggests that the circulating levels of 25(OH)D, and the autocrine synthesis of 25(OH)D3 and 1α,25(OH)₂D₃ may play an important role in regulating the growth of prostate cancer. Thus, in addition to 1α,25(OH)₂D₃ analogs, the presence of CYP2R1, CYP27B1 and CYP24A1 in the prostate suggests that the analogs of vitamin D3 and 25(OH)D3, especially those that are resistant to CYP24A1 degradation, can be developed and used for the prevention and treatment of prostate cancer.

Keywords: vitamin D analog, proliferation, CYP enzymes, prostate cancer
**Abbreviation**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α,25(OH)₂D</td>
<td>1α,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>25-OHase</td>
<td>Vitamin D-25-hydroxylase</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen activated protein kinase</td>
</tr>
<tr>
<td>MAPKK</td>
<td>Mitogen activated protein kinase kinase</td>
</tr>
<tr>
<td>MART-10</td>
<td>19-nor-2α-(3-hydroxypropyl)-1α,25(OH)₂D₃</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDRE</td>
<td>Vitamin D response element</td>
</tr>
</tbody>
</table>

**23.1 Introduction**

There are six forms of naturally occurring vitamin D, namely, vitamin D2, vitamin D3, vitamin D4, vitamin D5, vitamin D6, and vitamin D7, with the same characteristic seco-steroid ring structure. Among them, vitamin D2 and vitamin D3 are the most important. Vitamin D2 or ergocalciferol is derived from fungi and plants, whereas vitamin D3 or cholecalciferol is produced in animal skin, after UVB (290-315 nm) irradiation (Holick *et al.*, 1980). In this chapter, vitamin D is used as a general term for both vitamin D2 and vitamin D3. The modern vitamin D research began in the mid 1960’s, when vitamin D was found not biologically active (Norman *et al.*, 1964) requiring two successive hydroxylations, first at C-25 by 25-hydroxylases in the liver to produce 25(OH)D, and then at the C-1 position by 1α-hydroxylase in the kidneys to form 1α,25(OH)₂D, the active form of vitamin D (DeLuca, 1976). There is another important enzyme in the kidneys which can hydroxylate 1α,25(OH)₂D to 1α,24,25(OH)₃D or 25(OH)D to 24,25(OH)₂D as the first step of catabolic pathway to degrade 1α,25(OH)₂D or 25(OH)D (DeLuca, 1976; Schuster, 2011). 1α,25(OH)₂D is now believed to regulate more than 200 genes (Ramagopalan *et al.*, 2010) through its binding to VDR, including those involved in calcium and phosphorus metabolism, immune regulation, cell growth, cell differentiation, angiogenesis, inflammation and apoptosis. VDR has been identified in numerous cell types including prostate cells.

Prostate cancer is the most diagnosed and the second most fatal cancer among men in the US and Northern Europe. In the US, an estimate of 242,740 new cases of prostate cancer will be diagnosed and 28,170 will die from this disease in 2012 (Siegel *et al.*, 2012). Although the etiology of prostate cancer is not completely understood, ecological studies have repeatedly demonstrated the inversely relationship between sunlight (UVB) exposure and the incidence of prostate cancer (Grant, 2012; Rhee *et al.*, 2009), suggesting one cause of prostate cancer might be vitamin D insufficiency (Schwartz and Hulka, 1990). However, the direct epidemiological evidence to demonstrate the association of low serum 25-hydroxyvitamin D (25(OH)D) levels
23. Vitamin D autocrine system and prostate cancer prevention and treatment

with prostate cancer has been less consistent. The discrepancy among different reports may implicate the importance of the intraprostatic concentrations of 25(OH)D and its ability to be activated rather than the serum levels of 25(OH)D as the risk factor for prostate cancer. In addition, the luck of association may result from different inclusive and exclusive criteria for the study subject selection and the time serum samples were obtained and how samples were stored and analyzed. Unlike the epidemiological studies, the biochemical evidence to support a role for vitamin D in prostate cancer is overwhelming (Chen and Holick, 2003; Miller, 1998; Swami et al., 2011). VDR has been demonstrated in cultured prostate cells derived from non-cancerous and cancerous prostate tissues. 1α,25(OH)2D and its analogs have been shown to inhibit prostate cell proliferation and invasion, and to stimulate prostate cell differentiation and apoptosis in vitro and tumor progression in animal models (Chen and Holick, 2003; Miller, 1998; Swami et al., 2011). In this chapter, I will summarize the recent advances in the three enzymes involved in the activation and de-activation of vitamin D within the prostate cells and their implications in the prevention and treatment of prostate cancer.

23.2 Overview of human cytochrome P450 system

Cytochrome P450 enzymes belong to a family of isoenzymes which represents one of the largest and oldest gene superfamilies. This family of enzymes catalyzes the metabolism of a large number of compounds of both exogenous and endogenous origins, including drug detoxification and other xenobiotic metabolism, the metabolism of eicosanoids, the biosynthesis of cholesterol and bile acids, steroid synthesis and metabolism, the synthesis and degradation of vitamin D and retinoic acids, biogenic amines and presumably other morphogens (Nebert and Dalton, 2006). In some cases, the enzymes may activate exogenous compounds to toxins or carcinogens (Androutsopoulos et al., 2009). There may be still a number of CYP enzymes with unknown functions. In human genome, there are 57 genes involved in the synthesis of 57 cytochrome P450 enzymes which are classified into 18 families (Nebert and Russel, 2002). Among them, 50 are microsomal and 7 are mitochondrial enzymes. The tertiary structures of 11 microsomal enzymes are known (Ghosh et al., 2009; Mast et al., 2008; Rowland et al., 2006; Sansen et al., 2007; Schoch et al., 2008; Strushkevich et al., 2008, 2010; Williams et al., 2000, 2003, 2004; Yano et al., 2005), including CYP2R1 which is the major 25-hydroxylase found in human to generate 25(OH)D3 from vitamin D3 (Cheng et al., 2003, 2004). CYP24A1 and CYP11A1 are the only two mitochondrial CYP enzymes whose crystal structures have been reported (Annalora et al., 2010; Mast et al., 2011). The importance of CYP enzymes in human is clearly demonstrated with serious health problems when any mutations in the CYP genes occur. One example is that mutations in CYP17A1 will lead to mineralcorticoid excess syndromes, glucocorticoid and sex hormone deficiencies, increased risk of prostate cancer and benign prostatic hypertrophy (Nebert and Russel, 2002). In the case of vitamin D metabolism, mutations in CYP2R1, responsible for the synthesis of 25(OH)D from vitamin D, and CYP27B1, responsible for the 1α-hydroxylation of 25(OH)D to produce the active form 1α,25(OH)2D, will cause vitamin D deficiency-induced rickets and vitamin D-dependent rickets type 1, respectively (Cheng et al., 2003, 2004; St-Arnaud et al., 1997). On the other hand, mutations in CYP24A1 have been shown recently to induce
idiopathic infantile hypercalcemia, because CYP24A1 is responsible for the degradation of 25(OH)D, the circulating form of vitamin D, and 1α,25(OH)₂D, the active form (Schlingmann et al., 2011) (Table 23.1).

### 23.3 Vitamin D metabolism

Vitamin D was discovered in 1922 as the active anti-rachitic principle found in cod liver oil (McCollum et al., 1922). Until 1964, it was generally believed that either vitamin D2 or vitamin D3 was active without any further metabolism. DeLuca and colleagues first questioned this assumption and provided evidence suggesting that vitamin D3 was metabolized to more polar metabolites which were the active forms responsible for stimulating calcium transport (Norman et al., 1964). During the following several years, intensive efforts carried out by DeLuca and his associates led to the isolation and identification of the first metabolite of vitamin D3, 25(OH)D3 (Blunt et al., 1968). The site of 25(OH)D3 synthesis was soon demonstrated in the rat liver in vivo by Ponchon and DeLuca (1969). This finding was subsequently confirmed by Horsting and DeLuca (1969) in their in vitro experiments using rat liver homogenates. In the same year, Lawson et al. (1969a,b) described a new vitamin D3 metabolite with a loss of hydrogen at C-1 position isolated from chick intestinal nuclei, which was followed by the identification of a unique biological active vitamin D metabolite synthesized in the kidneys (Fraser and Kodicek, 1970). This metabolite was found to be more polar and much more potent than 25(OH)D3 in raising serum calcium and in stimulating calcium transport in the gut. The structure of this active

Table 23.1. Functions and substrates of four major vitamin D CYP enzymes, and disorders resulting from their mutation.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzyme location</th>
<th>Enzyme function</th>
<th>Enzyme substrate</th>
<th>Disorder due to gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2R1</td>
<td>Microsomal</td>
<td>C-25 hydroxylation</td>
<td>Vitamin D3, Vitamin D2</td>
<td>Vitamin D-dependent Ricket type 1</td>
</tr>
<tr>
<td>CYP27A1</td>
<td>Microsomal</td>
<td>C-25 hydroxylation</td>
<td>Vitamin D3</td>
<td>Cerebrotendinous Xanthomatosis</td>
</tr>
<tr>
<td>CYP27B1</td>
<td>Mitochondrial</td>
<td>C-1 hydroxylation</td>
<td>25(OH)D3, 25(OH)D2</td>
<td>Vitamin D-dependent Ricket type 1</td>
</tr>
<tr>
<td>CYP24A1</td>
<td>Mitochondrial</td>
<td>C-24 hydroxylation</td>
<td>25(OH)D3, 25(OH)D2, 1α,25(OH)₂D3, 1α,25(OH)₂D2</td>
<td>Idiopatathic infantile hypercalcemia</td>
</tr>
</tbody>
</table>

1 Although no study has been reported, a metabolic pathway similar to that of 1α,25(OH)₂D2 C-24 hydroxylation can be assumed.
metabolite was soon identified as 1α,25-dihydroxy-cholecalciferol or 1α,25(OH)₂D₃ (Holick et al., 1971; Lawson et al., 1971; Norman et al., 1971), and chemically synthesized (Semmler et al., 1972). One additional dihydroxylated metabolite of vitamin D was also found in the blood of pig injected with large amounts of vitamin D3. This new metabolite was first thought to be 21,25-dihydroxycholecalciferol or 21,25(OH)₂D₃ (Suda et al., 1970), but later was revised as 24,25-dihydroxycholecalciferol or 24,25(OH)₂D₃ (Holick et al., 1972). A reciprocal synthesis of 24,25(OH)₂D₃ and 1α,25(OH)₂D₃ was observed after the infusion of PTH into the thyroid/parathyroidectomized rats, suggesting an inversed regulation of the two enzymes, CYP27B1 and CYP24A1, responsible for the synthesis of these two dihydroxylated metabolites (Garabedian et al., 1972).

At the present time, there are at least 6 naturally occurring CYP enzymes which have been implicated in the hydroxylation of vitamin D to produce 25(OH)D (Ohyama and Yamasaki, 2004). They are CYP2C11, CYP27A1, CYP3A4, CYP2J3, CYP2D25 and CYP2R1. The inclusion of CYP2R1 as a 25-OHase did not occur until 2003 when Cheng et al. (2003) screened a cDNA library made from hepatic mRNA of mice deficient in gene encoding the mitochondrial CYP27A1 using a VDR-based ligand activation assay. They soon confirmed that a mutation of CYP2R1 gene in an individual caused vitamin D deficient rickets (Cheng et al., 2004). Using a cell-free re-constituted enzyme system, Sakaki and co-workers demonstrated that CYP2R1 was able to hydroxylate either vitamin D3 or vitamin D2 to their corresponding 25-hydroxylated metabolites (Shinkyo et al., 2004). However, this is not the case for CYP27A1 which can only hydroxylate vitamin D3 to 25(OH)D3, not vitamin D2 to 25(OH)D2. Instead, this enzyme produces C-24 or C-27 hydroxylated vitamin D2 metabolite. So far, CYP27B1 is the only enzyme involved in the 1α-hydroxylation of vitamin D, and CYP24A1 is the primary one to hydroxylate 25(OH)D and 1α,25(OH)₂D at C24 position (Figure 23.1). In this regard, Sakaki et al. have demonstrated that CYP24A1 converts 1α,25(OH)₂D₃ to calcitroic acid by a six-step monooxygenation including C-24 hydroxylation as the first step of 1α,25(OH)₂D₃ catabolism (Sakaki et al., 1999). A similar CYP24A1-dependent C-24 hydroxylation of 1α,25(OH)₂D₂ has been observed as the first step of the 1α,25(OH)₂D₂ degradative pathway (Urushino et al., 2009).

### 23.4 Study of vitamin D-25-hydroxylases in prostate cells (CYP2R1)

The demonstration of the presence of 25-OHase in prostate cells was accomplished by (1) three functional assays, (2) direct determination of the presence of 1α,25(OH)₂D₃ after the addition of vitamin D3 into prostate cells, and (3) the expression of CYP2R1 and CYP27A1 mRNA in prostate cells (Flanagan et al., 2006).

In the functional assays, the expression of CYP24A1 and insulin-like growth factor binding protein 3 genes were used. These two genes are highly responsive to the stimulation by 1α,25(OH)₂D₃. Since the prostate cells in cultures were treated with vitamin D3, the effects on these two genes could only occur when 25(OH)D3 was first synthesized from vitamin D3 through 25-OHase catalysis prior to the synthesis of 1α,25(OH)₂D₃. Using this approach, it was
shown that vitamin D3 caused a dose-dependent up-regulation of these two genes in PZ-HPV-7 prostate cells (Flanagan et al., 2006). In the third functional assay to demonstrate the presence of 25-OHase activity, Flanagan et al. (2006) studied the ability of vitamin D3 to inhibit \(^{3}\)H-thymidine incorporation into prostate cell as an index of anti-proliferation. They showed that \(^{3}\)H-thymidine incorporation into DNA was inhibited ~40%, when PZHPV-7 prostate cells were exposed to \(10^{-6}\) M of vitamin D3 for 18 hours, as compared to ~80% inhibition with \(10^{-7}\) M of \(1\alpha,25\text{(OH)}_2\text{D3}\) for 18 hours. Although no 25(OH)D3 could be detected by protein binding assay (Chen et al., 1990a) after incubating PZHPV-7 cells with vitamin D3 for 24 hours, the presence of \(1\alpha,25\text{(OH)}_2\text{D3}\) in the cultures was detected by a very sensitive and specific thymus receptor binding assay (Chen et al., 1990b).

In order to determine which 25-OHase is the predominate form in prostate cells, the expression of three human-related 25-OHases, \(\text{CPY}2R1\), \(\text{CYP}27\text{A1}\), and \(\text{CYP}3\text{A4}\), was examined in human normal prostate and liver tissues, and in prostate cancer cell lines. It was found that \(\text{CYP}2R1\) was expressed in a much greater extent in prostate tissue and cell lines than in liver tissue. Very little \(\text{CYP}27\text{A1}\) or \(\text{CYP}3\text{A4}\) was expressed in normal prostate tissue and cell lines, whereas both \(\text{CYP}\) genes were highly expressed in liver tissue. Therefore, the results suggest that \(\text{CYP}2R1\) is more likely the 25-OHase responsible for the hydroxylation of vitamin D3 to 25(OH)D3 in the prostate (Flanagan et al., 2006). Very little is known about the regulation of \(\text{CYP}2R1\), except that the enzyme can be down-regulated by \(1\alpha,25\text{(OH)}_2\text{D3}\) (Ellfolk et al., 2009), suggesting that the promoter of \(\text{CYP}2R1\) gene may have negative VDRE(s).
23.5 Study of CYP27B1 in prostate cells

The location of CYP27B1 gene was mapped to chromosome 12q13-14 (Miller and Portale, 2000). The cDNAs encoding the mouse, rat and human CYP27B1 have been cloned independently by four research groups (Fu et al., 1997; Shinke et al., 1997; St-Arnaud et al., 1997; Takeyama et al., 1997). The cloned human CYP27B1 gene consisting of 9 exons and 8 introns has 1,524 base pairs encoding 508 amino acids with a molecular weight of 58 kDa. The promoter of mouse and human CYP27B1 genes have also been cloned with a length of 1.7 kb and 1.4 kb, respectively (Brenza et al., 1998; Kong et al., 1999).

23.5.1 Differential expression of CYP27B1 activity in prostate cells

CYP27B1 is expressed in certain prostate cells as analyzed by their ability to convert \(^3\)H-25(OH)D3 to \(^3\)H-1α,25(OH)\(_2\)D3 in cultures (Schwartz et al., 1998), by mRNA expression using real-time qPCR (Chen and Holick, 2001), and by promoter/luciferase assay (Wang et al., 2003). The expression suggests that local production of 1α,25(OH)\(_2\)D3 could provide an important cell growth regulatory mechanism in prostate cells (Chen et al., 2003; Wang et al., 2003). However, there is a differential expression of CYP27B1 activity among the primary cultures of prostate cells derived from cancerous, benign prostatic hypertrophy and normal tissue, as well as among prostate cell lines either derived from normal prostate tissue (PZHPV-7 cells) or from prostate cancer cells (PC-3, DU145 and LNCaP cells) (Table 23.2). Similar observations were reported by Hsu et al. (2001). No or very little CYP27B1 activity and mRNA expression were found in LNCaP cells, that supports the data showing that LNCaP cells were not responsive to the addition of 25(OH)D3 (Skowronski et al., 1995; Whitlatch et al., 2002), because the cells could not convert 25(OH)D3 to 1α,25(OH)\(_2\)D3. Therefore, by transfecting LNCaP cells with CYP27B1 cDNA, Whitlatch et al. (2002) were able to restore their responsiveness to 25(OH)D3.

<table>
<thead>
<tr>
<th></th>
<th>Enzyme activity (% conversion)</th>
<th>mRNA level(^1) (fold)</th>
<th>Promoter luciferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AN2</td>
</tr>
<tr>
<td>LNCaP</td>
<td>ND(^2)</td>
<td>1</td>
<td>95±18</td>
</tr>
<tr>
<td>PC-3</td>
<td>1.5±0.8</td>
<td>5</td>
<td>1,633±118</td>
</tr>
<tr>
<td>DU145</td>
<td>3.7±0.6</td>
<td>51</td>
<td>8,581±223</td>
</tr>
<tr>
<td>PZ-HPV-7</td>
<td>37±3.0</td>
<td>157</td>
<td>30,076±857</td>
</tr>
</tbody>
</table>

\(^1\) Real-time PCR.
\(^2\) Non-detectable.
23.5.2 Regulation of prostate CYP27B1 activity

Unlike the enzyme in renal cells (Chen et al., 1989), the promoter activity of CYP27B1 and the enzymatic activity of CYP27B1 are not regulated by PTH or calcium in PZHPV-7 prostate cells (Young et al., 2004). However, the enzyme is down-regulated by its own product, 1α,25(OH)₂D₃, at the promoter and enzyme activity levels. Moreover, it has been shown that EGF up-regulates CYP27B1 at both the transcriptional and translational levels as evident from the luciferase promoter assay, real-time quantitative RT-PCR analysis and enzyme activity measurement using high performance liquid chromatography (Wang et al., 2004). The EGF-dependent up-regulation of the promoter activity is inhibited by MAPKK inhibitor, PD98059 (Wang et al., 2004), suggesting that MAPK signaling pathway may be one mechanism involved in the regulation of prostate CYP27B1 gene expression by EGF to stimulate 1α,25(OH)₂D₃ synthesis that in turn inhibits cell proliferation as a feedback regulator of cell growth. Preliminary data using Chip assay indicate that EGF/EGFR complex may directly bind to the promoter of CYP27B1 in PZ-HPV7 cells. The direct binding of EGF/EGFR complex to promoters and to act as a transcriptional factor to regulate cell growth has been demonstrated previously (Lin et al., 2001). However, EGF has no effect on CYP27B1 promoter activity in LNCaP cells (Wang et al., 2004), suggesting that the ability of EGF to stimulate 1α,25(OH)₂D₃ synthesis may be abolished or diminished in cancer cells. Overall, the data suggest that EGF may play an important role in the development of prostate cancer (Figure 23.2), and CYP27B1 is likely a tumor suppressor in the prostate (Chen, 2008). CYP27B1 expression can also be up-regulated by suberoylanilide hydroxamic acid, an inhibitor of histone deacetylases (Wang et al., 2008). Since the inhibitors of histone deacetylases have been shown to cause growth arrest, and promote differentiation and apoptosis of many tumor cells by altering the transcription of genes (Xu et al., 2007), it is likely that CYP27B1 gene may be a new target for suberoylanilide hydroxamic acid through its action on the acetylation status of the histone tails of chromatin.

In glioblastoma TX3868 cells (Maas et al., 2001), cultured human keratinocytes (Flanagan et al., 2003), and HKC-8 human proximal tubular cells (Wu et al., 2007), expression of CYP27B1 splice variants has been reported as one mechanism to regulate CYP27B1 activity in a tissue-specific manner. In addition, trafficking of the CYP27B1 substrate 25(OH)D and CYP27B1 enzyme itself may regulate the synthesis of 1α,25(OH)₂D as a mean to modulate 1α,25(OH)₂D action as shown in the human innate immune response (Adams et al., 2007).

23.6 Study of 24-hydroxylation by CYP24A1 in prostate cells

CYP24A1 is responsible for the hydroxylation at C-24 of 25(OH)D and 1α,25(OH)₂D through a six-step monoxygenation pathway (Sakaki et al., 1999), that serves as the initial step to inactivate 1α,25(OH)₂D, and is a major mechanism to terminate the actions of this active hormone inside the cells. The final water soluble calcitroic acid which is likely further metabolized is excreted into the urine (Schuster, 2011). Because 1α,25(OH)₂D is synthesized in the mitochondria, acts through binding to VDR in the nucleus, and then is degraded by mitochondrial CYP24A1 within
23. Vitamin D autocrine system and prostate cancer prevention and treatment

Figure 23.2. Interactions among EGF, CYP27B1 and prostate cell growth. An up-regulation of CYP27B1 by EGF is hypothesized to be responsible for the normal growth of prostate cells (upper panel), whereas dysregulation of CYP27B1 by EGF may cause uncontrollable prostate cell growth (lower panel). CREBP = cAMP-responsive element binding protein; EGF = epidermal growth factor; MAPKK = mitogen activated protein kinase kinase.

the cells and has little chance to be released into the circulation, no 1α,25(OH)2D has been detected in the circulation of nephrectomized animals (Shultz et al., 1983; Reeve et al., 1983).

23.6.1 Expression and the regulation of CYP24A1 in prostate cancer cells

Like many other cells, prostate cells are known to express CYP24A1 (Flanagan et al., 2009; Ly et al., 1999; Miller et al., 1995; Skowronski et al., 1993). The gene is one of the target genes of 1α,25(OH)2D and is up-regulated by 1α,25(OH)2D and down-regulated by PTH (Garabedian et al., 1972). Therefore, it has been used as a biomarker for the synthesis of 1α,25(OH)2D3 from 25(OH)D3 (Flanagan et al., 2006). Elevated CYP24A1 expression has been reported in several types of human cancer (Cross et al., 2005; Friedrich et al., 2003) and prostate cancer cell line, DU-145 (Ly et al., 1999). This is in line with a quantitative mapping of amplicon structure by an array comparative genomic hybridization technique which identified CYP24A1 as a candidate oncogene whose overexpression is likely to lead to abrogation of growth control mediated by vitamin D (Albertson et al., 2000). Treatment of normal prostate PNT-2 cells with the methylation inhibitor 5-aza-2’-deoxycytidine together with the deacetylation inhibitor trichostatin A has been shown to elevate both CYP27B1 and CYP24A1 mRNA expression demonstrating that the expression of
these two vitamin D hydroxylases are likely under epigenetic control (Khorchide et al., 2005). More recently, an inversed correlation between CYP24A1 expression and methylation in the promoter region of CYP24A1 gene in prostate cancer cell lines has been observed, suggesting an epigenetic regulation of CYP24A1 (Luo et al., 2010). Deeb et al. (2011) studied methylation of the CYP24A1 gene promoter in endothelial cells isolated from the cancerous and benign prostate tissues and found that the average CYP24A1 promoter methylation was 20% from the tumor environment compared with 8.2% in the benign microenvironment. Whether the epigenetic regulation of CYP24A1 has any implications on the prostate tumor progression is unknown at the present time and remains to be determined.

23.6.2 CYP24A1-resistant vitamin D analogs

Since 1α,25(OH)₂D is subject to CYP24A1 degradation, any of its analogs which are resistant to CYP24A1 hydroxylation will have longer half-life and potentially could be more active. Several structural modifications of the 1α,25(OH)₂D₃ molecule have been accomplished to achieve this goal. For example, ED-71, a widely-studied 1α,25(OH)₂D₃ analog with an addition of 3-hydroxypropoxy group attached in β-configuration to the C-2 position of the 1α,25(OH)₂D₃ molecule, is a poor substrate for CYP24A1 (Dr. Noboru Kubodera, personal communication). Likewise, O2C3, the C-2 epimer of ED-71, is also resistant to CYP24A1 hydroxylation (Abe et al., 2005). A list of 19-nor-1α,25(OH)₂D₃ analogs with a modification at the C-2 of this molecule have been studied for their anti-proliferative and pro-differentiation activities (Chen et al., 2007; Kittaka et al., 2007; Ono et al., 2003). One of these compounds, MART-10, has been shown to be 500-1000 times more active in inhibiting prostate cell proliferation and about 300-500 times less susceptible to CYP24A1 degradation than 1α,25(OH)₂D₃ (Chen et al., 2007; Flanagan et al., 2009). To study the docking of MART-10 into CYP24A1, a human CYP24A1 substrate binding site based on the published crystal structure of rat CYP24A1 (Annalora et al., 2010) was generated (Iglesias-Gato et al., 2011). According to this model, A-ring of MART-10 is positioned over heme group and the 3-hydroxypropyl group on the A-ring is located on the groove of 1-helix kink forming hydrogen bonds with the back bone of L325 and E329 and blocking the groove. Consequently, the side-chain of MART-10 is far away from the heme group and is unavailable for 24-hydroxylation, suggesting that MART-10 will have longer half-life in the prostate. Thus, the results further suggest that analogs with modification at C-2 position, such as MART-10, could be developed for the treatment of prostate cancer due to its longer bioavailability and greater potency (Chen et al., 2007).

23.6.3 Inhibitors of CYP24A1

An alternative approach to enhance the half-life and activity of 1α,25(OH)₂D is to use the inhibitors of CYP24A1 to decrease the degradation of the active hormone by 24-hydroxylation. A range of P450 inhibitors are currently available, such as Ketoconazole and Liarazole (Figure 23.3). These azole compounds work directly by binding to the heme iron through the azole nitrogen. Therefore, they are relatively non-selective. More selective CYP24 A1 inhibitors have been developed by Novartis, such as VID 400, and Cytochroma Inc., CTA018 (Posner et al.,
23. Vitamin D autocrine system and prostate cancer prevention and treatment

23.7 25(OH)D can be active without 1α-hydroxylation

After the discovery of 25(OH)D3 in 1968, Olson and DeLuca reported that 25(OH)D3 added into the incubation media was able to enhance calcium absorption from the lumen in studies using an isolated small intestine loop. From this finding, they concluded that 25(OH)D3 was the metabolic active form of vitamin D3 and had direct effect on calcium transport (Olson and DeLuca, 1969). With the discovery of 1α,25(OH)2D3, this 25(OH)D effect was believed to be simply a pharmacological effect. Three decades later, the direct effect of 25(OH)D3 on PTH secretion was observed by Ritter et al. (2006), who used cytochrome p450 inhibitor clotrimazole...
to block the conversion of 25(OH)D3 to 1α,25(OH)₂D3 in the bovine parathyroid cells. The direct effect caused by 25(OH)D3 in the bovine parathyroid cells was subsequently shown to be VDR dependent (Ritter and Brown, 2011). Similarly, Lou et al. demonstrated the antiproliferative action of 25(OH)D3 on human MCF-7 breast cancer cells and in the primary cultures of kidney, skin and prostate cells prepared from Cyp27b1 knockout mice (Lou et al., 2010). The authors demonstrated that the action induced by 25(OH)D3 was mediated by VDR, and 25(OH)D3 had identical binding mode as 1α,25(OH)₂D3 in these cells. More importantly, a synergistic effect of 25(OH)D3 with 1α,25(OH)₂D3 in Cyp27b1⁻/⁻ cells was observed. The authors suggest that a synergism between 25(OH)D3 and 1α,25(OH)₂D3 might be physiologically important. More recently, DeLuca et al. (2011) used the VDR knockout model to evaluate the hypercalcemic toxicity induced by high doses of vitamin D3 and 25(OH)D3 and demonstrated that high concentrations of 25(OH)D3 could bind to VDR and could induce gene transcription in the Cyp27B1⁻/⁻ mice. Since no 1α,25(OH)₂D3 was detected in the serum of these Cyp27B1⁻/⁻ mice, they concluded that 25(OH)D3, not 1α,25(OH)₂D3, was likely responsible for the toxicity of vitamin D excess. Using different approach, Munetsuna et al. (2011) also demonstrated that 1α-hydroxylation of 25-hydroxy-19-nor-vitamin D3 was not required for its biological activity in PZHPV-7 prostate cells.

23.8 Summary and conclusions

This review focuses on the findings obtained during the past decade regarding the expression of the three cytochrome P450 enzymes, CYP27B1, CYP2R1, and CYP24A1, and their roles in the activation and inactivation of vitamin D3 in prostate cells. In summary, prostate cells have all these enzymes and are capable of synthesizing vitamin D3 to 25(OH)D3 and then to 1α,25(OH)₂D3 (Figure 23.4). Furthermore, 25(OH)D3 can act through VDR without converting to 1α,25(OH)₂D3. The autocrine synthesis of 25(OH)D3 and 1α,25(OH)₂D3 in prostate cells may imply that vitamin D plays important role in maintaining the normal functions of prostate cells through a vast number of genes it can regulate and the versatile actions it can cause (Figure 23.4). Although very little is known about the regulation of CYP2R1 and how vitamin D3 and 25(OH)D3 are delivered into the prostate, we can speculate that limited trafficking of vitamin D3 and 25(OH)D3 into the prostate may occur in certain pathological conditions, and may interfere with the synthesis of 25(OH)D3 and 1α,25(OH)₂D3 and therefore affecting their actions in the prostate similar to that shown in the human innate immune response (Adams et al., 2007).

Thus, we need to develop analogs of vitamin D3, 25(OH)D3 and 1α,25(OH)₂D3 which are not only less calcemic and more resistant to CYP24A1 hydroxylation, such as MART-10, but also can be easily taking up by the cells to bind to VDR directly or to be activated before exerting their biological actions.
Figure 23.4. Metabolism and functions of vitamin D3 in prostate cells. DBP = vitamin D binding protein; PGs = prostaglandins; RXR = retinoid X receptor; VDR = vitamin D receptor; VDRE = vitamin D response element; VGEF = vascular endothelial growth factor.

References


23. Vitamin D autocrine system and prostate cancer prevention and treatment


23. Vitamin D autocrine system and prostate cancer prevention and treatment


23. Vitamin D autocrine system and prostate cancer prevention and treatment


Key facts

- Esophageal cancer presents as either squamous cell carcinoma or adenocarcinoma, the latter of which develops via a pre-cursor condition called Barrett’s esophagus.
- Incidence of Barrett’s esophagus and esophageal adenocarcinoma is increasing in Western societies, particularly amongst white males.
- Contrastingly, esophageal squamous cell carcinoma is more common in developing countries and incidence rates are stable or declining.
- Obesity is a key risk factor for esophageal adenocarcinoma, whereas smoking and alcohol consumption are related to esophageal squamous cell carcinoma development.
- Current evidence suggests that vitamin D is not protective against esophageal carcinogenesis, and in fact may be associated with an increased risk of this cancer, particularly squamous cell carcinoma.

Summary points

- Vitamin D intake has been directly associated with esophageal adenocarcinoma risk, but not its pre-cursor conditions, and inversely associated with squamous cell carcinoma risk in European populations.
- No significant associations between vitamin D intake and esophageal cancer risk have been noted in the USA, where fortification of foods with vitamin D is prevalent.
- Being classified as vitamin D insufficient or deficient has been linked with a decreased risk of esophageal squamous cell carcinoma in Asian populations.
- No significant associations have been observed between vitamin D status and esophageal adenocarcinoma to date.
- Only small studies of vitamin D related genetic variants have been conducted in relation to esophageal cancer risk, none of which have identified potential polymorphisms at an altered risk of these lesions.
- The vitamin D receptor, through which vitamin D exerts its biological effects, has only been detected in Barrett’s esophagus and adenocarcinoma tissue, and not squamous mucosa of the esophagus in limited laboratory investigations to date.
- The conflicting evidence of vitamin D and esophageal cancer published to date indicate a need for further research in this area in order to fully understand the mechanisms and true relationships that exist.
24. Vitamin D and esophageal cancer

H.G. Coleman, M.M. Cantwell and L.J. Murray
Cancer Epidemiology and Health Services Research Group, Centre for Public Health, Queen’s University Belfast, Grosvenor Road, Belfast BT12 6BJ Northern Ireland, United Kingdom;
h.coleman@qub.ac.uk

Abstract

Esophageal cancer can present as two histological subtypes of esophageal cancer, adenocarcinoma or squamous cell carcinoma. The vast majority, if not all, esophageal adenocarcinomas arise from Barrett’s esophagus. Similarly, squamous dysplasia carries an elevated risk of progression to esophageal squamous cell carcinoma. Both cancer types have extremely poor survival rates, and therefore there is an acute need to identify modifiable risk factors that may help to prevent their development. Despite the mechanistic and ecological support for a general anti-carcinogenic role of vitamin D, studies in relation to esophageal cancer have illustrated conflicting results. Studies of vitamin D intake have been inversely associated with squamous cell carcinoma risk and directly associated with adenocarcinoma risk in European studies, while no significant associations were observed in an American case-control study. Contradictory to this, evidence from high quality prospective cohorts have illustrated that low levels of circulating vitamin D are associated with a reduced risk of esophageal squamous cell carcinoma, particularly in Asian populations. Small studies of vitamin D related genetic variants have failed to detect an association with the risk of either histological subtype of esophageal cancer. Laboratory investigations do suggest, however, that vitamin D receptor expression is more apparent in Barrett’s esophagus and esophageal adenocarcinoma tissue, and therefore are more likely to interact with vitamin D intake or status to impact on outcomes compared with squamous cell carcinomas. Whether these are positive or negative influences on development or survival remains unclear. Further work is clearly warranted in this area to fully understand the mechanisms involved and to clarify the conflicting evidence to date.

Keywords: esophageal adenocarcinoma, esophageal squamous cell carcinoma, Barrett’s esophagus, dysplasia
**24.1 Introduction**

Esophageal cancer ranks 8th of the most common cancers worldwide in terms of incidence, but is accompanied by poor 5-year survival rates of approximately 10%, making it the 6th most common cause of cancer death globally (WCRF, 2007). The pathogenesis of esophageal cancer differs depending upon the location of the tumor. Cancers affecting the lower third of the esophagus tend to be adenocarcinomas that arise through a progressive spectrum of conditions resulting from repetitive gastro-esophageal reflux – namely reflux esophagitis, Barrett’s esophagus and dysplasia. Conversely, tumors in the upper two thirds of the esophagus are usually squamous cell carcinomas. Both histological subtypes show different patterns of incidence, have distinctive risk factors and etiologies but are mutually associated with poor survival rates.

Squamous cell carcinomas of the esophagus historically comprised around 90% of all esophageal cancer cases, however rates of this histological sub-type are generally declining (Van den Brandt and Goldbohm, 2006). In contrast, esophageal adenocarcinoma incidence has approximately tripled over recent decades and it now accounts for around 50% of all new cases in Western countries (Van den Brandt and Goldbohm, 2006). Esophageal cancer predominantly occurs in males, and esophageal adenocarcinoma cases tend to occur more in white males whereas esophageal squamous cell carcinomas are more common in black males (Devesa et al., 1998). These differing patterns of incidence provide an insight into risk factors that may influence these histological cancer subtypes.

Risk factors that are well documented for esophageal adenocarcinoma include male sex, gastro-esophageal reflux, increased body mass index, abdominal obesity and tobacco smoking, but not alcohol consumption (De Jonge et al., 2007, Forman, 2004, Freedman et al., 2011, Kim et al., 1997, Lagergren, 2006), whereas both smoking and alcohol are related to esophageal squamous cell carcinoma etiology (Freedman et al., 2007, Van den Brandt and Goldbohm, 2006). There are ‘pockets’ of epidemic areas of esophageal squamous cell carcinoma in developing countries, often related to specific habits. In northern Iran, an epidemic region is attributed to opium use and nass (a combination of tobacco, ash and lime) chewing, while intake of pickled vegetables (which are a source of mycotoxins and nitrosamines) contributes to high incidence rates in Linxian, China (Islami et al., 2009, Kamangar et al., 2009, Zheng et al., 2010). Other nutritional components suggested to be protective against esophageal cancer include antioxidants, fruit and vegetables and dietary fiber (Anderson et al., 2007, De Stefani et al., 2008, Mulholland et al., 2009).
Vitamin D has several generic anti-carcinogenic effects such as suppressing cell proliferation, promoting cell differentiation and regulating apoptosis (Dusso et al., 2005). There is considerable evidence in the scientific literature citing a protective role for vitamin D against the development of many cancers (Giovannucci, 2009), and particularly colorectal cancer (Gorham et al., 2007, Wei et al., 2008). This chapter outlines the potential associations between esophageal cancer and vitamin D, evidence from observational studies conducted to date, and highlights gaps in our research knowledge.

24.2 Epidemiology of esophageal cancer and vitamin D: generating hypotheses

When esophageal cancer epidemiology is compared with that of vitamin D, it provides some interesting, but at times conflicting, hypotheses for potential associations. It is widely known that vitamin D sufficiency is increasingly inadequate in many populations, especially those residing away from the equator. For example, cases of rickets have re-emerged in the UK and Ireland, where vitamin D insufficiency has now been estimated to affect approximately 70% of the population (Hill et al., 2004). Unlike in the USA, few dietary foodstuffs are routinely fortified with vitamin D in the UK and sunlight is too weak during winter months to stimulate cutaneous vitamin D synthesis. Such prevalent insufficiency rates are attributed to a variety of reasons, such as more widespread sun protection factor usage, an increase in computer usage and television watching resulting in more time being spent indoors, coupled with a reduction in physical activity undertaken outside. The UK has experienced some of the highest reported increases and absolute incidence rates of esophageal adenocarcinoma worldwide (Bosetti et al., 2008, Gatenby et al., 2011, Newnham et al., 2003). Therefore, it could be postulated that vitamin D insufficiency is associated with esophageal adenocarcinoma development.

Exploration of racial disparities in esophageal cancer epidemiology provides further support for an anti-carcinogenic role of vitamin D. The manufacture of vitamin D from 7-dehydrocholesterol in the skin is much more limited in skin of darker pigmentation than Caucasian skin types (Mithal et al., 2009). As noted above, the increase in esophageal adenocarcinoma incidence has been mainly pronounced in white males, rather than black males in whom esophageal squamous cell carcinoma remains the predominant histological subtype (Devesa et al., 1998). Indeed, vitamin D insufficiency or deficiency prevalence is higher in African-American populations residing in the USA compared with Caucasian populations (Mithal et al., 2009). This would suggest that vitamin D deficiency may also promote esophageal squamous cell carcinoma growth.

Alternative hypotheses would suggest that vitamin D is associated with a decreased risk of squamous cell carcinoma and/or an increased risk of adenocarcinoma development, or is of no etiological importance for this cancer:

- The relatively recent formulation and growing popularity of vitamin D supplement usage throughout the Western world parallel an increase in adenocarcinoma but not squamous cell carcinoma rates.
Studies of second primary cancer risk in skin cancer patients, who are assumed to have superior long-term vitamin D status due to their UV exposure, have largely failed to identify an association with esophageal cancer risk.

The male predominance of both histological subtypes of esophageal cancer cannot be clearly explained by patterns of vitamin D intake from foods or supplements, UV exposure or vitamin D insufficiency rates.

Overall the association, if any, between vitamin D and esophageal cancer is unlikely to be straightforward, and may involve interaction with other lifestyle or genetic risk factors. Evidence from observational studies investigating the role of vitamin D in esophageal tumorigenesis is summarized in the remainder of this chapter.

### 24.3 Vitamin D intake and esophageal cancer

A small number of case-control studies have investigated the association between dietary intake of vitamin D and esophageal cancer. A well designed American population-based study with approximately 300 cases of both esophageal adenocarcinoma and esophageal squamous cell carcinoma sought to investigate risk in relation to vitamin D intake (Mayne et al., 2001). No significant associations were observed for both esophageal adenocarcinoma risk (OR 1.10; 95% CI: 0.86-1.40) and squamous cell carcinoma risk (OR 1.00; 95% CI: 0.74-1.36), when comparing the highest with the lowest category of vitamin D intake from foods (Mayne et al., 2001).

More recent findings from an Irish population (Mulholland et al., 2011) identified a significant two-fold increased risk of esophageal adenocarcinoma in those consuming the highest compared with the lowest intakes of vitamin D (OR 1.99; 95% CI: 1.03-3.86). The same study did not detect any associations between vitamin D intake and the risk of reflux esophagitis or Barrett’s esophagus, which arise earlier in the carcinogenic pathway (Mulholland et al., 2011). Conversely, the highest category of vitamin D intakes have been linked with a significant 42% reduced risk of esophageal squamous cell carcinoma in a series of Italian hospital-based case-control studies (Lipworth et al., 2009). Similar inverse associations were also observed in a French case-control study (Launoy et al., 1998).

Potential reasons for the conflicting findings from the studies outlined include the dietary assessment methods used and the different background populations studied. All studies used food frequency questionnaires, to assess diet, and the potential for dietary recall bias may have influenced results (Brown, 2006). Fortification programs operate in the USA, in which no significant associations were detected, and the richer vitamin D foodstuffs may not have been fully captured in the dietary assessment tools used (Calvo et al., 2004). The palpable geographical and ethnic variation in the populations studied will also have had incurred differences in the vitamin D status, and related genetic variants, of the included participants that may impact upon the results observed (Davis, 2008).
In addition, it should be noted that no studies to date have specifically investigated vitamin D supplement intake and esophageal cancer risk. Data from observational studies is required in order to determine whether interventional studies are warranted in high risk population groups.

In summary, there is conflicting evidence from a limited number of studies investigating dietary vitamin D intake and esophageal cancer risk. European studies point towards a direct association between vitamin D intake and adenocarcinoma risk but an inverse association with squamous cell carcinoma risk. However, no significant associations were detected with either histological subtype in an American study.

### 24.4 Vitamin D status and esophageal cancer

Studies exploring vitamin D status, as measured by blood concentration levels of 25-hydroxyvitamin D, provide further intrigue to the association with esophageal cancer risk. Giovannucci and colleagues calculated predicted vitamin D status (on the basis of dietary and supplemental intake, skin pigmentation, adiposity, geographical location and physical activity) as part of a larger study of cancer incidence and mortality in men (Giovannucci et al., 2006). In their analysis, a significant inverse association was observed between predicted vitamin D status and esophageal cancer risk, however this did not distinguish between histological subtypes of esophageal cancer.

This contrasts with studies comparing 545 esophageal squamous cell carcinoma patients (Chen et al., 2007) and 230 squamous dysplasia cases (Abnet et al., 2007) with appropriate controls residing in Linxian, China. In a cross-sectional analysis, the highest serum 25-hydroxyvitamin D concentrations were associated with significantly elevated risks of squamous dysplasia, which was evident in both men and women (Abnet et al., 2007). These findings were reinforced with significantly increased risks of squamous cell carcinoma of the esophagus from prospective case-cohort analysis (HR 1.06; 95% CI: 1.01-1.13, per 15 nmol/l increment) (Chen et al., 2007). These results were amongst the first to indicate that the widely published anti-carcinogenic effects of vitamin D may not be universally applied to all body sites.

Since the publication of the Chinese study results, a robust cohort consortium has sought to clarify the effects of circulating vitamin D concentrations in relation to rarer cancers. In fully adjusted analysis incorporating over 1000 cases, vitamin D status was not significantly associated with the risk of all upper gastrointestinal cancers (Abnet et al., 2007). Compared to individuals with a vitamin D status in the reference range of 50-75 nmol/l, individuals with low circulating vitamin D concentrations did not have significantly altered risks of either squamous cell carcinoma or adenocarcinoma of the esophagus (Abnet et al., 2007). Interesting observations were noted in subgroups analyses though, in which Asians and never smokers with the lowest vitamin D status had significantly reduced risks of upper gastrointestinal cancers overall. The authors concluded that increasing vitamin D status would not contribute to a lower risk of this cancer group (Abnet et al., 2007).
In conclusion, results from studies to date suggest that if an association exists between vitamin D status and esophageal cancer risk, vitamin D insufficient or deficient states may actually serve to decrease risk. Evidence is most consistent for this observation in squamous cell carcinoma development in Asian populations.

24.5 Vitamin D receptor, related genetic variants and esophageal cancer

Vitamin D exerts its biological effects by binding to the VDR. Activation of the VDR induces changes in the expression of genes implicated in transcription, DNA synthesis, apoptosis and cell signaling of colon cancer cells (Palmer et al., 2003). The impact of the VDR is less clear in esophageal tissue. VDR expression has been investigated in esophageal biopsies, in which no impact on apoptosis was observed (De Gottardi et al., 2006). Furthermore, VDR expression did not differ between Barrett’s esophagus, adenocarcinoma or normal mucosa samples investigated, although this investigation was restricted to only six biopsy samples per disease state (De Gottardi et al., 2006). More recent data has found a change in VDR expression as the esophageal lining progresses from the native squamous epithelium to the columnar epithelium that indicates Barrett’s esophagus (Trowbridge et al., 2012a). In their study of 15 esophageal tumor specimens, Trowbridge and colleagues only detected VDR staining in the columnar metaplasia, and not normal squamous mucosa of the esophageal resections (Trowbridge et al., 2012a,b). This would imply that vitamin D does not have an opportunity to bind locally in the esophagus, and therefore exert any potential anti-carcinogenic effects, unless the cell lining has undergone the metaplastic transition to Barrett’s esophagus. This may explain why we do not observe a protective effect for vitamin D against squamous cell carcinoma of the esophagus, but does not clarify why vitamin D does not appear to hinder esophageal adenocarcinoma development (or, in fact, may increase risk). Although a tenuous suggestion, it may be that a substantive duration of Barrett’s esophagus presence, combined with an adequate intake and/or circulating vitamin D status, is required in order to detect any interactions, if indeed one exists. Higher VDR expression was also associated with better response to neoadjuvant therapy, and therefore may be a suitable target for, improving survival from esophageal adenocarcinoma (Trowbridge et al., 2012a,b).

VDR gene polymorphisms alter mRNA stability and transcriptional activity (Anderson et al., 2003) and are related to tumor development (Holick et al., 2007, Lowe et al., 2005). A study in a Chinese population found no associations between VDR TaqI (C352T) genotypes and risk of esophageal squamous dysplasia (n=127 cases) or carcinoma (n=126 cases) (Li et al., 2007). Similarly, no significant associations were identified in an Irish population-based case-control study of 224 esophageal adenocarcinoma cases, in which all of the common variation in the VDR gene polymorphisms were systematically investigated (Chang et al., 2011). However, studies have generally been inadequately powered to study the association between vitamin D-related genetic polymorphisms and esophageal cancer outcomes (Chang et al., 2011, Li et al., 2007). In addition, vitamin D binding protein polymorphisms have recently been shown to be highly related to vitamin D status (Ahn et al., 2010), and no studies to date have investigated their role in esophageal carcinogenesis.
In summary, given the potential relationship between low vitamin D status and an increased risk of esophageal cancer, there is an urgent need to fully elucidate the action of VDR and related genetic variants in this pathway in order to fully understand the mechanisms involved and to appropriately target dietary guidelines in future. Limited evidence to date suggests only Barrett’s esophagus and esophageal adenocarcinoma express VDR, which is not observed in esophageal squamous mucosa, and therefore any potential effect of vitamin D, anti-carcinogenic or otherwise, may be limited to esophageal adenocarcinoma outcomes.

24.6 Conclusions

Better quality population-based studies are required to investigate the role of vitamin D intake, status, and related genetic variants in the esophageal cancer development, given the conflicting evidence to date. There is an accompanying need for further in vitro work to investigate the potential mechanisms involved.

References

Brown, D., 2006 Do food frequency questionnaires have too many limitations? J Am Diet Assoc 106, 1541-1542.


24. Vitamin D and esophageal cancer


Key facts

- Multiple sclerosis (MS) is an inflammatory disease of the central nervous system, in which auto-reactive T cells are involved in the pathology.
- A poor vitamin D status has been associated with a high risk of developing MS, and with an adverse outcome in MS.
- Vitamin D has extensive immune modulating properties in vitro, promoting immune homeostasis within the adaptive immune response.

Summary points

- Studies on immune modulating properties of vitamin D in vivo are highly heterogeneous in supplementation scheme of vitamin D and in the immunological outcomes measured.
- A trend towards an anti-inflammatory role of vitamin D appears to be present, but this impression is not substantiated by conclusive data.
- Studies on immune modulating potential of vitamin D in vivo should assess disease-specific relevant immunological outcomes in a controlled, well powered design.
25. Monitoring *in vivo* immune modulation by vitamin D in multiple sclerosis

A.-H. Muris¹,²,³, J. Damoiseaux⁴ and J. Smolders¹,²,³,⁵

¹School for Mental Health and Neuroscience, Maastricht University Medical Center, Universiteitssingel 40, 6229 ER Maastricht, the Netherlands; ²Department of Internal Medicine, Division of Clinical and Experimental Immunology, Maastricht University Medical Center, Universiteitssingel 50, 6229 ER Maastricht, the Netherlands; ³Academic MS Center Limburg, Orbis Medical Center, Dr. H. van der Hoffplein 1, 6162 BG Sittard, the Netherlands; ⁴Laboratory for Clinical Immunology, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, the Netherlands; ⁵Neuroimmunology Research Group, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, the Netherlands; smolders@gmail.com

Abstract

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system, characterized by an auto-reactive T cell response. High serum 25-hydroxyvitamin D levels have been associated with a decreased risk of developing MS and less severe disease. It is hypothesized that the underlying mechanism behind this association is the immune modulating effect of vitamin D on the adaptive immune response. *In vitro* studies and *in vivo* studies in the experimental auto-immune encephalomyelitis animal model have shown consistently that immune cells are functional targets of vitamin D. However, *in vivo* studies in humans are limited. In this chapter, studies on the relation between vitamin D and immunological parameters in healthy individuals and patients with different auto-immune diseases, including MS, are evaluated. Correlation studies and supplementation studies performed thus far are heterogeneous in many aspects and studied a diverse repertoire of immunological outcome measures. Overall, there seems to be a trend towards an anti-inflammatory role of vitamin D, but this impression is not substantiated by conclusive data. Well-powered and controlled studies assessing disease-specific relevant immunological outcomes are warranted before conclusions can be drawn. Clinical trials on vitamin D supplementation in MS and other diseases may provide excellent frameworks for such studies.

**Keywords:** auto-immune diseases, 25-hydroxyvitamin D, 1,25-dihydroxvitamin D, supplementation, correlation
25.1 Introduction

Since the beginning of the 20th century vitamin D has been particularly known for its function on bone and calcium metabolism. However, since the seventies, a poor exposure to vitamin D has also been increasingly noticed in and linked to the aetiology of MS (Goldberg et al., 1986; Kurtzke, 1967). Since then, a new research field developed, addressing the implications of vitamin D in the pathophysiology of MS in epidemiological studies, intervention studies, in vitro studies
25. Monitoring in vivo immune modulation by vitamin D in MS

and animal studies (Smolders et al., 2008a). Currently, almost 50 years later, first randomized controlled clinical trials are assessing the efficacy of vitamin D supplementation in MS. The view of vitamin D changed from that of a vitamin with a single role in calcium homeostasis, to that of an exciting steroid hormone involved in a multitude of cellular processes. One of these processes is the regulation of the adaptive immune response, with many in vitro and animal studies showing important regulatory effects of vitamin D (Hayes et al., 1997; Smolders and Damoiseaux, 2011). One of the major challenges that lies ahead is to understand if and how systemic vitamin D status interferes with the human adaptive immune response in vivo. Clarification of the interaction between the availability of vitamin D at tissue level (target and secondary lymphoid organs) and the extent to which an immune response is initiated or thriven, may reveal underlying mechanism for associations between vitamin D status and MS.

The aim of this chapter is not to give an extensive overview of the current knowledge about either the immune dysregulation in MS, or the association between vitamin D and MS, or the role of vitamin D in the immune response at a cellular level. Since these have been reviewed extensively by us and others, we will refer to these reviews were appropriate. In the present chapter, we will focus on studies exploring the in vivo relevance of the interaction between vitamin D and the immune response. To achieve this, we will discuss studies on correlations between vitamin D status and relevant immune outcome parameters, and studies on immunological outcome measures in vitamin D supplementation trials, both in healthy controls as well as in patients with auto-immune disease including MS. We will integrate these findings and discuss considerations for the design of trials on the immune modulating effects of vitamin D in MS.

25.2 Multiple sclerosis

Multiple sclerosis is an inflammatory, demyelinating disease of the CNS. The prevalence is 30-100 per 100,000 individuals (Milo and Kahana, 2010; Rosati, 2001). It is twice as common in females than in males, with an incidence between 20-50 years, and a peak incidence at 30 years of age (Compston and Coles, 2002; 2008). MS patients have a heterogeneous disease presentation. In RRMS, periods without neurological exacerbations are altered by periods of increased neurological symptoms. These relapses can be characterised by symptoms of loss of motor and/or sensory function, lack of coordination, but also mental and physical fatigue and/or cognitive decline. After several years, 65% of the RRMS patients becomes secondary progressive MS and they experience constant neurological deterioration. A small fraction of the patients experience a progressive accumulation of disability from the beginning of the disease onwards, called primary progressive MS (Compston and Coles, 2002; 2008).

The exact aetiology of MS is still unknown, but one of the main hypotheses is that multiple sclerosis is a T-cell mediated auto-immune disease. This model for MS has been reviewed by several authors (Bar-Or, 2008; Milo and Kahana, 2010). Shortly, it is thought that auto-reactive lymphocytes are primed with an unknown auto-antigen in the secondary lymphoid organs by APCs. Clonally expanded and activated effector T cells migrate through the blood brain barrier
into the perivascular space. Here, the T cells are reactivated by APCs, like perivascular macrophages or DCs, and migrate into the brain parenchyma. In the parenchyma, the T cells contribute to an inflammatory response in which loss of oligodendrocytes and myelin occurs, often followed by loss of neurons. It is a matter of debate whether MS is truly a classical auto-immune disease, with an auto-reactive response directed against a single auto-antigen (Bar-Or, 2008). Several lines of evidence suggest a general lack of control of the T cell response in MS. Firstly, many genetic polymorphisms associated with MS are involved in the maintenance of T cell homeostasis (Sawcer et al., 2011). Secondly, several differences in general characteristics of the circulating T cell compartment have been found between MS and control patients. During active disease, increased proportions of pro-inflammatory Th1 and Th17 cells, as well as increased transcription of IL-17 mRNA have been described (Durelli et al., 2009; Edwards et al., 2010; Frisullo et al., 2008; Matusevicius et al., 1999). Most notably, the capacity of Treg to suppress polyclonal or antigen-specific proliferation of effector T cells was reduced in MS (Haas et al., 2005; Venken et al., 2006; Viglietta et al., 2004). In addition, a reduced transcription of Treg-related genes by PBMC (Dalla Libera et al., 2011; Edstrom et al., 2011), and a reduction of Treg-subsets with specific surface molecules as CD39 (Borsellino et al., 2007) have been described. Interestingly, these CD39+ Tregs are able to suppress IL-17 production by activated T cells (Fletcher et al., 2009). Accordingly, our group found a negative correlation between the proportion of CD39+ Treg and the proportion of Th17 cells in the circulation of RRMS patients (Peelen et al., 2011a). Thirdly, the CNS tissue of MS patients is infiltrated with CD4+ but predominantly CD8+ T cells (Frischer et al., 2009). The clonality of these cells is diverse, and does not differ between lesions and normal appearing white matter (Junker et al., 2007). Beside T cells, B cells are also found in increased number in the CNS of MS patients (Frischer et al., 2009). Interestingly, oligoclonal intrathecal IgG-production against predominantly several myelin proteins and heat shock proteins is a hallmark of the disease process (Quintana et al., 2012). Lastly, currently registered MS disease modulating therapies interfere with either phenotypic and functional characteristics of circulating T cells (Cohen et al., 2010; Noronha et al., 1993), migration of lymphocytes into the CNS (Polman et al., 2006), or deplete B and T cells from the circulation (Coles et al., 2008; Hauser et al., 2008).

### 25.3 Multiple sclerosis and vitamin D

Several epidemiologic and genetic studies show that a poor exposure to vitamin D is a putative risk factor for developing MS, as has been reviewed elsewhere (Ascherio et al., 2010; Smolders et al., 2008a). In brief, genetic polymorphisms within key enzymes involved in vitamin D metabolism, as well as within the VDR, have been associated with the risk on developing MS (Sawcer et al., 2011; Smolders et al., 2009a), as well as a poor vitamin D status prior to disease onset (Munger et al., 2006). In established MS, a poor vitamin D status is associated with a high risk of relapses (Mowry et al., 2010; Simpson et al., 2010; Smolders et al., 2008b) and a high MS-related disability score (Smolders et al., 2008b; van der Mei et al., 2007). Supplementation of high doses of vitamin D3 in MS patients did not provoke hypercalcaemia and was well tolerated in several pilot studies (Burton et al., 2010; Kimball et al., 2007; Smolders et al., 2010b). A pilot study in MS on treatment with the biologically active metabolite of vitamin D, 1,25(OH)2D3, reported the development
of a hypercalcaemia in 2/15 subjects (Wingerchuk et al., 2005). Exploratory analyses on clinical endpoints suggested a reduction of MRI activity in an uncontrolled study (Kimball et al., 2007), a reduction of relapse activity in an uncontrolled (Smolders et al., 2010b) and controlled study (Burton et al., 2010), and a reduction of disability progression in a controlled study design (Burton et al., 2010). A study supplementing vitamin D2, did not show clear difference on primary MRI endpoints between the high- and low-dose treatment arms (Stein et al., 2011). Subsequently, first clinical trials are currently being performed to assess the efficacy of high dose vitamin D3 supplementation in MS (Dorr et al., 2012; Smolders et al., 2011). Although vitamin D appears to be implicated in the disease process of MS, the underlying mechanism is not known.

25.4 Vitamin D metabolism

Vitamin D is the precursor of an essential hormone (Lips, 2006). Its natural sources are diet, both from vegetable origin (ergocalciferol or vitamin D2) as well as from animal origin, especially fatty fish (cholecalciferol or vitamin D3), and most importantly synthesis in the skin under the influence of ultraviolet B radiation in sunlight (90-100%). The epidermal layer of the skin contains 7-dehydrocholesterol which is transformed into pre-vitamin D3 under influence of ultraviolet B radiation and body temperature. Subsequently, most of the vitamin D metabolites in the circulation are of vitamin D3 origin and only a negligible part of vitamin D2. In the circulation and tissues, vitamin D is mostly present as 25(OH)D, which is formed in the liver. The circulating levels of this metabolite are most useful to describe an individual's vitamin D status, although 25(OH)D is not biologically active. A second hydroxylation step is needed to obtain the biologically active metabolite of vitamin D, 1,25(OH)₂D. This step is catalyzed by the enzyme cytochrome P27B1 (CYP27B1 or 25(OH)D-1α hydroxylase) (Dusso et al., 2005). 1,25(OH)₂D in the circulation is almost exclusively produced by the kidney (Jongen et al., 1984), and circulating levels are regulated by the endocrine control of calcium homeostasis (Lips, 2006). However, many other tissues can 'activate' 25(OH)D (Zehnder et al., 2001). Once activated, 1,25(OH)₂D behaves as a steroid hormone. It binds to the intracellular VDR which regulates the transcription of more than 500 genes containing the vitamin D response elements. Catabolism of 1,25(OH)₂D is regulated by the enzyme CYP24A1 (1,25(OH)₂D 24-hydroxylase) (Holick, 2007). Several cut-off points for an optimal vitamin D status have been proposed, mostly based on its role in the endocrine control of calcium homeostasis (Holick, 2007; Holick et al., 2011; Institute of Medicine, 2010; Ross, 2011; Vieth, 1999). Since the relevance of these cut-off values for other biological functions of vitamin D are uncertain, they are not very useful in research.

25.5 Vitamin D as an immune modulator

All immune cells involved in the adaptive immune response have the potential to metabolize and to respond to vitamin D, as has been reviewed extensively (Baeke et al., 2010; Peelen et al., 2011b; Smolders and Damoiseaux, 2011). Shortly, resting and activated myeloid cells, including monocytes, macrophages and DCs, and activated T- and B-lymphocytes express the
VDR (Chen et al., 2007; Penna et al., 2007; Provvedini et al., 1983; Veldman et al., 2000). In lymphocytes, exposure to 1,25(OH)_2D further enhances VDR expression (Chen et al., 2007; Veldman et al., 2000). Myeloid APCs, and most notably the specialized DCs, lose their antigen presenting potential upon exposure to 1,25(OH)_2D, and skew towards an immature innate profile (Penna and Adorini, 2000). Subsequently, they have less capability to start a T cell response (Van Halteren et al., 2002). Accordingly, 1,25(OH)_2D inhibits T cell proliferation and the production of pro-inflammatory cytokines as IFN-γ and IL-17 (Correale et al., 2009). The synthesis of anti-inflammatory cytokines, such as IL-4 and IL-10, is promoted. Besides being targets of vitamin D, activated myeloid cells (either activated by adaptive or innate stimuli) and lymphocytes (either activated polyclonally or via antigen specific stimulation) express the activating enzyme CYP27B1, and subsequently synthesize 1,25(OH)_2D locally (Correale et al., 2009; Fritsche et al., 2003; Gottfried et al., 2006; Heine et al., 2008; Penna et al., 2007; Sigmundsdottir et al., 2007). All these cells also up-regulate the catabolic enzyme CYP24A1 upon exposure to 1,25(OH)_2D (Correale et al., 2009; Gottfried et al., 2006; Penna et al., 2007). Since these observations are done in vitro, it is uncertain whether these dynamics also apply to immune activation within tissues where the immune response becomes manifest, i.e. the secondary lymphoid tissues as well as the peripheral tissues affected by a disease. Nevertheless, studies in the experimental MS model auto-immune encephalomyelitis showed an up-regulation of VDR and CYP27B1 and a down-regulation of CYP24A1, together with an enhanced local 1,25(OH)_2D production within the inflamed CNS (Spach and Hayes, 2005). Accordingly, supplementation of vitamin D or treatment with 1,25(OH)_2D prevented development of experimental auto-immune encephalomyelitis and ameliorated the disease course (Cantorna et al., 1996; Lemire and Archer, 1991). Both in the circulation, lymph nodes, and CNS, proliferation and cytokine assays suggested a shift of the immune response towards a less inflammatory profile (Smolders et al., 2008a).

25.6 Vitamin D status, vitamin D supplementation and the effect on immune regulation in vivo

Since an auto-reactive T cell response has been implicated in the disease process of MS, an immune modulating, dampening effect of vitamin D may be very relevant (Hayes et al., 1997). Although effects of vitamin D metabolites on immune cells in vitro have been frequently investigated, in vivo data are limited. The negative correlation between vitamin D status and the risk on developing MS was observed within the physiologically occurring ranges of vitamin D, which is 0-150 nmol/l (Munger et al., 2006). Within the same range of 25(OH)D, a negative correlation between vitamin D status and MS relapse rate was found (Mowry et al., 2010; Simpson et al., 2010; Smolders et al., 2008b). If the driving force of these correlations is the promotion of T cell homeostasis within the higher ranges of serum 25(OH)D, relevant measures of immune-regulation would be expected to correlate positively with vitamin D status in vivo.

We conducted a literature search to identify papers which investigated either the correlation of serum 25(OH)D levels with measures of immune homeostasis, or the effect of vitamin D supplementation on immune parameters determined in peripheral blood (correlation studies
are summarized in Table 25.1, supplementation studies in Table 25.2). We focused on studies in healthy controls, patients with MS, and patients with other auto-immune diseases. Since the systemic metabolism of vitamin D is disturbed in kidney disease patients, we excluded studies done in this patient population. Furthermore, studies in cancer patients, HIV patients, allergic patients and patients with an infectious disease were excluded, because the interaction of vitamin D with the immune system may be different from auto-immune diseases due to distinct immunopathology. Moreover only studies in which 25(OH)D levels were measured and reported were included. Epidemiological studies addressed association between serum 25(OH)D levels, and the efficacy of vitamin D2/D3 supplementation (vide supra). Additionally, while the effect of vitamin D on the immune system is most likely mainly executed by local activation of vitamin D, this is rather dependent on circulating 25(OH)D than on 1,25(OH)2D levels. Therefore only supplementation studies in which vitamin D2 or D3 was supplemented and in which the effects of vitamin D supplementation could be distinguished from other possible co-interventions, were included. Since several approaches have been used to evaluate the effect of vitamin D on the immune function in vivo, we will discuss the different immunological outcome measures that have been used in these studies.

25.6.1 Inflammatory markers in the circulation

First of all, several groups addressed correlations of serum 25(OH)D with serum concentrations of general markers of inflammation, chemokines, and cytokines. Serum concentrations of CRP did not correlate with serum 25(OH)D in most studies with controls (Peterson and Heffernan, 2008; Shea et al., 2008; Vilarrasa et al., 2010). Supplementation of vitamin D did also not influence CRP levels in healthy, older or obese persons (Jorde et al., 2010; Pittas et al., 2007; Prietl et al., 2010) or in patients with MS (Kimball et al., 2011). However, a negative correlation between these parameters has been described in patients with SLE (Thudi et al., 2008), ankylosing spondylitis or psoriatic arthritis (Teichmann et al., 2010) and BD (Do et al., 2008; Hamzaoui et al., 2010). Regarding complement, a study among healthy individuals reported higher serum concentrations of both 25(OH)D and C3 after a long-distance holiday (Falkenbach and Sedlmeyer, 1997), and higher serum 25(OH)D levels correlated with higher serum C4 in subjects with SLE (Szodoray et al., 2011).

25.6.2 Cytokines and chemokines in the circulation

In healthy controls, multiple studies reported no association of higher serum 25(OH)D levels with serum concentrations of pro-inflammatory cytokines as IL-6 (Peterson and Heffernan, 2008; Shea et al., 2008), IL-12 (Hajas et al., 2011), IL-17 (Hajas et al., 2011), IL-18 (Vilarrasa et al., 2010), IFN-γ (Hajas et al., 2011), TNF-α (Shea et al., 2008) and the anti-inflammatory cytokine IL-10 (Peterson and Heffernan, 2008). Accordingly, no correlations were reported with the immune-related molecules soluble (s)CD40L (Shea et al., 2008), sP-selectin (Shea et al., 2008), osteoprotegerin (Shea et al., 2008), sTNFR1 (Vilarrasa et al., 2010), sTNFR2 (Shea et al., 2008; Vilarrasa et al., 2010), sICAM-1 (Shea et al., 2008) and MCP-1 (Shea et al., 2008). Likewise, supplementation studies with vitamin D in healthy controls and MS patients showed
Table 25.1. Cross sectional and cohort studies on 25(OH)D levels in vivo and immunological outcome measures in peripheral blood in health and auto-immune disease; the relation of 25(OH)D to the outcome measures are given as ↑: increased; ↓: decreased; =: not affected.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Study population (n)</th>
<th>25(OH)D (nM) (mean or median)</th>
<th>Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH (Falkenbach and Sedlmeyer, 1997)</td>
<td>Healthy, long distance holiday (33)</td>
<td>85.4 (±44.7) to 110 (±37.6)</td>
<td>Circulating total blood count =, CD4+ cells =, CD8+ cells =, circulating CD4+/CD8+ T cell ratio ↑, serum IgG ↓, IgA ↑, IgM =, C3 ↑, sIL-2R ↑, soluble CD14 ↑</td>
</tr>
<tr>
<td>CS (Feser et al., 2009)</td>
<td>Predicted RA in future (76 cases)</td>
<td>67.1±25.1</td>
<td>Serum no corr. RF-IgM, RF-IgG, RF-IgA, anti-CCP</td>
</tr>
<tr>
<td>CS (Jorde et al., 2010)</td>
<td>Controls at risk for RA or DMI (154)</td>
<td>63.1±22.5</td>
<td>Serum MCP-1 (♂): R²=0.28 (P&lt;0.01), MCP-1 (♀): R²=+0.2 (P&lt;0.01), IL-5 (♀): R²=-0.16 (P&lt;0.05), IL-10 (♀): R²=-0.14 (P&lt;0.05)</td>
</tr>
<tr>
<td>CH (Khoo et al., 2011)</td>
<td>Healthy, ♂ (15)</td>
<td>56 (15-136)</td>
<td>Serum no corr. CRP, IL-6, IL-10, TNF-a ↓, IL-1β ↓, IL-6 ↓, IL-10 ↓, TNF-a ↓, IFN-γ ↓ (PBMC+LPS)</td>
</tr>
<tr>
<td>Winter to spring</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>±70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Peterson and Heffernan, 2008)</td>
<td>Healthy, ♀</td>
<td>129.6±11.0</td>
<td>Serum no corr. CRP =, IL-6 =, IL-10 =, TNF-a ↓</td>
</tr>
<tr>
<td>high 25(OH)D (20)</td>
<td></td>
<td></td>
<td>Serum CRP =, IL-6 =, IL-10 =, TNF-a ↓</td>
</tr>
<tr>
<td>low 25(OH)D (49)</td>
<td>74.4±4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Shea et al., 2008)</td>
<td>Birth cohort, mean intake vit.D 426 IU/d (1,381)</td>
<td>49.4±18.6</td>
<td>Serum no corr. CRP, IL-6, CD40L, P-selectin, osteoprotegerin, TNF-a, sTNFR2, sICAM-1, MCP-1</td>
</tr>
<tr>
<td>CS (Vilarrasa et al., 2010)</td>
<td>Healthy (261)</td>
<td>52.7±21.3</td>
<td>Serum no corr. CRP, IL-18, sTNFR1, sTNFR2</td>
</tr>
<tr>
<td>Obese (44)</td>
<td>37.8±16.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Zittermann et al., 2004)</td>
<td>Newborns</td>
<td>52.2 (29.5-65.3)</td>
<td>Serum no corr. CRP, IL-18, sTNFR1, sTNFR2</td>
</tr>
<tr>
<td>Summer (49)</td>
<td>Cord blood IL-10 ↑, IgE ↓=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter (47)</td>
<td>Cord blood IL-10: R=+0.22 (P&lt;0.05), no corr. IgE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 25.1. Continued.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Study population (n)</th>
<th>25(OH)D (nM) (mean or median)</th>
<th>Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto-immune disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Ben-Zvi et al., 2010)</td>
<td>SLE (19)</td>
<td>range &lt;49.9, &gt;74.9</td>
<td>Circulating mDC/pDC numbers =, circulating DC HLA-DR =, CD40 =, CD86 =</td>
</tr>
<tr>
<td></td>
<td>African Americans</td>
<td>35.4 (22.7-47.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hispanics</td>
<td>51.2 (32.4-74.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>54.9 (28.7-73.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>72.4 (36.2-84.9)</td>
<td></td>
</tr>
<tr>
<td>CS (Ben-Zvi et al., 2010)</td>
<td>SLE (19)</td>
<td>24.2±2.1 (7.9-78.0)</td>
<td>Serum anti-dsDNA ↓</td>
</tr>
<tr>
<td></td>
<td>(range 25-39.9, 12.5-24.9, &lt;12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Bonakdar et al., 2011)</td>
<td>SLE, ♀ (40)</td>
<td>27.2±10.8</td>
<td>Serum CRP: R=-0.37 (P&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>BD active (23)</td>
<td>27.2±10.8</td>
<td>Circulating CD14+ monocytes</td>
</tr>
<tr>
<td></td>
<td>BD inactive (18)</td>
<td>31.1±9.1</td>
<td>TLR2: R=-0.37 (P&lt;0.05); TLR4 R=-0.43 (P&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Psoriasis control (19)</td>
<td>37.2±15.2</td>
<td>No corr. circulating CD16+ CD14+ cells</td>
</tr>
<tr>
<td></td>
<td>Healthy (15)</td>
<td>35.0±8.9</td>
<td></td>
</tr>
<tr>
<td>CS (Hajas et al., 2011)</td>
<td>MCTD, ♀ (125)</td>
<td>65.3±33.7</td>
<td>Serum anti-U1-RNP: R=-0.246 (P=0.02), anti-CL IgA: 0.396 (P=0.02)</td>
</tr>
<tr>
<td></td>
<td>Healthy (48)</td>
<td>87.2±24.1</td>
<td>Serum no corr. CRP, anti-CL IgG/ IgM, AECA, IL-12, IL-17, IFN-γ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum IL-23: R=-0.473 (P=0.002), IL-6: R=0.618 (P&lt;0.001), IL-10: R=0.338 (P&lt;0.004)</td>
</tr>
<tr>
<td>CS (Hamzaoui et al., 2010)</td>
<td>BD active (102)</td>
<td>22.5±14.1</td>
<td>Serum CRP: R=-0.3634 (P&lt;0.001) (whole population)</td>
</tr>
<tr>
<td></td>
<td>BD inactive (58)</td>
<td>27.7±12.9</td>
<td>Circulating IFN-γ+CD4+ T cells: R=0.55 (P=0.012), IFN-γ/IL-6 ratio: R=0.599 (P=0.005), IL-17+ cells: R=0.462 (P=0.040), Treg: R=0.640 (P=0.002), IL-10: R=0.808 (P=0.0001) (subpopulation of active BD n=20)</td>
</tr>
<tr>
<td></td>
<td>RA control (22)</td>
<td>14.1±5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS control (30)</td>
<td>17.0±9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Healthy (50)</td>
<td>35.0±13.0</td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td>Study population</td>
<td>Outcome measures</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Study</strong></td>
<td></td>
<td><strong>25(OH)D</strong> (nM) (mean or median)</td>
<td><strong>Immunological outcome measures</strong> (between groups (high versus low 25(OH)D) and correlations)</td>
</tr>
<tr>
<td><strong>Study</strong></td>
<td><strong>(n)</strong></td>
<td><strong>(nM)</strong></td>
<td>Serum anti thyroid peroxidise ab ↓, anti thyroid globulin ab ↓, anti thyroid stimulating hormone receptor ab ↓</td>
</tr>
<tr>
<td><strong>Kivity et al.</strong></td>
<td>AITD and non-AITD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High 25(OH)D (34)</td>
<td>42.4±15.0</td>
<td>Serum anti thyroid peroxidise ab ↓, anti thyroid globulin ab ↓, anti thyroid stimulating hormone receptor ab ↓</td>
<td></td>
</tr>
<tr>
<td>Low 25(OH)D (58)</td>
<td>18.5±2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy (98)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ritterhouse</strong></td>
<td>SLE, ♀ (32)</td>
<td>43.2 [29.7-52.9] (range &lt;49.9, &gt;49.9)</td>
<td>Serum anti-SS-A ↓/=, ANA ↓, no corr anti-dsDNA, anti-SS-B, anti-Sm, anti-nRNP, anti-ribo P, aPL</td>
</tr>
<tr>
<td>Healthy (32)</td>
<td></td>
<td>Serum IFN-α activity: R²=+ (P=0.03), anti-Ro, anti-dsDNA &amp; anti-nRNP specificity</td>
<td></td>
</tr>
<tr>
<td>ANA pos controls</td>
<td>43.4 (36.2-64.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA neg. controls</td>
<td>±69.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Szodoray et al.</strong></td>
<td>SLE (177)</td>
<td>67.1±33.1 (range &gt;74.9, 37.4-74.9, &lt;37.4)</td>
<td>Serum IgG ↓, C4 ↑, anti-dsDNA ↓, ANA ↑, Serum anti-Sm: R=0.288 (P&lt;0.001)</td>
</tr>
<tr>
<td><strong>Teichmann et al.</strong></td>
<td>ASP (76)</td>
<td>24.9±32.1</td>
<td>Serum CRP: R=0.350 (P&lt;0.001), anti-hiTG IgA ↓</td>
</tr>
<tr>
<td>PsA (116)</td>
<td>48.4±46.4</td>
<td>Serum CRP =, ANA =, ↑ anti-dsDNA</td>
<td></td>
</tr>
<tr>
<td><strong>Thudi et al.</strong></td>
<td>SLE, ♀ (37)</td>
<td>76.6± 6.67 (range &lt;47.7, &gt;47.7)</td>
<td>Serum CRP =, ANA =, ↑ anti-dsDNA</td>
</tr>
<tr>
<td><strong>Knippenberg</strong></td>
<td>RRMS remission (23)</td>
<td>107.0 (77.0-128.0)</td>
<td>No corr. circulating B cell s, memory B cells, naive B cells, Breg</td>
</tr>
<tr>
<td>RRMS relapse (22)</td>
<td>66.5 (47.3-100.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy (30)</td>
<td>87.5 (67.5-95.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peelen et al.</strong></td>
<td>RRMS remission (32)</td>
<td>79.5 (63-107)</td>
<td>No corr. circulating IL17+CD4+T cells, trend towards lower vit D in patients with expansion Th17 cells</td>
</tr>
<tr>
<td>RRMS relapse (22)</td>
<td>64 (49-89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy (30)</td>
<td>87.5 (68-95)</td>
<td>Circulating CD39+ Treg (remission): R=−0.399 (P=0.024), not in MS relapse or healthy</td>
<td></td>
</tr>
</tbody>
</table>
### Table 25.1. Continued.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Study population</th>
<th>25(OH)D (nM) (mean or median)</th>
<th>Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (Royal et al., 2009)</td>
<td>RRMS (26)</td>
<td></td>
<td>Circulating Treg: R=0.44 (P=0.02), no corr. total naïve/memory CXCR3+ T cells or ratio</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>80.5±7.4</td>
<td>Untreated patients: circulating CXCR3+ naïve T cell R=0.49 (P=0.027), CXCR3+ naïve/memory T cell ratio R=0.61 (P=0.004)</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>73.5±6.9</td>
<td></td>
</tr>
<tr>
<td>CS (Smolders et al., 2009, 2010a)</td>
<td>RRMS (29)</td>
<td>54 (19-133)</td>
<td>No corr. circulating Th1 (R=0.34 (P=0.060))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%Th2, %IL-10+CD 4+ T cells, circulating Tregs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating Th1/Th2 ratio: R=0.44 (P=0.023)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating Treg suppressive capacity: R=-0.590 (P=0.002)</td>
</tr>
</tbody>
</table>

1 Study population in which relevant immunological outcome measure with respect to 25(OH)D levels were measured.

Abbreviations: 25(OH)D = 25-dihydroxy vitamin D; ab = antibody; AECA = anti-endothelial cell antibody; AITD = autoimmune thyroid diseases; ANA = antinuclear antibody; ASP = ankylising spondylitis; BD = Behcet’s disease; BMI = body mass index; Breg = regulatory B cell; C = complement component; CCP = cyclic citrullinated peptide; CH = cohort; CL = cardiolipin; CRP = C-reactive protein; CS = cross-sectional; DC = dendritic cell; DM = diabetes mellitus; dsDNA = double stranded DNA; htTG = human anti-tissue-transglutaminase; ICAM = intercellular adhesion molecule; IL = interleukine; MCP = monocyte chemoattractant protein; MCTD = mixed connective tissue disease; MS = multiple sclerosis; nRNP = nuclear ribonucleoprotein; RA = rheumatoid arthritis; RRMS = relapsing remitting multiple sclerosis; pERK = phospho-Erk; PL = phospholipid; PsA = psoriatic arthritis; ribo P = ribosomal P; RF = rheumatoid factor; RNP = ribonucleoprotein; SLE = systemic lupus erythematosus; Sm = Smith antigen; Th = T-helper; TLR = Toll-like receptor; TNFR = tumour necrosis factor-a receptor; Treg = regulatory T cell; ♂ = male; ♀ = female.
Table 25.2. Vitamin D supplementation studies and immunological outcome measures in peripheral blood in health and auto-immune disease; the effect on the outcome measures is given as ↑: increased; ↓: decreased; =: not affected.

| Study | Study design (duration) | Study population (n) + vitamin Dose (with weekly equivalents) | Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation) | Immunological outcome measures
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>OL (5 wks) (Adams et al., 2009)</td>
<td>Healthy low BMD &amp; 25(OH)D (7) 2 times 50,000 IU/w (100,000)</td>
<td>61.7±10.2 to 100.6±24.2</td>
<td>Circulating monocyte hCAP mRNA ↑</td>
</tr>
<tr>
<td></td>
<td>OL (1.5 wks) (Allen et al., 2012)</td>
<td>Healthy (4) D3 5,000 IU/d for 10 wks followed by 5,000/10,000 for 5 wks</td>
<td>38 (27-53) to 179.5</td>
<td>Supernatants IL-10 ↑ (PBMC+PPD/TT/DT/PHA) ↓ IL-17+ CD4+ T cells, = IL-10+ CD4+ T cells, = IL-10+ CD8+ T cells (PBMC+PHA) Supernatants IL-10 ↑ (total PBMC &amp; CD4+ depleted PBMC), IL-10= (CD4+PBMC); IL-17 ↓ (total PBMC &amp; CD4+ PBMC), IL-17= (CD4+ depleted PBMC) (sorted PBMC+PHA)</td>
</tr>
<tr>
<td></td>
<td>RCT, PC (22 wks) (Barnes et al., 2011)</td>
<td>Healthy 20-40 yrs: Control (56) D3 (47+55+53) 200, 400, 600 IU/d (1,400, 2,800, 4,200)</td>
<td>↑ 25(OH)D only for 400 or 600 IU/d</td>
<td>Serum IL-6 =, IL-10 =, TNF-α =, TGF-β =.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Healthy &gt;64 yrs: Control (54) D3 (48+52+48) 200, 400, 600 IU/d (1,400, 2,800, 4,200)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RCT, PC (3 mts) (Bock et al., 2011)</td>
<td>Healthy: Control (29) D3 (30) 140,000 IU/mt (35,000)</td>
<td></td>
<td>Circulating Treg ↑.</td>
</tr>
</tbody>
</table>
### Table 25.2. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design (duration)</th>
<th>Study population (n) + vitamin&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Dose (with weekly equivalents)</th>
<th>Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)</th>
<th>Immunological outcome measures&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT, PC (10 wks)</strong>&lt;br&gt; (Heine et al., 2011)</td>
<td>Healthy: Control (12)</td>
<td>25(OH)D&lt;sup&gt;3&lt;/sup&gt; (20) 2,000 IU/d (14,000)</td>
<td>28.4 to 80.3</td>
<td>Circulating total blood count =, monocytes =&lt;br&gt;Circulating B cell subsets =##</td>
<td>Serum IgA =, IgG =, IgM =, TT-specific IgA =, TT-specific IgG =&lt;br&gt;IgG &amp; IgA =##, TT-specific plasmablasts =, plasmablasts =##&lt;br&gt;Circulating TT-specific antibody secreting plasmablasts</td>
</tr>
</tbody>
</table>

| **RCT, PC (1 yr)**<br> (Jorde et al., 2011) | Obese: Control (105) | Baseline values not shown | Serum CRP =, IL-2 =, IL-4 =, IL-5 =, IL-10 =, IL-12 =, IL-13 =,<br>IL-17A =, IFN-γ =, ICAM-1 =, MCP-1 = [# and ##]. |## |<br>Supematants IL-2 =, IL-4 =, IL-5 =, IL-10 =, TNF-α =, IFN-γ =<br>(PBMC+TT/SEB antigen)##, ##. |

| **RCT, PC (6 wks)**<br> (Martineau et al., 2007) | Healthy TB contacts: Control (64) | 35.2 to 67.4 | Supernatants IFN-γ = (PBMC+TB antigens)##<br>In vitro anti-mycobacterial potential ↑ at 24h, = at 96h (whole blood+BCG) [# and ##]. |## |<br>Supematants IL-2 =, IL-4 =, IL-5 =, IL-10 =, TNF-α =, IFN-γ =<br>(PBMC+TT/SEB antigen)##, ##. |

| **RCT, PC (3 yrs)**<br> (Pittas et al., 2007) | Healthy ≥ 65 yrs: Control (161) | 76 (baseline) to? | Serum CRP =, IL-6 =##. |## |<br>Supematants IFN-γ = (PBMC+TB antigens)##<br>In vitro anti-mycobacterial potential ↑ at 24h, = at 96h (whole blood+BCG) [# and ##]. |

---

<sup>1</sup> D3 stands for 25-hydroxyvitamin D. 
<sup>2</sup> TT-specific refers to tuberculosis-specific. 
<sup>3</sup> The study indicates significant changes. 
<sup>4</sup> The study highlights significant and notable changes. 
<sup>5</sup> The study demonstrates a significant trend, with ++ indicating a stronger effect. 
<sup>6</sup> The study shows a notable increase, with ## highlighting a significant result.
<table>
<thead>
<tr>
<th>Study design (duration)</th>
<th>Study population (n) + vitamin(^1)</th>
<th>Dose (with weekly equivalents)</th>
<th>Study Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>OL (4 wks) (Prietl et al., 2010)</td>
<td>Healthy (46) D3</td>
<td>140,000 IU/2 wks (70,000)</td>
<td>Baseline: 59.7±32.2 wk4: 114.6±34.9 wk8: 144.8±37.7</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCT, PC (1 yr) (Bendix-Struve et al., 2010)</td>
<td>Crohn’s disease: Control (10)</td>
<td>61 (22-105) to 46 (27-97) (Δ-5 (67-33))</td>
<td>Circulating cell type distribution =</td>
</tr>
<tr>
<td></td>
<td>D3 (10)</td>
<td>1,200 IU/d (8,400)</td>
<td>In vitro proliferation CD4(^+) T cells ↑ (anti-CD3/CD28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Supernatants IL-6 ↑(##)/=, IL-4 ↑/=, IL-10 =, TNF-α =, IFN-γ = (monocyte-depleted PBMC+anti-CD3/CD28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating Treg =</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum CRP =, circulating Treg ↑. 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating Treg =</td>
</tr>
<tr>
<td>RCT, OL (52 wks) (Burton et al., 2010; Kimball et al., 2011)</td>
<td>RRMS/SPMS: Control (24)</td>
<td>83 (38-154) to 83±27</td>
<td>Serum CRP =, MMP-9 - [not ##], TIMP-1 - [not ##],</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OPN=, BAP=, Kallikrein 6=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum/ supernatant (PBMC+antigens) IL-1β =, IL-2 =, IL-4 =,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-5 =, IL-10 =, IL-12p40 =, IL-13 =, TNF-α =, IFN-γ = [## and ##]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In vitro proliferation PBMC (antigens: PHA =, TT=, CS=,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BLG=, BSA ̶, BSAp193 ̶, BSAp147 =, Tep96 =, GAD =,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GADp555 =, PI =, MBP =, EX-2 =, GFAP ̶, S100β ̶). 3, 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum TGF-b1 ↑ [## and ##]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating PBMC mRNA TNF-α =, IFN-γ =, IL-13 =,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-2 ̶/=</td>
</tr>
<tr>
<td>RCT, PC (6 mts) (Mahon et al., 2003)</td>
<td>MS: Control (22)</td>
<td>105.8±37.4 to 174.5±49.9</td>
<td>Serum TGF-β1 ↑ [## and ##]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circulating PBMC mRNA TNF-α =, IFN-γ =, IL-13 =,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-2 ̶/=</td>
</tr>
<tr>
<td>RCT, PC (6 mts) (Mosayebi et al., 2011)</td>
<td>RRMS: Control (33)</td>
<td>±25 to ±25</td>
<td>Supernatant IFN-γ =, TGF-β ̶, IL-10 ̶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ proliferative responses. 9</td>
</tr>
</tbody>
</table>

\(\Delta 25(OH)D\) (nM) (mean or median, pre- to post-supplementation) **Immunological outcome measures**

Table 25.2. Continued.
<table>
<thead>
<tr>
<th>Study design (duration)</th>
<th>Study population (n) + vitamin&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Dose (with weekly equivalents)</th>
<th>Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)</th>
<th>Immunological outcome measures&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>OL (12 wks)</td>
<td>RRMS (15) D3</td>
<td>20,000 IU/d (140,000)</td>
<td>50 (13-175) to 380 (151-535)</td>
<td>Circulating Treg =, Treg suppressive capacity =, IL10&lt;sup&gt;+&lt;/sup&gt;CD4&lt;sup&gt;+&lt;/sup&gt; T cells = , IFN&lt;sub&gt;γ&lt;/sub&gt;/IL4&lt;sup&gt;+&lt;/sup&gt; CD4&lt;sup&gt;+&lt;/sup&gt; T cells =</td>
</tr>
<tr>
<td></td>
<td>(Knippenberg et al., 2011; Smolders et al., 2010b, 2011)</td>
<td></td>
<td></td>
<td>Circulating B cells =, B cell subsets =, Serum IgM =, IgG &amp; IgA =, BAFF =, OPN =.</td>
</tr>
</tbody>
</table>

<sup>1</sup> Study population in which relevant immunological outcome measures with respect to 25(OH)D levels were measured.

<sup>2</sup> The effect on the outcome measures is given as ↑: increased; ↓: decreased; =: not affected; # pre- vs. post-supplementation in intervention group, unless otherwise stated; ## vitamin D supplementation vs. placebo.

<sup>3</sup> No adverse effects.

<sup>4</sup> = adverse events.

<sup>5</sup> No hypercalcemia or other adverse events.

<sup>6</sup> Well-tolerated.

<sup>7</sup> One mild asymptomatic hypocalcaemia.

<sup>8</sup> Annualized relapse rate =.

<sup>9</sup> No difference in EDSS nor in mean nr. of Gd-enhancing lesions.

Abbreviations: 25(OH)D = 25-dihydroxy vitamin D; BAP = bone-specific alkaline phosphatase; BAFF = B-cell activating factor; BCG = bycobacterium bovis bacille Calmette-Guérin; BLG = β-lactoglobulin; BMD = bone mineral density; BSA = bovine serum albumin; CRP = C-reactive protein; CS = casein; CTx = C-telopeptide; DT = diphtheria toxoid; EX-2 = exon 2 of MBP; GAD = glutamic acid decarboxylase; GFAP = glial fibrillary acidic protein; hCAP = human cathelicidin antimicrobial peptide; IFN = interferon; IL = interleukine; IM = intra muscular; MBP = myelin basic protein; MMP = matrix metalloproteinase; MS = multiple sclerosis; OL = open label; OPN = osteopontin; PHA = phytohemagglutinin; PC = placebo; PI = proinsulin; PPD = purified protein derivative; RCT = randomized controlled trial; RRMS = relapsing remitting multiple sclerosis; SEB = staphylococcal enterotoxin B; SI = stimulation index (MTT assay); TB = tuberculosis; Tep = transendothelial protrusion; TGF = tumor growth factor; Th = T-helper; TIMP = tissue inhibitor of metalloproteinase; TLR = toll-like receptor; TNF = tumor necrosis factor; Treg = regulatory T cell; TT = tetanus toxoid; Δ = difference.
no significant differences in serum IL-1β (Kimball et al., 2011), IL-2 (Jorde et al., 2010; Kimball et al., 2011), IL-4 (Jorde et al., 2010; Kimball et al., 2011), IL-5 (Jorde et al., 2010; Kimball et al., 2011), IL-6 (Barnes et al., 2011; Pittas et al., 2007), IL-10 (Barnes et al., 2011; Jorde et al., 2010; Kimball et al., 2011), IL-12 (Jorde et al., 2010; Kimball et al., 2011), IL-13 (Jorde et al., 2010; Kimball et al., 2011), IL-17 (Jorde et al., 2010), IFN-γ (Jorde et al., 2010; Kimball et al., 2011), TGF-β (Barnes et al., 2011), TNF-α (Barnes et al., 2011; Kimball et al., 2011) sICAM-1 (Jorde et al., 2010), MCP-1 (Jorde et al., 2010), C-telopeptide (Kimball et al., 2011), bone-specific alkaline phosphatase (Kimball et al., 2011), kallikrein 6 (Kimball et al., 2011), osteopontin (Kimball et al., 2011; Smolders et al., 2011) or B-cell activating factor (Knippenberg et al., 2011). Despite these negative findings, some studies reported correlations between vitamin D status and soluble molecules in serum. Higher serum levels of 25(OH)D have been reported to coincide with lower serum concentrations of TNF-α in healthy females (Peterson and Heffernan, 2008). In cord blood of newborns, serum 25(OH)D levels correlated positively with levels of IL-10 (Zittermann et al., 2004). This correlation was also observed in females with MCTD (Hajas et al., 2011) and active BD patients (Hamzaoui et al., 2010). Supplementation of vitamin D in patients with MS has been reported to induce increased serum levels of TGF-β (Mahon et al., 2003). Conversely, in otherwise healthy obese females, a negative correlation of serum 25(OH)D with serum IL-5 and IL-10 was reported (Jorde et al., 2010). A negative correlation with IL-6 and IL-23 has been reported in females with MCTD and healthy controls (Hajas et al., 2011; Shea et al., 2008). A long distance holiday induced both higher serum levels of 25(OH)D, sCD14, and sIL-2R (Falkenbach and Sedlmeyer, 1997). MCP-1 was positively correlated to 25(OH)D levels in obese females, but not in males (Jorde et al., 2010). Lastly, in MS patients, lower circulating concentrations of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 were reported after supplementation of vitamin D (Kimball et al., 2011).

25.6.3 Cytokine excretion by PBMC in supernatants

Of course, the cellular sources of cytokines detected in serum are uncertain. To identify cells within the PBMC fraction producing these cytokines, several groups analyzed the transcription profile of cytokines in PBMC directly ex vivo. Supplementation of vitamin D in healthy subjects increased expression of cathelicidin mRNA in monocytes (Adams et al., 2009), but did not differentially affect expression of TNF-α, IFN-γ, IL-2, or IL-13 mRNA in patients with MS (Mahon et al., 2003).

Additionally, several studies measured cytokine excretion in supernatants of specific cell cultures. Most studies cultured PBMC stimulated either with TLR-ligands (Khoo et al., 2011) or with polyclonal (Bendix-Struve et al., 2010) or antigen specific T cell stimuli (Heine et al., 2011; Kimball et al., 2011; Martineau et al., 2007). A higher vitamin D status correlated with a reduced IL-1β and TNF-α production upon TLR2 and TLR4 triggering, while a loss of IL-6, IL-10 and IFN-γ was only found with TLR4 triggering (Khoo et al., 2011). Upon T-cell stimuli, supernatant levels of IL-1β (Kimball et al., 2011), IL-2 (Heine et al., 2011; Kimball et al., 2011), IL-4 (Heine et al., 2011; Kimball et al., 2011), IL-5 (Heine et al., 2011; Kimball et al., 2011), IL-10 (Bendix-Struve et al., 2010; Heine et al., 2011; Kimball et al., 2011), IL-12 (Kimball et al., 2011), IL-13 (Kimball et
al., 2011), IFN-γ (Bendix-Struve et al., 2010; Heine et al., 2011; Kimball et al., 2011; Martineau et al., 2007; Mosayebi et al., 2011) and TNF-α (Bendix-Struve et al., 2010; Heine et al., 2011; Kimball et al., 2011), were not affected by supplementation of vitamin D, while TGF-β (Mosayebi et al., 2011) and IL-10 increased (Allen et al., 2012; Mosayebi et al., 2011) and IL-17 decreased (Allen et al., 2012). The positive effect on IL-10 was mainly due to an increased production of IL-10 by the CD4+ depleted PBMCs, while this cell population did not produce IL-17 (Allen et al., 2012).

A study on polyclonal stimulation of monocyte-depleted PBMC of patients with Crohn's disease reported a trend towards an increased concentration of IL-4 and IL-6 in the supernatants after supplementation (Bendix-Struve et al., 2010).

25.6.4 Proliferation of T cells in vitro

Another way to measure T cell responses to antigens, is to measure induction of proliferation in vitro. A study in Crohn's disease patients reported an increase of polyclonal T cell proliferation after supplementation of vitamin D (Bendix-Struve et al., 2010), while in MS patients proliferation was decreased (Mosayebi et al., 2011) or unaffected (Smolders et al., 2010a). Kimball et al. (2011) tested proliferative responses of T cells against a large panel of antigens in MS patients. After supplementation of vitamin D, proliferative responses against BSA, BSAp193, proinsulin, MBP and exon 2 of MBP were reduced compared with prior to supplementation.

25.6.5 Phenotyping PBMC directly ex vivo

Several studies analyzed circulating myeloid and lymphocyte subsets and phenotypes with flowcytometry. Two studies reported a negative correlation between serum 25(OH)D levels and monocyte expression levels of both TLR-2 (Do et al., 2008; Khoo et al., 2011) and TLR-4 (Do et al., 2008; Khoo et al., 2011). There was neither a correlation with the circulating proportions of myeloid and plasmacytoid DCs, nor with the expression of human leukocyte antigen-DR, CD40, or CD86 on these cells (Ben-Zvi et al., 2010). There was also no correlation with the proportion of CD14+CD16+ monocytes (Do et al., 2008). Vitamin D supplementation did not affect the total proportion of circulating monocytes (Heine et al., 2011).

Regarding lymphocytes, both B and T cell subsets have been characterized. A correlation was described of 25(OH)D levels with the percentages of total naïve (CXCR3+) and total memory (CXCR4+) CD4+ T cells in an MS cohort not treated with immune modulating therapies (Royal et al., 2009). In patients with BD, a positive correlation between 25(OH)D and total proportions of Treg was reported (Hamzaoui et al., 2010). Such a correlation was not confirmed in patients with MS (Smolders et al., 2010a), and was even contradicted by a negative correlation in another MS population (Royal et al., 2009). When assessing subsets of Tregs, lower circulating proportions of CD39+ Treg correlated with higher serum 25(OH)D levels in MS patients during disease remission (Peelen et al., 2011a). Additionally, the capacity of Treg to control polyclonal T cell proliferation was also improved in MS patients with higher serum 25(OH)D levels (Smolders et al., 2009). Supplementation of vitamin D has been reported to induce higher circulating proportions of Treg in healthy individuals (Bock et al., 2011; Prietl et al., 2010). Contrastingly, supplementation of
vitamin D did not result in higher proportions of naïve/ memory Treg in subjects with MS and Crohn’s disease (Bendix-Struve et al., 2010; Smolders et al., 2010b), and Treg suppressive capacity was also not significantly affected (Smolders et al., 2010b). Regarding T helper cell subsets, a study in BD patients reported negative correlations between serum 25(OH)D and the circulating proportion of Th1 and Th17 cells, and with the Th1/Th2-ratio (Hamzaoui et al., 2010). Although a study in MS patients could not detect correlations with the proportions of T helper cell subsets, the Th1/Th2 ratio correlated also negatively with serum 25(OH)D (Smolders et al., 2009). In a cross-sectional study among MS patients, serum 25(OH)D levels tended to be lower in patients with an expanded Th17 compartment (Peelen et al., 2011a). Supplementation of vitamin D in healthy subjects resulted in a decline of Th17 cells, while the number of IL-10+ T cells did not change (Allen et al., 2012). Supplementation of vitamin D in subjects with MS resulted in a decreased Th1/Th2-ratio, as well as an expanded proportion of IL-10+ T cells (Smolders et al., 2010b).

With respect to B cells, a study in SLE reported decreased B cell activation in subjects with high 25(OH)D levels (Ritterhouse et al., 2011). However, in subjects with MS, no correlation of serum 25(OH)D with circulating B cell subsets, including regulatory B cells, could be found (Knippenberg et al., 2011). Accordingly, supplementation of vitamin D did not affect B cell subset distribution in both subjects with MS and healthy control donors (Heine et al., 2011; Knippenberg et al., 2011). B cells are precursors to Ig-producing plasma cells. A study in SLE and in healthy individuals on long distance holiday reported lower total IgG concentrations in serum with increasing 25(OH)D level (Falkenbach and Sedlmeyer, 1997; Szodoray et al., 2011), which might be in line with the reduced B cell activation observed in the cross-sectional study (Ritterhouse et al., 2011). IgE production seems to be affected negatively by 25(OH)D (Zittermann et al., 2004), while IgA production was increased and IgM production was not correlated (Falkenbach and Sedlmeyer, 1997). On the other hand, other studies found no effect of vitamin D supplementation on total serum polyclonal IgG, IgM and IgA in healthy subjects and subjects with MS (Heine et al., 2011; Knippenberg et al., 2011). However, antigen specific antibody-production may be more relevant than the polyclonal compartment. Indeed, supplementation of vitamin D improved tetanus toxoid-specific IgG-response in tetanus toxoid vaccination (Heine et al., 2011). In several SLE cohorts contrasting results have been found regarding antinuclear antibody and anti-double stranded DNA antibody production (Bonakdar et al., 2011; Ritterhouse et al., 2011; Szodoray et al., 2011; Thudi et al., 2008). A negative correlation for serum 25(OH)D with antithyroid antibodies in thyroid disease patients (Kivity et al., 2011), with serum anti-human tissue transglutaminase IgA in ankylosing spondylitis and psoriatic arthritis patients (Teichmann et al., 2010) and a trend towards such a correlation with anti-SS-A-antibodies in SLE (Ritterhouse et al., 2011) have been reported, while there was a positive correlation with serum anti-Smith antibodies (Szodoray et al., 2011). In donors at risk for rheumatoid arthritis, there was no correlation of 25(OH)D with either rheumatoid factor or anti-cyclic citrullinated peptide antibodies (Feser et al., 2009). In patients with MCTD, serum anti-U1-ribonucleoprotein-antibody correlated negatively and serum anti-CL IgA positively with serum 25(OH)D, while no correlation with anti-CL IgG/IgM and anti-endothelial cell antibodies was found (Hajas et al., 2011).
25.7 Discussion and conclusion

Our literature search revealed evidently that studies done so far are heterogeneous in many aspects. Study populations range from healthy controls to at risk persons and patients with distinct auto-immune diseases. Moreover, most studies seem to be underpowered and have a lack of appropriate controls. Beside this, 25(OH)D status is not always properly reported. This is regrettable, since an effect of vitamin D on the immune system would be expected in the highest or higher than physiological ranges of 25(OH)D levels (Baeke et al., 2010). Without baseline 25(OH)D levels, it is difficult to discriminate between the effects of vitamin D in individuals with sufficient versus insufficient vitamin D status. Besides this, 25(OH)D assays have a great interassay variability (Lai et al., 2012) and cut-off points used for defining 25(OH)D insufficiency and sufficiency are not standardized. Furthermore, correlation studies have the potential bias of reverse causality: vitamin D status might not be responsible for the possible improvement in clinical and immunological outcomes in disease, but disability itself might influence the vitamin D status of individuals. Supplementation studies are not subjected to reverse causality, however, these also show great diversity in the supplemented compound (either vitamin D2 or vitamin D3) and in the dose and duration of supplementation. The supplemented doses range from doses which do not affect serum 25(OH)D levels significantly to doses which lead to supra-physiological serum 25(OH)D levels. These factors make the individual studies hardly comparable, and readily explains the many contradictive results. Finally, it is also important to keep a publication bias in consideration. In general, data suggest a trend towards a promotion of anti-inflammatory/regulatory cytokines and cells to be present, but this is certainly not substantiated by conclusive data.

On top of that, analyses of the studies thus far showed a diverse repertoire of immunological outcome measures. These outcome measures form a continuum, ranging from soluble markers of general inflammation which can be measured easily in large cohorts of study subjects, to labour-intensive, detailed assessment of specific cellular processes. We argue that the choice between either of these outcome measures should primarily be driven by the specific disease outcome that is to be studied. As described above, 1,25(OH)₂D interferes in vitro with proliferation and cytokine secretion by activated PBMC. These in vitro models reflect most likely the sites in the body where antigen presentation and immune activation take place, which are the secondary lymphoid organs and the tissues targeted by auto-immune diseases. In these tissues, the interaction of vitamin D with the immune response is unlikely to depend on either a single cell type or cytokine, but rather on a complex interplay between multiple vitamin D sensitive cells of the innate and adaptive immune system (Peelen et al., 2011b). When analyzing correlations between serum 25(OH)D levels and immunological parameters in the blood, one has to realize that there is a mismatch in space and time related to in vitro experiments. Immune cells in the circulation are on the move between target sites and lymphoid tissue, and soluble factors are derived from both specific immune responses of interest as well as from other cells. Most informative would be assessment of cells and cytokines in lymph nodes and target organs, but sampling of material is obviously not feasible in most diseases. Another issue to keep in mind is that effects of vitamin D on immune parameters, as described in this chapter, might be secondary to other effects of
vitamin D. For instance, the positive correlation between 25(OH)D and C4 in SLE might be due to an immune independent effect on disease activity; since SLE is an immune complex mediated disease associated with complement activation via the classical pathway, any reduction in disease activity will result in normalization of C4 levels. Similarly, the negative association of 25(OH)D levels with several auto-antibodies might not be due to a direct effect of vitamin D on B cells, but might be mediated via an effect on auto-antigen presentation by APCs. In addition, many auto-immune diseases are associated with infections (Kivity et al., 2009) causing inflammation and subsequent induction of auto-immune disease manifestations. Since vitamin D also affects the innate immune system via the production of beta-defensins and cathelicidins, this will reduce the infectious load and thereby the inflammatory response and eventually will affect the adaptive immune system. To substantiate an immune modulating effect of vitamin D in vivo, the best approach appears to be assessment of the endproducts of the immunological cascade in the circulation, which are most relevant to the disease or health outcome studied. For auto-immune diseases, analysis of auto-antibody titers or lymphocyte cell proliferative and cytokine responses against auto-antigens are more logical choices than assessment of polyclonal IgG levels, polyclonal T cell proliferation, or serum cytokine levels. In MS, where no single auto-antigen is present, studies in which immunological outcomes have been associated with disease in patient-control studies, or have been associated with exacerbation activity or remission of disease, seem most relevant. Ultimately, only well-powered, controlled studies in well defined cohorts measuring disease-specific relevant outcome measures will be most informative on a immune modulating potential of vitamin D.

Currently, several clinical trials are investigating the efficacy of vitamin D supplementation in MS and other auto-immune diseases. These trials provide an excellent framework to design potentially conclusive studies on the immune-modulating effects of vitamin D in vivo. It would be a missed chance not to address this issue in these studies, especially since insight in the working mechanism of vitamin D may reveal subgroups of patients which may benefit most from vitamin D supplementation. Additionally, a known working mechanism can facilitate hypothesis driven combination with existing treatment regimens, and may reveal a biomarker which allows titration of serum 25(OH)D levels to an adequate dose.

References


25. Monitoring in vivo immune modulation by vitamin D in MS


Falkenbach, A. and Sedlmeyer, A., 1997. Travel to sunny countries is associated with changes in immunological parameters. Photodermatology, Photoimmunology and Photomedicine 13, 139-142.


25. Monitoring in vivo immune modulation by vitamin D in MS


25. Monitoring in vivo immune modulation by vitamin D in MS


A.-H. Muris, J. Damoiseaux and J. Smolders


Zittermann, A., Dembinski, J. and Stehle, P., 2004. Low vitamin D status is associated with low cord blood levels of the immunosuppressive cytokine interleukin-10. Pediatric Allergy and Immunology 15, 242-246.
Vitamin D
in chronic diseases
Key facts

- Insulin resistance often presents with hyperglycemia despite elevated serum levels of insulin.
- Prolonged hypersecretion of insulin, as seen in insulin-resistant states, may lead to loss of pancreatic β-cell function and the eventual development of type 2 diabetes.
- Skeletal muscle is the primary site of glucose disposal, and therefore plays a key role in regulating whole-body glucose homeostasis.
- 25(OH)D 1α-hydroxylase is the enzyme responsible for converting 25-hydroxyvitamin D (25(OH)D), the precursor to the biologically active form, to 1α,25(OH)2D, the hormonally active form of vitamin D.
- 1α,25(OH)2D3, the active form of vitamin D, induces genomic responses through interaction with the nuclear vitamin D receptor, as well as non-transcriptional responses involving activation of transmembrane signal transduction pathways via a putative novel membrane receptor.

Summary points

- Epidemiological studies suggest that low serum 25(OH)D is associated with reduced insulin sensitivity.
- Results from clinical trials and animal studies support that vitamin D intervention may improve insulin resistance.
- Vitamin D may influence insulin sensitivity through its ability to enhance muscle mass and function.
- Expression of the 25(OH)D 1α-hydroxylase enzyme in extra-renal tissues relevant to insulin sensitivity such as muscle, adipose tissue and pancreas may allow for local conversion of 25(OH)D to 1α,25(OH)2D, leading to autocrine/paracrine responses.
- There is evidence to suggest that 1α,25-dihydroxyvitamin D (1α,25(OH)2D) regulates insulin sensitivity at key points in the insulin signaling pathway including increased protein expression of the insulin receptor and tethering and fusion of the GLUT4 glucose transporters to the cell membrane, thereby enhancing insulin signaling and facilitating glucose uptake.
- Parathyroid hormone (PTH) and 1α,25(OH)2D are major regulators of serum calcium. Elevated serum parathyroid hormone is associated with impaired insulin sensitivity, and high serum 25(OH)D may enhance insulin sensitivity through its ability to lower serum PTH.
- Increased adiponectin and osteocalcin are associated with improved insulin sensitivity and secretion, and studies suggest a positive relationship between these and serum 25(OH)D.
- Vitamin D is essential for insulin synthesis and secretion in pancreatic β-cells.
26. Vitamin D and insulin sensitivity

B. Larrick, S. Donkin and D. Teegarden
Interdepartmental Nutrition Program, Purdue University, West Lafayette, IN 47907, USA; teegarden@purdue.edu

Abstract

Insulin resistance is characterized by an insufficient response of the body to insulin secretion, and is associated with a cluster of metabolic disorders that are collectively referred to as the insulin resistance syndrome. Features associated with insulin resistance include glucose intolerance, hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and hypertension, among others, and together these increase the likelihood of developing type 2 diabetes. Evidence from epidemiological, clinical, and tissue- and cell-based studies suggest a role of vitamin D in the prevention of insulin resistance. Serum levels of 25-hydroxyvitamin D, an indicator of vitamin D status, have been associated with improved glucose homeostasis, and data from clinical intervention studies support that treatment with 1α,25-dihydroxyvitamin D, the hormonally active form of vitamin D, may improve insulin sensitivity. Investigations of the mechanisms by which vitamin D improves insulin sensitivity suggest that vitamin D may have direct effects on insulin synthesis and secretion, as well as insulin-sensitizing effects on target tissues. Defining the mechanisms by which vitamin D may improve insulin sensitivity and glucose homeostasis is critical to understanding how vitamin D may be effective in preventing the development of insulin resistance, and its progression to type 2 diabetes.

Keywords: vitamin D, 1α,25(OH)2D, insulin resistance, homeostatic model assessment
26.1 Vitamin D metabolism

Vitamin D3 (cholecalciferol) is a biologically inert prohormone made endogenously from 7-dehydrocholesterol in the skin upon exposure to ultraviolet light. To become an active hormone, vitamin D3 is metabolized first in the liver to its major circulating form 25(OH)D, which circulates bound to serum vitamin D binding protein. In the kidney 25(OH)D is converted to its hormonal form, 1α,25(OH)₂D, which functions through the nuclear VDR (DeLuca, 2004). Formation of 25(OH)D by hepatic vitamin D-25-hydroxylase (CYP27) appears to be only loosely regulated, and is used as an indicator of vitamin D status. However, conversion of 25(OH)D to 1α,25(OH)₂D by renal 1α-hydroxylase (CYP1α) is under tight regulation, influenced by serum PTH, 1α,25(OH)₂D, and calcium levels (Jones et al., 1998). Degradation of 1α,25(OH)₂D is induced via a negative-feedback mechanism, initiated by 24-hydroxylation of 1α,25(OH)₂D by 24-hydroxylase (CYP24). 24-Hydroxylation results in the metabolism of 1α,25(OH)₂D to calcitriol acid, which is eliminated primarily through the bile (DeLuca, 2008) (Figure 26.1).

Though the activity of renal 1α-hydroxylase is tightly controlled by PTH, 1α-hydroxylase enzymes have been identified in several extra-renal tissues, including skin, colon, lung, bone, prostate, intestine, pancreatic islets, vasculature, liver, brain, muscle and adipose tissue, among others, which may allow for local production of 1α,25(OH)₂D (Hewison et al., 2007; Li et al., 2008;
26. Vitamin D and insulin sensitivity

Zehnder et al., 2001). Additionally, these extra-renal 1α-hydroxylase enzymes do not appear to be regulated by PTH (Flanagan et al., 2003; Young et al., 2004), suggesting that elevated 25(OH)D status may lead to local production of 1α,25(OH)2D independent of calcium status, potentially yielding autocrine/paracrine responses such as acute improvements in insulin resistance.

1α,25(OH)2D induces genomic responses through interaction with the nuclear VDR, as well as non-transcriptional responses involving activation of transmembrane signal transduction pathways via a putative novel membrane receptor, including activation of the adenylyl cyclase-cAMP-protein kinase A and phospholipase C-diacylglycerol (DAG)-inositol trisphosphate-PKC signal transduction pathways and the Ca2+ messenger system (Boland, 2011). 1α,25(OH)2D is involved in biological processes such as cell proliferation, differentiation, apoptosis, and muscle contraction (Boland et al., 2005), and an emerging body of evidence also suggests the involvement of 1α,25(OH)2D in the improvement of insulin sensitivity, which will be the focus of this chapter.

26.2 Insulin resistance

Insulin resistance is characterized by a subnormal response of the body to insulin, and is an early predictor of type 2 diabetes. Under normal conditions, circulating levels of blood glucose
are under tight homeostatic regulation. When blood glucose levels rise in response to dietary glucose, insulin secretion is stimulated in pancreatic β-cells to promote peripheral glucose uptake. Conversely, when concentrations of blood glucose are too low, glucagon secretion from pancreatic α-cells is stimulated to maintain normoglycemia (Erkelens, 2001; Muniyappa et al., 2008).

Initiation of the insulin signaling cascade begins when insulin binds to the insulin receptor (Taniguchi et al., 2006). The insulin receptor belongs to the family of receptor tyrosine kinases, which, in addition to the insulin receptor, includes insulin-like growth factor-1, vascular endothelial growth factor, fibroblast growth factor, and epidermal growth factor (Fantl et al., 1993; Ullrich and Schlessinger, 1990). The insulin receptor consists of two extracellular α-subunits, each of which are linked to one of two intracellular β-subunits having tyrosine kinase domains. In insulin-responsive tissues, binding of insulin to the α-subunit of the insulin receptor induces allosteric interactions between the two receptor dimers. This allows for increased tyrosine kinase activity of the insulin receptor β-subunits, causing autophosphorylation of tyrosine residues in the insulin receptor, and subsequent phosphorylation of IRS-1 and IRS-2 (Taniguchi et al., 2006).

Both IRS-1 and IRS-2 have phosphotyrosine-binding and pleckstrin-homology domains, and serve as docking platforms for other signaling proteins that have SH2 domains (Taniguchi et al., 2006). These phosphotyrosine- and pleckstrin-homology binding domains have an affinity for phosphatidylinositol lipids and proteins such as PKC, and are highly involved in cell signal transduction pathways (White, 2002). At least twenty tyrosine residues have been identified as insulin-stimulated phosphorylation sites, including Tyr-460, -608, -628, -895, -939, -987, -1172, and -1222 (numbers correspond to rat IRS-1 sequence) (Sun et al., 1993). Following phosphorylation of several tyrosine residues within the IRS proteins the intracellular signaling molecule PI3K, which has a SH2 domain and can associate with IRS-1, is activated. This results in the subsequent generation of the second messenger phosphatidylinositol-3,4,5-trisphosphate which binds phosphoinositide-dependent protein kinase 1, resulting in the downstream activation of PKB/Akt and PKC (Bomfeldt and Tabas, 2011; Henriksen et al., 2011; Schwanstecher and Schwanstecher, 2011; Taniguchi et al., 2006; White, 2002).

This signaling event results in glucose uptake via GLUT4 glucose transporters in both skeletal muscle and adipose tissue, however the effects of this insulin-stimulated signaling event differ between muscle and adipose tissue. In adipocytes, in a non-insulin-stimulated state intracellular GLUT4 vesicles travel along a microtubule network covering the entire plasma membrane, to which the vesicles periodically tether in a loose, temporary fashion. In response to insulin, GLUT4 vesicles tether tightly and fuse to the plasma membrane, increasing the concentration of membrane GLUT4 transporters and allowing for glucose uptake (Lizunov et al., 2005). In muscle, the major site of glucose disposal, insulin stimulates local fusion of GLUT4 vesicles that are already 'pre-tethered' to the sarcolemma, or muscle cell membrane, allowing for glucose uptake, rather than stimulating the recruitment of intracellular GLUT4 vesicles (Lizunov et al., 2012).
Systemic actions of insulin include inhibition of hepatic gluconeogenesis and glycogenolysis, inhibition of FFA release from adipose tissue, and stimulation of fatty acid, protein, and triglyceride biosynthesis (Brown and Goldstein, 2008; Muniyappa et al., 2008; Reaven, 2005a; Schwanstecher and Schwanstecher, 2011) and are mediated through the signaling molecules described above. Insulin binding to its receptor also activates other signaling pathways including mitogen-activated protein kinase, which is involved in mitogenesis, survival, and cell differentiation (Avruch, 1998; Taniguchi et al., 2006). There are several points in the insulin signaling cascade at which disruption in signal transduction may occur in insulin resistance, such as negative regulation of the insulin receptor, of the IRS proteins, and of PKB/AKT kinases. Negative regulators of the insulin receptor include receptor serine phosphorylation and protein tyrosine phosphatases, which dephosphorylate critical tyrosine residues thereby reducing the receptor’s activity. Additionally, suppressor of cytokine signaling proteins sterically hinder the interaction between the insulin receptor and IRS proteins, and ligand-induced internalization and degradation of the insulin receptor both contribute to impaired insulin sensitivity. Sustained inhibition of IRS proteins may occur via ubiquitin-targeted proteosome-mediated degradation (White, 2002), and IRS proteins may undergo serine phosphorylation at Ser-24, -267, -302, -307, -318, -332, -357, -408, -522, -612, -632, -662, -731, -789, and -1099/1100 (Weigert et al., 2008) in response to insulin, free fatty acids, and cytokines resulting in short-term inhibition of insulin-induced cell signaling (White, 2002). Hyperinsulinemic states may also interfere with insulin signaling by reducing expression of IRS-1 (Schwanstecher and Schwanstecher, 2011; Taniguchi et al., 2006).

Disruptions in insulin receptor signaling, or chronic activation of insulin receptor pathways in response to elevated FFA, glucose, insulin, cytokines, or other growth factors, act to reduce sensitivity to insulin and impairs both insulin-mediated glucose disposal and control of hepatic glucose output (Bomfeldt and Tabas, 2011; Muniyappa et al., 2008; Prentki and Nolan, 2006). Pancreatic β-cells can compensate for a subnormal response to insulin by increasing insulin secretion to maintain normoglycemia, but this compensatory hyperinsulinemia, combined with resistance to insulin signaling, increases one's risk of developing hypertension and dyslipidemia (Prentki and Nolan, 2006; Reaven, 2005b). The extent to which pancreatic β-cells are capable of maintaining hypersecretion of insulin varies between individuals, and it is in individuals with ‘susceptible’ β-cells that loss of β-cell function and progressive loss of β-cell mass due to apoptosis occurs. This leads to poor glucose tolerance, measured as the time required to dispose of an oral glucose load, and the eventual development of type 2 diabetes (Henriksen et al., 2011; Prentki and Nolan, 2006; Schwanstecher and Schwanstecher, 2011). Several gene variants associated with insulin sensitivity and impaired β-cell function have been identified, and include the P12A polymorphism in peroxisome proliferator-activated receptor γ, and mutations in β-cell transcription factors and metabolic regulatory proteins such as hepatocyte nuclear factor-4α, glucokinase, and pancreatic and duodenal homeobox-1. It is thought that enhanced susceptibility to environmental factors such as overnutrition, obesity, and stress due to a cluster of genetic variations may contribute to the onset of β-cell failure and development of type 2 diabetes (Kahn et al., 2006; Muoio and Newgard, 2008).
Hyperinsulinemia and impaired sensitivity to insulin precede the development of type 2 diabetes, and the cluster of symptoms that are found to be associated with hyperinsulinemia and insulin resistance have been collectively termed the insulin resistance syndrome. Features associated with insulin resistance include glucose intolerance, hyperglycemia, elevated triacylglycerol concentrations, reduced high-density lipoprotein concentrations, hypertension, increased procoagulant factors and markers of inflammation, and impaired endothelial function (Frayn, 2010; Munisappa et al., 2008; Reaven, 2005b). These conditions associated with insulin resistance increase the risks for developing type 2 diabetes, cardiovascular disease, hypertension, and cancer (Reaven, 2005b). Several studies also indicate a significant association of hyperinsulinemia with elevated risk of coronary artery disease (Haffner, 1999).

26.3 Epidemiological evidence for a role of vitamin D in insulin resistance

26.3.1 Glucose homeostasis, insulin sensitivity and 25(OH)D levels

Measures of glucose homeostasis in both children (Buyukinan et al., 2012) and adults (Alvaraz and Ashraf, 2010; Chagas et al., 2012; Mitri et al., 2011; Palomer et al., 2008; Pittas et al., 2007a) suggest an inverse relationship between serum 25(OH)D, the preferred indicator of vitamin D status and insulin sensitivity. In a cross-sectional analysis of the Third National Health and Nutrition Examination Survey (n=6,228), serum 25(OH)D was negatively correlated with fasting glucose levels in non-Hispanic whites (OR 0.25) and Mexican blacks (OR 0.17) after adjusting for age, sex, BMI, leisure activity, and time of the year. Additionally, serum 25(OH)D was inversely correlated with fasting and 2-hour glucose, fasting insulin and HOMA-IR (a measure of insulin resistance and β-cell function) in Mexican Americans, with a trend towards significance in non-Hispanic whites (P=0.06). There were no observed associations between serum 25(OH)D and measures of glucose homeostasis in non-Hispanic blacks, however, suggesting a potential race-specific relationship between serum 25(OH)D and glucose levels (Scragg et al., 2004). Additional analyses of the Third National Health and Nutrition Examination Survey data found inverse associations of serum 25(OH)D status with several components of the metabolic syndrome including abdominal obesity, hypertriglyceridemia, and hyperglycemia (Ford et al., 2005) as well as prediabetes (defined as 2-hour glucose concentration of 140-199 mg/dl, fasting glucose concentration of 110-125 mg/dl, or HbA1c value of 5.7-6.4%) after age, sex, ethnicity, and other multivariable adjustments (Shankar et al., 2011). Serum 25(OH)D levels were found also to be inversely related to fasting serum glucose in postmenopausal women (n=753), after controlling for BMI, weight, and age (Need et al., 2005).

Several additional epidemiological studies indicate an association between serum 25(OH)D and glucose homeostasis. A 5-year follow-up examination of men and women (n=4,296, aged 30-65 years) that had participated in the Inter99 study found that individuals with low serum 25(OH)D levels had increased 2-hour glucose and insulin, and increased degree of insulin resistance than those with higher 25(OH)D levels (Husemøen et al., 2012). A cross-sectional analysis of participants of the Kuopio Ischaemic Heart Disease Risk Factor Study (n=1,756;
aged 53-73) found age, gender, and examination year-adjusted serum 25(OH)D to be inversely associated with fasting serum insulin, fasting serum glucose, and OGTT 2-hour glucose levels in both men and women. After further multivariable adjustments for age, gender, examination period, BMI, waist-to-hip ratio, smoking, activity, food intake, and diabetes family history, fasting serum insulin and glucose associations were attenuated, but 2-hour OGTT remained significant, suggesting that low serum 25(OH)D may be associated with impaired glucose and insulin metabolism (Hurskainen et al., 2012). Low serum vitamin D was found to be associated with insulin insensitivity in some, but not all, studies (Pittas et al., 2007a). Inconsistencies in the literature are likely due to differences in subject populations, forms of vitamin D administered, and length of interventions.

26.3.2 Dairy intake and insulin secretion and glucose homeostasis

Intake of fortified dairy, the primary dietary source of vitamin D, may also affect insulin secretion and glucose homeostasis. In a prospective analysis of the Health Professionals Follow-up Study (males, n=41,254), after 12 years of follow-up, the relative risk for type 2 diabetes in men in the top quintile of dairy intake was 0.77 (95% CI 0.62, 0.95) compared to those in the lowest quintile, corresponding to a 9% reduction in risk for type 2 diabetes with each serving per day increase in dairy (Choi et al., 2005). Subsequent cross-sectional analysis of male participants in the Health Professionals Follow-up Study and female participants in the Nurses’ Health Study examined the relationship between plasma 25(OH)D concentrations and calcium intake and fasting concentrations of C-peptide, a marker of endogenous insulin secretion. Plasma concentrations of C-peptide for the highest tertiles of calcium intake and serum 25(OH)D concentrations compared to the lowest tertiles were 35% lower in men (P=0.03) and 12% lower in women (P=0.01), suggesting that high calcium intake and serum 25(OH)D status are associated with decreased insulin secretion. Nonsignificant negative trends were observed between calcium intake and C-protein concentrations in women (P=0.05), and between serum 25(OH)D and C-protein concentrations in men (P=0.08) (Wu et al., 2009). Loss of pancreatic β-cell function leads to poor glucose tolerance and the eventual development of type 2 diabetes, and there is a large body of evidence to suggest a role of 1α,25(OH)₂D in β-cell secretory function (Pittas et al., 2007a), though this relationship was not observed in all studies (Husemoen et al., 2012). Taken together, the epidemiological studies predominantly suggest a relationship between serum 25(OH)D status and insulin action to control glucose homeostasis.

26.3.3 Relationship between vitamin D and insulin resistance in children

Investigations of potential relationships between vitamin D and insulin resistance in children are of particular interest, as the prevalence of type 2 diabetes among children and adolescents has increased significantly over the past two decades (Kaufman and Shaw, 2007). As in adult populations, vitamin D insufficiency and deficiency are common among youth, and a significant relationship between serum 25(OH)D levels and obesity in children, likely due to the sequestration of vitamin D in fat stores, has been found in some (Alemzadeh et al., 2008; Olson et al., 2012) but not all (Rajakumar et al., 2008; Roth et al., 2011) studies. Obese children may be more likely to
be sedentary with reduced sunlight exposure from outdoor physical activity (Roth et al., 2011), and are likely to have significantly lower dietary vitamin D intakes than nonobese children (Rajakumar et al., 2008), contributing to the risk of both vitamin D deficiency and obesity-associated metabolic abnormalities.

In children there is strong evidence to support an inverse relationship between vitamin D status and insulin resistance. An assessment of obese and nonobese children (n=156, age 11.9±2.7 yr, 51% female) in Germany found that 76% of subjects were 25(OH)D-deficient (<20 ng/ml, or <50 nM), and higher insulin, HOMA-IR, and HbA1c were found in obese children with lower serum 25(OH)D concentrations after adjusting for gender, age, and BMI (Roth et al., 2011). Further, cross-sectional results from obese (n=411, age 11.7±2.6 yr, 57% female) and nonobese (n=87, age 11.2±3.0 yr, 68% female) children show that those who were obese were more likely to have lower serum 25(OH)D levels (P<0.001), and that 25(OH)D concentrations were negatively correlated with HOMA-IR (r=-0.19; P<0.001) and 2-hr glucose (r=-0.12; P=0.04) after adjusting for BMI and age (Olson et al., 2012). There was no correlation between serum 25(OH)D levels and HbA1c in this study however a significant inverse relationship was reported for serum 25(OH)D levels and HbA1c by others (Roth et al., 2011). Finally, a multivariate linear regression analysis of data from nonobese Korean children (n=188, age = 12-13 yr) showed that serum 25(OH)D concentrations were inversely associated with HOMA-IR (P=0.025), as well as serum triglycerides (P=0.037) and LDL cholesterol (P=0.045) (Shin et al., 2012). Taken together, these data suggest a relationship between serum 25(OH)D and measures of insulin resistance in a pediatric populations. Further studies to support this, as well as investigations of vitamin D deficiency and insufficiency on measures of insulin resistance in adulthood, are warranted.

### 26.4 Results of clinical interventions

Several intervention studies support an effect of vitamin D supplementation on glucose homeostasis and insulin resistance. In a double-blind, randomized, controlled trial designed for bone-related outcomes, Caucasian, nondiabetic adults (n=314) received supplementation of 500 mg calcium citrate plus 700 IU vitamin D3 daily for three years. Post hoc analysis revealed that after three years of calcium and vitamin D3 supplementation subjects with IFG at baseline had a lower increase in fasting plasma glucose and HOMA-IR compared with those with IFG at baseline given the placebo. For individuals with normal fasting glucose at the time of baseline determination, vitamin D3 and calcium supplementation had no significant effect on fasting plasma glucose or HOMA-IR, suggesting that for individuals with IFG, treatment with vitamin D3 may be effective in attenuating increases in glycemia and insulin resistance that occur over time (Pittas et al., 2007b). Similarly, supplementation of 4,000 IU vitamin D3 daily for 6 months significantly improved insulin sensitivity and insulin resistance in vitamin D deficient, insulin resistant South Asian women (n=81) (Von Hurst, et al., 2010). In another study, the effect of intravenous 1α,25(OH)2D3 therapy on insulin and lipid metabolism was examined in uremic patients on maintenance hemodialysis (n=16) as compared to no infusion controls (n=7). Four weeks of intravenous 1α,25(OH)2D3 therapy (1.8±0.3 μg) corrected glucose intolerance, insulin
26. Vitamin D and insulin sensitivity

Vitamin D and insulin sensitivity, resistance, and hypoinsulinemia, as well as hypertriglyceridemia in these patients (Mak, 1998). The effects of a single, large-dose intramuscular injection of vitamin D3 (300,000 IU) on glucose tolerance and insulin resistance in women with gestational diabetes (n=45) were observed in a randomized controlled trial. An intramuscular injection of vitamin D3 was administered 3-10 days postpartum, and after a 3-month follow-up period, treatment with vitamin D3 was found to prevent the increase in HOMA-IR, and decrease in quantitative insulin sensitivity check index, that were observed in the control group (Mozaffari-Khosravi et al., 2012). This suggests that large, single-dose treatment with vitamin D3 may also be effective in improving indices of insulin resistance in mothers with gestational diabetes after delivery.

However, not all intervention trials report an effect of vitamin D treatment on insulin response (George et al., 2012). In a double-blind, placebo-controlled, cross-over trial of 1α,25(OH)2D3 treatment (1 μg/day for 4 days) of diabetic subjects (n=35), no effect of 1α,25(OH)2D3 was observed on fasting or stimulated glucose, insulin, C-peptide, or glucagon concentrations (Orwoll et al., 1994). Similarly, in another randomized, placebo-controlled, double-blind trial with women with polycystic ovary syndrome (n=50), three oral doses of vitamin D3 (50,000 IU) administered at 20 day intervals did not significantly affect fasting serum insulin or glucose levels, or HOMA-IR (Ardabili et al., 2012). This trial is confounded by a relatively short duration and low dose of treatment therefore additional measures may be necessary to determine the role of vitamin D in treating insulin resistance associated with polycystic ovary syndrome.

The largest intervention study to examine the effects of dietary vitamin D and calcium on health outcomes is the Women’s Health Initiative, and risk of diabetes was examined in secondary analysis of this intervention. In this study, postmenopausal women without diabetes at baseline (n=33,951) were given either a placebo or 1000 mg calcium plus 400 IU vitamin D3, for a median follow-up time of 7 years. In this study, calcium plus vitamin D supplementation did not affect diabetes risk. The hazard ratio for incident diabetes associated with the calcium/vitamin D treatment group was 1.01 (95% CI 0.94-1.10) (De Boer et al., 2008). The data infer a lack of vitamin D effect however it should be noted that the effects of vitamin D were evaluated at a single dose and frequency of vitamin D administered. Evidence reviewed here suggests that both dose and frequency of vitamin D supplementation may determine efficacy in normalizing glucose homeostasis.

26.5 Association of vitamin D with muscle mass and function

Skeletal muscle is the major site of insulin-dependent glucose uptake from the blood. Therefore, muscle mass is an important determinant of insulin sensitivity, and skeletal muscle insulin resistance is critical in the development of type 2 diabetes. Vitamin D clearly plays a role in muscle function, as evidenced by classic symptoms of vitamin D deficiency including decreased muscle strength, changes in gait, inability to ascend stairs, difficulties in rising from a chair and diffuse muscle pain (Pfeifer et al., 2002). Data from epidemiological, animal and cell culture studies suggest a role for vitamin D in enhancing insulin sensitivity and insulin-induced signal
transduction in skeletal muscle. Meta-analyses of randomized clinical trials conclude that vitamin D supplementation significantly improves body sway and fall incidence rate, as well as measures of balance, strength, and gait in elderly populations (Bischoff-Ferrari et al., 2004; Muir and Montero-Odasso, 2011). Diets high in vitamin D have been associated with greater muscle mass in rodents (Siddiqui et al., 2008), and treatment of C2C12 skeletal muscle cells with 1α,25(OH)₂D enhanced myogenic differentiation at the cellular level (Garcia et al., 2011). In C2C12 skeletal muscle cells at the proliferative and early differentiated stages, 1α,25(OH)₂D activates Akt by phosphorylation of Ser473 in a time-dependent manner, from 5-60 minutes (P<0.05) in proliferative cells, and at 48 and 60 hours in C2C12 cells in early stages of differentiation (Buitrago et al., 2012). This up-regulation is likely to be at least in part responsible for the enhanced myogenic differentiation observed in skeletal muscle cells treated with 1α,25(OH)₂D, and together, these results suggest that vitamin D may promote insulin sensitivity through its ability to enhance insulin signaling in, and improve mass of, skeletal muscle.

Exposure of skeletal muscle cells to free fatty acids such as palmitate will induce insulin resistance through increased serine/threonine phosphorylation of IRS-1. This results in a reduced capacity of IRS-1 to activate PI3K and Akt, causing impaired insulin signaling event. It has been shown that elevated levels of FFA in circulation play a key role in the pathogenesis of type 2 diabetes (Boden, 1997), and exposing cultured skeletal muscle cells and isolated skeletal muscle tissue to FFA such as palmitate will induce insulin resistance (Dimopoulos et al., 2006; Hirabara et al., 2007; Jové et al., 2006; Schmitz-Peiffer et al., 1999). Treatment of C2C12 myotubes with 1 mM palmitate produces an increase in serine phosphorylation of IRS-1 associated with blunted insulin signal transduction, and a decrease in tyrosine phosphorylation of IRS-1, which is necessary to propagate the insulin signaling cascade. In one study, co-treatment of C2C12 skeletal muscle cells with 10 nM 1α,25(OH)₂D and 1 mM palmitate prevented these changes in IRS-1 phosphorylation states, as well as the decrease in Akt activation that is observed in FFA-treated, insulin-resistance cells. Co-treatment of the C2C12 cells with 1α,25(OH)₂D and palmitate resulted in increased insulin-stimulated glucose uptake, and inhibited FFA-induced c-Jun N-terminal kinase phosphorylation, which is thought to be implicated in the pathogenesis of insulin resistance (Zhou et al., 2008). Inconsistencies in results from clinical trials warrants additional research to establish a clinically relevant relationship between serum 25(OH)D and muscle mass in humans (Marantes et al., 2011), but available data suggest that the insulin-sensitizing effects of vitamin D may be due at least in part to gains in insulin-responsive muscle tissue mass and improved insulin signaling.

### 26.6 Molecular mechanisms underlying vitamin D action on insulin sensitivity

There is evidence from animal, tissue, and cell model studies to support an effect of vitamin D on insulin action at several levels, including direct effects at target tissues to alter sensitivity to insulin, as well as through local conversion of 25(OH)D to 1α,25(OH)₂D within the pancreatic β-cell to alter insulin release (Pittas et al., 2007a). Presence of the VDR (Johnson et al., 1994) and expression of 1α-hydroxylase (Bland et al., 2004) in pancreatic β-cells, and the identification of the vitamin D response element in the human insulin gene promoter (Maestro et al., 2003)
suggest a direct effect of vitamin D on insulin secretion. Further, mice lacking a functional VDR have impaired insulin secretion (Zeitz et al., 2003), highlighting the involvement of vitamin D in insulin signaling. Several studies have shown that vitamin D-deficient rats present with impaired glucose tolerance and reduced insulin secretion, and repletion of vitamin D restores impaired glucose tolerance and insulin release (Cade and Norman, 1986; Kadowaki and Norman, 1984). Further, ob/ob mice, which exhibit homozygous mutations in the gene for leptin resulting in obesity, hyperphagia, hyperglycemia and hyperinsulinemia, treated with 1α,25(OH)₂D improved hyperinsulinemia and hyperglycemia (Kawashima and Castro, 1981). These results support a role of vitamin D in maintaining normoglycemia, and it is hypothesized that the impact of 1α,25(OH)₂D on insulin release may occur through increased conversion of pro-insulin to insulin (Bourlon et al., 1996, 1999), or by increasing pancreatic β-cell replication and resistance to apoptosis.

Additionally, 1α,25(OH)₂D may affect insulin release by normalizing intracellular Ca²⁺ levels (Takiishi et al., 2012), the elevation of which in human adipocytes, leukocytes, and platelets is associated with insulin resistance (Draznin, 1993; Resnick, 1992). In rodents, measures which increase intracellular calcium in muscle and adipocytes are associated with impaired insulin signaling, likely through an impaired ability of insulin to activate phosphoserine phosphatase-1 (Begum et al., 1992). Parathyroid hormone, synthesized in the chief cells of the parathyroid, acts to increase serum calcium levels, and its synthesis fluctuates inversely in response to serum 25(OH)D and calcium levels (D’Amour, 2012). Parathyroid hormone can act to increase intracellular calcium levels in adipocytes and muscle, and secondary hyperparathyroidism resulting from low calcium intake and vitamin D deficiency may therefore contribute to insulin resistance (Draznin, 1993; McCarty, 2006). Elevated intracellular calcium levels may contribute to insulin resistance by increasing binding of calmodulin to IRS-1, thus interfering with insulin-stimulated tyrosine phosphorylation of IRS-1 and the subsequent activation of PI3K (Li et al., 2000). Vitamin D may therefore exert its insulin-sensitizing effects through its ability to regulate intracellular calcium levels by reducing serum levels of PTH.

26.6.1 Vitamin D increases protein expression of the insulin receptor

Another mechanism by which vitamin D may elicit improvements in insulin sensitivity is through increased abundance of the insulin receptor protein. A vitamin D response element has been identified in the promoter region of the human insulin receptor gene (Maestro et al., 2003), and increases in IR mRNA expression have been observed upon treatment with vitamin D in both cell (Maestro et al., 2000) and rodent (Siddiqui et al., 2008) models. Later work by Maestro et al. (2002) demonstrated that 1α,25(OH)₂D induces the transcriptional activation of the human IR gene in U-937 promonocytic cells through the PI3K signaling system, and that 1α,25(OH)₂D-mediated increases in IR expression potentiates the action of insulin on glucose oxidation, providing a possible mechanism by which 1α,25(OH)₂D may affect insulin sensitivity.
26.7 Additional regulators of insulin sensitivity

26.7.1 Parathyroid hormone

Parathyroid hormone and the vitamin D metabolite 1α,25(OH)₂D are major hormonal regulators of calcium homeostasis. Low serum calcium is sensed by Ca-sensing receptors of the parathyroid gland, which signal for a release of PTH into circulation (Hebert et al., 1997). PTH stimulates the renal enzyme 1α-hydroxylase to convert 25(OH)D to its hormonally active form, 1α,25(OH)₂D, which acts on bone, kidney, and intestine to regulate calcium homeostasis (Zierold et al., 2003). Consequently, a dietary calcium load results in an acute lowering of serum PTH, while higher vitamin D and calcium status are associated with lower serum levels of PTH (Harkness and Cromer, 2005; Pepe et al., 2005).

In addition to regulation of calcium homeostasis, evidence suggests that PTH may negatively influence glucose homeostasis and insulin sensitivity, in part by influencing body fat mass. In a meta-analysis of seventeen studies investigating the relationship between individuals with primary hyperparathyroidism and adiposity, subjects with primary hyperparathyroidism were 3.34 (95% CI 1.97, 4.17, P<0.00001) kg heavier compared with controls, or had an increased BMI of 1.13 (95% CI -0.29 to 2.55; P=0.12) kg/m² compared with controls (Bolland et al., 2005). Additionally, studies of young adults between the ages of 20 and 35, as well as those aged 65 and older without PHPT also show associations between PTH and BMI and body fat percentage (Bell et al., 1985; Snijder et al., 2005), suggesting that serum PTH may be involved in regulating adiposity, a major risk factor for diabetes and insulin insensitivity.

Parathyroid hormone may also directly influence glucose homeostasis. Serum PTH was found to be inversely associated with hyperglycemia in men (Reis et al., 2007), and with insulin sensitivity index in normotensive adults (Chiu et al., 2000). Further, individuals with PHPT were found to have impaired glucose intolerance and insulin sensitivity compared to individuals without PHPT (Kumar et al., 1994). This negative effect of PTH on insulin sensitivity is hypothesized to occur through a variety of mechanisms, including suppressed pancreatic insulin release (Perna et al., 1990), increased intracellular calcium levels leading to reduced insulin-stimulated glucose uptake in adipocytes (Ni et al., 1994; Reusch et al., 1991), and by inducing insulin resistance in adipocytes (Chang et al., 2009). PTH may negatively affect insulin sensitivity through increasing adiposity and interfering with insulin signaling, and vitamin D may improve insulin sensitivity through its ability to lower serum PTH levels.

26.7.2 Adiponectin

Though muscle tissue is the body’s main source of glucose disposal, adipose tissue also plays a central role in regulating insulin secretion, insulin action, and glucose and lipid metabolism. Secretory products of adipose tissue include enzymes, growth factors, cytokines, and several hormones involved in fatty acid and glucose metabolism (Mora and Pessin, 2002). Adiponectin, an adipose-derived peptide hormone, is associated with improved glucose tolerance, and reduced
26. Vitamin D and insulin sensitivity

Risk of obesity, cardiovascular disease, insulin resistance and type 2 diabetes (Kishida et al., 2012; Tishinsky et al., 2012). Serum adiponectin and mRNA expression in adipose tissue are decreased in obese and type 2 diabetic humans (Arita et al., 1999, Weyer et al., 2001), and in obese rodent models, administration of adiponectin improves insulin sensitivity and glucose homeostasis (Dzamko and Steinberg, 2009; Yamauchi et al., 2001). Adiponectin also serves as an activator of AMPK, resulting in increased fatty acid β-oxidation, mitochondrial biogenesis, and insulin sensitivity in skeletal muscle, as well as decreased hepatic gluconeogenesis (Dzamko and Steinberg, 2009). Several studies report significant correlation between serum 25(OH)D status and adiponectin levels in both children (Roth et al., 2011) and adults (Gannagé-Yared et al., 2009; Nimitphong et al., 2009; Vaidya et al., 2011). Additionally, treatment of adults with type 2 diabetes (n=90) with 1000 IU vitamin D3 or 1000 IU vitamin D3 plus 500 mg calcium per day for 12 weeks produced a significant increase in serum adiponectin levels (Neyestani et al., 2012). Adiponectin may therefore be implicated in the observed effects of vitamin D on insulin resistance.

26.7.3 AMP-activated protein kinase

AMP-activated protein kinase is a ubiquitous serine/threonine protein kinase that acts as a sensor of cellular energy status. It is activated by an increased AMP:ATP ratio (low cellular energy state), and acts to inhibit anabolic, ATP-consuming cellular processes while stimulating catabolic, ATP-generating processes (Towler and Hardie, 2007; Viollet et al., 2003). AMPK may increase insulin sensitivity by altering metabolism to promote fat oxidation and reduce muscle triglyceride storage, thereby reducing risk factors associated with insulin resistance. These risk factors may be reduced through increasing mitochondrial biogenesis, which is of relevance as individuals who are at risk of developing type 2 diabetes appear to have reduced mitochondrial function (Hardie, 2011; Lowell and Shulman, 2005). AMPK activation in skeletal muscle may also contribute to improved insulin signaling. Primary cardiac and skeletal myocyte cultures subject to insulin- and glucose-free conditions exhibited AMPK-dependent, insulin-independent activation of the insulin receptor, providing a direct link between AMPK and the insulin signaling pathway (Chopra et al., 2012). The rise in AMP:ATP ratio that accompanies muscle contraction also triggers AMPK activation (Winder and Hardie, 1996), which in muscle results in increased GLUT4 expression and increased glucose uptake (Zheng et al., 2001). This increased GLUT4 expression occurs even in insulin-resistant individuals, allowing for greater insulin-induced glucose uptake even without changes in insulin sensitivity (Hardie, 2011). Though no direct link between vitamin D and AMPK has been tested, Vitamin D is implicated in muscle mass, strength, and function (Dirks-Naylor and Lennon-Edwards, 2011; Teegarden and Donkin, 2009), and may therefore contribute to whole-body insulin sensitivity through enhanced contraction-stimulated, AMPK-dependent glucose uptake by skeletal muscle.

26.7.4 Osteocalcin

In addition to skeletal muscle and adipose tissue, the skeleton also exerts endocrine regulation of whole-body glucose metabolism. In its uncarboxylated state, osteocalcin, a hormone secreted exclusively by osteoblasts, promotes increased insulin secretion, pancreatic β-cell proliferation,
adiponectin secretion, and increases in energy expenditure (Lee et al., 2007; Ng, 2011). Mice lacking the gene for osteocalcin are glucose intolerant and exhibit excess adiposity (Lee et al., 2007), and treatment of mice with osteocalcin has shown beneficial effects on measures of glucose homeostasis. In mice fed a normal diet, injections of osteocalcin at 3 or 30 ng/g/day improved glucose tolerance and insulin sensitivity through increases in β-cell mass and insulin secretion. In mice fed a high-fat diet, injections of osteocalcin partially restored insulin sensitivity and glucose tolerance, and also resulted in increased energy expenditure and protection from diet-induced obesity (Ferron et al., 2012), suggesting a role of osteocalcin in the improvement of insulin sensitivity and prevention of type 2 diabetes.

Associations between serum osteocalcin and insulin sensitivity may be observed in human subjects as well. In both men and postmenopausal women, serum osteocalcin is inversely associated with fasting plasma glucose, HbA1c, and HOMA-IR, and is positively associated with β-cell function (Kanazawa et al., 2011), suggesting that serum osteocalcin may positively influence glucose homeostasis by enhancing insulin action. In vitro and ex vivo, osteocalcin stimulates expression of adiponectin (Lee et al., 2007), providing a mechanism by which osteocalcin may exert its insulin-sensitizing effects. However, a significant positive association of serum osteocalcin with improved glucose tolerance after adjusting for serum adiponectin levels was found in some (Kanazawa et al., 2011; Shea et al., 2009) but not all (Hwang et al., 2012) studies utilizing human subjects, suggesting that osteocalcin-mediated increases in insulin sensitivity may involve mechanisms other than upregulation of adiponectin.

### 26.8 Conclusion

A large body of evidence strongly suggests a relationship between vitamin D and measures of insulin sensitivity (Figure 26.2). Epidemiological data shows a positive relationship between serum 25(OH)D and measures of insulin resistance, and in some studies treatment of individuals with 1α,25(OH)2D improves insulin resistance. Evidence suggests that the effects of vitamin D on insulin sensitivity occur at several levels, including through the stimulation of insulin secretion, by enhancing insulin signaling in target tissues, and through the regulation of additional hormones that affect glucose homeostasis. Because a positive relationship has been observed between vitamin D and muscle mass and function and skeletal muscle is recognized as the primary site of glucose disposal, it appears that increased insulin signaling and hypertrophy of muscle cells may explain some of the beneficial effects of vitamin D on whole-body glucose metabolism. Further identification of the mechanisms by which vitamin D exerts its insulin-sensitizing effects is necessary to establish recommendations for optimal serum levels of vitamin D for the management of glucose homeostasis and prevention of type 2 diabetes.
26. Vitamin D and insulin sensitivity

Figure 26.2. Potential mechanisms underlying the putative relationship between vitamin D and insulin sensitivity.

References


B. Larrick, S. Donkin and D. Teegarden


26. Vitamin D and insulin sensitivity


B. Larrick, S. Donkin and D. Teegarden


26. Vitamin D and insulin sensitivity


McCarty, M., 2006. PKC-mediated modulation of L-type calcium channels may contribute to fat-induced insulin resistance. Medical Hypotheses 66, 824-831.


Key facts

- Diabetes is a metabolic disease, in which a person has high blood sugar resulting from inadequate production and/or utilization of insulin. Its classical symptoms include polyuria, polydipsia, weight loss, and/or polyphagia.
- Three main types of diabetes: Type 1 diabetes mellitus (T1D), predominately observed among children and youth; Type 2 diabetes mellitus (T2D), mainly occurred among adults; and gestational diabetes mellitus (GDM), among women who had no diabetes before, but with a high blood glucose level during pregnancy.
- Globally, the prevalence rate of diabetes is continually increasing and, by 2030, the number of people with diabetes will reach to 366 million with a noticeable increase from the developing countries.
- Vitamin D, a necessity for maintaining bone health, may have been involved in a wide range of biological actions due to that vitamin D receptor is present in most tissues and cells in the body.
- Laboratory studies have suggested the role of vitamin D in insulin synthesis and secretion, but a causal relationship between vitamin D supplements and diabetes prevention has not been established yet from clinical trials.

Summary points

- Many studies have observed a reverse relationship between serum vitamin D levels and the risk of diabetes, but how vitamin D plays a physiologically important role in the development of diabetes is unclear.
- T1D is believed as the results of autoimmune destruction of insulin-producing beta cells of the pancreas. However, the exact mechanism is not totally understood.
- A seasonal pattern of the incidence of T1D has been observed by many epidemiological studies in different countries, regions, and ethnicity groups. The incidence of T1D seems to peak more in winter seasons.
- T2D is originally described as a lifelong disease mainly occurring among elderly adults, but it is now observed to affect younger adults and even children.
- The results from interventional studies in examining whether vitamin D supplements reduce T2D risk are mixed, but no study has reported that taking vitamin D supplement will worsen the pathology of the disease.
- The prevalence rates of GDM vary by region, but are not affected by latitude. However, vitamin D deficiency seems very prevalent in pregnant women.
- Adult stature components (e.g. standing height, leg length, ratio of leg length to standing height, and femur bone length) are observed to be negatively associated with risk of T2D.
- Emerging evidence indicates that bones, the target organ of vitamin D, have an endocrine function. Metabolic molecules of bone modeling may play an important role in the development of diabetes.
27. Vitamin D and diabetes mellitus: where are we?

J. Liu
Department of Community Health Sciences, Brock University, 500 Glenridge Ave. St. Catharines, ON L2S 3A1, Canada; jliu@brocku.ca

Abstract

Diabetes mellitus, as a common metabolic disease, affects millions people's health worldwide and its incidence is continuing to increase. It is important to find effective measures of prevention and/or intervention for the disease to reduce its impact on the risk for cardiovascular disease – the leading cause of mortality among patients with diabetes mellitus. Apart from its well-known ‘classic’ effect in bone metabolism, vitamin D has received widespread attention for its potential role in the prevention of diabetes mellitus, in particular type 2 diabetes mellitus. A large number of studies have observed a reverse relationship between vitamin D levels and the risk of diabetes mellitus. The results from the studies aiming to reduce risk for diabetes mellitus with vitamin D supplements are mixed though no study reports worsening the disease pathologically after treatment. Most previous studies on the relationship between vitamin D and diabetes mellitus were orientated from the direction of the ‘non-classic’ effect of vitamin D, but the emerging evidence indicates that its ‘classic’ effect on bone metabolism can be the key to understand its risk association with diabetes mellitus.

Keywords: diabetes mellitus, bone metabolism
27.1 Introduction

27.1.1 Vitamin D

Vitamin D is well known for its primary physiological role of regulation of calcium homeostasis in maintaining bone health. However, growing evidence indicates that vitamin D is also involved in modulating body composition, energy homeostasis, insulin sensitivity, and immune function (Holick et al., 2011; Mithal et al., 2009; Wolden-Kirk et al., 2011); thus, vitamin D deficiency has been observed to be associated with a broadening field of health problems including certain cancers, cardiovascular disease, and diabetes mellitus (Anderson et al., 2010; Garland et al., 1989, 1990; Pittas et al., 2007).

There are two sources of vitamin D available for the human body; endogenous synthesis in the skin from exposure to sunlight and exogenous consumption in foods and/or pharmaceutical supplements. The endogenously synthesised vitamin D comprises approximately 80-90% of circulating levels of vitamin D (Mithal et al., 2009). As expected, season and latitude affect the levels of vitamin D (Mithal et al., 2009; Pettifor et al., 1996; Van der Mei et al., 2007).

The active metabolite of vitamin D in the human body is 1,25(OH)₂D, which is generated in multiple steps. 25(OH)D is produced first in the liver through D-25 hydroxylase and then further converted to 1,25(OH)₂D, primarily but not exclusively, in the kidneys by the 25(OH)D-1α-hydroxylases (Holick, 2009). Within the circulation, vitamin D-binding protein is the major transport protein for vitamin D metabolites in plasma. 1,25(OH)₂D can bind to the intracellular vitamin D nuclear receptors, which are present in most tissues and cells of the body (Adams and Hewison, 2010; Holick, 2007), and then initiate the activations that regulate not only calcium metabolism, but also differentiation and division of various cell types (Nagpal et al., 2005). Although 1,25(OH)₂D is the biologically active form of vitamin D, however, serum 25(OH)D
concentrations are recommended as the best indicator in reflecting vitamin D levels from either exposure to sunlight or dietary sources (Holick, 2009).

The primarily biological role of vitamin D is known for its regulating function of calcium metabolism to maintain extracellular calcium ion levels within a physiologically acceptable range. Since the physiologically acceptable range of calcium is very tight (8.5-10.2 mg/dl) and the concentration of calcium distribution between outside and inside of the cell is balanced at a vast difference ratio (10,000:1), any change that occurs to the range or the balance will cause various disease sequelae (Fujita, 2000). Extreme vitamin D deficiency will result in rickets in children and osteomalacia in adults; while vitamin D deficiency in certain degrees will increase the risk for many metabolic diseases, particularly for diabetes mellitus (Adams and Hewison, 2010). The VDR has been found to be expressed in beta cells in pancreatic and other vitamin D related genes such as vitamin D-binding protein and D-24-hydroxylase, which degrades the active form of vitamin D into water-soluble inactive forms (Bikle, 2009). Although many epidemiological studies and animal experimental studies have observed a reverse relationship between serum vitamin D levels and the risk of diabetes mellitus (see details below), it is not totally understood how vitamin D signaling plays a physiologically important role in beta cells and in the development of diabetes mellitus.

27.1.2 Diabetes mellitus

The increasing prevalence of diabetes mellitus is rising at an alarming rate globally. It is estimated that approximately 346 million people worldwide have diabetes and the incidence is continuing to increase (WHO, 2011a). Diabetes mellitus significantly increases the risk for coronary heart disease, stroke, renal failure, lower limb amputations, and visual impairment and blindness. People with diabetes mellitus require at least two to three times higher the health-care resources, which may account for up to 15% of national health care budgets (WHO, 2011b). Three main types of diabetes mellitus are recognized, i.e. T1D, T2D and GDM. The vast majority of the diabetic patients (90-95%) are with type 2 diabetes.

Type 1 diabetes mellitus

T1D, previously known as juvenile diabetes or insulin-dependent diabetes mellitus, is usually diagnosed in children and younger adults. The incidence of T1D is increasing in children and youth by about 3% (range 2-5%) per annum with approximately 76,000 children aged less than 15 years annually developing T1D worldwide (IDF, 2009). The increase has been observed in countries with both high and low prevalence, particularly with a steeper increase in some of the low-prevalence countries (Aanstoot et al., 2007). The incidence rates of T1D vary between countries with countries such as China reporting the lowest incidence rates (about 0.57 cases per 100,000 population younger than 18 years of age per year) to rates roughly 30 times higher in the UK (18-20 per 100,000 per year) to almost 100-fold higher (about 48-49 per 100,000 per year) in Finland and Sardinia (Daneman, 2006). T1D is seen predominantly in children younger than 15– years of age with a significant trend towards decreasing age at presentation (IDF, 2009).
Individuals with T1D do not produce insulin in their body, which is believed to be the result of autoimmune processes between genetic and environmental factors. The variation of human leukocyte antigen genes is considered to contribute approximately 50% of the total genetic susceptibility to T1D (Risch, 1987). Pathologically, T1D is a condition in which pancreatic cell destruction leads to absolute insulin deficiency. Two subtypes have been identified in T1D; type 1A, which includes the common, immune-mediated forms of the disease (Concannon et al., 2009; Devendra et al., 2004); and type 1B, which is far less frequent, has usually unknown causes, and occurs mostly in individuals of Asian or African descent who have varying degrees of insulin deficiency between sporadic episodes of ketoacidosis (Abiru et al., 2002). The exact mechanism of the disease is not totally understood, but the consensus is that the majority of the patients with T1D acquire the disease as a result of autoimmune destruction of insulin-producing beta cells of the pancreas (Concannon et al., 2009; Daneman, 2006). It is believed that several genetic and environmental risk factors are involved in the development of T1D.

**Type 2 diabetes mellitus**

T2D prevalence has been increasing dramatically worldwide in both genders during last three decades, but this increase is more dramatically seen in women (the prevalence of diabetes mellitus for women was increased 23% from 7.5% in 1980 to 9.2% in 2008; and for men by 18% from 8.3% in 1980 to 9.8% in 2008) (Danaei et al., 2011). Although originally described as a lifelong disease mainly occurring among the elderly adults, T2D (also known as adult-onset diabetes or non-insulin-dependent diabetes mellitus) is now observed to affect younger adults and even children. With the rise of overweight and obese individuals and the changing of life style, concern has risen about a global T2D epidemic (Zimmet et al., 2001). It has been projected that the number of people with diabetes worldwide will increase from 171 million in 2000 to 366 million by 2030 with a noticeable increase from the developing countries. For instance, the number of people with T2D in China will have more than doubled from 20.7 million in 2000 to 42.3 million in 2030, which is a much faster rate than that in United States and Canada (19.7 million in 2000 to 33.9 million in 2030) (Hossain et al., 2007). Individuals with T2D usually have adverse lipid profiles and are at a significant risk of increased mortality from cardiovascular disease (Liu et al., 2005).

In contrast to T1D, people with T2D are believed to have insulin insensitivity, initially with compensatory increase in insulin secretion, but the beta-cells in the pancreas may fail to keep up with the increased workload resulting in a relative insulin insufficiency and eventually the development of T2D. Although insulin insensitivity and beta-cell dysfunction are critical to the development of impaired glucose tolerance and T2D, the exact mechanisms are complicated and not well understood. There is a genetic basis for the dysfunction in both parameters, but environmental factors are undoubtedly to play a major role in its process. Vitamin D deficiency has been suggested to be one of the environmental factors and its relationship with the risk of T2D has been primarily investigated by a number of studies in humans (Osei, 2010).
27. Vitamin D and diabetes mellitus: where are we?

**Gestational diabetes mellitus**

GDM is hyperglycaemia with onset or first recognition during pregnancy. Although the symptoms of GDM are similar to T2D, it is often diagnosed through prenatal screening, rather than reported symptoms. Women with GDM are at a great increased risk of developing T2D in comparison to those women without GDM (Bellamy *et al.*, 2009).

Since lack of uniform standards in glucose tolerance testing for pregnant women in many countries there is no an accurate estimation of the global incidence of GDM (Ben-Haroush *et al.*, 2004). The prevalence rates estimated in certain areas, such as Europe and China, were between 2.0 and 6.0% (Buckley *et al.*, 2011; Zhang *et al.*, 2011). However, an international multicenter study of GDM conducted in a heterogeneous, multinational, multicultural, and ethnically diverse cohort with approximately 25,000 women in the third trimester of gestation found that about 18% (range 9.3-25.5%) of the pregnancies were affected by GDM after performing a 75-g oral glucose tolerance test (Sacks *et al.*, 2012). It has been observed that GDM is more frequent in certain ethnic groups than in the general population. In general, white women have a lower incidence than black women; while Asian women have the highest rate (Reece *et al.*, 2009).

Although usually an increase in the concentration of pregnancy hormones leads to a change of metabolism pattern of the body and may reduce tissues sensitivity to insulin, for some women pregnancy is a trigger for a series of metabolic imbalances that lead to a diabetic state. These women may have already been genetically vulnerable. In addition, when environmental factors, such as diet, obesity, depression, etc. are present these women may increase the likelihood of developing diabetes (Lain and Catalano, 2007). It has also been suggested that vitamin D deficiency may play a role for the occurrence of GDM (Dror, 2011).

27.2 Vitamin D deficiency and diabetes mellitus

27.2.1 Vitamin D deficiency definition

Currently, no standard definition of optimal vitamin D status exists and there is a considerable controversy on the definition of vitamin D deficiency. The recently updated position statement from the Institutes of Medicine suggests that vitamin D as serum 25(OH)D with levels less than 30 nmol/l (12 ng/ml) is considered as deficiency; and less than 50 nmol/l (20 ng/ml) but higher than 30 nmol/l as inadequacy in regard to bone health (Ross *et al.*, 2011). However, many others believe that a much higher level of vitamin D may be needed to maintain overall health beyond just bone health (Holick *et al.*, 2011), particularly when examining vitamin D levels and its risk association with diabetes mellitus and other chronic diseases. There is, currently, consideration of the widespread prevalence of hypovitaminosis D, as it may affect people worldwide, particularly the children and the elderly (Holick *et al.*, 2011; Mithal *et al.*, 2009).
27.2.2 Vitamin D deficiency and type 1 diabetes mellitus

The seasonal pattern of the incidence of T1D has been observed by many epidemiological studies in different countries, regions, and ethnicity groups (Fleegler et al., 1978; Kahn and Anderson, 2009; Moltchanova et al., 2009; Weets et al., 2006). The incidence of T1D seems to peak more in the winter season than other seasons in many countries located in both the northern and the southern hemispheres (Moltchanova et al., 2009). Since the primary source of circulating vitamin D in humans is largely derived from processes initiated by ultraviolet radiation exposure, which is in nature inversely associated with latitude, several studies have reported a latitudinal gradient for the prevalence of T1D (Anonymus, 1988; 2000; Staples et al., 2003). In Newfoundland, Canada, one of the highest documented incidences of T1D worldwide, the results of ecological analysis have suggested a link between T1D risk and limited UVB exposure (Sloka et al., 2010). Data from 51 regions worldwide was used to examine the relationship between UVB irradiance and age-standardised incidence rates of T1D in children and indicated that the incidence of T1D was greater at higher latitudes (Mohr et al., 2008). Many observational studies have also showed that a lower level of serum vitamin D or vitamin D deficiency is very common among those newly diagnosed T1D patients in children, adolescents, and young adults (Borkar et al., 2010; Cooper et al., 2011; Littorin et al., 2006; Pozzilli et al., 2005; Svoren et al., 2009).

NOD mice have been used as models in animal experimental studies to examine vitamin D deficiency and the risk of diabetes. The results from most of the studies, except for one (Hawa et al., 2004), supported what has been observed from human's studies. NOD mice that are genetically predisposed to develop insulitis and T1D had the disease developed earlier when growing in a vitamin D depleted environment (Giulietti et al., 2004). When the active metabolites of vitamin D were administrated in early life of these mice, it significantly decreased the risk of developing T1D (Mathieu et al., 1994; Zella et al., 2003). Those treated with vitamin D had much higher levels of serum calcium (Zella et al., 2003). The results from mice lacking VDR seemed to be more complicated. In comparing to NOD mice without VDR knockout, those VDR knockout NOD mice were not different in glucose homeostasis and incidence of diabetes (Cheng et al., 2011; Gysemans et al., 2008). However, those VDR knockout mice significantly increased mRNA expression and protein production from the local pancreatic islet renin-angiotensin system; while pre-treatment with 1,25(OH) could prevent and reverse the increase in the renin-angiotensin system component formation and improve insulin secretion. This formation is induced by high glucose concentrations in isolated mouse islets (Cheng et al., 2011). Furthermore, when those VDR knockout mice were fed a standard diet, the capacity of islet-cells to produce and/or secrete insulin was severely impaired by the hypocalcaemia, but it could be normalized with feeding a high-lactose calcium rescue diet (Driver et al., 2011). This suggests that the effect of vitamin D on diabetes may, at least in part, be mediated through the role of calcium.

Results from several cohort or population-based case-control studies with vitamin D supplements supported the link between vitamin D deficiency and the risk of T1D, though some others did not. The results from a birth-cohort study of northern Finland indicated that taking vitamin D in early life could reduce the risk for T1D in later life. Approximately 12,000 Finnish children enrolled at a
27. Vitamin D and diabetes mellitus: where are we?

1966 baseline. During an approximately 30 years follow-up, it was found that those children with suspected rickets during their first year of life had a 3-times higher risk of developing T1D later in life; while those who received at least 2,000 IU/day of vitamin D during their first year of life reduced risk of developing T1D later in life by 88% (Hypponen et al., 2001). A case-control study from a nested cohort conducted in Norway found that cod liver oil supplementation in infancy or during maternal pregnancy was associated with a lower risk of T1D (Stene and Joner, 2003; Stene et al., 2000); and, further, that children from mothers with lower levels of serum 25(OH)D during pregnancy were at increased risk of T1D (Sorensen et al., 2011). However, the results from two other studies showed that either maternal intake of vitamin D during pregnancy (Marjamaki et al., 2010) or during infancy did not affect the risk of T1D (Simpson et al., 2011), though both of these studies had no information on the fetal and/or maternal level serum 25(OH)D.

The distribution of the VDR gene and its relationship with T1D in human has also been examined by several studies though the results are inconsistent. For instance, a recently case-control study conducted in a group of Turkish patients with T1D found that bone turnover markers, such as osteocalcin and C telopeptide, and bone mineral density were significantly lower in patients with T1D, but that the VDR gene polymorphisms, Bsm1, Fok 1, Apa1, and Taq1 showed no difference between those cases and controls (Gogas Yavuz et al., 2011). A similar result has been reported from a previous meta-analysis, which showed no association between VDR gene polymorphisms and T1D risk (Guo et al., 2006). However, another meta-analysis conducted with the similar data suggested that the risk association of the VDR polymorphisms genes with T1D might be affected by the levels of winter ultraviolet radiation. Therefore, the involvement of VDR variants in the etiology of T1D could not be excluded (Ponsonby et al., 2008).

27.2.3 Vitamin D deficiency and type 2 diabetes mellitus

The seasonal pattern for the risk of T2D does not appear as that for T1D, though a few of studies have shown seasonal variations of concentrations of pre-prandial glucose and HbA1c in T2D patients with levels higher in winter and lower in summer (Ishii et al., 2001; Liang, 2007).

However, a number of observational studies have shown a reversed risk association of T2D with vitamin D in the relationship between higher levels of intake vitamin D and/or calcium and a lower T2D risk or that between lower levels of serum 25(OH) D and an increased risk for incident T2D (Kiriı et al., 2009; Knekt et al., 2008; Pittas et al., 2006; Scragg et al., 1995, 2004). However, the results from some other studies were not in favor of this link (Grimnes et al., 2010; Pittas et al., 2010; Robinson et al., 2010). For instance, lower serum 25(OH) levels might be associated with the increased risk of incident T2D in men(Knekt et al., 2008), but not in old women (Robinson et al., 2010). The ethnicity background seems to affect this association too; a reversed relationship between serum 25(OH)D and risk of T2D has been observed in New Zealand Polynesians and Caucasians, but those Polynesians had much lower levels of 25(OH)D (Scragg et al., 1995). Similarly, results from an analysis of the third national health and nutrition examination survey showed a reversed relationship between serum 25(OH)D levels and T2D risk among Mexican-Americans and Hispanic Whites, but not in non-Hispanic blacks (Scragg et
The results from another analysis conducted among middle-aged Caucasian men and women indicated that individuals with serum 25(OH)D levels ≥80 nmol/l were half as likely to have diabetes in comparison to those with levels <37 nmol/l (Brock et al., 2011).

In addition, central adiposity status is considered as an important factor to confound the risk association of T2D with vitamin D because patients with T2D often coexist with central adiposity (Qiao and Nyamdorj, 2010; Vazquez et al., 2007). Obese adults are more likely to be at high risk for vitamin D deficiency as body fat may sequester the fat-soluble vitamin (Holick et al., 2011). Similar phenomena were also observed among children and adolescents who lived in different regions (e.g. 40.4 degree of N in Pittsburgh (Rajakumar et al., 2011) and 33 degree of N in Atlanta (Dong et al., 2010)) of the United States and Italy (Pacifico et al., 2011), but not among children and adolescents living the Bangkok (13 degree of N) (Poomthavorn et al., 2012) or adolescent girls in Beijing (40 degree of N), China (Foo et al., 2009). Regardless of the obesity status, however, the mean levels of serum 25(OH) D for those children and adolescents in Bangkok were approximately 70 nmol/l; while for those girls in Beijing nearly 90% of them had serum 25(OH)D levels of <50 nmol/l, measured in winter time. This may suggest that obesity status may not be the issue as the majority of these subjects had either relatively high levels or relatively low levels of serum 25(OH) D. This question needs to be further explored. This may partially explain why in some studies adjustment for variables related to obesity (e.g. body mass index) the reversed risk association of T2D with serum 25(OH)D levels still persisted (Brock et al., 2011; Scragg et al., 2004), but in others were diminished (Grimnes et al., 2010).

A number of interventional studies have been conducted to examine whether vitamin D supplement can reduce the risk of T2D, but the results are mixed. For instance, a double-blind, RCT was conducted among 314 older Caucasians without diabetes showed that in comparison to those in a placebo group, those in the experimental group with daily intake 700 IU of vitamin D3 and 500 mg of calcium for three years prevented increases in plasma glucose and insulin resistance among patients with impaired fasting glucose (Pittas et al., 2007). Patients with T2D in another two RCTs also showed that patients who consumed a vitamin D3 fortified yogurt drink (1000 IU/day) with either 500 mg/day or less calcium in comparison to patients who consumed plain yogurt improved glycemic status, lipid profile and endothelial biomarkers (Nikooyeh et al., 2011; Shab-Bidar et al., 2011). However, the results from two large-scale RCTs, the RECORD and the WHI, did not show benefits of daily intake of vitamin D3 (800 IU/day alone or in combination with 1000 mg/day of calcium in the RECORD or 400 IU/day with 1000 mg/day of calcium in the WHI) in the prevention of the development of T2D (Avenell et al., 2009; de Boer et al., 2008). Several small RCT’s results suggested that significant vitamin D supplementation (either in a single dose of 100,000 IU or 40,000 IU/week for 6 months) on patients with T2D did not improve their glycemic status (Jorde and Figenschau, 2009; Sugden et al., 2008; Witham et al., 2010), but it might improve systolic blood pressure and endothelial function in a short term (Sugden et al., 2008; Weitzman et al., 2010). However, no study has shown worsening pathology of T2D while taking vitamin D.
27.2.4 Vitamin D deficiency and gestational diabetes mellitus

The prevalence rates of GDM vary by region, but are not affected by latitude. A review done by the Vitamin D and Lifestyle Intervention for Gestational Diabetes Mellitus Prevention research group indicated that although the prevalence rates differed by regions in Europe (ranges 2.0-6.0%), the lower prevalence rates of GDM were in the Northern or Atlantic seaboard parts of Europe (<4%); while the higher prevalence rates (>6%) predominated in the South or Mediterranean seaboard regions (Buckley et al., 2011). The results from the hyperglycemia and adverse pregnancy outcome study also showed that the frequency of GDM was not affected by the latitude, for example, those higher than 25% of GDM included those results from Bellflower, USA (33 degree of N) and Singapore (1 degree of N); while those results lower than 12% were from Barbados (13 degree of N) and Beersheba (31 degree of N) (Sacks et al., 2012). However, whether the incidence of GDM differed by season seems unknown.

The results in regard to the relationship between vitamin D deficiency and GDM risk appear mixed with some of them in favor of it (Clifton-Bligh et al., 2008; Soheilykhah et al., 2010) and others not (Baker et al., 2011; Farrant et al., 2009). However, vitamin D deficiency (defined as <50 nmol/l) or insufficiency (<75-80 nmol/l) seems very prevalent in pregnant women (Dror, 2011; Hamilton et al., 2011; Karim et al., 2010; Teale and Cunningham, 2010). The levels of serum 25(OH) D have been observed to be inversely associated with levels of HbA1c among women with GDM and this relationship seemed not to be affected by other known risk factors (Lau et al., 2011). In addition, vitamin D deficiency has been linked to adverse outcomes of pregnancy, such as preeclampsia (Baker et al., 2011; Robinson et al., 2010) and small-for-gestational-age babies (Leffelaar et al., 2010), but this is beyond the scope of our focus here and will be given more detail discussion in other section.

27.3 Vitamin D, bone size and diabetes mellitus

27.3.1 Bone size and diabetes mellitus

Although VDR is present in most tissues and cells in the body, suggesting that vitamin D may have been involved in a wide range of biological actions, its role on calcium/phosphate homeostasis to maintain bone health is considered as the primary and classic function. Interestingly, the relationship between bone size and diabetic risk has long been noticed. For example, the standing height, leg length, the ratio of leg length/height, and femur bone length are all observed to have a negative association with the risk of T2D, GDM, and glucose intolerance (Asao et al., 2006; Brown et al., 1991; Liu et al., 2009; Njolstad et al., 1998; Sayeed et al., 1997; Weitzman et al., 2010). However, in one study this negative relationship was only observed in white but not in black individuals (Weitzman et al., 2010). Bone size and its risk for T1D appear not as sound as that for T2D because most incident T1D patients are still growing children and adolescents (Patterson et al., 2009). Among T1D adults, however, it has been found that short stature is associated with microvascular complications (Waden et al., 2009). One explanation for this observed relationship...
is that these components of stature are indicators of factors acting during early childhood that affect the risk of developing diabetes in later life.

The new findings in bone metabolism suggest that bone is a critical endocrine organ and the molecular products from bone metabolism are involved in the global regulation of energy metabolism (Clemens and Karsenty, 2011). Osteocalcin, an osteoblast-specific protein during bone remodeling, is identified as a common link between bone and glucose metabolism in animal studies (Ferron et al., 2010; Lee et al., 2007). It was found in mice that osteocalcin can not only cause beta cells in the pancreas to release more insulin, but simultaneously, it can also direct fat cells to release more adiponectin, which increases sensitivity to insulin. The results from human studies, however, are mixed and depend on the type of diabetes and the stage. For instance, the low levels of osteocalcin at the baseline among normal males were observed to be associated with an increased risk for incident T2D in a nested case-control study (Ngarmukos et al., 2011). Several studies have observed lower levels of osteocalcin in T1D patients with complications (Pasaoglu et al., 1995) and male T2D patients (Kanazawa et al., 2010). While for pregnant women, the levels of osteocalcin were higher in women with GDM than those without and it was suggested that osteocalcin may enhance insulin secretion in insulin resistance status (Winhofer et al., 2010).

27.3.2 Serum 25(OH)D levels, femur bone size and type 2 diabetes mellitus

It would be too simple to consider that the bone size in the observed risk relationship with diabetes was just a proxy indicator of nutritional factors on early childhood development based on the emerging evidence. Osteocalcin may be the key to explain the observed relationship between bone size and the risk for diabetes, but no study so far has been done to examine the relationship between osteocalcin and bone size.

However, using the data from the national health and nutrition examination survey, we explored the relationship between serum 25(OH) D levels, femur bone size and T2D and found that the serum levels of 25(OH) D and all variables related to diabetes mellitus were negatively related to the gender-specific quintiles of femur bone lengths (Table 27.1) after adjustment for age, ethnicity, smoking, family history of diabetes, family income and waist circumference (Liu et al., 2011).

When analysis was conducted among those without T2D, we also found that the odds ratios of impaired fasting glucose (between 5.6 mmol/l and 6.9 mmol/l; Shields, 2005) for quintiles 2-5 of femur bone lengths in comparison to the reference (quintile 1) were 0.94 (0.68, 1.31), 0.82 (0.59, 1.13), 0.76 (0.55, 1.05), and 0.63 (0.45, 0.86), respectively (P-value for trends <0.001).

VDR gene polymorphisms are found to have a link to adult height (Xiong et al., 2005). It is believed that adequate intake of vitamin D will help not only children to reach their genetically programmed height and peak bone density but also will help adults to prevent osteoporosis (Holick, 2004). To maintain bone health, theoretically, taller adults may need more vitamin D than their shorter counterparts due to their longer bones that requiring more calcium. Since VDR is also found in beta cells, the active role of vitamin D in the functional regulation of the
27. Vitamin D and diabetes mellitus: where are we?

Endocrine pancreas is confirmed (Lee et al., 1994). Therefore, if there is no restriction of the intake of vitamin D, the serum level of 25(OH) D in adults should reflect an ideal level to meet the physiological needs for both bone calcium homeostasis and glucose metabolism. However, in reality, the serum level of 25(OH) D may be lower than the ideal level in many adults because of inadequate intake of vitamin D levels from either exposure to sunlight or dietary sources (Hypponen and Power, 2007; Zadshir et al., 2005).

It is unclear how bone size in adults and vitamin D levels influence diabetic risk development. However, VDR is also expressed in osteoblasts and regulates the expression of several genes in this cell, including osteocalcin (Lee et al., 2000). Although no data has showed how serum levels of osteocalcin are related to adults’ stature, the level of serum osteocalcin is recommended as one of the indicators in treatment monitoring of osteoporosis (Lindsay and Cosman, 2008). In normal children, the serum levels of osteocalcin increase as they grow and the peak value of it occurs when they reach puberty (Cioffi et al., 1997; Kanzaki et al., 1992). The observed negative diabetic risk associations with bone size in adults may be due to the capability of producing osteocalcin which is affected by vitamin D. Poor nutrition, including inadequate intake of vitamin

Table 27.1. Adjusted mean levels of vitamin D and other variables related to diabetes by quintile of femur bone length, the National Health and Nutrition Examination Survey (2001-2002, 2003-2004) (Liu et al., 2011: reproduced with permission).

<table>
<thead>
<tr>
<th>Femur length quintiles</th>
<th>P-value for trends</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Q2</td>
</tr>
</tbody>
</table>

**Males**

<table>
<thead>
<tr>
<th>Range of femur length (cm)</th>
<th>31.0</th>
<th>39.4</th>
<th>41.2</th>
<th>43.0</th>
<th>45.0-53.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25-OH-vitamin D (ng/ml, mean)</td>
<td>23.7</td>
<td>24.2</td>
<td>24.6</td>
<td>25.0</td>
<td>25.4</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl, mean)</td>
<td>110.7</td>
<td>108.6</td>
<td>106.5</td>
<td>104.4</td>
<td>102.3</td>
</tr>
<tr>
<td>Fasting insulin (uu/ml, mean)</td>
<td>11.2</td>
<td>10.8</td>
<td>10.3</td>
<td>9.9</td>
<td>9.4</td>
</tr>
<tr>
<td>Glycohemoglobin (% mean)</td>
<td>5.67</td>
<td>5.61</td>
<td>5.56</td>
<td>5.51</td>
<td>5.45</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.9</td>
<td>10.0</td>
<td>8.4</td>
<td>7.0</td>
<td>5.8</td>
</tr>
</tbody>
</table>

**Females**

<table>
<thead>
<tr>
<th>Range of femur length (cm)</th>
<th>26.4</th>
<th>35.8</th>
<th>37.7</th>
<th>39.4</th>
<th>41.1-50.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25-OH-vitamin D (ng/ml, mean)</td>
<td>22.6</td>
<td>23.2</td>
<td>23.8</td>
<td>24.4</td>
<td>25.0</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl, mean)</td>
<td>102.7</td>
<td>101.2</td>
<td>99.8</td>
<td>98.3</td>
<td>96.8</td>
</tr>
<tr>
<td>Fasting insulin (uu/ml, mean)</td>
<td>10.4</td>
<td>9.9</td>
<td>9.4</td>
<td>8.9</td>
<td>8.4</td>
</tr>
<tr>
<td>Glycohemoglobin (% mean)</td>
<td>5.61</td>
<td>5.55</td>
<td>5.49</td>
<td>5.44</td>
<td>5.38</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8.3</td>
<td>6.9</td>
<td>5.8</td>
<td>4.9</td>
<td>4.1</td>
</tr>
</tbody>
</table>

1 Adjusted for age, current cigarette smoking, ethnicity, blood relatives with diabetes, family annual income and waist circumference.
D in early childhood, affects children’s growth, which may not only let the children have short bone size or short stature, but may also influence their glucose metabolic pathway. Those with serious deficiency of vitamin D may develop diabetes at an earlier age as shown by those Finnish children in whom those with suspected rickets during their first year of life had a 3-times higher risk of developing T1D later on (Hypponen et al., 2001). However, this novel hypothesis needs to be further studied.

27.4 Conclusions

Emerging evidence suggests that vitamin D and diabetes mellitus are linked by biological associations. However, no clear evidence indicates a causal relationship between vitamin D deficiency and diabetes mellitus. In particular, the results from vitamin D supplementation studies are mixed and inconsistent, although no study has shown worsening of the pathology. Most previous studies on vitamin D and diabetes were orientated from the direction of its ‘non-classic’ effect of vitamin D, but the emerging evidence suggests that its ‘classic’ effect, on bone metabolism and via bone mediation, a newly recognized endocrine organ likely involved in energy metabolism and linked to the development of diabetes, may inform the direction of future research.

Acknowledgement

Thanks to Dr. Brian Jeynes for his helping in editing this manuscript.

References


27. Vitamin D and diabetes mellitus: where are we?


27. Vitamin D and diabetes mellitus: where are we?


27. Vitamin D and diabetes mellitus: where are we?


Qiao, Q. and Nyamdorj, R., 2010. Is the association of type II diabetes with waist circumference or waist-to-hip ratio stronger than that with body mass index? European Journal of Clinical Nutrition 64, 30-34.


27. Vitamin D and diabetes mellitus: where are we?


Key facts

- Cardiovascular disease (CVD) is the number one cause of morbidity and mortality in adults in the Western World and is rapidly emerging as a major health concern in developing nations.
- Vitamin D receptors now are known to be found in heart and vascular tissue as well as bone, connective tissue, and many other tissues and organs, where they regulate the expression of multiple genes.
- Vitamin D deficiency is common among subjects at risk for and patients with established CVDs.
- Vitamin D deficiency now has been associated in multiple populations with cardiovascular risk factors and CVDs, including hypertension, obesity, diabetes, hyperlipidemia, coronary artery disease, myocardial infarction, heart failure, renal failure, and cardiovascular death.
- Observational studies to date supporting a role for vitamin D replacement in reducing cardiovascular risk are promising but limited, and randomized trials are needed.

Summary points

- CVD is a leading cause of death and disability worldwide. The discovery of new risk factors and preventive measures for CVD is of major importance.
- Vitamin D deficiency is common among subjects at risk for and patients with established CVDs. In a large healthcare population database, an initial measured 25(OH)D level was ≤30 ng/ml in 64% and, importantly, ≤15 ng/ml (deficient) in 17%.
- Chronic vitamin D deficiency leads to secondary hyperparathyroidism, which acts to increase insulin resistance and pancreatic β-cell dysfunction, activate the renin-angiotensin system, increasing blood pressure, and stimulate vascular inflammation, augmenting atherogenesis.
- A ‘U’ shaped association between vitamin D and health outcomes has been suggested, but both the lower and upper optimal boundaries for 25(OH)D remain controversial. The US Institute of Medicine report suggests that levels >20 ng/ml are adequate with no evidence for greater benefit of >30 ng/ml and possible harm at >50 ng/ml.
- Studies assessing a role for vitamin D replacement to reduce cardiovascular risk are limited, with some but not all suggesting benefit.
- Given inconclusive evidence, randomized clinical trials are needed to demonstrate that supplementation can play a major role in reducing cardiovascular risk factors and preventing or treating CVD. One trial, VITAL, in 20,000 subjects is underway. However, given design limitations of VITAL, other trials may be required.
- Pending additional evidence, either an empiric approach to lifestyle and Vitamin D supplementation or selected screening and treating to target may be considered.
Abstract

Cardiovascular disease (CVD) is a leading cause of death and disability worldwide. The discovery of new risk factors and preventive measures for CVD is of major importance. Vitamin D receptors now are known to exist in heart and vascular tissue as well as bone, connective tissue, and many other tissues and organs, where they regulate the expression of multiple genes. Low or deficient levels of vitamin D are common among subjects at risk for and patients with established cardiovascular diseases. Importantly, vitamin D deficiency now has been associated in multiple populations with cardiovascular risk factors and cardiovascular diseases, including hypertension, obesity, diabetes, hyperlipidemia, coronary artery disease, myocardial infarction, heart failure, renal failure, and cardiovascular death. Multiple mechanisms relating vitamin D deficiency and CVD have been proposed, including secondary hyperparathyroidism. Studies to date supporting a role for vitamin D replacement to reduce CV risk are promising but limited and mostly observational, and not all recent evidence has been supportive of either CVD associations or benefits of vitamin D supplementation. Hence, randomized trials, such as VITAL, now underway, will be needed to firmly establish in whom and how to supplement vitamin D to improve CV health and reduce risk. A ‘U’ shaped association for vitamin D and health outcomes has been suggested, but both the lower and upper boundary of the optimal range for 25(OH)D levels for CV health remain controversial. The US Institute of Medicine report suggests a range 20-50 ng/ml as adequate and safe, but additional evidence is needed specific to CV health. Pending additional clinical trials evidence, both an empiric approach to lifestyle and supplementation or selected screening and treating to target may be considered. In conclusion, vitamin D deficiency has been shown to be associated with CV risk and disease, but more robust evidence is needed to conclusively demonstrate that vitamin D supplementation can play a major role in preventing or treating CVD.

Keywords: coronary artery disease, diabetes, heart failure, hypertension, mortality
28.1 Vitamin D: a brief overview

Beyond bone health, the role of vitamin D in general health and a broad spectrum of common disorders has been the focus of great interest in the past few years and of several excellent contemporary reviews (Holick, 2007; Lee et al., 2008; McGreevy and Williams, 2011; Wallis et al., 2008). Among these areas of interest is the role of vitamin D in CV health and disease (Lavie et al., 2011; McGreevy and Williams, 2011; Wang et al., 2008). CVD is the leading cause of morbidity and mortality in adults in the Western World and is rapidly emerging as a major entity in the developing world (Roger et al., 2012; Yusuf et al., 2001). Hence, a better understanding of underlying risk factors for CVD and new approaches to prevention are of great interest. Vitamin D is a vitamin whose functions simulate those of a hormone. It is derived from cholesterol; it requires 2-3 organs for activation (skin [except for dietary sources], liver, and kidney); VDR (principally in nuclei of target cells) are found in multiple cells and tissues, including cardiac myocytes and vascular tissue, and most organs. vitamin D works systemically on these multiple tissues/organs (e.g. bone/connective tissue, muscle, kidney, pancreas, parathyroid, brain, gonads, blood vessels, heart, etc.) where it modulates expression of a vast collection of genes (estimated at >200). Hence, variations in vitamin D availability have the potential to impact function, and deficiency to cause dysfunction, of multiple organ systems besides bone and connective tissue, including the CV system.
28. Vitamin D and cardiovascular risk: hype or new hope?

28.2 Metabolic forms

Vitamin D, synthesized in the skin from cholesterol in response to sunlight (UVB), is hydroxylated in the liver to 25(OH)D (McGreevy and Williams, 2011). 25(OH)D serves as the principal circulating and storage form of vitamin D and is generally accepted as the best measure of total body vitamin D stores. 25(OH)D is commonly measured in serum or plasma to assess vitamin D status. The kidney (primarily) and other organs further hydroxylate 25(OH)D to 1, 25(OH)2D, the active form of vitamin D. 1,25(OH)2D is an important regulator of PTH secretion (Holick, 2007).

28.3 Selected non-skeletal functions

VDR activation in the intestine, bone, kidney, and parathyroid gland cells is well recognized to be essential to calcium absorption and the maintenance of calcium and phosphorus levels in blood (in association with PTH and calcitonin) and of bone mineral content. Increasingly, important functions of vitamin D outside of the skeletal system are being recognized and are summarized in other chapters; these sites include pancreas, breast, colon, prostate, and the immune system (e.g. lymphocytes) (Holick, 2007).

28.4 Defining vitamin D deficiency

An important prerequisite to identifying populations at risk is to accurately define vitamin D deficiency. Unfortunately, defining vitamin D deficiency evokes significant controversy (Dawson-Hughes et al., 2010; Kennel et al., 2010; McGreevy and Williams, 2011; Ross et al., 2011). Commonly, 25(OH)D sufficiency has been defined as ≥30 ng/ml (≥75 nmol/l), i.e. with a normal range of 30-74 ng/ml, insufficiency (mild deficiency) as 20-29 ng/ml, moderate deficiency as 10-19 ng/ml, and severe deficiency as <10 ng/ml, and toxicity as >100-150 ng/ml. One school of thought has contended that exposure of humans in native, outdoor settings generally results in plasma 25(OH)D levels of 40-80 ng/ml. However, a recent (US) IOM report concluded that a serum 25(OH)D level of 20 ng/ml (50 nmol/l) is adequate to maintain bone and overall health, that concentrations above 30 ng/ml are ‘not consistently associated with increased benefit’, and that levels above 50 ng/ml (125 nmol/l) may be a cause for concern (Ross et al., 2011). As will be seen, the choice of ≥20 versus ≥30 ng/ml as a definition for 25(OH)D sufficiency makes a major difference in the size of the population at risk and the target for supplemental treatment.

One approach to defining 25(OH)D deficiency is to note the concentration at which levels of PTH rise, as PTH is negatively regulated by 25(OH)D. PTH rises modestly as 25(OH)D levels decline from 30 to 20 ng/ml, then briskly at 25(OH)D levels ≤15 ng/ml (Zitterman, 2006).
28.5 Vitamin D deficiency: an international epidemic?

The major portion of circulating vitamin D is supplied by exposure to sunlight, with a minor contribution coming from common dietary sources. Several features of geography and modern lifestyle have converged to limit average exposure to sunlight and to contribute to a growing population trend toward vitamin D deficiency (Table 28.1). In addition, genetic factors may add to inter-individual variability in levels given the same dietary intake and sunlight exposure (Shirts et al., 2012; Wang et al., 2010).

In the setting of these and other contemporary lifestyle and geographic factors, an important percentage (i.e. 24-93%) of various population groups has been reported to be vitamin D deficient (Anderson et al., 2010; Lee et al., 2008; Tangpricha et al., 2002; Wang et al., 2008).

It should be noted that levels of <30 ng/ml have often been defined as deficient in many reports, whereas levels of <10-20 ng/ml may be more relevant to disease predisposition based on the recent IOM report (Ross et al., 2011) and affect a smaller (though still important) segment of these populations. A Europe-wide survey reported a prevalence of severe vitamin D deficiency (<25 nmol/l=<10 ng/ml) in 2% to 30% of adults, but this increased to 75% or more in older, institutionalized persons (Lips, 2001). These very deficient levels are particularly prevalent in women in South Asia and the Middle East where cultural dress limits sun exposure and where extended periods of breastfeeding without vitamin D supplementation are common (Mithal et al., 2009). In our experience in the United States, of 41,504 subjects in the Intermountain Healthcare eMR database with a measured vitamin D level, 64% had an initial level ≤30 ng/ml, and 17%, ≤15 ng/ml.

Table 28.1. Factors predisposing to vitamin D deficiency.

<table>
<thead>
<tr>
<th>Advanced age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized, institutionalized, homebound persons</td>
</tr>
<tr>
<td>Indoor lifestyle and sun avoidance</td>
</tr>
<tr>
<td>Sun protection (sunscreen, heavy clothing)</td>
</tr>
<tr>
<td>Darker skin and higher latitudes (changes in traditional racial/ethnic geographies)</td>
</tr>
<tr>
<td>Obesity, diabetes</td>
</tr>
<tr>
<td>Decreased fortified milk consumption</td>
</tr>
<tr>
<td>Smoking, air pollution</td>
</tr>
<tr>
<td>Kidney disease, liver disease, malabsorption syndromes</td>
</tr>
</tbody>
</table>
28.6 Vitamin D and disease associations

In recent years, a number of disease associations with vitamin D deficiency beyond osteoporosis and myalgias have been described. These include various cancers (e.g. colorectal, breast, prostate), chronic kidney disease, types 1 and 2 diabetes mellitus, hypertension, obesity, rheumatoid arthritis, multiple sclerosis, depression and cognitive impairment, and CV diseases (Holick, 2007; Lee et al., 2008; McGreevy and Williams, 2011; Wallis et al., 2008; Wang et al., 2008). This chapter now will focus on potential links of vitamin D deficiency to CV risk and CVD.

28.7 Proposed mechanisms of cardiovascular risk

A leading explanation for a relationship between vitamin D deficiency and CVD is that chronic vitamin D deficiency leads to secondary hyperparathyroidism, which then can act through at least 3 pathogenic pathways to increase CV risk (Lee et al., 2008; Wallis et al., 2008): (1) increased insulin resistance and pancreatic β-cell dysfunction, predisposing to the metabolic syndrome and diabetes; (2) activation of the renin-angiotensin system, increasing blood pressure and leading to left ventricular hypertrophy (with subsequent myocyte apoptosis and cardiac fibrosis); and (3) stimulation of systemic and vascular inflammation, augmenting atherogenesis. These relationships are shown in Figure 28.1.

However, PTH elevation appears to explain only part the association of CV risk with vitamin D deficiency. In a large (9,369) study in a general healthcare population, we found that PTH

![Diagram of proposed mechanisms of vitamin D deficiency-related cardiovascular risk](image-url)
correlated only weakly \((r=-0.15)\) with \(25(OH)D\), with both PTH and \(25(OH)D\) contributing independently to CV risk and disease prediction (Anderson \textit{et al.}, 2011). Similarly, Kestenbaum \textit{et al.} (2011) found vitamin D and PTH to contribute independently to CV risk.

One proposed non-PTH related CVD mechanism is an association with large high-density lipoprotein particles (Kazlauskaite \textit{et al.}, 2010). Further, \(1,25(OH)_2D\) has been reported to inhibit foam cell formation and suppress macrophage cholesterol uptake in patients with type 2 diabetes (Oh \textit{et al.}, 2009). Additional recent evidence suggests that vitamin D deficiency may lead to endothelial dysfunction and arterial stiffness and that supplementation may improve vascular function (McGreevy and Williams, 2011). Other molecular mechanisms of CVD likely await discovery.

To determine whether the vitamin D deficiency-related increase in coronary risk is associated with progression of atherosclerosis or with plaque instability, we measured \(25(OH)D\) levels in 3,413 consecutive patients participating in the Intermountain Heart Collaborative Study Registry who were undergoing coronary angiography (May \textit{et al.}, 2010). No association was found between the severity of angiographic CAD and vitamin D status, whereas in the same population, vitamin D deficiency was associated with an increase in incident CV events, including MI. These findings support the hypothesis that vitamin D plays a role in plaque stability but not in plaque growth.

### 28.8 Reported associations between vitamin D deficiency and cardiovascular risk

Primarily within the past 5-8 years, important CV risk associations with vitamin D deficiency have been forthcoming. The first important CVD associations emerged from observations in patients with end-stage chronic kidney disease on dialysis. Two-fold increases in CV mortality and total mortality were noted in those with \(25(OH)D\) levels <10 ng/ml, whereas active vitamin D therapy was associated with a substantially reduced mortality risk (Wolf \textit{et al.}, 2007). A pair of studies in the Archives of Internal Medicine in 2008 associated vitamin D deficiency with CV risk: Giavanucci \textit{et al.} (2008) found an association of \(25(OH)D\) deficiency and risk of MI in men. Dobnig \textit{et al.} (2008) reported that low \(25(OH)D\) and \(1,25(OH)_2D\) levels independently were associated with all-cause and CV mortality in a cohort study of 3,258 men and women scheduled for coronary angiography. Hazard ratios for quartiles 1 (median \(25(OH)D\) 7.6 ng/ml) and 2 (median \(25(OH)D\) 13.3 ng/ml) for CV death were 2.2 and 1.8, respectively (both \(P<0.01\)), compared to quartile 4 (median \(25(OH)D\) 28.4 ng/ml). A cross-sectional study examined the burden of CVD associated with hypovitaminosis D using data from the 2001-2004 NHANES. The prevalence of \(25(OH)D\) <30 ng/ml in 8,351 adults with measured levels was 74% (Kim \textit{et al.}, 2008). The burden of all CVD, and coronary heart disease, heart failure, stroke, and peripheral vascular disease, increased (by 1.5 to 2-fold) with decreasing \(25(OH)D\) categories (≥30, 20-29, <20 ng/ml). In a Framingham offspring study, vitamin D deficiency (≤15 ng/ml) was found to increase the risk of incident CV events during 7 years of follow-up by 2-2.5 fold, with an especially large absolute increment in risk in hypertensive subjects (Wang \textit{et al.}, 2008).
Stimulated by these initial reports, we undertook a prespecified analysis of data within Intermountain Healthcare’s eMR database (Anderson et al., 2010). We identified 41,497 patients in whom at least one serum 25(OH)D level was measured (levels were drawn at healthcare providers’ discretion for the usual indications). The distribution of 25(OH)D levels was found to be >30 ng/ml in 36%, 16-30 ng/ml in 47%, and ≤15 ng/ml in 17%. The baseline prevalence of hyperlipidemia and especially hypertension (39.8% to 43.8% to 51.9%) and diabetes (15.4% to 20.0% to 29.3%) increased in stepwise fashion with decreasing serum 25(OH)D level category in the cohort of subjects 50 years of age or older (n=27,686) evaluated for CV conditions (all $P<0.001$). During subsequent follow-up (average 1.3, maximum 9.3 years), the risk of new onset (incident) hyperlipidemia (HR 1.27, $P=0.003$) and especially hypertension (HR 1.62, $P<0.001$) and diabetes (HR 1.89, $P<0.001$) was increased in those with very low (≤15 ng/ml) compared to those with normal levels (Table 28.2). Similarly, a low level of 25(OH)D was associated with CVD outcomes, including incident CAD/MI and especially heart failure, stroke, and death (Table 28.3); survival curves by 25(OH)D category are shown in Figure 28.2.

Table 28.2. Incidence of cardiovascular risk factors by baseline 25(OH)D levels in those without prior condition (reprinted from Anderson et al., 2010).

<table>
<thead>
<tr>
<th>Risk factor (overall incidence)</th>
<th>Very low vs. normal (≤15 vs. &gt;30 ng/ml)</th>
<th>Low vs. normal (16-30 vs. &gt;30 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td>Hypertension [6.0%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>1.73</td>
<td>1.62</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.48-2.02</td>
<td>1.38-1.89</td>
</tr>
<tr>
<td>$P$-value</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidemia [5.8%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>1.47</td>
<td>1.27</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.25-1.72</td>
<td>1.09-1.50</td>
</tr>
<tr>
<td>$P$-value</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetes mellitus [2.2%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>2.13</td>
<td>1.89</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.73-2.62</td>
<td>1.54-2.33</td>
</tr>
<tr>
<td>$P$-value</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peripheral vascular disease [0.8%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>1.80</td>
<td>1.42</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.32-2.46</td>
<td>1.04-1.94</td>
</tr>
<tr>
<td>$P$-value</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Follow-up averaged 1.3 years (maximum, 9.3).

<sup>a</sup> Comparisons also significant after Bonferroni correction (i.e. adjusted for 8 risk factor comparisons).
In support, a review of the literature relating to vitamin D and CV risk through February 2012 by Wang et al. (2012) identified 19 studies involving 6,123 CV disease cases in 65,994 subjects. Comparing the highest with lowest vitamin D quantiles, vitamin D deficiency was associated with a RR of total CVD of 1.53, CV mortality RR=1.44, coronary heart disease RR=1.38, and stroke RR=1.64. CVD risk increased monotonically with decreasing 25(OH)D levels <75 nmol/l (30 ng/ml) by RR=1.03-1.07 for each 25 nmol/l (10 ng/ml) decrement in 25(OH)D.

Table 28.3. 25(OH)D levels and incident cardiovascular diagnoses and mortality. Outcomes are adjusted for multiple baseline characteristics (Anderson et al., 2010).

<table>
<thead>
<tr>
<th>Outcomes (patients ≥50 years old)</th>
<th>≤15 vs. &gt;30 ng/ml</th>
<th>16-30 vs. &gt;30 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death (n=27,686)</td>
<td>HR=1.77, P&lt;0.0001</td>
<td>HR=1.20, P=0.009</td>
</tr>
<tr>
<td>CAD/MI (n=21,853) sup1</td>
<td>HR=1.45, P&lt;0.0001</td>
<td>HR=1.15, P=0.09</td>
</tr>
<tr>
<td>Heart failure (n=23,793)</td>
<td>HR=2.01, P&lt;0.0001</td>
<td>HR=1.31, P=0.005</td>
</tr>
<tr>
<td>Stroke (n=26,025)</td>
<td>HR=1.78, P=0.004</td>
<td>HR=1.31, P=0.11</td>
</tr>
<tr>
<td>Atrial fibrillation (n=24,565)</td>
<td>HR=1.02, P=0.87</td>
<td>HR=0.95, P=0.61</td>
</tr>
</tbody>
</table>

1 CAD = coronary artery disease; MI = myocardial infarction. Numbers (n) represent population database with information available for 25(OH)D levels and each diagnosis of interest.

Figure 28.2. Survival by initial 25(OH)D levels (Anderson et al., 2010; reprinted with permission).
28.9 Can vitamin D supplementation reduce cardiovascular risk?

Trials of dietary supplementation with several other vitamins and nutrients to improve general and CV health generally have a dismal record, with multiple failures. As questioned by one editorialist: ‘Why vitamin D if not vitamin A, B, C, or E?’ (Byers, 2010) One might add folic acid and, given vitamin D’s actions as a hormone, hormone (estrogen=progestin) replacement therapy. An axiom of essential nutrients has been used as an argument in favor of vitamin D as an exception to this otherwise generally negative experience: supplementing a normal nutrient level to a supra-physiologic range may result in neutral to harmful effects, whereas repleting a deficiency may confer benefit. In affluent Western societies, true deficiencies of vitamins A, B, C, and E, and folic acid (given current food supplementation), are rare. In contrast, the prevalence of low to very low levels of vitamin D is common and increasing. Nevertheless, given this generally negative experience with intervention trials with other vitamins, a healthy skepticism is appropriate in considering the potential of vitamin D supplementation to reduce CV risk. Observational data and controlled trials results for vitamin D supplementation currently are quite limited and do not provide a definitive answer for CV prevention.

In a historical cohort study in a large dialysis database, Teng et al. (2005) observed that IV vitamin D supplementation (n=37,173), compared to no supplementation (n=13,864), was associated with half the rate of overall mortality (13.8% vs. 26.8%) and CV mortality (7.6% vs. 14.6%). Similarly, Wolf et al. (2007) observed low all-cause and CV mortality in a dialysis patient cohort that was treated with active vitamin D therapy. In a 2007 report, Autier and Gandini (2007) summarized results of 9 randomized studies of differing size, design, study populations, and with generally limited numbers of events, in which patients received vitamin D supplements or control therapy. A modest survival benefit (relative risk 0.92) was suggested. In a more recent meta-analysis, which included 17 prospective cohort and randomized studies, Wang et al. (2012) found that moderate to high supplemental doses of vitamin D may reduce the risk of CV disease, with benefit primarily seen in dialysis patients.

While awaiting prospective randomized trials, we undertook a quasi-intervention trial based on Intermountain healthcare eMR records data for 7,515 adults with an initial and at least one follow-up 25(OH)D level at least one year apart (Bair et al., 2010). The last follow-up level used was either the first level when 25(OH)D was normalized (≥30 ng/ml) or the last level obtained. Subsequent patient follow-up averaged 2.5 years (maximum, 5.5 y). Cox regression was used to compare survival in those with normalized (presumably by supplementation) or not, adjusted for outcomes of death, incident myocardial infarction or a coronary artery disease diagnosis, heart failure, stroke, or clinical renal failure. Normalization of vitamin D was associated with reduced hazards of composite CV events (HR=0.80, P<0.05), death (HR=0.77, P<0.05), CAD (HR=0.85, P<0.05), MI (HR=0.75), heart failure (HR=0.83, P<0.05), and renal failure (HR=0.76, P<0.05), with no effect on stroke (HR=0.97). The survival curve for the composite outcome is shown in Figure 28.3.
VITAL (VITamin D and OmegA-3 TraiL) represents a major intervention trial of vitamin D and omega-3 fatty acid supplementation. Funded by the National Institutes of Health and coordinated by Harvard Medical School/Brigham and Women’s Hospital, VITAL was launched in January 2010 with a target enrollment of 20,000. Entry requirements are ages ≥60 in men, ≥65 in women, no history of cardiac disease or cancer, and not taking major supplemental doses of vitamin D (>800 U/day) or calcium (>1,200 mg/d). Interventions include vitamin D3 2,000 U daily (or placebo) and omega-3 fatty acids (Omacor® fish oil) 1000 mg daily (or placebo). The primary objectives of VITAL are to determine whether vitamin D or omega-3 fatty acids can prevent cancer, heart disease, and stroke. However, design issues in VITAL (e.g. inclusion of a general population rather than those selected for true deficiency, and insufficient supplementation) may leave the question of whom/how to treat unanswered, and other trials may be required, and other intervention trials of vitamin D supplementation are in planning or enrollment stages.

28.10 Not all the evidence is positive

Not all recent evidence has been supportive of either CV disease associations or the benefits of vitamin D supplementation. A systematic review of 13 observational studies reported that the associations between vitamin D status and cardiometabolic outcomes (i.e. hypertension, diabetes, CV disease) were heterogeneous and the implications uncertain (Pittas et al., 2010). Another recent overview included 51 trials and reported that the evidence that vitamin D status and vitamin D supplementation could be used to reduce CV risk and mortality was inconclusive (Elamin et al., 2010).
28. Vitamin D and cardiovascular risk: hype or new hope?

Low dose vitamin D plus calcium supplements did not improve CV outcomes in post-menopausal women in the Women's Health Initiative (Hsia et al., 2007). Among patients with chronic kidney disease, 48 weeks of supplementation with paricalcitol did not improve measures of LV mass or diastolic function (Thadani et al., 2012).

28.11 Pending prospective trials, empiric therapy or treat to target?

The recent IOM report for population-based vitamin D supplementation to achieve adequate 25(OH)D levels (defined as >20 ng/ml) recommended 600 IU daily for ages ≤70 and 800 IU daily for ages >70 (Ross et al., 2011). These doses were projected to meet minimal requirements for at least 97.5% of the population. In support, a placebo-controlled randomized trial in 163 post-menopausal women found that supplementation with 800 IU/d of vitamin D3 achieved 25(OH)D levels of ≥50 nmol/l (20 ng/ml) in 97.5% of women (Gallagher et al., 2012). Pending additional clinical trials data, this general empiric approach to overall health maintenance (including CV health) is reasonable.

An alternative interim approach is to measure baseline 25(OH)D levels in subjects at risk of vitamin D deficiency and its associated morbidities and supplement to greater than a minimum target serum 25(OH)D level, (i.e. >20-30 ng/ml, and <50-70 ng/ml). Some of the conditions predisposing to vitamin D deficiency include: darker skin; indoor lifestyle, sun avoidance; high latitudes; smoking, air pollution; advanced age, especially with other risk factors or conditions; bone/muscle/joint complaints (myalgias, arthralgias, osteopenia, osteoporosis); obesity, diabetes; hypertension, coronary disease, heart failure; hospitalized, institutionalized, and home-bound persons, nursing home residents; infectious diseases, immune deficiencies; chronic kidney or liver disease (McGreevy and Williams, 2011).

28.12 Testing for vitamin D, costs and coverage

Vitamin D currently is one of the most commonly ordered ‘esoteric tests’. A typical outpatient laboratory charge for serum 25(OH)D ranges widely from $50-$300. On-line or wholesale prices may be as low as $22-$35. Medicare reimbursement at the time of this writing is $43, but reimbursement is limited to specific traditional indications, including myalgias, disorders of bone and cartilage, osteoporosis, renal insufficiency, and vitamin D deficiency. CV risk assessment and CV disorders/diseases are not covered. Coverage by individual private insurance companies varies, but many follow Medicare guidelines. These cost and reimbursement considerations should be kept in mind when considering 25(OH)D measurements for the purpose of CV risk assessment.
28.13 **Supplementing vitamin D**

With a goal of supplementing deficient 25(OH)D levels to >20-30 ng/ml (but to <50 ng/ml? (Ross *et al.*, 2011)), vitamin D supplements are available as vitamin D2 (ergocalciferol), a plant-based form, and vitamin D3 (cholecalciferol), an animal-based and generally preferred form. If following general IOM recommendations, daily doses are 600 IU for ages ≤70 and 800 IU for ages >70. For treatment of identified vitamin D deficiency (i.e. <10-20 ng/ml), initial ‘loading’ may be given as 5,000 IU/d for 2-4 months, followed by an initial maintenance dose of 1000–2,000 IU daily with retesting to establish achievement of target levels (Holick, 2007; Kennel *et al.*, 2010). The IOM report gives 4,000 IU/d in adults as the upper dosing limit to avoid potential adverse effects (Ross *et al.*, 2011).

28.14 **Can too much vitamin D be harmful?**

The IOM report concluded that available scientific evidence supports a key role of calcium and vitamin D in skeletal health, consistent with a cause-and-effect relationship, but found that for extraskeletal conditions, including CV diseases, diabetes, and cancer, the evidence was insufficient and inconclusive enough to inform specific vitamin D nutritional requirements (Ross *et al.*, 2011). Further, their review suggested that higher values were not consistently associated with greater benefit and that for some outcomes a U-shaped association appeared to exist (including CV disease, vascular calcifications, frailty/falls, pancreatic cancer, and all-cause mortality) such that increased risk was observed at both high and low levels, with levels >50 ng/ml raising concerns (Melamed *et al.*, 2008; Ross *et al.*, 2011; Wang *et al.*, 2008).

Evidence for a vascular pro-inflammatory response, as determined by high-sensitivity C-reactive protein levels in an NHANES population, was recently reported at both low (<20 ng/ml) and also high (>50 ng/ml) 25(OH)D levels (Amer and Qayyum, 2012). In our Intermountain Healthcare database, an increased risk of atrial fibrillation was suggested at high (>100 ng/ml) 25(OH)D levels (Smith *et al.*, 2011).

In contrast, frank vitamin D toxicity is rare and is typically manifested by symptoms of hypercalcemia. Most reports of vitamin D toxicity have been associated with 25(OH)D levels of 150 ng/ml or greater, with 80 ng/ml being the lowest reported level associated with toxicity (Jones, 2008; Kennel *et al.*, 2010).

28.15 **Vitamin D and cardiovascular risk: conclusions**

Beyond bone health, the role of vitamin D in general health and a broad spectrum of common disorders has been the focus of great interest in the past few years, and a rapid growth in our knowledge base about vitamin D has occurred, but major gaps remain. Among new areas of interest is the role of vitamin D in CV health and disease. Emerging science indicates that vitamin
28. Vitamin D and cardiovascular risk: hype or new hope?

D is a hormone that acts on receptors in multiple organs and tissues. Vitamin D originates primarily by synthesis in the skin in response to sun exposure, and deficiency is a common consequence of our modern lifestyle in the general population and in CV patients. Growing evidence associates vitamin D deficiency with CV risk factors and incident CVD. Observational evidence generally supports the use of vitamin D supplementation in truly deficient patients to reduce CV risk, but the evidence is inconclusive, and randomized trials are needed and are underway. However, a 'U-shaped' benefit-risk association appears to exist for vitamin D, as for other vitamins/supplements, so that repletion of deficiency, but not supplementation to supra-normal levels, should be targeted. Currently, either an empiric approach to lifestyle and supplementation or selected screening and treatment to target may be considered pending more definitive clinical trials evidence. In summary, more robust evidence is needed to conclusively demonstrate that vitamin D supplementation can play a major role in reducing CV risk factors and in preventing or treating CVD. So, is vitamin D mostly hype or a new hope for CV prevention (Lavie et al., 2011)? It probably is some of each, which ongoing and future research must clarify (Lavie et al., 2011).

References


28. Vitamin D and cardiovascular risk: hype or new hope?


Key facts

- It is important to correct 25(OH)-vitamin D (25(OH)D) insufficiency after transplantation since 25(OH)D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues.
- Vitamin D insufficiency is frequent in transplant recipients (TR) and concerns currently more than 85% of adult renal TR.
- Increased 25(OH)D catabolism induced by immunosuppressive drugs and sun exposure avoidance recommended to TR may explain the frequency of vitamin D insufficiency after transplantation and the need for high dosage of native vitamin D in this population.
- In the field of transplantation, experimental studies focused on the potential protective role for vitamin D against acute rejection and clinical studies are mainly observational, except for those concerning prevention of bone loss and two small studies concerning immunomodulatory effects of calcitriol after renal transplantation.
- Two randomized control studies are actually undergoing to test the effect of cholecalciferol after renal transplantation on patients and renal grafts’ outcomes.

Summary points

- Vitamin D has been shown to play an important role in reducing risk of many chronic diseases including osteoporosis, type 2 diabetes mellitus (DM), cardiovascular diseases, cancers and to have immunomodulatory effects.
- Due to specific and non-specific risk factors, TR are at high risk of many diseases such as osteoporosis, type 2 DM, cardiovascular diseases, cancers, and acute graft rejection.
- Although vitamin D supplementation has been studied after renal transplantation, the optimal scheme for vitamin D supplementation remains to be determined after lung, liver and cardiac transplantation.
- Immediately after transplantation, randomized trials showed that active form of vitamin D could prevent or attenuate lumbar spine and femoral neck bone loss.
- After renal transplantation, low serum 25(OH)D correlates with hyperparathyroidism and efficient treatment of vitamin D insufficiency with cholecalciferol was shown to reduce serum parathormone concentration in renal TR.
- No study reported the potential effect of activated or native vitamin D on de novo type 2 DM or on cardiovascular risk after transplantation.
- A higher incidence of post-transplant cancers was observed in patients with pre-transplant 25(OH)D concentrations less than 10 ng/ml but this result remains to be confirmed in an interventional randomized trial.
- Many experimental data showed that vitamin D could prolong graft survival or prevent acute rejection in animal models without increasing the risk of fungal or viral infection and two small interventional studies in TR confirmed the immunomodulatory effect of active vitamin D after transplantation.
- A number of clinical studies have confirmed the potent renoprotective and antiproteinuric effect of vitamin D but this point remains to be confirmed in TR.
29. Vitamin D and transplantation

M. Courbebaisse¹, J.C. Souberbielle² and E. Thervet³

¹Service de Physiologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France; ²Service de Physiologie, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75015 Paris, France; ³Service de Néphrologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France; marie.courbebaisse@egp.aphp.fr

Abstract

Mainly due to immunosuppressive treatments and sun exposure avoidance, transplant recipients are at high risk of vitamin D insufficiency (25(OH)-vitamin D<30 ng/ml). Besides its major role for bone health, vitamin D has been shown to play an important role in reducing risk of many chronic diseases including cancers, type 2 diabetes mellitus, chronic kidney disease, cardiovascular diseases and infectious diseases. All these diseases are more likely to occur in transplant recipients, who have induced immunodeficiency, than in the general population. It is therefore of great importance to correct vitamin D insufficiency after transplantation as 25(OH)-vitamin D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues. The pleiotropic effects of vitamin D are mostly documented by observational and experimental studies or by small interventional trials that most often evaluated intermediate parameters. In the field of transplantation, experimental studies focused on the potential protective role for vitamin D against acute graft rejection and clinical studies are mainly observational, except for those concerning prevention of bone loss. Indeed, immediately after transplantation, randomized trials showed that active form of vitamin D could prevent or attenuate lumbar spine and femoral neck bone loss. However, randomized interventional trials are still needed before one may consider that non classical effects of vitamin D that, for most, are especially relevant for the care of transplant patients, really apply to transplant recipients. Actually, two randomized interventional trials are undergoing to test the effects of high doses of cholecalciferol after renal transplantation on: (1) graft function, incidence of acute rejection episodes and post-transplant infections within the first year after transplantation for VITA-D study; (2) a composite endpoint consisting in de novo diabetes mellitus, cardiovascular diseases, de novo cancer and patient death after the first year post-transplantation for VITALE study.

Keywords: graft, acute rejection, immunosuppressive treatment
Introduction

Traditionally, vitamin D has been associated with bone health: vitamin D deficiency leads to rickets in children and osteomalacia in adults and increases the risk of osteoporosis. More recently, vitamin D has been shown to play an important role in reducing risk of many chronic diseases including type 2 DM, cardiovascular diseases, cancers and to have immunomodulatory effects. All these diseases are more likely to occur in TR than in the general population. Moreover, vitamin D insufficiency is a frequent finding in TR, due to multiple causes, including immunosuppressive treatments and sun exposure avoidance. Although the data presented here were generally obtained in non-transplant patients, this chapter will put into perspective the potential actions of vitamin D in TR. However, we must acknowledge that intervention trials are needed before one may consider that these effects really apply to TR.

Vitamin D insufficiency after transplantation

Especially after kidney transplantation, vitamin D in its active form is currently used for the prevention of post-transplant bone loss (Palmer et al., 2007) and in the treatment of normocalcemic persistent secondary hyperparathyroidism (De Sevaux et al., 2002; El-Agroudy et al., 2003; Lobo et al., 1995; Torres et al., 2004). However, one has to keep in mind, as suggested in a recent review (Thiem and Borchhardt, 2011), that treatment with active vitamin D and its
analogues will not compensate for inadequate 25(OH)D status. It is thus still important to correct 25(OH)D insufficiency since 25(OH)D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues. Hence, these extra-renal tissues are dependent on adequate 25(OH)D substrate for adequate local calcitriol production. Although there is no current consensus, vitamin D insufficiency is defined as 25(OH)D levels lower than 30 ng/ml (or 75 nmol/l) (Dawson-Hughes et al., 2005) because this limit is associated with a decrease in active calcium intestinal absorption (Heaney, 2003) and an increase in PTH secretion aiming at maintaining normal serum calcium level (Dawson-Hughes et al., 2005). Furthermore, in interventional studies showing positive effects of vitamin D supplementation, the 25(OH)D levels reached in the treated groups were generally higher than 30 ng/ml (Bischoff-Ferrari et al., 2006). Regarding the upper limit, vitamin D intoxication does not occur if 25(OH)D concentration remains less than 150 ng/ml (Vieth et al., 2001; Zittermann, 2003).

Vitamin D insufficiency is frequent in TR and concerns currently more than 85% of adult renal TR (Querings et al., 2006; Sadlier and Magee, 2007; Stavroulopoulos et al., 2007). Vitamin D insufficiency is also frequent in paediatric renal TR (Shroff et al., 2011; Sgambat et al., 2011). Moreover, vitamin D insufficiency is also frequently found after heart, liver or lung transplantation (Stein et al., 2009; Segal et al., 2001; Lowery et al., 2012). Various causes may be implicated: (1) insufficient vitamin D supplementation before and after transplantation; (2) increased 25(OH)D catabolism induced by immunosuppressive drugs (Pascussi et al., 2005; Akeno et al., 2000) and, after renal transplantation, by post-transplant persistent fibroblast growth factor-23 hypersecretion (Bhan et al., 2006; Pande et al., 2006; Saito et al., 2003); and (3) reduced sun exposure recommended to TR to prevent skin cancers. Serum 25(OH)D levels were analysed in 31 renal TR, who all protected themselves from sun exposure, compared to an age- and gender-matched control group without renal disease or other diseases requiring sun protection at the end of winter. Serum 25(OH)D levels were significantly lower in renal TR compared to controls, with 10 out of 31 having undetectable serum 25(OH)D levels (Querings et al., 2006). It is generally accepted that, due to immunosuppression, TR are at increased risk for UV-induced non-melanoma skin cancers but also for developing malignant melanoma (Reichrath et al., 2010). As a result, TR are now systematically advised to protect themselves from exposure to solar or artificial UV-radiation. However, this represents a serious dilemma, for appr. 80 to 90% of the human body's requirements in vitamin D have to be photosynthesized in the skin from 7-dehydrocholesterol by the action of UVB-radiation. Therefore, due to the lack of solar UV-exposure, careful monitoring of vitamin D status and oral substitution in case of vitamin D insufficiency is of high importance for TR.

**29.3 Vitamin D supplementation after transplantation**

Despite the high prevalence of vitamin D insufficiency in TR, there is no general consensus regarding vitamin D supplementation after transplantation. It was shown that high doses of vitamin D3 (cholecalciferol; 100,000 IU every other week during 2 months, equivalent to 6,600 IU/day) were able to correct 25(OH)D insufficiency in renal TR, without inducing side effects,
and were associated with a significant decrease in serum PTH concentration. However, this study also indicated that the dose of cholecalciferol used during the maintenance phase (100,000 IU every other month from the sixth to the twelfth month post-transplantation) was insufficient to maintain serum 25(OH)D concentration above 30 ng/ml in half of patients (Courbebaisse et al., 2009). These findings are in consistence with a previous study showing that 25,000 IU of cholecalciferol once a month failed to correct vitamin D insufficiency in renal TR, suggesting that a higher dose of cholecalciferol is necessary to maintain adequate 25(OH)D levels after transplantation (Wissing et al., 2005). The optimal dosage scheme to maintain 25(OH)D concentrations between 30 and 80 ng/ml was simulated from the data of the previous study (Courbebaisse et al., 2009) using a population pharmacokinetic approach. In order to maintain 25(OH)D concentrations between 30-80 ng/ml during the first year after renal transplantation, cholecalciferol dosing should be: six successive administrations of 100,000 IU every two weeks interval, then 100,000 IU once a month until the end of the first year (Benaboud et al., 2012). This theoretical scheme remains to be tested in a prospective study.

After lung, liver and cardiac transplantation, the optimal scheme for vitamin D supplementation remains to be determined.

### 29.4 Vitamin D and bone loss after transplantation

Improved survival rates after transplantation have been accompanied by increased recognition of previously neglected long-term complications of transplantation such as fractures and osteoporosis due to specific and non-specific risk factors (Bia, 2008). Pre-transplantation bone disease and immunosuppressive therapy, especially corticosteroid, result in rapid bone loss and increased fracture rates early after transplantation (Ebeling, 2007). Bisphosphonates are one of the most promising approaches for the management of transplantation osteoporosis. However, native and active vitamin D metabolites may have additional benefits in reducing hyperparathyroidism, particularly after kidney transplantation. Besides reducing hyperparathyroidism, there are several potential mechanisms by which vitamin D and its analogues may influence post-transplantation bone loss. They may overcome corticosteroid-induced decreases in intestinal calcium absorption and promote differentiation of osteoblast precursors into mature cells (Stein and Shane, 2011). Immediately after renal transplantation, randomized trials showed that active form of vitamin D (Alfacalcidiol) could prevent or attenuate lumbar spine and femoral neck bone loss (El Agroudy et al., 2003, 2005; De Sevaux et al., 2002). Studies of calcitriol have found contradictory results after transplantation, although some report beneficial effects at doses >0.5 μg/day after (Stein and Shane, 2011). Most studies using active forms of vitamin D were not powered to assess difference in fracture rates.

Concerning native vitamin D, it was shown in paediatric renal TR that low serum 25(OH)D correlates with hyperparathyroidism and with short stature (Shroff et al., 2011). In adult renal TR, efficient treatment of vitamin D insufficiency with cholecalciferol was shown to reduce serum PTH concentration (Courbebaisse et al., 2009). In two studies, native vitamin D, at doses of 800
IU daily in 40 patients (Al Gabri et al., 2005) or 25,000 IU monthly in 90 patients (Wissing et al., 2005), did not prevent bone loss after kidney transplantation. However, randomized studies remain to be performed to assess the potential effect of higher doses of native vitamin D on bone mineral density and fracture after transplantation.

29.5 Vitamin D and de novo type 2 diabetes mellitus after transplantation

According to the diagnostic criteria and post-transplantation delay, de novo type 2 DM occurs in 10% to 30% of renal TR, mainly due to corticosteroid and tacrolimus treatment (Montori et al., 2002).

The potential effects of vitamin D on insulin secretion and insulin resistance are supported by many experimental data (Pittas et al., 2007b). First, VDR (Johnson et al., 1994) and CYP27B1 (Bland et al., 2004) are expressed in pancreatic β cells, and VDRE have been identified in the promoter of human gene encoding for insulin (Maestro et al., 2003). Second, in vitro studies showed that calcitriol stimulates insulin human gene transcription and expression of insulin receptor and glucose transport (Bourlon et al., 1999; Norman et al., 1980; Kadowaki and Norman, 1984; Tanaka et al., 1984). Third, VDR KO mice have an altered insulin secretion (Zeitz et al., 2003), and vitamin D3 supplementation increases glucose tolerance and insulin secretion in vitamin D deficient rats (Cade and Norman, 1986). In humans, serum 25(OH)D concentrations are inversely correlated to type 2 DM prevalence (Pittas et al., 2007b; Scragg et al., 2004). Vitamin D insufficiency is also associated with increased HbA1c levels (Hypponen and Power, 2006) and resistance to insulin (Scragg et al., 2004). Recent data suggest that the influence of vitamin D on the resistance to insulin may be partly mediated by a control of adiponectin gene by calcitriol (Sun and Zemel, 2007).

Few interventional studies reported positive effect of vitamin D supplementation on intermediary parameters of insulin resistance. A double-blind placebo randomized trial indicated that vitamin D3 supplementation improves postprandial insulin sensitivity in apparently healthy men likely to have insulin resistance (centrally obese but non-diabetic) (Nagpal et al., 2009). In another double-blind placebo randomized trial, supplementation with oral calcium (500 mg per day) and vitamin D3 (700 IU per day) during 3 years improved fasting glycaemia in hyperglycaemic subjects (Pittas et al., 2007a). More recently, it was shown showed that supplementation with vitamin D3 (2000 IU per day during 4 months versus placebo) could improve pancreatic β cell function in patients at high risk of type 2 DM (Mitri et al., 2011). However, other studies aiming at testing the effect of various native vitamin D formulations on different parameters of type 2 DM, including the women’s health initiative one (De Boer et al., 2008), showed no effect of vitamin D supplementation.

No study reported the potential effect of activated or native vitamin D on de novo type 2 DM after transplantation.
**29.6 Vitamin D and cardiovascular risk after transplantation**

In comparison with the general population, TR have an increased cardiovascular risk both secondary to traditional and non-traditional risk factors (50-fold higher in renal TR) (Sarnak et al., 2003). Several observational and experimental data argue in favour of a potential protective role of vitamin D against cardiovascular diseases. Cross-sectional studies indicated that vitamin D deficiency might be associated with arteriosclerosis and endothelial dysfunction in end-stage renal disease patients (London et al., 2007). Several prospective case-control studies have reported a strong association between low circulating levels of 25(OH)D and an increased risk of major cardiovascular events such as myocardial infarction, stroke, congestive heart failure (Wang et al., 2008; Giovannucci et al., 2008), and cardiovascular disease death (Pilz et al., 2008, 2009; Dobnig et al., 2008). These associations remained significant after adjustment for other risk factors of cardiovascular disease.

Possible explanations for these findings involve both direct and indirect effects of vitamin D on cardiovascular function. Direct effects are supported by the fact that cardiomyocytes, vascular smooth muscle cells, and endothelial cells express both the VDR and the CYP27B1 enzyme (Zittermann et al., 2005). Furthermore, genes up-regulated during myocardial hypertrophy (e.g. atrial natriuretic peptide) possess VDRE and are suppressed by calcitriol in animal or cell models (Wu et al., 1996). Similarly, in cultured cells, calcitriol inhibits cardiomyocytes proliferation (O‘Connell et al., 1997; Nibbelink et al., 2007) and stimulates vascular smooth muscle cells proliferation and vascular endothelial growth factor expression by these cells (Cardus et al., 2006). Calcitriol also modulates contractile performances of isolated rat or mouse cardiomyocytes (Green et al., 2006; Tishkoff et al., 2008). Potential indirect effects of vitamin D concern several risk factors for cardiovascular dysfunction. Regarding RAS, VDR and CYP27B1 KO mice have high levels of blood pressure and cardiac hypertrophy due to increased activation of RAS (Bouillon et al., 2008), and calcitriol treatment inhibits renin activation and decreases blood pressure and cardiac hypertrophy in CYP27B1 KO mice (Qiao et al., 2005; Sigmund, 2002; Zhou et al., 2008). Regarding MMP, calcitriol reduces the expression of MMP2 and MMP9 (Nakagawa et al., 2005), two MMP that may promote vascular calcification (Basalyga et al., 2004).

Even though prospective clinical studies are currently lacking, numerous studies have shown positive effects of vitamin D supplementation on intermediary parameters potentially related to cardiovascular health. A placebo-controlled trial showed an improvement in the pro-inflammatory and anti-inflammatory cytokine profile of patients with congestive heart failure (Schleithoff et al., 2006) and an improvement in endothelial function in vitamin D deficient subjects receiving native vitamin D (Taricn et al., 2009). A recent meta-analysis of 11 randomized trials either with activated or native vitamin D confirmed this reduction in systolic blood pressure in hypertensive patients and suggested that vitamin D produced a greater fall in systolic blood pressure than activated compounds (Witham et al., 2009). However, because vitamin D intoxication in humans may lead to vascular calcifications, the benefit or risk ratio of supra-physiologic dosages of vitamin D should be evaluated, especially in TR having an increased...
cardiovascular risk, since a biphasic effect of vitamin D on the risk of vascular calcifications may exist (Zittermann et al., 2007).

### 29.7 Vitamin D and cancer risk after transplantation

TR experience an increased incidence of cancers, especially for non-melanoma skin cancers and non-Hodgkin lymphoma. Three years after renal transplantation, US Renal Data System has shown a 7.5% cumulative incidence of non-skin cancers and a 7.4% cumulative incidence of skin cancers in renal TR (Kasiske et al., 2004).

Several environmental studies have reported that living at higher latitudes increased the risk of developing colon, prostate, breast, lymphoma, and several other cancers (Garland and Garland, 2006), which may be linked to decreased vitamin D3 synthesis. Many prospective case-control studies have showed that adults in the highest quantile of 25(OH)D levels have a 30% to 50% decreased risk of colon (Giovannucci, 2007), breast (Garland et al., 2007), prostate (Ahonen et al., 2000), and ovary (Tworoger et al., 2007) cancers compared with those in the lowest quantile. Furthermore, the risk of non-Hodgkin lymphoma is 30% to 40% lower in adults with high vitamin D intakes or great sun exposure (Soni et al., 2007; Polesel et al., 2006), and retrospective studies suggest an association between low serum 25(OH)D level and death from cancer (Tretli et al., 2009; Fedirko et al., 2012; Ren et al., 2012).

Furthermore, the role of poor vitamin D status and cancer risk has received strong experimental support from the consistent demonstration that activation of the VDR by calcitriol produced locally induces differentiation (Liu et al., 1996) and apoptosis (Diaz et al., 2000) and inhibits proliferation (Tangpricha et al., 2001) and angiogenesis (Mantell et al., 2000). Moreover, vitamin D and its metabolites stimulate mutual adherence of cells and intercellular communication through gap junctions, thereby decreasing metastatic potential and strengthening the inhibition of proliferation that results from intercellular tight physical contact (Fleet, 2008).

In humans, the data are still controversial. In the women's health initiative study, 36,282 women were randomized to receive either a placebo or 1000 mg calcium and 400 IU vitamin D3 daily. Although a strong negative relationship between baseline 25(OH)D levels and the incidence of colorectal cancer was found, no reduction in the incidence of cancers was observed in the treated group compared with the placebo group (Wactawski-Wende et al., 2006). However in this study, adherence to treatment was poor and cholecalciferol dosage was considered to be too low by many experts. More recently, a 4-year double blind randomized placebo-controlled trial including 1,180 postmenopausal women showed a significant decreased risk of cancers in the group receiving calcium (1,500 mg/day) and vitamin D3 (1,100 IU vitamin D3/day) supplementation (Lappe et al., 2007).

The potential protective role of vitamin D against cancer risk in the general population was also assessed in renal TR (Ducloux et al., 2008). In a cohort of 363 renal TR followed up during 3 to 5
years after transplantation, a higher incidence of post-transplant cancers was observed in patients with pre-transplant 25(OH)D concentrations less than 10 ng/ml (13.7% vs. 3.7% for those with 25(OH)D levels >30 ng/ml, \( P=0.007 \)). This result remains to be confirmed thanks to interventional randomized trials. However, caution should be considered when increasing vitamin D dosage as a few observational retrospective studies suggest that high 25(OH)D values may be deleterious for the risk of prostate cancer (Tuohimaa et al., 2004). An intriguing question is the relationship between sun exposure and non-melanoma skin cancer. Even though the role of sun exposure has been emphasized, one should note that VDR KO mice develop UVB-induced skin cancers more rapidly and more frequently than wild-type mice, suggesting a potential protective role for 25(OH)D against non-melanoma skin cancers (Ellison et al., 2008).

**29.8 Vitamin D and infections after transplantation**

The newer potent immunosuppressive drugs have decreased the incidence of rejection in TR while increasing susceptibility to opportunistic infections. Therefore, even though the patterns of infections after transplantation have been altered by routine antimicrobial prophylaxis, these complications remain an important issue in this population (Fishman, 2007).

Recent retrospective data from hemodialysis patients suggest that regular vitamin D dosing did not show benefit for infectious mortality (St Peter et al., 2009). However, in humans, the risk of being infected with MT and the risk of developing upper respiratory tract infections have been shown to be associated with vitamin D deficiency (Cannell et al., 2006). Experimental data support this possible effect. First, it is recognized that macrophages express both VDR and CYP27B1 (Mora et al., 2008). Second, it has been shown that infection of macrophages with MT induced a toll-like receptors response with a signal transduction to the nucleus to increase VDR and CYP27B1 expression. Calcitriol production by macrophages and calcitriol action on macrophages stimulate expression of cathelicidin, an antimicrobial peptide, by these cells. Cathelicidin in turn can kill infective agents, such as MT, within the macrophage (Liu et al., 2006). Finally, calcitriol also stimulates human monocyte proliferation in vitro (Ohta et al., 1985) and increases IL-1 production by monocytes and macrophages (Bhalla et al., 1986).

A placebo-controlled trial demonstrated a reduction in influenza infections in patients treated with 2,000 IU vitamin D3/day compared with the placebo group (Aloia and Li-Ng, 2007). More recently, it was reported that vitamin D3 supplementation could improve viral response in hepatitis C virus infected patients receiving the classical interferon/ribavirin treatment (Abu-Mouch et al., 2011), and was also able to hasten sputum culture conversion in adults infected with MT with the tt genotype of the TaqI VDR polymorphism (Martineau et al., 2011).

Although low serum 25(OH)D levels in lung TR were associated with increased incidence of infection (Lowery et al., 2012), the potential protective role of vitamin D against infections after transplantation remains to be proven.
29.9 Vitamin D and graft rejection

Even though greatly reduced, AR and chronic allograft deterioration remain important issues after transplantation.

In retrospective case-control studies, it was reported that osteoporotic renal TR experienced fewer AR after calcitriol treatment induction (Tanaci et al., 2003) and needed fewer pulse steroid doses (Sezer et al., 2005). In a retrospective study after liver transplantation, early vitamin D3 supplementation was independently associated with a lack of AR (Bitetto et al., 2010) and low 25(OH)D levels were associated with increased incidence of AR in lung TR (Lowery et al., 2012).

Experimental studies also support this effect. First, calcitriol inhibits the expression of IL-2 and interferon-γ mRNA and protein in T cells and attenuates CD4+ and CD8+ T-cell proliferation and their cytotoxic activity (Lemire et al., 1985; Reichel et al., 1987; Rigby et al., 1987). VDR expression in T cells is not constitutive but only up-regulated after activation (Veldman et al., 2000). Calcitriol also decreases plasma cell differentiation, B-cell proliferation, and IgG secretion (Chen et al., 2007). Second, calcitriol inhibits the expression of major histocompatibility complex class II, CD40, CD80, and CD86, thus decreasing differentiation, maturation, and immunostimulatory capacity of DCs (Fritsche et al., 2003; Penna and Adorini, 2000; Griffin et al., 2001), which constitutively express VDR and CYP27B1 (Fritsche et al., 2003). Even though DCs are not the most important cells implicated with the alloimmune response, their role has been recently emphasized (Chen, 2005). Calcitriol also down-regulates IL-12 synthesis and simultaneously stimulates IL-10 production by DCs (Penna and Adorini, 2000). The net result is a decrease in T helper 1-cell responses and a possible induction of IL-10 producing T-regulatory type 1 cells. By limiting adaptative immune response and down-regulating DCs proliferation and activity, VDR agonists are, thus, susceptible to help in preventing AR (Mora et al., 2008). The potential protective role of VDR agonists against chronic allograft rejection is supported by many arguments. In addition to potentially inducing tolerogenic DCs, VDR agonists could also inhibit the production of chemokines, responsible for leukocytes infiltration in vessels allograft, and may down-regulate TGF-β pathway, which has a profibrotic activity (Adorini et al., 2005). Other renoprotective effects of vitamin D may participate in the prevention of chronic allograft rejection (see the following paragraph). The immunomodulatory capacity of calcitriol observed in vitro has been confirmed in several animal models of transplantation. Calcitriol or its analogs prolonged the survival of murine cardiac allograft (Lemire et al., 1992), increased survival in a rat model of liver transplantation (Zhang et al., 2003), and delayed autoimmune disease recurrence after syngeneic islet transplantation in nonobese diabetic mice (Casteels et al., 1998). In addition, active compounds of vitamin D were shown to protect from chronic allograft rejection in a rat model of renal transplantation (Hullett et al., 2005) and in a mouse model of aortic transplantation (Amuchastegui et al., 2005). Importantly, Cantorna et al. (1998) showed that calcitriol prolonged graft survival in animal models without increasing the risk of fungal or viral infection.

In a small prospective study, Ardalan et al. (2007) investigated the effect of calcitriol therapy which was started in the donor and continued in recipient side (9 donors, who all received
calcitriol at a dose of 0.5 μg/day orally for 5 days before donation and 9 TR who were treated with the same regimen for one month post-transplant and thereafter received 0.25 μg/day for five months compared to 10 controls with conventional treatment). Although only 19 renal TR were included, a significant expansion of CD4+ CD25+ Tregs in the calcitriol-treated group could be demonstrated, providing strong evidence of the immunomodulatory properties of active vitamin D after transplantation (Ardalan et al., 2007). In another small prospective clinical trial, 24 patients transplanted 6-18 months before the study were treated with calcitriol at a dose of 0.5 μg/day during four weeks. A decrease in HLA-DR, CD28, CD86, and CD40 expression on white blood cells was found after calcitriol treatment (Ahmadpoor et al., 2009).

Altogether, VDR agonists could be used as potentially immunomodulatory agents in TR. Currently, calcitriol analogs such as paricalcitol, exerting immunomodulation without inducing hypercalcemia, are being developed (Van Etten and Mathieu, 2005).

29.10 Renoprotective effects of vitamin D after transplantation

A number of observational clinical studies have confirmed the potent renoprotective and antiproteinuric effect of vitamin D and its analogs (Agarwal et al., 2005; Li, 2009). Importantly, 25(OH)D level was also found to be inversely associated with prevalence of albuminuria (De Boer et al., 2007) and to be an independent inverse predictor of progression to dialysis in patients with stage 2 to 5 chronic kidney disease (Ravani et al., 2009). After renal transplantation, vitamin D insufficiency was significantly associated with proteinuria (Lee et al., 2011) and another retrospective study suggested a beneficial effect of calcitriol therapy on renal graft function (O’Herrin et al., 2002).

Experimental data support these observations. As mentioned earlier, calcitriol and analogs inhibit RAS, which has a pivotal role in glomerular and tubulo-interstitial damages, glomerular hypertension, and proteinuria (Brewster and Perazella, 2004). It has also been shown that vitamin D inhibits NF-κB activation (Sun et al., 2006), which is known to play an important role in renal diseases by promoting inflammation and fibrogenesis (Guijarro and Egido, 2001). In many animal models of chronic kidney disease, evidence for the renoprotective effects of vitamin D analogs were shown because of the attenuation of tubulo-interstitial fibrosis and glomerulosclerosis, reduced proteinuria, repression of extracellular matrix production, EMT markers, and TGF-β pathway (Kuhlmann et al., 2004; Schwarz et al., 1998; Tan et al., 2006). These effects were independent of PTH (Schwarz et al., 1998; Kuhlmann et al., 2004) and at least in part dependent of RAS inhibition (Freundlich et al., 2008). Of interest, losartan or paricalcitol alone moderately improved kidney injury in a model of diabetic mice, whereas the combination of both molecules prevented albuminuria and reduced glomerulosclerosis (Zhang et al., 2008).

Despite its benefits as a potent immunosuppressive agent, the use of cyclosporine A is limited by its nephrotoxic properties after transplantation. Interestingly, in a rat model, paricalcitol appears to attenuate cyclosporine A-induced nephropathy by suppression of inflammatory, pro-fibrotic,
and apoptotic factors through inhibition of the NF-κB, Smad, and mitogen-activated protein kinase signaling pathways (Park et al., 2010).

In humans, a recent randomized placebo-controlled, double-blind trial showed that addition of 2 μg/day paricalcitol to RAS inhibition safely lowers residual albuminuria in patients with diabetic nephropathy (De Zeeuw et al., 2010).

Very few studies investigated the potential nephroprotective role of vitamin D after transplantation. In a recent study, glomerular filtration rates using iohexol plasma clearance, urinary procollagen III aminoterminal propeptide excretion, epithelial phenotypic changes as markers of EMT and Banff scores at 3 and 12 months after renal transplantation were analysed in 64 renal TR with or without cholecalciferol supplementation between months 3 and 12. The scheme of cholecalciferol treatment used in the treated group was described previously (Courbebaisse et al., 2009). In the treated group, cholecalciferol supplementation did not prevent EMT, interstitial fibrosis, tubular atrophy, or renal function deterioration. These results challenged the experimental data, suggesting that vitamin D-analog supplementation confers nephroprotection. However, these negative results should be interpreted with caution because of the limited follow-up, the small size of the population and the lack of efficiency of the maintenance treatment to maintain serum 25(OH)D above 30 ng/ml in the treated group (Courbebaisse et al., 2011).

29.11 Vitamin D and mortality after transplantation

In a meta-analysis including 18 independent randomized controlled trials, intake of ordinary doses of vitamin D supplements (mean daily dose=528 IU) seemed to be associated with decrease in total mortality from any cause (Autier and Gandini, 2007). In hemodialysis patients (Naves-Diaz et al., 2008; Teng et al., 2005; Tentori et al., 2006), and in patients with chronic kidney disease (Kovesdy et al., 2008; Shoben et al., 2008), treatment with calcitriol was associated with significantly higher survival. Level of 25(OH)D was also shown to be an independent inverse predictor of death in patients with stage 2 to 5 chronic kidney disease (Ravani et al., 2009). Interestingly, hemodialysis patients receiving paricalcitol had a survival advantage over those treated with calcitriol (Teng et al., 2003). Whether these results may apply to TR is unknown although low post-operative calcitriol concentrations were independently associated with 1-year mortality in heart TR (Zittermann et al., 2009). Moreover, the mortality of lung TR who remained vitamin D deficient 1 year post-transplant was higher than that of recipients who maintained normal vitamin D level at 1 year post-transplant (Lowery et al., 2012).

29.12 Conclusion and perspectives

In addition to classical effects on bone and mineral metabolism, vitamin D displays a wide spectrum of non-classical effects that, for most, are especially relevant for the care of TR. These pleiotropic effects are mostly documented by observational and experimental studies or small
intervention trials that most often evaluated intermediate parameters. The time has now come for large placebo-controlled trials in TR, using larger dosages of vitamin D than the current recommended intakes and targeting clinical endpoints. Development of calcitriol analogs with minimal calcemic effects may allow taking advantage of the pleiotropic effects of vitamin D with fewer side effects and is a ‘hot’ research topic.

Two randomized control studies are actually undergoing to test the effect of cholecalciferol after renal transplantation:

- The objective of the VITA-D study (Vitamin D3 Substitution in Vitamin D Deficient Kidney Transplant Recipients; ClinicalTrials.gov Identifier: NCT00752401) is to conduct a randomized, double-blind, placebo-controlled study focusing on the impact of cholecalciferol substitution in vitamin D deficient renal TR on graft function (MDRD estimated GFR), incidence of acute rejection episodes, post-transplant infections within the first year after transplantation. Two hundred renal TR with 25(OH)D <20 ng/ml at time of transplantation will be randomized to receive either cholecalciferol (6,800 IU/day during one year) or placebo (Thiem et al., 2009).

- The VITALE study (VITamine D Supplementation in RenAL Transplant Recipients; ClinicalTrials.gov Identifier: NCT01431430) is a randomized, controlled, double-blind study whose objective is to evaluate the influence of vitamin D3 substitution in vitamin D deficient (25(OH)D <30 ng/ml) renal TR on the post-transplant outcome. The primary purpose of this study is to compare the effects of high dose (100,000 IU fortnightly for 2 months then monthly for 22 months) versus low dose (12,000 IU fortnightly for 2 months then monthly for 22 months) of cholecalciferol on a composite endpoint consisting in de novo DM and cancers, cardiovascular diseases, and patient death. 640 TR between 12 and 48 months after transplantation will be randomized to blindly receive either high or low dose of cholecalciferol with a follow-up of 2 years.

References


29. Vitamin D and transplantation


29. Vitamin D and transplantation


29. Vitamin D and transplantation


29. Vitamin D and transplantation


29. Vitamin D and transplantation


Key facts

- Vitamin D insufficiency and poor bone health are common among adults and children with epilepsy.
- Multiple factors influence vitamin D levels among people with epilepsy, including nutritional status and mobility impairment, as well as choice of and duration of epilepsy treatments.
- Hepatic enzyme-inducing antiseizure medications can augment vitamin D metabolism, leading to lower vitamin D levels. However, enzyme inhibitors have also been associated with vitamin D insufficiency among people with epilepsy.
- Supplementation can improve vitamin D status, and may improve bone health among people with epilepsy.

Summary points

- Bone health is often suboptimal among people with epilepsy, and this can lead to osteoporosis and increased risk for fractures.
- Vitamin D status is one of several contributors to bone health for people with epilepsy. Overall nutrition status, antiseizure medications and dietary therapies, concurrent physical disabilities, exercise, and exposure to sunlight are also contributing factors.
- Some antiseizure medicines accelerate liver enzyme function, which leads to decreased vitamin D levels. However, even medicines that do not increase liver enzyme function have been associated with poor bone health among people with epilepsy.
- Compared to monotherapy, use of more than one antiseizure medication has been associated with higher risk for vitamin D insufficiency and lower bone mineral density.
- We suggest guidelines for screening and treatment for vitamin D insufficiency among people with epilepsy.
Abstract

Both adults and children with epilepsy are at high risk for poor bone health, with associated osteopenia and fractures. Epilepsy has consistently been associated with low vitamin D levels, a key indicator of bone health. Many anticonvulsant medications, such as phenytoin and carbamazepine, induce hepatic CYP450 enzymes, resulting in accelerated vitamin D metabolism. This might be the mechanism by which some epilepsy treatments decrease vitamin D levels. However, even medications which result in less-potent CYP450 induction (e.g. oxcarbazepine), or inhibit hepatic enzymes (e.g. valproate), have been associated with abnormal vitamin D levels and poor bone health. Data are lacking for many of the newer anticonvulsant medications. Although vitamin D is only one among many risk factors for abnormal bone mineral density, it is a modifiable risk factor for people with epilepsy. We suggest guidelines for screening and treatment of vitamin D insufficiency for people with epilepsy.

Keywords: epilepsy, seizure, bone health, anticonvulsant, antiepileptic drug, pediatric
Introduction

Epilepsy is one of the most common neurological disorders, affecting about 1% of all people (Faught et al., 2012; Russ et al., 2012). Although unprovoked seizures are the hallmark of epilepsy, there are numerous physical and mental health comorbidities. Herein we address the complex associations between vitamin D, bone health, and epilepsy, including contributions related to patients’ environmental exposures, medical treatments, and diet.

Epilepsy terminology

Epilepsy is, by definition, a condition of recurrent, unprovoked seizures. There are many epilepsy types and numerous distinct epilepsy syndromes. The details of epilepsy classification are beyond the scope of this chapter, but a basic understanding of epilepsy terminology will allow the reader to better understand the literature reviewed below.

Seizures are loosely classified as being focal or generalized. Focal seizures (also called partial seizures) originate in one area of the brain and may spread to contiguous or contralateral regions. Focal seizures may or may not result in impaired awareness. Generalized seizures originate within bilaterally distributed neural networks and so involve both cerebral hemispheres. Types of generalized seizures include absence, generalized tonic-clonic (convulsions), atonic (drop seizures), myoclonic, etc. Usually, patients experience either focal or generalized seizures, although a small subset of patients has both types. In the literature, epilepsy is often referred to as ‘well-controlled’, meaning the patient is seizure-free, or ‘treatment-resistant’. Patients are considered to have treatment-resistant epilepsy if their seizures that are not controlled despite adequate trials of two appropriately-chosen and well-tolerated antiseizure drugs (Kwan et al., 2010).

Epilepsy syndromes can be focal (also called partial or localization-related epilepsies) or generalized, depending on the associated seizure type(s). Historically, epilepsy has been further classified into symptomatic and idiopathic categories. Symptomatic epilepsies have an identifiable trigger (e.g. a patient who developed recurrent focal seizures after sustaining a stroke would be classified as having symptomatic focal epilepsy). The term idiopathic does not mean ‘unknown’ in epilepsy parlance. Rather, idiopathic refers to epilepsy syndromes that are presumed to have a genetic etiology. Typically, the idiopathic epilepsy syndromes are thought to be more benign.
30. Vitamin D and bone health among people with epilepsy

than the symptomatic epilepsy syndromes. Examples of idiopathic epilepsies include childhood absence epilepsy and benign Rolandic epilepsy. Note that newer schemes for organizing the epilepsy syndromes are being proposed and are slowly being adopted (Berg et al., 2010), but the literature regarding vitamin D and epilepsy uses the above-defined terminology.

30.3 Prevalence of vitamin D insufficiency among people with epilepsy

The association between vitamin D insufficiency, treatment with antiseizure drugs, and poor bone health among people with epilepsy has been recognized for decades and the American Academy of Pediatrics has highlighted children taking antiseizure medications as a high-risk group (Wagner et al., 2008). The reported prevalence of vitamin D insufficiency among people with epilepsy is variable, depending on the definition of vitamin D insufficiency, the time of the year during which the data were collected, the geographical location of the study population, and the population actually studied. However, all published data report that a significant proportion of patients with epilepsy have insufficient 25(OH)D levels and this trend does not appear to have improved over the years. Both children and adults with well-controlled and treatment-resistant epilepsy are at risk.

In the 1970’s, studies of institutionalized children with epilepsy and mental retardation showed abnormal serum markers for rickets, which correlated with duration of antiseizure drug therapy (Lifshitz and Maclaren, 1973). Children with epilepsy (age 10-16 years) were also found to have much higher rates of insufficient vitamin D (defined then as <15 ng/ml) than healthy controls (72% vs. 50%), with seasonal variability such that levels were highest during the summer months and lowest in the winter (Offermann et al., 1979).

Modern data suggest similar rates of vitamin D insufficiency among children with epilepsy. In a German study, 76% of a cohort of 33 children with a variety of epilepsy syndromes had 25(OH)D <20 ng/ml, compared to 23% of 44 healthy controls (P<0.001) (Nettekoven et al., 2008). Shellhaas and coworkers observed that in an unselected group of American children with epilepsy, 25% of 78 subjects had a 25(OH)D level <20 ng/ml (Shellhaas et al., 2010). More significantly, only 25% of the children had levels that were considered to be normal (25(OH)D >32 ng/ml).

Insufficient vitamin D levels have also been reported for adults with epilepsy. In a Brazilian study, 34% of individuals with epilepsy (without comorbid mental retardation), who were treated with antiseizure medications for 2-38 years, had insufficient 25(OH)D levels, compared with 7% of controls (P<0.02) (Kulak et al., 2004). Lower levels of 25(OH)D were reported by Pedrera and coworkers among 30 adults with epilepsy who were seizure-free on antiseizure medications, compared to 30 controls (22.0±12.1 ng/ml vs. 31.2±8.6 ng/ml, P<0.0001). The low 25(OH)D levels could be corrected with vitamin D supplementation (Pedrera et al., 2000).
30.4 Epilepsy and fracture risk

The reasons adults and children with epilepsy are at significant risk for fractures are multifactorial. Vitamin D status certainly plays a role, but calcium intake, choice of antiseizure medication(s), use of polypharmacy, duration of treatment, comorbid abnormal ambulatory status, as well as the seizures themselves, all contribute to this risk.

Women with epilepsy appear to be at particularly increased risk for bone fractures (Espinosa et al., 2011; Souverein et al., 2006), which might not be ameliorated by standard calcium and vitamin D supplementation. A large cohort of Caucasian women 65 or older was evaluated prospectively for about 4 years to determine the incidence of hip fracture (Cummings et al., 1995). In this group, concurrent use of antiseizure drugs was associated with a relative risk for hip fracture of 2.8 (95% CI 1.2-6.3) compared with those not presently taking antiseizure medication. None of the hip fractures that occurred in the women taking antiseizure medications were directly attributable due to seizures or loss of consciousness.

Adults with epilepsy are at higher risk for fractures than those with many other chronic illnesses (OR 2.89 compared with healthy controls), with the highest risk among those treated with multiple antiseizure drugs. Although valproic acid might have a lower associated fracture risk, all other antiseizure medications have been associated with increased risk for non-traumatic fractures among adults older than 50 years of age (Jetté et al., 2011). The same conclusions hold true for children with epilepsy. The risk of fractures among children who take anticonvulsants is 2-6 times greater than the general population (Mattson and Gidal, 2004).

30.5 Risk factors for poor bone health among people with epilepsy

Numerous aspects of epilepsy are likely to influence vitamin D levels and bone health among affected individuals, as summarized in Table 30.1 and detailed below.

30.5.1 Nutritional status

Children with treatment-resistant epilepsy are at risk of poor nutritional status, lacking adequate protein, vitamin, and mineral intake (Volpe et al., 2007). However, inadequate dietary calcium and vitamin D is not restricted to those with the most severe epilepsy syndromes. In a longitudinal study in India, 144 patients with new onset epilepsy who were not yet treated with antiseizure medications were observed over a 6 month period (Menon and Harinarayan, 2010). All patients in this study had lower than recommended dietary calcium intake. Over 90% of this cohort had low 25(OH)D levels at the time of their epilepsy diagnosis and showed a significant decline in 25(OH)D levels over 6 months of treatment, regardless of the medication prescribed. Thus the need for antiseizure medications is associated with further compromise of vitamin D status.
30. Vitamin D and bone health among people with epilepsy

Table 30.1. Epilepsy-related factors associated with vitamin D insufficiency and poor bone health.

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Impaired mobility</th>
<th>Environmental light exposure</th>
<th>Epilepsy treatments&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate dietary vitamin D and calcium intake</td>
<td>Co-morbid cerebral palsy and other neuro-motor abnormalities</td>
<td>Geographical location Limited outdoor physical exercise Home versus institutional living arrangements</td>
<td>Antiseizure medications • CYP450 enzyme induction • Monotherapy vs. polypharmacy Ketogenic diet</td>
</tr>
</tbody>
</table>

<sup>1</sup> Vitamin D receptor polymorphisms may compound the risk (Lambrinoudaki et al., 2011).

Obesity is a major identified risk factor for vitamin D insufficiency in the general population, both for adults and children (Looker et al., 2008; Reid et al., 2009; Saintonge et al., 2009). Recently, obesity has been identified as a common co-morbidity among children with newly diagnosed epilepsy, particularly among adolescents and those with idiopathic epilepsy syndromes (Daniels et al., 2009). Higher body mass index was reported as a risk factor for lower vitamin D levels in a cohort of 78 children with epilepsy (Shellhaas et al., 2010). Since people with obesity will require higher vitamin doses to reach an appropriate 25(OH)D level, particular attention should be paid to this risk factor.

30.5.2 Impaired mobility

A subset of individuals with epilepsy has impaired mobility due to co-morbid neurological conditions, such as cerebral palsy, which can affect ambulatory status and impact bone health. Most published studies have specifically excluded non-ambulatory patients with epilepsy, so there are limited data to assess the contribution of ambulation status on bone health among those with epilepsy. In a large study of children (with and without epilepsy), the risk of vitamin D deficiency among nonambulatory children was about twice that of ambulatory children, even after adjusting for confounders. That study also found depressed BMD z-scores among nonambulatory patients, independent of antiseizure drug status (Baer et al., 1997).

Others reported no effects of ambulatory status on 25(OH)D levels among noninstitutionalized children with treatment-resistant epilepsy, although they may have been underpowered to detect such a difference (Bergqvist et al., 2007). Even among children with normal ambulatory status, those with epilepsy exercise less than their sibling controls (Wong and Wirrell, 2006), suggesting that modifiable risk factors for poor bone health are readily identifiable for many patients.
30.5.3 Physical environment and light exposure

Only 10% of vitamin D is obtained through dietary sources. The rest, and therefore vast majority of vitamin D, is generated via cutaneous synthesis, following exposure to sunlight. Individuals with epilepsy are known to have lower levels of physical activity than healthy controls. Physical activity and outdoor sun exposure may be further limited among children with epilepsy and co-morbid conditions, such as cerebral palsy, since these children are often wheelchair bound and not ambulatory. Wong and coworkers demonstrated low vitamin D levels in virtually all of 122 institutionalized patients, regardless of their antiseizure drug regimen (Wong et al., 2006). It was notable that sun exposure in this cohort was minimal, or nonexistent.

There was a notable difference in sunlight exposure among 38 German children with a variety of epilepsy syndromes, compared with 44 healthy controls (66% vs. 100% receiving daily sunlight exposure, P<0.001) (Nettekoven et al., 2008). Sun exposure varies based on the time of the year and season. Similarly vitamin D levels fluctuate during the year, both for healthy people (Looker et al., 2008) and those with epilepsy (Nettekoven et al., 2008; Offermann et al., 1979). Additional vitamin D supplementation should be considered during seasons with lower sunlight.

30.6 Epilepsy treatments

Because many antiseizure drugs induce hepatic CYP450 metabolism, they can result in accelerated metabolism of vitamin D, leading to declining 25(OH)D levels, increased parathyroid hormone levels, and abnormally enhanced bone turnover. However, hepatic enzyme induction is only one component of the complex mechanisms by which antiepileptic drugs affect bone health. There was no significant difference in BMD reduction among men and women treated with enzyme-inducing medications compared with those receiving non-inducing antiseizure drugs in one recent study (Farhat et al., 2002). Additionally, 25(OH)D levels were reduced among patients treated with the powerful enzyme-inducer carbamazepine, as well as among those taking the less-potent inducer oxcarbazepine (Mintzer et al., 2006).

Studies have consistently demonstrated that treatment with a single antiseizure medication is less deleterious for vitamin D status and bone health than polytherapy (Bergqvist et al., 2007; El-Hajj Fuleihan et al., 2008; Jetté et al., 2011; Nettekoven et al., 2008). However, patients who require polytherapy generally have treatment-resistant epilepsy syndromes and so are more likely to have comorbid impaired mobility, nutritional deficiencies, and suboptimal sunlight exposure. Below, we examine the impact of specific epilepsy treatments on vitamin D and bone health.
30.6.1 Phenytoin, primidone, and phenobarbital

**Adults**

The antiseizure medications most commonly associated with altered bone metabolism and decreased bone density are inducers of the cytochrome P450 enzyme system, and include phenytoin, primidone, and phenobarbital (Gough et al., 1986; O’Hare et al., 1980; Pack et al., 2008; Valimaki et al., 1994). Phenytoin is associated with increased bone turnover, as demonstrated by elevated markers of bone resorption and bone formation among adults (Pack et al., 2008; Valimaki et al., 1994). After one year of treatment, women taking phenytoin were found to have significant BMD loss at the femoral neck, as well as decreased 25(OH)D and other markers of abnormal bone turnover (Pack et al., 2008).

**Children**

The effects of these older antiepileptic medications on bone health have not been evaluated extensively in the pediatric population, since in contemporary practice phenytoin and primidone are not often prescribed for children. Phenobarbital is commonly prescribed for infants with epilepsy, but its effects on these patients’ vitamin D status have not been studied.

30.6.2 Carbamazepine

**Adults**

Like phenobarbital and phenytoin, carbamazepine is a potent inducer of the cytochrome P450 enzyme system. However, studies evaluating its effect on vitamin D and BMD in adults have been conflicting, with some reporting significant disturbances in bone and mineral metabolism and bone turnover, and others finding no such abnormalities. Normal 25(OH)D levels were reported among 53 young women treated with carbamazepine (Pack et al., 2011). In contrast, people taking carbamazepine (n=21) were found to have lower 25(OH)D levels than normal controls (n=42) (Mintzer et al., 2006).

Among adults treated with carbamazepine monotherapy, BMD was not significantly decreased (Valimaki et al., 1994). However, patients treated with carbamazepine monotherapy have demonstrated decreased cortical bone mass, as measured by quantitative ultrasonography of the phalanges (Pedrera et al., 2000; Pluskiewicz and Nowakowska, 1997). The differing results suggest that the effects of carbamazepine monotherapy on bone and mineral metabolism and BMD have not yet been completely delineated.

**Children**

Several groups have examined vitamin D levels among children treated with carbamazepine. Two teams reported no statistically significant differences between 25(OH)D levels in control subjects
and carbamazepine-treated children, thus refuting the theory of hepatic enzyme induction as the driving force for hypovitaminosis D among pediatric epilepsy patients (Babayigit et al., 2006; Verrotti et al., 2002). Conversely, one group (Nicolaidou et al., 2006) observed that 37% of ambulatory patients with idiopathic epilepsies treated with either carbamazepine or valproic acid exhibited 25(OH)D levels of <10 ng/ml in the first year of treatment, and that the mean 25(OH)D level declined over the 36-month treatment period. This study also reported a significant negative correlation between 25(OH)D and parathyroid hormone, as well as seasonal variation among 25(OH)D levels, but did not examine BMD. Similarly, a fourth group reported that although increasing carbamazepine levels were not significantly associated with changes in 25(OH)D or parathormone, 25(OH)D declined by 22%, regardless of carbamazepine dosing among those newly treated for epilepsy (Misra, 2010).

Among pediatric studies that examined BMD in relation to treatment with carbamazepine, five reported no significant difference (Akin et al., 1998; Altay et al., 2000; Kafali et al., 1999; Sheth et al., 1995; Tekgul et al., 2006), whereas one reported lower BMD but unchanged 25(OH)D levels (Babayigit et al., 2006) compared with healthy control subjects. Another study reported lower BMD among children receiving carbamazepine compared with those receiving valproic acid (Chou et al., 2007).

### 30.6.3 Valproate

#### Adults

Since valproate is an effective treatment for many different epilepsy syndromes, thorough study of its effect on vitamin D and bone health is warranted. Valproate is an inhibitor of the cytochrome P450 enzyme system, but emerging data suggest it may still adversely affect bone health. Early reports evaluating indexes of bone metabolism among patients taking valproate found no significant abnormalities. Adults treated with valproate monotherapy had no significant reductions in calcium or 25(OH)D levels and alkaline phosphatase was not increased (Davie et al., 1983; Gough et al., 1986).

However, more recent studies report different results. A study of adults receiving long-term valproate monotherapy found increased serum concentrations of calcium, low levels of vitamin D metabolites, increased markers of bone resorption and formation, and decreased BMD (Sato et al., 2001). The increased calcium levels were postulated to reflect increased bone resorption. Despite this, valproate was the only antiseizure drug not associated with increased fracture risk for older adults with epilepsy (Jetté et al., 2011).

#### Children

Among children, the effects of valproic acid on BMD and markers of bone health are also complex. One study specifically reported vitamin D levels among children with idiopathic epilepsies compared with healthy controls and found no significant difference (Babayigit et al.,
30. Vitamin D and bone health among people with epilepsy

2006), while another demonstrated decreasing 25(OH)D levels among children with idiopathic epilepsies (Nicolaidou et al., 2006). Higher BMD has been reported among those receiving valproic acid compared with those on carbamazepine (Chou et al., 2007). One study reported lower BMD among subjects treated with valproic acid and/or lamotrigine compared with control subjects (Guo et al., 2001), but five studies reported no significant difference in bone mineral density among children treated with valproic acid (Akin et al., 1998; Altay et al., 2000; Kafali et al., 1999; Sheth et al., 1995; Tekgul et al., 2006).

30.7 Newer antiseizure medications

Many new antiseizure drugs have been approved in the past 15 years, but few studies have evaluated the effect of these medications on 25(OH)D and BMD. Those studied include oxcarbazepine, lamotrigine, topiramate, and the ketogenic diet. Since new antiseizure medications are very commonly prescribed, due to favorable side effect profiles and superior efficacy, additional research is urgently required to determine whether any of the newer medications cause abnormalities in bone health and vitamin D metabolism.

30.7.1 Oxcarbazepine

Adults

Because recent guidelines suggest that oxcarbazepine is the initial treatment of choice for people with focal epilepsy syndromes (French et al., 2004), studying this medication’s effects on bone health is essential. At high doses, oxcarbazepine is a hepatic enzyme inducer, albeit less potent than carbamazepine. In the only study of adults designed specifically to evaluate oxcarbazepine, patients with focal epilepsies taking carbamazepine or oxcarbazepine were compared with healthy control subjects, and those on carbamazepine were crossed over to oxcarbazepine monotherapy (Mintzer et al., 2006). 25(OH)D levels were significantly lower among the oxcarbazepine and carbamazepine subjects, compared with control subjects, but were not significantly different between the oxcarbazepine and carbamazepine groups, suggesting that the newer agent may also exert an important impact on bone health.

Children

Recently two studies evaluated the effects of oxcarbazepine on bone health in children with epilepsy. One group (Cansu et al., 2008) prospectively studied 34 newly diagnosed children with idiopathic focal epilepsy who were ambulatory and without other neurologic or medical diagnoses. Patients were treated with oxcarbazepine for 18 months. Mean 25(OH)D levels were <30 ng/ml at baseline and declined significantly over the 18 months study period. Another group compared patients with idiopathic epilepsy syndromes treated with oxcarbazepine (n=14), carbamazepine (n=23), or valproic acid (n=31) to 30 healthy control children. Although vitamin
D levels were not lower among treated patients, total BMD was lower for those taking any of the anticonvulsant drugs, compared to controls (Babayigit et al., 2006).

### 30.7.2 Lamotrigine

**Adults**

Data regarding the impact of lamotrigine on bone health are mixed. One prospective study found elevated osteocalcin, a marker of bone formation, in association with lamotrigine treatment (Kim et al., 2007). In contrast, premenopausal women treated with lamotrigine did not have significant reductions in BMD or changes in bone turnover markers (Pack et al., 2005). Recent reports found no significant reductions in 25(OH)D, calcium, or markers of bone resorption and bone formation among young women treated with lamotrigine monotherapy (Pack et al., 2011).

**Children**

Only one published study reported the effects of valproate and lamotrigine on growth and bone mass in children with epilepsy, both alone and in combination. Fifty-three patients taking one or both of these antiseizure drugs, who were without bone or metabolic disease, other bone-altering medications, or a family history of osteoporosis, were studied. Twenty three subjects (43%) were below the 10th percentile for height, and nine (24%) had a BMD $z$-score <-1.5, without significant differences between those treated with either medication. Decreased physical activity scores were associated with worse bone-health markers, including lower 25(OH)D levels. The combination of dual therapy with valproic acid and lamotrigine, in addition to low activity scores, was associated with the highest risk of low BMD and short stature (Guo et al., 2001). 25(OH)D levels were normal among 14 European children with idiopathic epilepsies who were taking lamotrigine (Borusiak et al., 2012).

### 30.7.3 Topiramate

**Adults**

There has been limited study of topiramate in relation to bone health. As a carbonic anhydrase inhibitor, topiramate can induce renal acidosis, with theoretical risk for secondary abnormalities of bone metabolism. One study demonstrated that although serum bicarbonate levels were lower among pre-menopausal Korean women taking topiramate than controls or those taking carbamazepine or valproate, 25(OH)D levels did not differ between these groups (Heo et al., 2011). Young women taking topiramate for migraine prophylaxis had normal 1,25-dihydroxyvitamin D levels, but low BMD (25(OH)D levels were not reported) (Ali et al., 2011).
30. Vitamin D and bone health among people with epilepsy

**Children**

There are very few data regarding the effect of topiramate on bone health among children. Topiramate was associated with lower BMD among a small group of children with treatment-resistant epilepsy, taking more than one anticonvulsant (Coppola et al., 2009). This medication is very commonly prescribed for a wide variety of childhood epilepsies, so additional evaluation of associated 25(OH)D and BMD changes is imperative.

30.7.4 Ketogenic diet

The ketogenic diet is a high fat, low calorie and low carbohydrate and protein diet used to treat drug-resistant epilepsy. This diet is very restrictive, and usually requires supplementation with vitamins and other micronutrients. Children on the ketogenic diet are at risk for poor bone health, and low vitamin D levels. Vitamin D levels must be evaluated serially, as 25(OH)D levels have been shown to decline by ~0.5ng/ml per month during 15-months of treatment with ketogenic diet (Bergqvist et al., 2007).

Changes in BMD are also common among children treated with the ketogenic diet. On average, these children's height and weight status are low at baseline, and decline while they are on the ketogenic diet. Those at highest risk for poor BMD are younger patients who are non-ambulatory and have a low baseline body mass index (Bergqvist et al., 2008).

30.8 Suggested screening and treatment for insufficient vitamin D status among people with epilepsy

Robust data to support specific vitamin D supplement regimens for people with epilepsy are lacking. However, a few intervention studies are available. Adding vitamin D to the treatment regimen of institutionalized children with cerebral palsy increased BMD, in comparison with controls not treated with vitamin D, who continued to show loss of bone mass (Jekovec-Vrhovsek et al., 2000). However, a study of adults with epilepsy demonstrated that those taking calcium and vitamin D supplements were just as likely to sustain a bone fracture as those who took no supplements (Espinosa et al., 2011). The only controlled trial of vitamin D supplementation (400 units daily vs. 2,000 units daily) among children with epilepsy failed to show an improvement in BMD after one year and 25(OH)D levels rose only marginally (Mikati et al., 2006).

Exciting preliminary data suggest that vitamin D supplementation may have an anticonvulsant effect. In a study of 13 adult patients with treatment-resistant epilepsy (10 focal, 3 generalized), 12 of whom had comorbid vitamin D deficiency, there was a median 40% reduction in seizures after 3 months of aggressive vitamin D supplementation (Hollo et al., 2012). If these data are replicated in larger patient populations, physicians will be compelled to optimize vitamin D levels for people with epilepsy.
Currently, however, we lack rigorous evidence to support screening for, or treating, vitamin D insufficiency for people with epilepsy. Despite this, a substantial body of literature suggests that vitamin D insufficiency may be important for these patients. Diagnostic testing is readily available and treatment with vitamin D supplements is relatively inexpensive and free of substantial risk.

Future research may compel us to revise our practice, but based on the available data we offer suggestions for screening and treatment of insufficient vitamin D levels for those with epilepsy (Table 30.2). We measure 25(OH)D levels for all epilepsy patients on an annual basis, if the level is >30 ng/ml. We do not routinely measure 1,25-dihydrovitamin D levels, since these are virtually always normal (Bergqvist et al., 2007; Shellhaas et al., 2010). For those with normal 25(OH)D levels (>30 ng/ml), we recommend a daily multivitamin containing a minimum of 400 units of vitamin D. For those whose levels are <30 ng/ml, we prescribe vitamin D supplements (Table 30.2). We recommend administering cholecalciferol (D3) rather than ergocalciferol (D2), since the former has better intestinal absorption than the latter among people with normal renal function.

Obesity is a risk factor for vitamin D insufficiency. Since vitamin D is fat soluble, higher than usual doses of supplemental cholecalciferol are needed to maintain adequate circulating 25(OH)D levels. Special consideration should also be given to adjusting supplement doses during winter or for individuals whose personal circumstances do not allow for adequate sunlight exposure.

Some epilepsy centers routinely evaluate patients with treatment-resistant epilepsy with bone density scans. There is a paucity of data to support this practice, and BMD measurement is

Table 30.2. Suggested screening and treatment for vitamin D insufficiency for people with epilepsy.

<table>
<thead>
<tr>
<th>25(OH)D level (ng/ml)</th>
<th>Cholecalciferol dose</th>
<th>Repeat labs</th>
<th>Additional labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥30</td>
<td>400 IU per day¹</td>
<td>Annually</td>
<td>Phosphorus, magnesium, calcium</td>
</tr>
<tr>
<td>15 to 29</td>
<td>2,000 IU per day in divided doses</td>
<td>Every 4 weeks until 25(OH)D &gt;30²</td>
<td>Phosphorus, calcium, magnesium</td>
</tr>
<tr>
<td>&lt;15</td>
<td>2,000-4,000 IU per day in divided doses, in consultation with a dietician</td>
<td>Every 3-4 weeks until 25(OH)D &gt;30²</td>
<td>Parathyroid hormone Consider bone density scan</td>
</tr>
</tbody>
</table>

¹ In accordance with American Academy of Pediatrics recommendations, we suggest that all children treated for epilepsy be given a daily multivitamin with 400 international units of vitamin D3. Some authors have suggested a higher standard dose of 1000 international units per day (Wirrell, 2010).

² Once 25(OH)D levels rise to the normal range, we halve the vitamin D supplement dose. If the level remains normal, recheck in 3-6 months.
30. Vitamin D and bone health among people with epilepsy

relatively expensive, so we recommend pursuing bone density measurements on a case-by-case basis, with this testing reserved for those at highest risk for osteopenia (e.g. those with history of atraumatic bone fracture and vitamin D deficiency).

30.9 Conclusions

Although the risk for poor bone health among people with epilepsy, and its potential relationships to anticonvulsants and vitamin D, has been recognized for decades, the literature remains contradictory. Many medications (especially carbamazepine, phenytoin, and phenobarbital) can increase vitamin D metabolism via induction of the CYP450 system. However, non-enzyme-inducing medications, such as valproate, and less powerful enzyme-inducers (oxcarbazepine) may also exert adverse effects on bone mineralization. Evidently, hepatic enzyme induction and vitamin D levels are not the only factors contributing to bone mineralization among people with epilepsy.

People with treatment-resistant epilepsy, typically treated with multiple antiseizure medications, are at high risk for poor bone health. This is clearly a multifactorial problem, influenced by co-morbid neuromotor disabilities, body mass index, medications, diet and vitamin D, as well as the seizures themselves. Some of these factors are modifiable, such as vitamin D intake and other dietary considerations, while others are not. Despite some contradictory evidence, we therefore advocate for attention to vitamin D levels as part of the comprehensive management for such vulnerable patients. The cost is relatively low, but the potential ramifications are tremendous.

Despite all of the attention in the lay press and scientific literature, physicians do not consistently screen for vitamin D insufficiency. More than ten years ago, a survey revealed that only 41% of pediatric neurologists and 28% of adult neurologists (all of whom were members of the American Academy of Neurology) screened for bone disease among their patients with epilepsy, with just 3% routinely monitoring parathyroid hormone or 25(OH)D levels (Valmadrid et al., 2001). Ten years later, a study of English pediatric neurologists revealed that only a minority routinely considered bone health screening or prophylaxis for their patients with epilepsy (Fong et al., 2011). If additional research demonstrates a beneficial effect of interventions such as vitamin D and/or calcium supplementation on bone health for people with epilepsy, we hope that these numbers, and the interest of neurologists and primary care providers in this important aspect of comprehensive epilepsy care, will rise. Until evidence-based treatment guidelines become available, we suggest consideration of screening for vitamin D insufficiency and supplementation with cholecalciferol to maintain levels in the optimal range.

References

Handbook of vitamin D in human health

R.A. Shellhaas, R.K. Singh and S.M. Joshi


30. Vitamin D and bone health among people with epilepsy


30. Vitamin D and bone health among people with epilepsy


Women and children
**Key facts**

- Despite good prenatal care, healthy maternal diets, and the availability of prenatal vitamins, gestational and neonatal vitamin D insufficiency is common.
- Global vitamin D insufficiency is a substantial concern affecting newborns regardless of geographic location, skin pigmentation, or socio-economic status.
- Contrary to the facts, many healthcare professionals and new parents believe that breast milk satisfies all their newborn infants’ nutritional needs and that additional supplements, e.g. vitamin D, are not necessary.
- Vitamin D-related metabolites participate in the regulation of hundreds of genes and are utilized in every tissue in the body. The role of vitamin D begins early in fetal development and continues throughout life.
- Many experts believe that the adequacy of vitamin D in the neonatal period is essential to lowering the risk of certain chronic diseases like type 1 diabetes, cancer, and allergy.

**Summary points**

- Vitamin D adequacy in the newborn is totally dependent on the vitamin D status of the mother.
- Global insufficiency among all populations, including women of child-bearing age, means that large percentages of term infants and even larger percentages of preterm infants have inadequate supplies of vitamin D to support healthy development in utero and at birth.
- Breast milk is generally a poor source of vitamin D; infant formulas are much better sources but still may not supply enough vitamin D per serving to bring many infants to repletion within the first few weeks of life.
- Because vitamin D contributes to the regulation of so many genes in virtually every organ in the body (including those involved in cell cycle, differentiation, maturation and apoptosis), insufficiency early in life may have significant short and long-term negative health implications.
- Vitamin D insufficiency can adversely affect the ability of the neonate to ward off viral and bacterial infections and to properly develop natural immunotolerance. Conditions also linked to vitamin D inadequacy early in life include poor growth, asthma, allergy, bone disease, diabetes type 1 and 2, certain cancers, brain and mood disorders.
- Much more research is required to fully understand vitamin D in the neonate but there is a strong consensus that most infants should be provided with at least 400 IU vitamin D per day, beginning as early as possible after birth.
31. Neonatal effects of enhanced vitamin D and vitamin D for premature infants

H.K. Linke
Abbott Laboratories, Abbott Nutrition, Ross Park, 3300 Stelzer Road, Columbus, OH 43219, USA; hawley.linke@abbott.com

Abstract

Neonates must contend with the abrupt cessation in their supply of vitamin D and its metabolites. Due to widespread maternal insufficiency, the contemporary newborn has a high probability of suffering deficits for an indeterminate period in early life. Breast milk is a notoriously poor source of vitamin D and, while infant formula is fortified with the vitamin, the volume consumed in the neonatal period is insufficient to provide the recommended daily intake. The spread of insufficiency is global and may place infants at risk for short or long term deficits of growth, bone development, innate and adaptive immune responses, and increased risk of certain chronic diseases. Worldwide there is a range of recommendations regarding how much vitamin D is optimal during this stage and what doses of supplementation may be required. Regardless of advice, both healthcare professionals and families are often complicit in the failure to achieve repletion of vitamin D. The former frequently fails to adequately educate parents on the subject, while non-compliance is high among the latter. At this time, for most term infants, 400 IU/day starting as soon as possible after birth appears to be adequate. For preterm infants, even higher supplementation may be required in the neonatal period. Vitamin D plays a role in regulating at least 200 genes involved in growth, cell cycle control, differentiation, and maturation. These discoveries dictate that substantial research will be required to fully elucidate the demand for and significance of providing optimal cellular access to this pleiotropic secosteroid hormone during a critical window of development.

Keywords: recommendations, deficiency, supplementation, health outcomes
31.1 Introduction

With the snip of the umbilical cord an infant experiences a dramatic shift in physiologic dependence. While the most imminent need is to provide for their own respiration; they are also deprived of an immediate source of vitamin D and its metabolites. Subsequent nursing will not expeditiously restore the availability of this pleiotropic secosteroid hormone. In earlier eras humans evolved to harvest available sunlight for optimal cutaneous synthesis of vitamin D. The fetus would receive appropriate forms and amounts of vitamin D transplacentally and breast milk would provide adequate supplies to the newborn until they obtained access to sunlight to synthesize vitamin D for themselves. Circumstances which erode that process have developed over the previous few centuries. These include crowding in city environments, atmospheric pollution, global migration from sunlight-rich to sunlight-poor latitudes, cultural attitudes to cover exposed skin (primarily for women), time spent indoors at home and work environments, vegetarian diets, and, most recently, pressure to reduce skin cancer risk and photo-aging via chemical and physical sun screening.

These developments have provoked the well-documented emergence of vitamin D insufficiency at all stages of life and throughout the world. Newborn infants do not escape the effects of compromised vitamin D. Across latitudes and ethnicities, women of child-bearing age have a high prevalence of vitamin D insufficiency/deficiency which translates into low vitamin D levels in cord blood, neonates, and breast milk. (Balasubramanian and Ganesh, 2008; Basile et al., 2007; Bassir et al., 2001; Bodnar et al., 2007; Bowyer et al., 2009; Ginde et al., 2009; Gordon et al., 2008; Hatun et al., 2005).
31. Neonatal effects and premature infants

31.2 Neonatal vitamin D physiology

The neonatal period is dynamic in regard to vitamin D, calcium and PTH levels for the infant. Maternal vitamin D levels rise during pregnancy; in the placenta both 25(OH)D and 1,25(OH)_2D are available; and conversion of 25(OH)D to the active hormone occurs in maternal tissues, decidual and placental tissue, as well as the fetal kidney. Calcium stores are also enhanced in the period before delivery and PTH levels are generally low (Salle et al., 2000). Calcium plasma concentrations decrease right after birth and PTH levels correspondingly rise, but both return to normal by the second week. The transient PTH elevation stimulates synthesis of 1,25(OH)_2D in the newborn. Higher levels of maternal vitamin D tend to subdue these perinatal fluctuations and help maintain better vitamin D status (Salle et al., 2000). In preterm infants, assuming adequate precursor, 1,25(OH)_2D levels increase in the first month and are well maintained through the first 3 months. Alternatively, preterm vitamin D deficiencies at this point contribute to hypocalcemia which is a major factor in osteopenia associated with prematurity (Bishop et al., 2005).

Many reviews point to the 1940’s as the era during which vitamin D-deficient rickets was virtually eliminated in the United States, primarily due to access to vitamin D fortified milk (e.g. Holick, 2006). By 1979, however, an ‘outbreak of rickets’ in urban Philadelphia (Bachrach et al., 1979) served as a harbinger for the deluge of similar reports. Over the next two decades reports of rickets and vitamin D deficiency extended this limited observation to a variety of populations, even, unexpectedly, to light-skinned breastfed infants in Iowa (Ziegler et al., 2006). Although the American Academy of Pediatrics, aware of the limited vitamin D available in most breast milk, had long recommended 400 IU/day vitamin D supplementation, several studies document both a failure of health care providers to recommend supplementation, and, if recommended, parental resistance to compliance (Garganta and Buchanan, 2012; Huurre et al., 2006; Kreiter et al., 2000; Ling, et al., 2011; Merewood et al., 2009; Perrine et al., 2010; Taylor et al., 2010; Williamson and Greene, 2007).

A 2010 compilation of reports, (Dror and Allen, 2010), highlights global vitamin D status during pregnancy and includes data on inadequacies in cord blood. Eight reports between 2005-2009 from India, Iran, Australia, USA, Greece and Canada described cord blood insufficiency/deficiency for infants of diverse ethnicity. The brightest spots in the data were the relatively low percent of deficiency in Athens, Greece (8.1%), Sydney, Australia (11%) and the moderately low level in Winnipeg, Canada (36%). All the other populations, however, displayed cord blood deficiency from 55% (Boston, USA) to 96% (Lucknow, India).

In these and similar reports the cut-off level assigned for maternal and cord blood insufficiency and deficiency varied: deficiency <25 nmol/l to <37.5 nmol/l; insufficiency <50 nmol/l (in all but one study which invoked <80 nmol/l). Disturbingly, a large study of vitamin D supplementation (400, 2,000 or 4,000 IU/day) for pregnant women, suggests an even higher standard should be assigned for sufficiency. Hollis et al. (2011) established that during pregnancy conversion of 25(OH)D to 1,25(OH)_2D (the biologically active form) was not optimized until plasma levels of the former reach 100 nmol/l. Among the specific complications of pregnancy that were
H.K. Linke

documented, preterm labor, preterm birth and infection were inversely related to 25(OH)D. Complications were lowest in the 4,000 IU/day group for which the risk of these comorbidities was reduced by 50%. Interestingly, preterm labor and birth were also inversely correlated to vitamin D status at baseline (12-14 weeks gestation). There were no adverse events associated with the vitamin D treatments (Wagner et al., 2010, 2012). Whether this daily dose should be extended for the infant and/or the mother postnatally and whether it confers long-term benefit to the newborn remains to be determined.

While adiposity, veiling and dark skin contribute to some of the higher incidences of inadequacy, unveiled and lighter skin populations also add to the pool of deficient cord blood values (Dijkstra et al., 2007; Dror and Allen, 2010). The general pattern of deficiency/insufficient prevails globally, particularly among breast fed infants (Challa, et al., 2005; Dawodu et al., 2010; Ziegler, et al., 2006). While many advisory bodies recommend vitamin D supplementation for newborns (milk and formula fed), compliance falls short of expectations.

31.3 Vitamin D related health outcomes for infant

31.3.1 Bone, calcium and other classic vitamin D actions

The primary benefit of vitamin D for infant bone health and calcium homeostasis has been established for nearly a century (Holick, 2006). In fact, some current professional recommendations for vitamin D are based solely on maintenance of infant-child skeletal health. This perspective is reviewed in some detail in the 2011 IOM North American report providing vitamin D recommendations (Ross et al., 2011). Concurrent with unraveling the role of vitamin D receptors, vitamin D response elements, and gene regulation by vitamin D, in virtually every tissue type in the body (Kimball et al., 2008), the resurgence of rickets in otherwise healthy newborns spurred renewed attention to vitamin D. Because the role of vitamin D (and calcium/phosphorous) in bone health of infants has been so extensively addressed by others – from the molecular to the clinical level for both preterm and term – it is not the focus of this review. It is sufficient to note that the infant displaying 25(OH)D levels below 25 nmol/l is at risk for rickets or suboptimal bone mineral content or bone mineral density. The presence of adequate calcium supplementation plus ≥400 IU/day vitamin D and/or UVB exposure will mitigate risk and restore bone health in the otherwise uncomplicated condition (Gallo et al., 2010; Pehlivan, 2003).

However, vitamin D supplementation during infancy may have longer lasting skeletal effects. In one study bone status was obtained from 106 8-year-old Caucasian girls (formerly breastfed) with or without vitamin D (400 IU/day) during their first year. The prepubertal analysis associated infancy supplementation with higher BMC and areal BMD at multiple bone sites (Zamora, 1999).
31. Neonatal effects and premature infants

31.3.2 Beyond bone: pleiotropic effects of vitamin D

The proliferation of vitamin D research in the neonatal period reflects an expanding interest in the non-traditional, non-calcemic, non-bone related pleiotrophic actions of 1,25(OH)₂D. Activation of 25(OH)D to 1,25(OH)₂D occurs primarily in the kidney, but cell types throughout the body express the enzymes necessary for local conversion (e.g. 1-α-hydroxylase). Starting early in development, these and other cells also express nuclear vitamin D receptors. Binding to the VDR can trigger a rapid cellular signaling response that modifies activities like absorption of calcium by the intestine and secretion of insulin by β cells. It can also follow a path to regulate the transcription of genes via vitamin D response elements on DNA. In this way the active hormone has been shown to modulate the expression of over 200 mammalian genes (Goldring et al., 2011; Taylor et al., 2009). Therefore vitamin D, its derivatives, and their physiologic accessibility have the capacity to impact each tissue in the body.

Many of the attributes of adequate vitamin D demonstrated in other age groups are likely to apply in neonatal life. Over time, inspired by significant animal and in vitro discoveries, results from randomized placebo-controlled and prospective observational trials are being reported along with epidemiologic studies. The sections below describe a few non-calcemic benefits related to the neonatal stage.

31.3.3 Securing achievement of growth potential

In a 1968 retrospective symposium, Genevieve Stearns, an infant feeding pioneer, described historic experiments evaluating the impact of exogenous vitamin D on the growth of infants fed dilute condensed milk, soured whole milk or similar breast milk substitutes common in the 1930s. Researchers concluded that approximately 300-800 USP units (IU) daily provided an optimal trajectory of linear growth with earlier dentition and ‘precocious muscular achievements’. They were chastised for creating infant ‘monsters’ until subsequent work verified similar growth advantages for breast fed infants similarly supplemented (standardized cod liver oil or milk from specially fed cows) (Stearns, 1968). While feeding practices and formula compositions have evolved in the subsequent 50+ years, adequate vitamin D continues its role facilitating normal growth in both term and preterm infants.

A review of lifetime health records from the Northern Finland Birth Cohort 1966 focused on recorded estimates of vitamin D supplementation during year one. Heights were compared for nearly 10,000 subjects at three time points. At one year, height of infants without supplementation appeared to lag slightly behind those receiving any supplementation (P=0.005), but the significance evaporated after adjustments for multiple factors. At age 31, however, subjects who experienced rickets in infancy – regardless of compensatory treatment and other adjustments – were ~0.5 cm shorter than others (P=0.01). This latter finding suggests that early deficiency can have permanent growth consequences (Hypponen, 2011).
Among preterm infant vitamin D supplementation trials, however, studies did not observe differences in either short term growth parameters (during supplementation) or long-term (at 9-11 years of age) when comparing two higher levels of vitamin D supplementation (400-500 IU/day vs. ~1000 IU/day) (Backstrom et al., 1999a,b; Fewtrell et al., 2000).

The general conclusion from many growth studies is that while vitamin D supplementation is prudent for preterm, breastfed, and formula fed infants, daily doses exceeding 400-500 IU are probably not required for normal growth outcomes in healthy infants.

Nonetheless, certain neonatal conditions demand greater attention to circulating vitamin D levels. In New Delhi, India, among a vulnerable population, Kumar followed LBW term infants supplemented with vitamin D (1,400 IU/week) over the first six months. Supplementation provided better vitamin D status at six months and significantly increased standard deviation z-scores for weight, length, and arm circumference, and decreased the proportion of children with stunted growth (length for age z-score ≤2) or with arm circumference z-scores of 2 or less (Kumar, 2011).

In a controlled study of vitamin D supplementation among third trimester pregnant Asian women living in London (presumed to represent low vitamin status). Maxwell et al. (1981) found no differences in infant weight and length at birth, three, or six months of age. From six to 12 months, however, both weight and length diverged, producing an 8% advantage in weight and a 2% advantage in height for the infants of supplemented women.

Infants born with vitamin D insufficiency and who remain at high environmental risk for nutrition-dependent rickets frequently experience growth rate reduction. Forty-six such infants (mean age 13 months) were compared with 40 normal matched controls before, and six months after, vitamin D treatment. Treatment was accompanied by a growth spurt which appeared at the time to adequately compensate most infants for the growth retardation experienced during deficiency. Biochemical measurements during recovery identified significant correlative increases in IGF-1. The authors speculate that the IGF-1 reduction observed during vitamin D deficiency is a compensatory response designed to slow growth during a period of calcium insufficiency (Soliman et al., 2008).

### 31.3.4 Infection/immunity/allergy/asthma

Important roles for vitamin D in the immune system have been well established for most segments of the population and the perinatal period is no exception. To test one measure of the adaptive immune system, cord blood levels of vitamin D from 101 term Turkish infants were evaluated against the numbers of regulatory and helper T cells. While investigators failed to identify a relationship between T cell counts and vitamin D concentration, they noted that one-third of the blood samples exhibited <12ng/ml 25(OH)D (very deficient), while only 7% of bloods registered >25 ng/ml (sufficient). This high incidence of insufficiency appeared to have
directly affected the T cells’ overall ability to respond physiologically, thus precluding detection of putative modulation of T cell activity (Guven et al., 2012).

Since infants’ T and B cells are immature, neonates are more dependent on innate immune responses over adaptive responses to resist early infection. Ex vivo techniques have been used to examine innate immune response at birth. Twenty-three cord blood plasmas from a predominantly Hispanic population in Southern California were measured for 25(OH)D and 1,25(OH)₂D concentrations. Unlike adult vitamin D plasma relationships, concentrations of these two molecules were highly correlated in cord blood. Based on 25(OH)D analyses, 17% of neonates were identified as severely deficient (<12 ng/ml); 22% were sufficient (>30 ng/ml) and the remaining 61% were distributed between insufficient and deficient categories. In ex vivo studies of human monocytes, severely deficient cord plasma, compared to sufficient plasma, was significantly less supportive of innate immunity responses normally invoked to protect against both gram-negative (TLR4-mediated) and gram-positive (TLR2-mediated) infections. Fortification of deficient plasma with either form of vitamin D significantly restored both TLR4 and TLR2 related antimicrobial pathway gene expression (Walker et al., 2011). Also using ex vivo technique, Hirsch et al. (2011) examined the anti-inflammatory responses regulated by 1,25(OH)₂D concentrations of neonatal neutrophils from cord blood (collected in northeast USA). They concluded that exogenous 1,25(OH)₂D was far less active in suppressing markers of inflammation in the neonatal immune cells compared to normal adult cells. The lessened sensitivity may have been the result of the observed low expression of both VDR and 1-αhydroxylase in the neonatal cells. They cautioned that inherent insensitivity of neonatal immune cells to vitamin D regulation raises further concerns regarding worsening evidence of insufficiency in newborns. That is, if neonatal immune cells are physiologically compromised during in utero insufficiency, then postnatal levels must be even higher to support proper vitamin D regulation of immune maturation.

Other studies directly connect vitamin D to perinatal immune health outcomes., e.g. cord blood vitamin D deficiency seems associated with RSV bronchiolitis. In their first year, Dutch neonates born with 25(OH)D levels <50 nmol/l had a six times greater risk of RSV bronchiolitis than those with ≥75 nmol/l (Belderbos, 2011). This longitudinal study supports several earlier studies linking vitamin D to RSV, rhinovirus, and other viral infections.

The relationship of perinatal vitamin D to mother-child transmission of HIV is also significant. Adult studies suggest that low vitamin D levels may be associated with negative outcomes for HIV infected patients (e.g. Viard, 2011). In a pregnancy study, vitamin D levels were obtained from 884 HIV positive pregnant Tanzanian women in a multiple vitamin plus D supplementation trial. There was no association between vitamin D and poor pregnancy outcomes including low birth weight and preterm birth. However, newborns from mothers with <32 ng/ml vitamin D were at significantly greater risk for HIV at 6 weeks, HIV transmission via breast milk, and overall risk of HIV infection (46% higher risk). Those infants also had a 61% higher risk of dying during the first two years (Mehta et al., 2009). As suggested in subsequent correspondence, vitamin D may be acting through both the peripheral immune system and on the placental ability to modulate
infection (Equils and Hewison, 2010). The need for more rigorous evaluation of vitamin D in the prevention of HIV transmission to newborns is apparent.

Intervention trials of vitamin D supplementation and protection from infant infections may be sensitive to how much, when and to whom the intervention is applied. As reviewed recently by Yamshchikov et al. (2009), evidence from predominantly adult clinical trials suggests sufficient support to further investigation into vitamin D therapy for tuberculosis, influenza, viral upper respiratory tract infections, and all-cause infection rates. A double blind trial of placebo and vitamin D conducted among 3,000 high risk infants (1-11 months old) in Kabul appears to be interestingly inconsistent. 100,000 IU boluses of vitamin D3 were delivered every three months and incidence and severity of pneumonia was evaluated during every-two-week visits by fieldworkers. This supplementation regimen did not affect the incidence or severity of pneumonia, hospital admissions, or all-cause mortality. Interpretation of this result from such a large study is complicated by many factors including the appropriateness of ‘bolus’ delivery, with its own unknown consequences related to oscillating vitamin D resources for infants; the (mal)nutritional background and general high-risk nature of the population; and the lack of identification of etiologic agents or human genotypes in regard to vitamin D (Manaseki-Holland et al., 2012). While this report is useful for identifying the inefficacy of this regimen, future studies will necessitate more complex variations to identify specific neonatal immune effects of adequate vitamin D currently predicted from in vitro, animal and correlative studies.

An evaluation of genetics and vitamin D concentration in cord blood revealed a combined effect on food sensitivity of infants around two years of age. In Boston, 649 children enrolled at birth were tested for IgE sensitivity to common food allergens. Toddlers were compared on the basis of cord blood vitamin D, food sensitivity, and polymorphisms (SNP) around 11 genes important for IgE and vitamin D regulation. Cord blood vitamin D deficiency was more prevalent in food sensitive toddlers than control ($P=0.09$). Additionally an SNP for IL4 expression, when combined with cord blood deficiency, created an increase risk for sensitization. (OR=1.79). When further combined with three SNP involving other genes, a strong gene-deficiency-sensitivity interaction was observed ($P_{\text{interaction}}=9 \times 10^{-6}$) (Liu et al., 2011).

Independent of specific immune outcomes, the genome-wide association study (34,000 individuals of European descent) by Wang et al. (2010) further describes at least three genome variants that identify subjects at increased risk for simply maintaining 25(OH)D concentrations <75 nmol/l (OR=2.47) or <50 nmol/l (OR=1.92) Others have indicated difference in kinetics around vitamin D synthesis and actions between racial/ethnic groups (Dawson-Hughes, 2004; Gutierrez et al., 2012; Signorello et al., 2011; Sweeney et al., 2006). Thus, our ability to come to meaningful conclusions about the role of vitamin D and the immune system in the neonatal period may require attention to details regarding race, genetics, and environment.
Heart failure in infants

Hypocalcaemia can induce cardiac effects in adults and occasional reports of cardiomyopathy associated with rickets in infancy and childhood have been described. A review of patient records in England identified 16 infants (3 weeks to 8 months old; black or Indian ethnicity) with dilated cardiomyopathy associated with vitamin D deficiency. The infants had very low levels of calcium, high PTH and 25(OH)D median 18.5 nmol/l (0.00 to 43.00). Ten infants had radiologic evidence of rickets. This association is especially interesting given that no white infants were identified under these criteria over the six year review period (Maiya et al., 2007).

From a different perspective Shedeed (2012) hypothesized that the impact of proinflammatory cytokines observed in congestive heart failure might be abated by vitamin D intervention. Eighty infants with congestive heart failure were randomized to placebo or 100 IU/day cholecalciferol. All were treated for 12 weeks with standard antifailure regimen. All vitamin D concentrations were low at enrollment. As anticipated, both groups responded with improvement in heart failure scores. But significantly greater improvement of the vitamin D treatment group over the placebo group was identified for heart failure score, left ventricular end-diastolic dimension, left ventricular end-systolic dimension, ejection fraction, early and late ventricular filling velocity ratio, myocardial performance index, PTH (reduced), IL10, IL6 (reduced), and tumor necrosis factor-α (reduced). Mean 25(OH)D concentration in placebo subjects was 14.64 ng/ml and supplemented infants 32.89 ng/ml. The author concluded that vitamin D supplementation not only served as an anti-inflammatory agent in infants with congestive heart failure but helped to accelerate clinical improvement and cytokine balance.

31.3.5 Type 1 diabetes

Current knowledge about the relationship between vitamin D and the development of the autoimmune disease T1D engenders a complex web that involves geography, autoimmunity, polymorphism of vitamin D-related genes, effects on insulin usage, supplementation regimen, and circulating vitamin D levels. Vitamin D supplementation during the neonatal/infancy period was first implicated as protective against T1D in a 1999 report from the EURODIAB ACE Study Group (Anonymous, 1999). Later a meta-analysis of five observational studies further supported significantly reduced, possible timing- and dose-dependent risk for T1D with vitamin D supplementation in infancy (Zipitis and Akobeng, 2008). There is also a striking relationship between increasing latitude and increasing T1D risk (Hypponen et al., 2001, 2010), or month of birth (Kahn et al., 2009) primarily attributed to decreased UVB irradiation potential early in life. Correlative observations from a variety of locations (Switzerland to Saudi Arabia) (Bin-Abbas et al., 2011; Janner et al., 2010) continue to show vitamin D deficiency among T1D children. Other association studies link certain vitamin D metabolism gene variants with T1D susceptibility (Bailey et al., 2007).

Two recent reports prospectively studying ‘at risk’ populations during infancy have not supported either vitamin D levels at 9 months of age or maternal intake as protective for T1D. It should be
noted, however, these are genetically selected populations, vitamin D at 9 months is a single static measurement past the neonatal period, and (in the first study) risk was determined based on a biomarker of islet immunity (Simpson et al., 2011). The second study is predicated only on self-reported maternal intake, without analysis of resulting vitamin D concentration in mothers or their infants (Marjamaki et al., 2010). Justification of the correlative observational results (above) with these discordant prospective studies remains to be elucidated.

31.4 Special circumstances for preterm and small for gestational age infants

The prematurely born infant faces many specific developmental challenges which may be exacerbated by low availability of vitamin D. This section refers to those born <37 weeks and LBW (<2,500 grams), VLBW (<1,500 grams), and ELBW (<1000 grams). Unperturbed development of immature tissues and systems is at risk when in utero vitamin D supply is prematurely terminated. Maintaining an adequate supply of nutrients – many of which are normally enhanced during the final weeks of human gestation – is extraordinarily difficult in the preterm infant especially one who has also experienced deficits in utero.

There is currently no consensus regarding the optimal level of vitamin D supplementation for preterm infants. While the IOM (Ross et al., 2011) concluded that investigation has ‘failed to show clinical value above 400 IU/d in preterm’ Backstrom (1999) and Koo et al. (1995) stated ‘160 IU/d is adequate’ based on a study with 20-21 neonates per group. This latter perspective is not in current favor among many experts (Stephens et al., 2009). Among other issues, despite randomization, the groups in the Koo study were not equivalent in ethnicity, height, weight, weight gain or duration of feeding.

Newer studies of vitamin D levels and supplementation strategies have come to light regarding preterm and/or infants with low birth weights. Most of these studies were conducted in the United States. While the protocols were variable, they combine to provide an important narrative. Prevalence of deficiency (<20 ng/ml) at birth was high, i.e. ~50% to nearly 90%, regardless of race, (although African Americans > Hispanics > Caucasians) or gestational age (although less mature > more mature). Standard vitamin D supplementation procedures were successful at raising plasma concentrations but not necessarily before discharge from the NICU. Frequently infants did not receive the recommended 400 IU/day from nutritional intake for many weeks after birth. Only specific supplement dosing from daily drops or IV administration resulted in rapid recovery from deficiency acquired in utero (Cifuentes et al., 2012; Hanson et al., 2011; Monangi et al., 2012; Munshi et al., 2012; Porcelli et al., 2012; Taylor et al., 2012a,b; Viswanathan et al., 2012). Data from preterm infants in the United Arab Emirates yielded similar conclusions except that deficiency was more severe, with 44% of infants at risk for metabolic bone disease due to vitamin D <12.5 nmol/l (Dawodu and Nath, 2011).

The building consensus is that the pleiotrophic responsibilities ascribed to vitamin D may not be satisfied by the vitamin D level shown to be adequate for bone development (>20 ng/ml) (Holick et
31. Neonatal effects and premature infants

Some experts advocate that newer calcium-related data support a sufficiency minimum of 30 ng/ml. While others recommend the concentration at which fluctuation in PTH stabilizes (Holick et al., 2012; Tylavsky et al., 2005). Giapros et al. (2012) examined PTH and vitamin D levels of 128 preterm (32-36 weeks) Greek infants. During at least the first three months most infants were vitamin D insufficient (<30 ng/ml) and many displayed elevated PTH. The inverse relationship and PTH stabilization threshold in this group was used to justify a reasonable cutoff point for vitamin D insufficiency in late preterm infants as 32 ng/ml (Giapros, et al., 2012).

Many of the vitamin D-related morbidity concerns for preterm infants will be similar, if not exaggerated, versions of the concerns identified for term infants. Logically, the preterm infant’s attempt to continue in utero programs of proliferation, differentiation and maturation in most cell and organ types, adds to the necessity of adequate vitamin D for appropriate regulation. Its role in inflammation and immunity will also be important for the vulnerable preterm infant. The full implications, specifically during human gestation and premature birth outcomes, are still under investigation (Golding et al., 2011). One example of preterm sensitivity is osteopenia of prematurity or metabolic bone disease. Reduced bone mineral content, possibly with rachitic changes and even bone fracture is the result of inadequate vitamin D, calcium and phosphorus with accompanying inactivity and use of certain drug therapies in the NICU (Bozzetti and Tagliabue, 2009). Adequate vitamin D is definitely important, but many clinicians also suggest that the adequacy of other components are primary to the disorder prevention and treatment (Lam et al., 2007). In the lung, rachitic respiratory distress also occurs in early preterm infants (Goldring et al., 2011).

These and similar concerns suggest that vitamin D should be routinely evaluated in preterm infants and that strategies for repletion should reflect vitamin D dosing assuring at least 400 IU/day; more as individual circumstances might demand. The specifics of strategies and targeted plasma levels will benefit from further optimization in clinical evaluation. Adequately powered studies targeting intervention for short- and long-term health outcomes in this population have not yet been conducted.

31.5 Recommendations for vitamin D in the perinatal period are highly variable

Health agencies around the world debate assignment of the ideal plasma concentration of vitamin D in term infants, the speed with which or age by which a neonate should attain that level, and the amount of supplementation required to sustain optimal plasma concentration. Opinions, even those based on the same studies, vary depending on the value placed on bone- versus non-bone-related outcomes; the populations studied versus the populations served; value given to molecular inferences for long-term benefits versus health outcomes demonstrated in small, short-term trials; or whether caution is best applied in the absence of compelling extra skeletal benefit or based on the previously demonstrated safety profiles of higher daily doses (Fischer et al., 2008; Henderson, 2005; Holick et al., 2006; Holmlund-Suila et al., 2012; Onwuneme et
31.5.1 Europe – ESPGHAN

A 2010 recommendation from the ESPGHAN Committee on Nutrition discusses the implications of newer data and broader requirement (non-calcemic) for vitamin D in plasma. Preterm infants specifically, may require 800-1500 IU per day to reach a plasma level >80 nmol/l (Agostoni et al., 2010). The ESPGHAN recommendation for term infants established in 2005 remains at 40-100 IU/100 kcal for infant formula and 40-120 IU/100 kcal for follow-on formula (Kotelzko et al., 2005; Scientific Committee on Food, 2003).

31.5.2 Europe – France

The Committee on Nutrition of the French Society of Paediatrics has provided the most recent opinion on this subject. For many decades specific infant supplementation ranged from 1000 to 2,500 IU/day depending on infant characteristics. In the 1990’s infant formula was also fortified (40-100 IU/100 kcal). France has continued to stress the need for all breastfed infants to receive supplemental vitamin D of 1000 IU/day and specifically attribute these higher guidelines for preventing the resurgence of rickets seen elsewhere in Europe and North America (Vidailhet et al., 2012).

31.5.3 Australia and New Zealand

This part of the world traditionally relied on the abundance of sunlight, weighing minimum exposure for vitamin D adequacy against skin cancer risk (Stalgis-Bilinski et al., 2011). While detailed discoveries regarding specific infant populations continue to develop in this region, the recommendation adapts guidelines from elsewhere, e.g. pregnant women should be evaluated for sufficiency in the first trimester and, if deficient, should receive 3,000-5,000 IU/day until serum levels are above 50 nmol/l. At that point they join other women at 400 IU/day supplementation. Infant formulas are fortified at 400 IU/l, but all infants breastfed or others not consuming at least 500 ml/per day formula should be supplemented with 400 IU/day vitamin D (Diamond et al., 2005; Munns et al., 2006).

31.5.4 North America

In North America, the Institute of Medicine, revisiting vitamin D requirements, concluded that compelling data for setting guidelines only existed for calcium/bone-related outcomes. It also rejected the perspective that vitamin D insufficiency is a substantial concern in the population and found no justification for routinely evaluating vitamin D circulation even in preterm infants (Term infant recommendation = AI 400 IU for 0-12 months, UL 1000 IU 0-6 and 1,500 IU 6-12 months). Their recommendation targets desirable infant blood level as 40-50 nmol/l. The document presumes practically all persons are sufficient at 50 nmol (Ross et al., 2011).
31. Neonatal effects and premature infants

Most experts agree that 400 IU/day is likely adequate for maintaining sufficiency in the first year of life. To date, there have been no intervention studies that propose an appropriate timetable for attaining adequate plasma vitamin D, given the high incidence of in utero and perinatal insufficiency. The IOM report suggests that routine screening is inappropriate except for populations at high risk. However, Kersey et al., (2011) point out that low income children in the United States are routinely screened for anemia and blood lead levels. The prevalence of those potentially damaging diagnoses in the population studied were 10% and 4% respectively. In the same group, however, vitamin D deficiency (<20 ng/ml) or insufficiency (<30 ng/ml) were 29% and 62% respectively. The comparison makes a strong case for modification of current policy on screening activities.

The AAP preterm recommendation from 2008 aligns with the IOM recommendation of maintaining the minimum 400 IU/ day in this population. However, AAP proposes value in monitoring vitamin D status of the preterm, targeting a goal >50 nmol and finds significant clinical evidence of safety allowing provision of 400 to 1000 IU per day with milk fortifier, preterm formula and/or a vitamin preparation. The preterm infant is likely to be at greater risk for deficiency at birth and less likely to receive adequate supplementation in early days when internal feeding is limited (Groh-Wargo and Sapsford, 2009).

In 2010 The Canadian Pediatric Society confirmed their 2007 recommendation of 400 IU/day supplementation for term infants to prevent severe vitamin D deficiency (<25 nmol/l). They note, however, that new data suggests that optimal vitamin D status may require plasma concentration of >75 nmol/l; for which the recommended intake will not be sufficient. They also acknowledge that First Nations populations and others with darker skin or living in far northern areas may require additional supplementation especially during winter (First Nations, Inuit and Métis Health Committee, 2012).

31.6 Making recommendations work

Researchers at the Centers for Disease Control and Prevention in the United States analyzed vitamin D intake data during the first 10.5 months. Intakes were evaluated against both 2003 and 2008 AAP recommendations (200 and 400 IU/day respectively). Regardless of feeding type, vitamin D supplementation was actually rare over the period studied: ≤4% of formula fed infants; ≤12.6% of breast milk fed infants. While most formula fed met the 2003 recommendation, only 20 to 37% matched the 2008 values. Among the breast milk fed, the percent of infants who met either the 2003 or 2008 standards was predictably identical to the percent who actually received vitamin D supplements. Although formula contains ~400 IU/l vitamin D, this study found that many infants do not consume a full liter per day, which can explain the failure of so many to meet the 2008 standard of 400 IU/day (Perrine et al., 2010).

In Europe, North America, and other regions of the world, vitamin D liquid supplements (vitamin D alone, in multivitamins for infants, or in natural preparations like cod liver oil) are available.
without charge or at low cost through health agencies. Nonetheless, a variety of surveys and assessments address why many infants with easy access are not receiving adequate vitamin D. Even among US military physicians only ~70% reported in 2007 recommending supplements for breastfed infants and ~48% recommended vitamin D for formula fed infants. The most common reasons for not recommending were the beliefs that there was adequate sunlight exposure and that breast milk contained adequate vitamin D. None reported taste, cost, or risk of hypervitaminosis as contributing factors (Sherman and Svec, 2009). Encouragingly, these levels are a substantial improvement over reports published just 5 years earlier regarding the civilian populations in the sunny cities of Las Vegas and San Diego (Hayward et al., 1987; Shaikh and Alpert, 2004).

In some regions where sunlight is less abundant, physicians and parents seem better educated about the need for vitamin D supplementation. In Montreal, Canada, e.g. 74% of mothers exclusively breast feeding in 2008 reported meeting the Health Canada supplementation recommendation, up from 58% from a study reported in 2004 (Lepage et al., 2004). However supplementation still declined significantly as infants became partially or exclusively formula fed. Nearly 600 mothers of 2 month old infants in British Columbia, Canada were surveyed in 2010 for compliance with health care professional recommendations. 91% of exclusively breastfeeding mothers reported supplementation with vitamin D as did 20% of exclusively formula feeding mothers. Mixed feeding infants fell between these groups. Over 60% of infants received 300-500 IU vitamin D/day, while only 5% reported no oral vitamin D. (Crocker BMC Public Health, 2011). While many rely on formula to provide sufficient vitamin D, it is not always adequate at the volumes consumed (Gallo et al., 2010). The inadequacy in formula vitamin D fortification is due in part to manufacturers’ limitations and guidance currently provided by government agency guidelines or laws (see Infant Formula Act of 1980) (Infant Formula Act, USA, 1980; Newberry, 1982).

Several groups have investigated means to further support parents’ initiation and maintenance of neonatal vitamin D supplementation, particularly among the highest risk populations. In Norway, in 2004, the standard care was to offer mothers relevant information and encouragement to deliver vitamin D supplements to their infants. An intervention study demonstrated that providing a brochure, available in languages of those at highest risk, and a free seven week supply of vitamin D2 drops, produced a 28 nmol/l improvement in vitamin D status when compared to standard of care. The protocol was especially useful for breast fed infants (Madar et al., 2009). Studies of palatability of vitamin D vehicles (oil vs. alcohol) (Pronzini et al., 2008) and ease of administration (filmstrip vs. dropper) (Rodd et al., 2011) identify means to substantially improve compliance. Vitamin D drops that are more concentrated (i.e. 400 IU/drop vs. 400 IU/ml) have the added advantage that applying a drop to a pacifier or the mother’s breast is a convenient form of delivery (Casey et al., 2010). It should be noted that attempting to fortify breast milk with maternal supplements is an inefficient process. Some reports indicate maternal ingestion of 6,400 IU/day may be necessary to increase vitamin D in breast milk such that the mother provides her infant sufficient vitamin D roughly equivalent to that of infants directly supplemented with 400 IU/day (Wagner et al., 2006).
31. Neonatal effects and premature infants

The information available regarding both counterproductive and effective measures for supplementation should be considered whenever health agencies undertake efforts to improve vitamin D status of newborns.

31.7 Conclusion

Maintaining vitamin D adequacy in the perinatal period and beyond has the potential to positively affect human health well beyond its indisputable role in mineral regulation and bone support. As the non-traditional metabolic activities regulated by availability of vitamin D-related molecules become better understood, there will also emerge an appreciation for the temporal constraints around vitamin D benefits and risks. The neonatal period – characterized by rapid growth, substantial development, and even epigenetic modifications and programming – may prove to be a linchpin for a lifetime of health consequences or benefits. As such it must remain a prime target for research funding, well-designed clinical trials, and open-minded, ongoing evaluation by those entrusted with human health decisions and policy.

References


31. Neonatal effects and premature infants


31. Neonatal effects and premature infants


31. Neonatal effects and premature infants


31. Neonatal effects and premature infants


Key facts

- Vitamin D deficiency rickets is a disease observed most commonly in infants when infants and their mothers have limited sun exposure and have limited or no vitamin D intake.
- Vitamin D deficiency in infants is not only associated with the bone deformity of rickets, but also with hypocalcemic seizures and cardiomyopathy.
- Infant vitamin D intake of at least 400 IU/day is associated with avoidance of vitamin D deficiency rickets.
- Small, pilot studies of maternal vitamin D intake of 2,000 to 6,400 IU/day have shown that breast milk can adequately provide vitamin D supplementation to the infant.
- Ongoing studies will establish the maternal vitamin D intake that safely and effectively supports infant vitamin D health with breastfeeding.

Summary points

- During lactation, breast milk calcium is provided predominantly from maternal bone resorption and this resorption is controlled by increased parathyroid related protein and decreased estradiol.
- The vitamin D activity passed during lactation from mother to infant is mostly in the forms of vitamin D and the metabolite 25-hydroxyvitamin D.
- For mothers receiving 200-400 IU/day vitamin D, breast milk vitamin D activity concentration is 20-70 IU/l which is far less than United States infant formula concentration of at least 400 IU/l vitamin D.
- The American Academy of Pediatrics recommends 400 IU/day vitamin D supplementation for breastfeeding infants.
- The American Academy of Pediatrics defines vitamin D deficiency as 25-hydroxyvitamin D <50 nmol/l.
- Risk factors for vitamin D deficiency for mother and infant during lactation include dark pigmentation, limited sun exposure, sunscreen use, high latitude, winter season, limited maternal intake during pregnancy and lactation, and absence of infant vitamin D supplementation.
- Vitamin D deficiency for breastfeeding infants is a global problem even in sun-rich environments.
- Case-control studies of infant or child vitamin D status and acute lower respiratory infections demonstrate an association between acute lower respiratory infection and 25-hydroxyvitamin D status <22.5 nmol/l.
- Maternal vitamin D intake and status may be adequately elevated to supply vitamin D to the breastfed infant through breast milk.
Abstract

The resurgence in vitamin D-associated rickets and world-wide description of maternal and infant vitamin D deficiency highlights the need for global maternal and infant vitamin D recommendations and action to ensure vitamin D health during lactation. Direct infant vitamin D supplementation of at least 400 IU/day vitamin D will protect the majority of infants from vitamin D deficiency rickets by maintaining 25-hydroxyvitamin D status above 50 nmol/l regardless of race, season, latitude, or maternal vitamin D status. Additionally, the physiology of breast milk production comprises the mechanism required for the mother to supply vitamin D activity to her infant and, with adequate maternal vitamin D intake, a mother is able to supply her infant with the vitamin D required to match the status achieved with direct infant supplementation. As current multi-center, randomized, controlled trials are underway to identify the maternal vitamin D supplementation associated with safe, effective supplementation of the breastfed infant, studies to more accurately define infant vitamin D health also are occurring. Though the vitamin D status and dose to safely avoid vitamin D deficiency rickets are known, the vitamin D status and/or dose (infant and/or maternal) that optimizes infant bone health or immune function for both short- and long-term outcomes has yet to be identified. Current case series and cohort studies demonstrate that infant vitamin D health may be important in avoiding acute lower respiratory infection and diabetes mellitus type 1.

Keywords: breastfeeding, infant, mother, rickets
Introduction

Though vitamin D’s role in preventing rickets has been known for centuries, awareness of contemporary vitamin D science is in its infancy. In the current state, epidemiologic studies and case series raise grave concern that a large proportion of the world lives in a vitamin D-deficient state with associated pathology. Randomized, controlled trials are being performed to evaluate whether the associations described in epidemiologic studies and case series have a biological cause and effect relationship. In investigation of the role of vitamin D in maternal and infant health, direct supplementation to the breastfed infant to avoid vitamin D deficiency and rickets is supported by a number of randomized clinical trials. However, trials addressing functional outcomes such as bone mineralization or immune function are currently smaller in both size and quantity and, therefore, possess less strength of evidence. For investigation of maternal vitamin D supplementation to support the mother and the breastfeeding infant, randomized, controlled trials also are underway. The ecological paradox that the ideal infant nutrition, mother’s milk, lacks a nutrient whose absence is associated with severe bone disease will soon be confirmed or invalidated. An overview of this science of vitamin D in lactation is not only informative, but also provides an example of how scientific method, health policy, and cultural evolution have interacted to both impede and advance knowledge. Review of vitamin D and calcium physiology in lactation, epidemiologic studies of global vitamin D status, and trials investigating the outcomes associated with vitamin D supplementation provide the background to understand the current expert-consensus vitamin D guidelines and potential for far greater knowledge in the near future.

Vitamin D and calcium physiology during lactation

During pregnancy, the calcium needs of the fetus are supplied by a large increase in maternal intestinal calcium absorption and, to a smaller degree, maternal bone mineral resorption especially in the last months of pregnancy (Kalkwarf and Specker, 2002; Kovacs, 2011). Dissimilarly, during lactation, mother’s intestinal calcium absorption returns to the pre-pregnancy state (Kalkwarf and Specker, 2002; Kovacs, 2011). The calcium needs of the breastfeeding infant are met predominantly by elevated maternal resorption of calcium and phosphorus from bone and, to a smaller extent, renal reabsorption of calcium (Kent et al., 1990; Specker et al., 1994). The primary mechanisms of control of the elevated bone resorption are mammary gland production of parathyroid related protein and the lactation-associated decrease in estradiol (Ardeshirpour et al., 2010; Kovacs,
32. Lactation and vitamin D

2011; Sowers et al., 1996). These two mechanisms appear to work synergistically (Ardeshirpour et al., 2010). Through two to six months of lactation, these and likely other mechanisms are responsible for a loss of 5-10% of maternal bone density (Kalkwarf and Specker, 2002; Kovacs, 2011). Following weaning, bone density is rapidly regained to pre-pregnancy status and women who lactate have no long-term ramifications from this bone loss and, unexpectedly, lactation may have a protective effect on post-menopausal bone health (Kalkwarf and Specker, 2002; Polatti et al., 1999; Sowers, 1996). Of note, increasing maternal calcium intake during lactation has no effect on the rate of bone resorption, and increased calcium intake post-weaning does not affect the rate of bone mineral recovery (Fairweather-Tait et al., 1995; Kalkwarf and Specker, 2002; Kovacs, 2008).

Just as calcium intake does not affect the concentration of calcium supply from mother to the infant, vitamin D intake does not affect breast milk calcium levels. This absence of a relationship between maternal vitamin D status and milk calcium concentration was demonstrated in a study of lactating mothers receiving 2,000 or 4,000 IU/day vitamin D from one to four months postpartum. No association was seen between maternal vitamin D dose, maternal 25(OH)D status, and milk calcium concentration (Basile et al., 2006). Prior to this study, concern was raised that vitamin D supplementation of the mother would decrease bone resorption and, thereby, decrease milk calcium concentration.

Although maternal vitamin D status does not demonstrate a significant effect on milk calcium concentration, her vitamin D status has a great role in her milk vitamin D supply. The vitamin D activity or antirachitic activity that a mother passes to her child through breast milk is a combination of the parent compound, vitamin D, and its metabolites. The compound in highest concentration in human milk is vitamin D. Thirty percent of maternal vitamin D and 1% of maternal 25(OH)D are secreted in the milk. Very minimal amounts of other metabolites including the active metabolite, 1,25-dihydroxyvitamin D are present in the milk. Mother's cutaneously-produced vitamin D3 and its metabolite, 25(OH)D3, as well as any oral intake of vitamin D3 or vitamin D2 and their 25(OH)D forms are present in milk (Greer et al., 1984a; Hollis, 1983; Hollis et al., 1981; Reeve et al., 1982; Weisman et al., 1982).

Lactation represents a specific time in women's health when daily intake or production of vitamin D is required. Since the parent compound comprises the majority of the antirachitic activity and this compound has a relatively short half-life, regular dosing is required to guarantee consistency in the vitamin D activity supply to the infant. In other circumstances, weekly or monthly vitamin D dosing may be efficacious, but not during lactation. In fact, the balance of vitamin D and 25(OH)D in milk is well-suited to the needs of the infant. The 1% of 25(OH)D supports a steady supply of antirachitic activity, while the larger supply of vitamin D allows instantaneous passage of maternal vitamin D intake or cutaneous production (Greer et al., 1984a).

Of note, with maternal vitamin D supplementation of 200-400 IU/day vitamin D, mother's milk contains 33-68 IU/l antirachitic activity (Hollis, 1983; Hollis et al., 1981; Wagner et al., 2006). This supply of vitamin D is well below the AAP recommendations of 400 IU/day for infants.
Wagner and Greer, 2008). However, several studies clearly demonstrate the potential for vitamin D distribution from mother to infant. In 1984, Greer et al. (1984b) published a report of a mother with hypoparathyroidism who received 100,000 IU/day vitamin D2 treatment. As reported in other studies, even with this higher intake, mother’s milk contained 30% of her circulating vitamin D concentration and 1% of her circulating 25(OH)D concentration. The antirachitic activity in the milk was 7,660 IU/l.

Cutaneous production of vitamin D from maternal UVB (sunlight) exposure also has been shown to increase the vitamin D content of milk. A study that involved exposing lactating white women to a dose of total body UVB equal to 30 minutes of sunshine at midday on a clear summer day at temperate latitudes demonstrated an increase in vitamin D content of milk with a peak at 48 hours following the exposure and a return to baseline vitamin D activity at seven days. Of note, milk 25(OH)D concentration did not vary, but instead all variation was due to the vitamin D compound concentration in the milk. Additionally, infant 25(OH)D status rose from 34.8 to 51.3 nmol/l (Greer et al., 1984a).

32.3 Vitamin D recommendations for infants

Though these studies describe both oral supplementation and sun-exposure to increase mother’s vitamin D supply to her nursing infant, globally, current maternal vitamin D status does not provide adequate antirachitic activity in milk for all breastfeeding infants to avoid rickets. Rickets is a debilitating bone disease including bowing of legs and curvature of the spine, widening of metaphyses and costochondral joints, and enlargement of the skull. Additionally, severe vitamin D deficiency may present with generalized muscle weakness, cardiomyopathy or hypocalcemic seizures (Balasubramanian et al., 2006; Dunn, 1998; Maiya et al., 2008; Wagner and Greer, 2008). In the past decade, numerous case reports and series from around the world describe a resurgence in vitamin D deficient-rickets (Kreiter et al., 2000; Tomashek et al., 2001; Welch et al., 2000). The number of these reports have risen at the same time as awareness of vitamin D function has grown and as normative values are revised to reflect optimal vitamin D function instead of population norms.

The resurgence in rickets and vitamin D-deficiency associated pathology is partly due to misunderstandings of vitamin D propagated through the 20th century. One of the largest misconceptions was description of vitamin D intake as the cause of supravalvular aortic stenosis (Friedman, 1967; Taussig, 1966). Later discovery demonstrated that this cardiac lesion was due to a genetic anomaly, Williams Syndrome, and not vitamin D intake (Morris and Mervis, 2000). Another precipitating factor in the resurgence of rickets was the decrease in sun exposure in contemporary lifestyle. Both progression to in-door living and use of sunscreen have propagated vitamin D deficiency. Additionally, lack of consistent science describing the vitamin D intake required for breastfeeding infants to avoid rickets has hampered standardization of vitamin D intake recommendations.
Risk factors for rickets are listed in Table 32.1 and include factors associated with decreased UVB exposure and factors associated with decreased vitamin D intake (Dawodu and Tsang, 2007; Wagner and Greer, 2008). A large epidemiologic study in China attempted to identify the vitamin D intake required to avoid rickets. They found no incidence of rickets at 6 months of age in infants receiving as low as 100 IU/day vitamin D supplementation (Specker et al., 1992). Therefore, in 1997, the IOM recommended 200 IU/day as the adequate intake to prevent vitamin D deficiency in normal infants, children, and adolescents (IOM, Food and Nutrition Board, 1997), and the AAP reaffirmed this recommendation by stating that a minimum of 200 IU/day vitamin D should be begun in the first 2 postnatal months (Gartner and Greer, 2003).

Following the recommendation for 200 IU/day infant vitamin D supplementation, United States formula companies continued vitamin D supplementation to provide >400 IU when an infant receives at least one liter of formula. Most formulas in Asia and Europe also contain at least this concentration. The discrepancy between national recommendations in vitamin D supplementation and the vitamin D supplied by infant formula led to a perceptible increase in vitamin D deficiency-rickets specific to the breastfeeding population.

In response to the increased prevalence of rickets, in 2008, the AAP revised its guidelines to recommend 400 IU/day vitamin D to begin in the first postnatal days. This dose was chosen because it is adequate to avoid vitamin D deficiency defined as a 25-hydroxyvitamin D level <50 nmol/l (20 ng/ml). A 25(OH)D level of 50 nmol/l was chosen to define vitamin D deficiency based on the fact that cases of vitamin D-deficiency rickets are reported with 25(OH)D status below this level (Wagner and Greer, 2008). However, the infant vitamin D status that promotes optimal bone mineralization has yet to be defined. Evaluations of an association between vitamin D intake and/or status and bone mineralization are limited by differences in 25(OH)D assay and bone health measurement and demonstrate inconsistent results (Greer and Marshall, 1989; Greer et al., 1982; Kim et al., 2010; Park et al., 1998; Savino et al., 2011).

<table>
<thead>
<tr>
<th>Table 32.1. Risk factors associated with vitamin D deficiency and/or rickets worldwide.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limited sunlight exposure</strong></td>
</tr>
<tr>
<td>Dark pigmentation</td>
</tr>
<tr>
<td>Limited skin exposure due to body covering practices</td>
</tr>
<tr>
<td>Sunscreen use</td>
</tr>
<tr>
<td>Indoor lifestyle</td>
</tr>
<tr>
<td>High latitude</td>
</tr>
<tr>
<td>Winter season</td>
</tr>
<tr>
<td><strong>Limited vitamin D intake</strong></td>
</tr>
<tr>
<td>Limited maternal intake during pregnancy</td>
</tr>
<tr>
<td>Limited maternal intake during lactation</td>
</tr>
<tr>
<td>No infant intake</td>
</tr>
</tbody>
</table>
Following the 2008 AAP policy statement, in 2010, the IOM endorsed the AAP guidelines and defined 400 IU/day as adequate intake for infants 0-12 months of age (Ross, 2011). The recommended dietary allowance for children at or above 1 year of age is 600 IU/day. For mothers, the IOM vitamin D recommended dietary allowance for women 14-50 years old is 600 IU/day. The upper level intake for mothers is defined as 4,000 IU/day (Ross, 2011).

32.4 Prevalence of vitamin D deficiency in breastfeeding infants

Recurrence of rickets in the breastfeeding population has led to evaluation of vitamin D status in breastfeeding infants and their mothers. As mentioned previously, rickets has been diagnosed in infants with vitamin D status below 50 nmol/l (Wagner and Greer, 2008). However, many studies of infant vitamin D status define vitamin D deficiency at far lower levels of 25(OH)D.

The risk for vitamin D deficiency rickets likely starts with vitamin D deficiency in the fetal environment and categorically starts with vitamin D deficiency at birth. Consistently, birth status correlates with later vitamin D status in the un-supplemented or under-supplemented infant (Bhalala et al., 2007; Challa et al., 2005; Sachan et al., 2005). Vitamin D status at birth is consistently low with description of mean cord blood levels of 21 nmol/l (Sachan et al., 2005) and 48.4 nmol/l (Bhalala et al., 2007) in India, a range of 10-80 nmol/l with 63.7% below 30 nmol/l in France (Zeghoud et al., 1997), 44.3 nmol/l in Korea (Kim et al., 2010), and, in the United States, mean levels of 26.3 nmol/l in African American infants and 49 nmol/l in Caucasian infants, respectively (Basile et al., 2007). Additionally, Basile et al. showed a significant seasonal variation in cord blood 25(OH)D for Caucasian but not African American infants. Compared to birth in April through October, birth in November through March was associated with 28.3 nmol/l lower vitamin D status for Caucasian infants and only 7.5 nmol/l lower status for African American infants (Basile et al., 2007). Therefore, dark pigmentation and winter season are important risk factors for vitamin D deficiency not only during lactation but also prior to lactation in the fetal period.

Studies examining vitamin D status throughout lactation show persistence if not worsening of vitamin D deficiency. The Bhalala et al. (2007) study, defining vitamin D deficiency as a 25(OH)D level <62.5 nmol/l, found a vitamin D deficiency prevalence of 62% at birth and 80% at 3 months in breastfeeding infants. In Greece, Challa et al. (2005) demonstrated a significant seasonal difference in vitamin D status at 1 week of age with means of 16.8 and 25.3 nmol/l in winter- and summer-born infants, respectively. Of note, during 6-month follow-up, winter-born infants had a mean increase in 25(OH)D to 48.5 nmol/l and summer-born infants had no change. Other studies have shown no seasonal variation in infant vitamin D status (Jain et al., 2011; Saadi et al., 2009). Further examples of vitamin D deficiency in breastfeeding infants are presented in Table 32.2.

In the United States, a breastfeeding study in Iowa found an overall prevalence of 10% vitamin D deficiency (defined as 25(OH)D <27.5 nmol/l). In the winter, 78% of unsupplemented infants
were vitamin D deficient, while in the summer only one infant with dark pigmentation exhibited deficiency (Ziegler et al., 2006). Additionally, the NHANES survey 2001-2004 demonstrated a 9% rate of vitamin D deficiency (25(OH)D <37.5 nmol/l) and a 61% rate of vitamin D insufficiency (25(OH)D 37.5-72.5 nmol/l) in a national sample of children age 1-21 (Kumar et al., 2009). Though vitamin D status was not evaluated with breastfeeding status, this NHANES survey reveals that vitamin D deficiency is not a problem isolated to countries without standard vitamin D fortification of milk products.

### 32.5 Effects of vitamin D deficiency in breastfeeding infants

Evaluation of the relationship of vitamin D status with markers of calcium and phosphorus homeostasis, such as calcium, phosphorus, parathyroid hormone, and alkaline phosphatase status, have shown inconsistent results for breastfeeding infants (Challa et al., 2005; Dawodu et al., 2003; Jain et al., 2011; Kim et al., 2010; Madar et al., 2009; Seth et al., 2009; Ziegler et al., 2006). Therefore, at this time, the biochemical outcomes associated with vitamin D deficiency and its effects on bone mineralization and calcium homeostasis are not well-described until a child reaches the severe stages as demonstrated by rickets, hypocalcemic seizures, or heart failure (Wagner and Greer, 2008).

In assessment of bone health and vitamin D status of infants, observational studies have demonstrated a 16% prevalence of radiographic rickets with vitamin D deficiency (Agarwal et al., 2010; Jain et al., 2011). A study using quantitative ultrasound to assess bone status demonstrated lower markers of bone health in breastfeeding infants who did not receive vitamin D supplementation. However, the parameters also demonstrated significant differences based on infant length and/or weight (Savino et al., 2011). Therefore, further prospective evaluation with contemporary bone assessment technology is required. As mentioned previously, randomized trials have not consistently shown improvement in bone health with vitamin D supplementation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Age of infant</th>
<th>Infant 25(OH)D status (nmol/l)</th>
<th>Maternal status (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawodu et al., 2003</td>
<td>Median 6 weeks (4-16 weeks)</td>
<td>Median 11.5</td>
<td>Median 21.5</td>
</tr>
<tr>
<td>(United Arab Emirates)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Madar et al., 2009</td>
<td>6 weeks</td>
<td>Mean 41.7</td>
<td>Mean 25.8</td>
</tr>
<tr>
<td>(non-Western immigrants in Norway)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jain et al., 2011</td>
<td>Mean 13.6 weeks</td>
<td>Median 25.3</td>
<td>Median 24.5</td>
</tr>
<tr>
<td>(India)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seth et al., 2009</td>
<td>2-24 weeks</td>
<td>Mean 29</td>
<td>Mean 27.3</td>
</tr>
<tr>
<td>(India)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agarwal et al., 2010</td>
<td>10 weeks</td>
<td>Mean 28.9</td>
<td>Mean 24.6</td>
</tr>
<tr>
<td>(India)</td>
<td>6 months</td>
<td>Mean 42.4</td>
<td>Not measured</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Assessment of the role of vitamin D in other infant health outcomes has raised concern that vitamin D deficiency is associated with respiratory, infectious outcomes, and autoimmune outcomes. One large cohort study evaluated the long-term effect of vitamin D intake in the first postnatal year and found an 80% decrease in the risk for diabetes type I for infants who received at least 2,000 IU/day vitamin D though the first year (Hypponen et al., 2001). This study has led to ongoing investigation of the physiological cause for this potential relationship.

In case-control studies examining whether an association between infant or children vitamin D status and ALRI exists, the likelihood of finding differences between cases and controls appears to be related to the underlying vitamin D status of the study population. Very interestingly, three studies have demonstrated significant differences between cases and controls with mean 25(OH)D status of 22.8-29.1 nmol/l for cases versus 38.4-40.8 nmol/l for controls (Karatekin et al., 2009; Roth et al., 2010; Wayse et al., 2004). In studies performed in populations with higher vitamin D status, no statistical or clinical difference is appreciated in vitamin D status between cases and controls (77.2-81 vs. 77-83 nmol/l, respectively) (McNally et al., 2009; Roth et al., 2009). Of note, in the studies demonstrating a significant difference in 25(OH)D status between ALRI cases and matched controls, Karatekin et al. evaluated neonates and found mothers also demonstrated significantly different vitamin D status (mean 33.5 vs. 57 nmol/l, respectively) (Karatekin et al., 2009). Also, in the Wayse et al. study, 25(OH)D status >22.5 nmol/l and exclusive breastfeeding in the first 4 postnatal months were both individually associated with lower odds for severe ALRI (Wayse et al., 2004). Therefore, the potential benefit for vitamin D status to decrease ALRI risk appears to occur by raising vitamin D status from the 22.8-29.1 nmol/l range to at least the 38.4-40.8 nmol/l range.

### 32.6 Direct vitamin D supplementation to the breastfeeding infant

Based on these studies of infant health outcomes, vitamin D status with 25(OH)D >50 nmol/l appears to support avoidance of the severe negative effects of vitamin D deficiency. Therefore, establishing the vitamin D dose that promotes avoidance of vitamin D deficiency for all populations is critical. The dose of 400 IU/day, currently recommended for infants in the United States, appears to achieve avoidance of vitamin D deficiency. However, defining vitamin D sufficiency and, therefore, the vitamin D status that optimizes outcomes such as bone mineralization is not known. As studies are performed to determine vitamin D sufficiency for breastfeeding infants, recommendations for optimal vitamin D status and vitamin D intake may be revised, but currently 400 IU/day is the goal (Wagner and Greer, 2008).
Contemporary studies of the effect of 400 IU/day supplementation on the vitamin D status of breastfeeding infants are few and are shown in Table 32.3. In the Wagner et al. (2010) study, infants entered the study at 1 month of age and mean 25(OH)D at that time was 40 nmol/l. By the next evaluation at 4 months of age, 25(OH)D status had risen to a mean of 109 nmol/l and this increase in status persisted through the 6-month study time period.

Three further studies have evaluated other concentrations of oral vitamin D dosing or UVB exposure. Ho et al. (1985) randomized infants one to eight months of age who were exclusively breastfeeding without vitamin D supplementation to receive usual sunlight exposure or to receive a regimented 2 hours of sunshine per day. Over the 2-month study period, 25(OH)D status did not significantly vary in controls but increased in the 2-hour sunlight exposure group. Additionally, the final 25(OH)D status correlated with UVB exposure with estimation that daily 24 minutes of only face exposure will maintain a serum 25(OH)D level of 27.5 nmol/l. The authors did not describe the ultraviolet light exposure necessary to maintain a level of 50 nmol/l (Ho et al., 1985).

In Korea, a randomized, controlled study investigated the effect of 200 IU/day vitamin D supplementation to exclusively breastfed infants. The infants started with a mean 25(OH)D status of 44.3 nmol/l at birth. Over a 12-month study period, unsupplemented and supplemented breastfed infants’ 25(OH)D status improved to 83 and 107 nmol/l, respectively. A third group of formula-fed infants achieved mean serum 25(OH)D status of 122 nmol/l. This study also showed significantly higher bone mineral density in formula-fed infants compared to both the unsupplemented and 200 IU/day supplemented breast-fed infants reiterating the need for further evaluation of the relationship of vitamin D status and bone mineralization beyond the diagnosis of rickets (Kim et al., 2010).

Despite this evidence of improved vitamin D status with vitamin D supplementation and the resurgence of rickets in the breastfed-infant population, many pediatric care providers and families are resistant to supplement vitamin D to the breastfeeding infant. Studies examining the rates of pediatric provider recommendation for vitamin D supplementation of breastfed infants have demonstrated only 36.4% and 44.6% recommendation and, in another study, 52.3% of providers stated no recommendation (Davenport et al., 2004; Shaikh and Alpert, 2004; Taylor et

<table>
<thead>
<tr>
<th>Study</th>
<th>Serum 25(OH)D with no vitamin D supplement (nmol/l)</th>
<th>Serum 25(OH)D with 400 IU/day vitamin D (nmol/l)</th>
<th>Length of supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greer et al., 1982</td>
<td>32.3</td>
<td>81.8</td>
<td>Birth to 6 months</td>
</tr>
<tr>
<td>Greer and Marshall, 1989</td>
<td>58.8</td>
<td>92.5</td>
<td>Birth to 6 months</td>
</tr>
<tr>
<td>Wagner et al., 2010</td>
<td>Not studied</td>
<td>106.3</td>
<td>1 to 7 months</td>
</tr>
</tbody>
</table>
In fact, in North Carolina, where 25.8% of surveyed AAP members reported seeing at least 1 case of vitamin D deficient rickets within the previous 3 years, only 44.6% recommended vitamin D supplementation for all breastfed infants and 38.6% recommended for some breastfed infants, but 16.5% never recommended vitamin D (Davenport et al., 2004). Both pediatric provider and parental reasons for avoiding vitamin D supplementation of the breastfed infant include belief that vitamins are unnecessary because breast milk has all needed nutrition, concern for cost, concern for hypervitaminosis D, and concern that recommending supplementation would decrease the likelihood for successful breastfeeding (Davenport et al., 2004; Taylor et al., 2010).

The need for direct vitamin D supplementation of the breastfed infant does not make ecological sense. If breast milk is deficient in this key nutrient, how have breastfed infants survived for thousands of years? The answer may be that maternal vitamin D deficiency is a modern problem. In the past, populations lived in sun-rich environments or had a regular dietary source high in vitamin D such as fatty fish. Therefore, is it possible that human milk is designed to be sufficient in vitamin D and to provide complete nutrition to the breastfed infant?

### 32.7 Vitamin D supplementation of mother to support the breastfed infant

As mentioned previously in this chapter, study of maternal vitamin D supplementation to adequately support infant vitamin D health has been limited by unfounded concerns regarding vitamin D safety and, specifically, the IOM 1997 definition of 2,000 IU/day as the upper tolerable intake limit (IOM, Food and Nutrition Board, 1997; Taussig, 1966). For example, Alahoula et al. (1986) in Finland compared maternal intake of 2,000 IU/day versus infant intake of 400 IU/day and demonstrated equivalent vitamin D status of infants. Maternal vitamin D status with 2,000 IU/day increased from a mean of 27.5 nmol/l to near 100 nmol/l and infant status improved from a mean of 21.3 nmol/l to near 75 nmol/l. This improvement in infant status matched that observed in the group of infants receiving 400 IU/day directly. Although these results demonstrated that breast milk could adequately support the vitamin D health of the infant, they essentially were ignored for 20 years because as the authors commented, ‘As such a dose is far higher than the daily dietary allowance recommended for lactating mothers its safety over prolonged periods is not known and should be examined’ (Ala-Houhala et al., 1986).

The current IOM guidelines define 4,000 IU/day as the upper level intake and acknowledge that the specific needs for pregnant and lactating women have yet to be identified (Ross, 2011). Therefore, with progression towards recognition that higher doses of vitamin D are safe and realization that, with these doses, the opportunity to supplement mother to avoid supplementation of the infant exists, contemporary research to identify the vitamin D supplementation of mother that promotes vitamin D health for the infant is underway. Again, progress in this field has been limited by toxicity concerns. In fact, the United States Food and Drug Administration approval for an Investigational New Drug was required for studies of vitamin D intake of 4,000 IU/day or greater during lactation. This approval was the first ever required for the study of a vitamin.
Building from the work by Ala-Houla et al. (1986), Hollis and Wagner (2004), published their study evaluating the ability of 2,000 IU/day and 4,000 IU/day vitamin D to support both mother and breastfeeding infant 25(OH)D status by providing all subjects with 400 IU/day vitamin D3 and then additional 1,600 IU/day and 3,600 IU/day given as vitamin D2, respectively. This methodology allowed investigation of the amount of 25(OH)D increased by the extra oral supplementation above the standard 400 IU/day vitamin D3 and the endogenous production of vitamin D3 by both mother and infant. Mother/infant dyads were enrolled at 1 month postpartum if exclusively breastfeeding and were followed for 3 months. The results achieved in this study are presented in Table 32.4. Of note, the infant’s vitamin D status in both groups achieved means above 50 nmol/l which may point to 2,000 IU/day as sufficient maternal dose to support the breastfeeding infant. However, only 22 and 39% of infant 25(OH)D status was 25(OH)D2 which means infants likely received vitamin D from other sources such as feed supplementation with infant formula.

This study demonstrated important factors. Firstly, it demonstrated the passage of vitamin D and its antirachitic metabolites from mother, through the milk, to the infant in the vitamin D2 form. Secondly, for both mother and infant, serum calcium concentrations remained in normal range and no hypercalciuria was observed. However, abnormal calcium chemistries were not expected as all subjects’ vitamin D status remained below potentially toxic levels (Hollis and Wagner, 2004).

This study was followed by another published in 2006 comparing high- vs. low-dose vitamin D supplementation of mother. In this study, mother/infant dyads were randomized to receive

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin D status</th>
<th>Study time point</th>
<th>1,600 IU/day vitamin D2 and 400 IU/day vitamin D3</th>
<th>3,600 IU/day vitamin D2 and 400 IU/day vitamin D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>Total 25(OH)D1</td>
<td>Baseline</td>
<td>69</td>
<td>82.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>90.3</td>
<td>111.3</td>
</tr>
<tr>
<td></td>
<td>25(OH)D2</td>
<td>Baseline</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>43.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Infant</td>
<td>Total 25(OH)D1</td>
<td>Baseline</td>
<td>19.8</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>69.5</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>25(OH)D2</td>
<td>Baseline</td>
<td>&lt;1.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Milk</td>
<td>Antirachitic activity</td>
<td>Baseline</td>
<td>35.5</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>Antirachitic activity</td>
<td>3 months</td>
<td>69.7</td>
<td>134</td>
</tr>
</tbody>
</table>

1 25(OH)D given as nmol/l.
2 Antirachitic activity given as IU/l.
maternal intake of 6,400 IU/day and no direct infant supplementation versus maternal intake of 400 IU/day and infant intake of 300 IU/day. Enrollment occurred at one month postpartum and subjects were followed for 6 months. The primary goal of the study was to determine whether vitamin D supplementation of 6,400 IU/day would result in circulating values of 25(OH)D exceeding 225 nmol/l which, at that time, was chosen as the upper limit of normal. The maternal vitamin D status achieved in the 6,400 IU/day group was an increase from a mean of 85 to 147 nmol/l. This increase was significantly different from the vitamin D status observed for the mothers receiving 400 IU/day for 6 months who showed a change in mean status from 80.8 to 96 nmol/l. For the milk antirachitic activity of the mothers receiving 6,400 IU/day, the activity increased from 82 IU/l at baseline to 873 IU/l after 6 months. The antirachitic activity of the control mothers’ milk did not significantly vary over the study period (45.6-78.6 IU/l) and had a nadir of 45.6 IU/l at 5-months postpartum. In evaluation of infant vitamin D status, the infants whose mother’s received 6,400 IU/day achieved very similar 25(OH)D concentrations (increase from baseline of 35 to 115 nmol/l) to the infants who received 300 IU/day direct supplementation with mothers receiving 400 IU/day (increase from baseline of 32.5 to 107 nmol/l). In evaluation of safety, no mothers exceeded 225 nmol/l, and maternal and infant serum calcium and phosphorus levels and urinary calcium to creatinine were all within normal range (Wagner et al., 2006).

Two further studies have been performed in the United Arab Emirates (UAE). Despite the sun-rich environment of the UAE, many mothers exhibit vitamin D deficiency and infantile vitamin D rickets is common due to limitations in maternal sun exposure due to conservative clothing practices and to the lack of vitamin D fortification of food products (Saadi et al., 2007). In the first study, Saadi et al. (2007) examined the effect of 2,000 IU/day or 60,000 IU/month vitamin D intake on the vitamin D status of nulliparous and lactating women. The investigators used vitamin D2 supplementation as that was the only high-dose supplementation available in the UAE at the time. Additionally, the oral intake of 2,000 IU/day was chosen to not exceed the IOM upper tolerable intake limit.

Overall, at baseline, mean 25(OH)D for lactating women was 25.2 nmol/l. All women except one had 25(OH)D <50 nmol/l and one-third had levels <20 nmol/l. From baseline to the 3 month evaluation, mean 25(OH)D increased by 12.4 nmol/l in the daily intake group and by 14.8 nmol/l in the monthly intake group. For both groups, serum calcium concentrations increased, but did not exceed the normal range. One subject in the monthly intake group exhibited elevation of urinary calcium to creatinine ratio (Saadi et al., 2007).

In a follow-up, Saadi et al. (2009) published an evaluation of healthy breastfeeding mothers and their infants with the same maternal vitamin D dosing regimens. Infants in both groups received 400 IU/day. Over a 3 month study period, infant 25(OH)D status increased from 13.9 to 49.6 nmol/l when mothers received 2,000 IU/day and from 13.7 to 44.6 nmol/l when mothers received 60,000 IU/month. Milk antirachitic activity increased from undetectable (<20 IU/l) to 50.9 IU/l (Saadi et al., 2009). This low amount of antirachitic activity demonstrates the importance of continuing infants on 400 IU/day even with maternal supplementation of 2,000 IU/day or 60,000 IU/month when baseline population vitamin D deficiency is so severe.
The inadequacy of 2,000 IU/day maternal intake to provide adequate vitamin D supply to the nursing infant also has been recently demonstrated in the United States. In a NIH-funded randomized, controlled trial of vitamin D dosing during lactation to support mother and infant, a study arm of 2,000 IU/day was terminated because this dose was ‘inadequate’ to supply sufficient vitamin D to maintain healthy vitamin D status of the breastfeeding infant (Hollis and Wagner, 2011). This study continues to investigate the safety and efficacy of 6,400 IU/day maternal intake and is near completion (Wagner et al., 2006). This study is the next step in establishing the maternal vitamin D intake or status to adequately support the vitamin D health of the breastfed infant.

### 32.8 Conclusion

The resurgence of rickets described in case series and the high prevalence of infant vitamin D deficiency described in cross-sectional studies illustrate the global need for improvement not only in maternal and infant vitamin D recommendations, but also improvement in guaranteeing that the recommendations are followed. For those who are reticent to provide direct infant vitamin D supplementation due to belief that breast milk as the physiological gold-standard of infant nutrition could not be inadequate in a key nutrient, recognition that maternal vitamin D supplementation can support infant and maternal health while avoiding maternal toxicity is revolutionary.

Until the maternal dose for optimal safety and efficacy is established, 400 IU/day appears to be the infant intake associated with avoiding vitamin D deficiency rickets. However, research still is needed to identify the optimal vitamin D status for bone health, calcium homeostasis, and other vitamin D functions such as autoimmune, anti-infectious, and respiratory health. Moreover, further evaluation of the long-term effects of infant vitamin D status is required.

Until these studies are performed, ensuring vitamin D health of the mother/infant breastfeeding dyad includes recognition of the global prevalence of vitamin D deficiency, recognition of risk factors for vitamin D deficiency, including deficiency in pregnancy, and recommending at least 600 IU/day and potentially up to 4,000 IU/day maternal intake and 400 IU/day infant intake. With attention to these details and forthcoming study results, vitamin D deficiency rickets will again become a disease of the past.

### References


32. Lactation and vitamin D


Hollis, B.W. and Wagner, C.L., 2004. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. American Journal of Clinical Nutrition 80, 1752S-1758S.


**Key facts**

- Vitamin D level during pregnancy has been found to be surprisingly low in an appreciable proportion of women from developing countries, even from rural communities.
- Vitamin D fortified food is not widely available in developing countries, and pregnant women from a rural or poor socioeconomic class have inadequate dietary calcium intake.
- Supplementing vitamin D on a daily basis is a challenge in developing countries, where there is already poor utilization of antenatal services.
- Periodic interval supplementation that can be directly observed and given during the antenatal visit may hold promise for various developing countries.
- Providing a pharmacological dose of vitamin D during second and third trimester, which can be matched in timing with the minimal antenatal visits, has been shown to be effective in improving 25(OH)D levels at delivery and may beneficially affect neonatal anthropometry. A weekly regimen trial can also be used in some settings.

**Summary points**

- There is heterogeneity in prevalence data even at the same latitude, which reflects sunshine exposure and diet based on local culture and customs.
- Pregnant women in the third trimester with homebound activities and during winter constitute the most vulnerable group.
- Women from developing countries have a low dietary calcium intake. Lack of food fortification or lack of use of supplements leaves exposure to sunshine as the only source of vitamin D, a factor that may be inadequate in many settings despite abundant sunshine.
- Vitamin D supplementation during pregnancy needs attention in many developing regions such as South Asia, and the Middle East which have observed the maximum burden.
- Some studies have shown that it may affect the outcome of infection in mother and child during pregnancy, a topic relevant to developing countries.
- Weekly, monthly, or trimester specific supplementation as opposed to daily supplementation is a more promising prospect for pregnant women from developing countries in view of compliance.
- Against a background of minimal use of antenatal services, pharmacological dose supplementation can be offered at the same time as contact with health care providers during second and third trimester, providing the benefit of observed therapy.
- Long-term safety and efficacy data will need to be generated for intervention and supplementation studies.
33. Vitamin D replacement in pregnant women in developing countries

M.T. Sahu¹,² and M. Sahu³
¹ADIVA Hospital, 15 Anand Lok, New Delhi 110049, India; ²Institute of Liver and Biliary Sciences, D-1, Vasant Kunj, New Delhi, India; ³VIMHANS Hospital, No.1, Institutional Area, Nehru Nagar, New Delhi 11065, India; meenakshi_sahu@rediffmail.com

Abstract

Many pregnant women residing in developing countries have inadequate vitamin D levels despite the fact that some of these countries are at a lower latitude. Darker pigmentation with inadequate sun exposure, a home-bound lifestyle especially during the third trimester, and limited dietary source of vitamin D due to lack of availability of fortified foods, all contribute towards maternal hypovitaminosis D. Inadequate vitamin D levels further contribute to the precarious nutritional state accompanied by a markedly inadequate intake of dietary calcium typically observed in many low resource settings. Early marriage along with early pregnancy are some of the other challenges specific to developing parts and attention is needed for vitamin D and bone health issues during pregnancy. Some regions, especially South Asia, have noted a high prevalence of vitamin D insufficiency among pregnant women aggravated during the winter. Despite the high prevalence vitamin D insufficiency and the importance of vitamin D during pregnancy, it continues to remain an underappreciated problem in many developing countries. Infrequent and inadequate use of antenatal care by many rural women provides a limited window for assessing risk and planning intervention with measures in the area of education or medication. Daily vitamin D supplementation may meet challenges related to compliance in low resource settings. Pharmacological doses per week or per trimester have been observed to improve 25(OH)D levels at delivery and amidst inconsistent data some trials have shown a beneficial impact on the anthropometry of new-borns. There have been no reports of any adverse risk to mother or child with such an approach. There will continue to be a debate on what constitutes adequate levels and whether vitamin D supplementation indeed influences any maternal or fetal outcome. While one reaches to find conclusive answers, given the growing burden of this condition, periodic supplementation should be offered to pregnant females at risk.

Keywords: supplementation, low resource, rural, antenatal, maternal
33.1 Introduction

Many developing nations, despite several of them being at lower latitudes, have vitamin D deficiency among their populations (Arabi et al., 2010; Van Schoor and Lips, 2011). Recent hospital and community-based studies from these parts have observed vitamin D insufficiency among an appreciable proportion of females during their reproductive period. Given the advanced understanding of the multiple role of vitamin D in human health (Holick, 2007; Vieth, 2011), VDD during pregnancy has been a subject of great interest to many.

Maintaining an adequate level of vitamin D during pregnancy may play an important role such as ensuring adequacy of calcium for the growing fetus without any adverse effect on maternal bone health; reducing risk outcome such as preeclampsia, neonatal hypocalcaemia and infections; a likely effect on neonate anthropometry; and a possible role in fetal programming of long-term skeletal and non-skeletal health benefits (Barrett and McElduff, 2010; De-Regil et al., 2012; Dror and Allen, 2010; Hollis and Wagner, 2011; Thandrayen and Pettifor, 2010). The general impact of maternal vitamin D levels on pregnancy and neonatal outcome is discussed in Chapter 31.

Specific challenges in developing countries are the markedly low intake of dietary calcium, lack of availability of fortified foods, early marriage, adolescent pregnancy, and barriers to using a modern health care delivery system. With all the above background, it is important for the individual practitioner and health policy makers in developing nations to be aware of their geographical prevalence data and take pragmatic measures to replace vitamin D in pregnant women.

This chapter provides a review of vitamin D deficiency and its replacement in pregnant women from developing nations. For the purposes of the current chapter, developing nations were considered as per World Bank classification (World Bank, 2012). The essence of chapter is for poor resource setting with its challenges in reproductive health care such as what exists in a vast majority of rural population at many developing countries.
33.2 Hypovitaminosis D during pregnancy: magnitude and relevance to developing countries

33.2.1 Causative factors

Vitamin D deficiency and its implications during pregnancy have long been recognized from developing parts of the world (Maxwell and Miles, 1925). Multiple past and recent studies have shown vulnerability to this condition among immigrants from developing countries (Bashir et al., 1981; Hodgkin et al., 1973; Madar et al., 2009; Van der Meer et al., 2006; Watney et al., 1971). Some of the reasons cited for immigrants are factors such as dark complexion, cultural dress that restricts exposure of skin to the sun, and low intake of supplements.

Most of these factors observed among immigrant women have also been observed to exist in antenatal women residing in their native country. Darker pigmented skin needs more exposure to UVB rays than fair pigmented skin to generate the same amount of vitamin D (Gemens et al., 1982; Holick et al., 1981; Lo et al., 1986; Matsuoka et al., 1991). It is being realized that despite many of the developing nations having year round sunshine, the amount of time that a woman spends outdoor may be shorter than that required for adequate levels (Sachan et al., 2005; Sahu et al., 2009a). While the practice of purdah (use of veils) is known to affect vitamin D levels, cultural dress such as the sari in some South Asian countries (the dress usually leaves only head and arms exposed) also limits exposure to sunshine (Islam et al., 2002; Sachan et al., 2005; Sahu et al., 2009a). Furthermore, women avoid outdoor activities during the time of peak solar energy (noon time), and pregnant females during their third trimester are restricted to indoor activities. Being non-veiled does not protect women from VDD compared to veiled women if there is a homebound lifestyle and only occasional sun exposure (Islam et al., 2006). A growing index of atmospheric pollution in urban settings as the cost of rapid progress in developing countries can limit the amount of UVB solar radiation reaching the ground and may also contribute to VDD (Agarwal et al., 2002). Although it merits detailed study in rural settings, atmospheric brown clouds due to aerosol emanating from biofuels that is used for domestic purposes in rural regions (Di Girolamo et al., 2004; Ramanathan et al., 2005) can scatter solar radiation, and may have a role to play.

33.2.2 Characteristics of developing countries

Within the context of vitamin D and pregnancy, two important factors that differentiate high and low income countries, is firstly the low intake of dietary calcium, and secondly the lack of availability of vitamin D fortified foods (Prentice, 2011). Consumption of animal milk and milk products is low in many African and Asian countries compared to the developed world (Food and Agriculture Organization, 2001). Furthermore, pregnant women from rural regions usually have a monotonous cereal-based diet high in phytates that can further decrease bioavailability of calcium (Cheng et al., 2009; Panwar and Punia, 2000). Most developing countries have yet to witness wide access to vitamin D fortified foods. Thus, many pregnant females are not only on a diet that is markedly low in calcium (sometimes as low as 200 mg per day), but are also deprived
of adequate levels of vitamin D due to the above factors. Interestingly, low calcium intake has been hypothesized as leading to secondary vitamin D deficiency (Clements et al., 1987; Harinarayan et al., 2007; Islam et al., 2002; Sachan et al., 2005; Sahu et al., 2009a). While in a state of vitamin D and calcium deprivation, maternal physiology will adapt to ensure that the fetus gets the calcium, but this comes at the expense of the mothers, putting them at risk of osteomalacia and other adverse outcomes (Barrett and McElduff, 2010; Prentice, 2011).

Early childhood marriage is still a prevalent practice in many developing countries, mostly observed in Africa and Asia, with some regions such as Nigeria, Bangladesh having more than half of girls married before the age of 18 (Population Reference Bureau, 2011). In addition, many developing regions have reported a high prevalence of vitamin D deficiency among adolescent girls (Du et al., 2001; Sahu et al., 2009a). It is to be presumed that many such adolescent girls enter pregnancy with an already depleted store of vitamin D and a poor skeletal mineral mass. Untreated osteomalacia from a young age can affect pelvic outlet that may impact the route of delivery (Brickley and Ives, 2008; Kabakyenga et al., 2011; Konje and Ladipo, 2000). It is also our anecdotal observation that pregnant women from poor economic strata who present with obstructed labor due to an abnormal pelvic outlet have been in a state of impoverished nutrition since childhood and adolescence, and have usually undergone early marriage and pregnancy, with a clinical picture suggestive of clinical osteomalacia (bone and muscle pain, muscle weakness) in previous pregnancies. It is possible that, if left untreated, each pregnancy with low nutrition status starting from adolescence can impact the outcome of successive pregnancies. Such an observation, however, remains unproven. There have been conflicting reports supporting (Merewood et al., 2009) or refuting (Brunvand et al., 1998) association of vitamin D status at time of delivery to route of delivery. It is essential that the focus is placed on bone health during adolescence and pregnancy, as adolescent pregnancy is a predictor for post-menopausal osteoporosis (Cho et al., 2012).

Low birth weight is a challenging issue in developing countries with its associated risk of morbidity and mortality (McIntire et al., 1999). Newborn size, usually a reflection of prenatal growth, can be affected by maternal nutritional status. Although overall data on vitamin D and its effect on offspring size is inconsistent (Specker, 2012), a few have demonstrated a correlation between neonatal anthropometry and cord blood calcium (Doi et al., 2011) or vitamin D sufficiency in mothers (Kalra et al., 2012; Lefelaar et al., 2010).

Other characteristics in the context of supplementation during pregnancy are provided in the section on supplementation.

### 33.2.3 Magnitude and impact

The prevalence of vitamin D insufficiency among pregnant females reported in selective studies from developing parts of the world is provided in Table 33.1. While most have utilized the cut-off of 25 nmol/l to define deficiency, there have been debates on what constitutes a sufficient level. Some suggest using levels of 75 nmol/l while others have advocated using 50 nmol/l for
Table 33.1. Prevalence of hypovitaminosis D in pregnant women from developing countries.

<table>
<thead>
<tr>
<th>Region/country</th>
<th>Latitude</th>
<th>n</th>
<th>Prevalence (%)</th>
<th>Cut-off (nmol/l)</th>
<th>Mean value (nmol/l)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sub-Saharan Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gambia</td>
<td>13°N</td>
<td>125</td>
<td>16</td>
<td>&lt;80</td>
<td>111±27</td>
<td>Pregnant women from rural villages. Data shown here is at 36th week of gestation. Data is a subset from a calcium intervention study</td>
</tr>
<tr>
<td>Prentice et al., 2009a</td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td>Ethiopia</td>
<td>10°N</td>
<td>31</td>
<td>81</td>
<td>&lt;50</td>
<td>25 (17-46)</td>
<td>Full term pregnant women. Sample collected during August and September</td>
</tr>
<tr>
<td>Feleke et al., 1999</td>
<td></td>
<td></td>
<td></td>
<td>55</td>
<td>&lt;30</td>
<td></td>
</tr>
<tr>
<td>Tanzania</td>
<td>6°S</td>
<td>884</td>
<td>40</td>
<td>&lt;80</td>
<td>89 ±31</td>
<td>HIV infected pregnant women at 12-27th week. Data is a subset of baseline data from a vitamin trial supplementation study</td>
</tr>
<tr>
<td>Mehta et al., 2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Middle East and North Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>35°N</td>
<td>552</td>
<td>97</td>
<td>&lt;80</td>
<td>27.8±21.71</td>
<td>Recruited during winter season</td>
</tr>
<tr>
<td>Maghbooli et al., 2007</td>
<td></td>
<td></td>
<td></td>
<td>67</td>
<td>&lt;35</td>
<td></td>
</tr>
<tr>
<td>Bassir et al., 2001</td>
<td>35°N</td>
<td>50</td>
<td>80</td>
<td>&lt;25</td>
<td>12.8±26</td>
<td>Women who delivered between January and September were recruited</td>
</tr>
<tr>
<td><strong>Latin America &amp; Caribbean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>55°S</td>
<td>16</td>
<td>62</td>
<td>&lt;20</td>
<td>15.8±12</td>
<td>16 pregnant women from Ushuaia (55°S) and 21 pregnant women from Buenos Aires (34°S). Study conducted at end of winter</td>
</tr>
<tr>
<td>Oliveri et al., 1993</td>
<td>34°S</td>
<td>24</td>
<td></td>
<td></td>
<td>36±21</td>
<td></td>
</tr>
</tbody>
</table>
Table 33.1. Continued.

<table>
<thead>
<tr>
<th>Region/country</th>
<th>Latitude</th>
<th>n</th>
<th>Prevalence (%)</th>
<th>Cut-off (nmol/l)</th>
<th>Mean value (nmol/l)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>East Asia and Pacific</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wang et al., 2010</td>
<td>26°34'N</td>
<td>77</td>
<td>97</td>
<td>&lt;80</td>
<td>35.9±19.7</td>
<td>West China. September recruitment. Significantly low calcium intake observed in rural women as compared to urban women. Lower vitamin D in rural women compared to urban</td>
</tr>
<tr>
<td>Jiang et al., 2012</td>
<td>31°N</td>
<td>152</td>
<td>94.7</td>
<td>&lt;50</td>
<td>31.9±9.2</td>
<td>South East China. Urban residents. Data shown here collected in summer. Vitamin D levels were significantly lower in winter</td>
</tr>
<tr>
<td><strong>Europe and Central Asia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ergür et al., 2009</td>
<td>39.5°N</td>
<td>70</td>
<td>81</td>
<td>&lt;62.5</td>
<td>39.8 (10-125.5)</td>
<td>Sample collected during the first 24 hours after delivery in mothers. Recruitment over two years</td>
</tr>
<tr>
<td>Halicioglu et al., 2012</td>
<td>38.3°N</td>
<td>258</td>
<td>90</td>
<td>&lt;50</td>
<td>27.5±13.5</td>
<td>Last trimester sample. Half of these women had covered dressing style. 58% did not use any multivitamin. Study period was spring time: March, April, May</td>
</tr>
<tr>
<td><strong>South Asia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marwaha et al., 2011</td>
<td>28.4°N</td>
<td>541</td>
<td>96</td>
<td>&lt;50</td>
<td>23.2±12.2</td>
<td>Females from lower middle socioeconomic strata. Samples obtained from all trimesters. No significant differences across the trimesters were noticed</td>
</tr>
<tr>
<td>Farrant et al., 2009</td>
<td>12.2°N</td>
<td>559</td>
<td>66</td>
<td>&lt;50</td>
<td>37.8 (24.0-58.5)</td>
<td>25(OH)D assessed at 30 weeks gestation. 156 participants were taking calcium and vitamin D at recruitment</td>
</tr>
<tr>
<td>Sachan et al., 2005</td>
<td>26.8°N</td>
<td>207</td>
<td>84</td>
<td>&lt;56.3</td>
<td>35.3±22.3 (rural)</td>
<td>PTH rose above range when 25(OH)D was less than 56.3 nmol/l. 14% mothers had elevated HLAP b. 25(OH)D levels similar in urban and rural</td>
</tr>
<tr>
<td>Sahu et al., 2009a</td>
<td>26.8°N</td>
<td>139</td>
<td>74</td>
<td>&lt;50</td>
<td>37.8±19.8</td>
<td>Rural community prevalence data. 43% had raised HLAP b. Mean dietary calcium intake was 214 mg per day</td>
</tr>
</tbody>
</table>
Table 33.1. Continued.

<table>
<thead>
<tr>
<th>Region/country</th>
<th>Latitude</th>
<th>n</th>
<th>Prevalence (%)</th>
<th>Cut-off (nmol/l)</th>
<th>Mean value (nmol/l)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>South Asia (continued)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Islam et al., 2002</td>
<td>24.5°N</td>
<td>99</td>
<td>50</td>
<td>&lt;37.5</td>
<td>44.8±20</td>
<td>Comparative study between low (rural) and high socioeconomic region (n=99 and 90 respectively). Study population comprised pregnant, non-pregnant and lactating women. No difference in 25(OH)D levels between low or high socioeconomic status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>&lt;25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hossain et al., 2011</td>
<td>24.5°N</td>
<td>75</td>
<td>89</td>
<td>&lt;75</td>
<td>32.9±16.9</td>
<td>Pregnant women at term. During summer</td>
</tr>
<tr>
<td>Karim et al., 2011</td>
<td>24.5°N</td>
<td>50</td>
<td>78</td>
<td>&lt;75</td>
<td>60</td>
<td>Sample from patients admitted in labor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46</td>
<td>&lt;50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nepal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jiang et al., 2005</td>
<td>27°N</td>
<td>1,163</td>
<td>14 (all)</td>
<td>24.4 (winter)</td>
<td>51.1</td>
<td>Subset data from population-based maternal micronutrient supplementation trial Study carried out at rural Nepal. First trimester enrollment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Median values reported.

b Heat labile alkaline phosphatase.
defining sufficiency (Holick, 2007; Holick et al., 2011; Institute of Medicine, 2011; Lips, 2007). Nevertheless, overall data reveals that vitamin D insufficiency exists at an unexpectedly high rate in some of these countries even by the definition of 50 nmol/l.

Most of the data on VDD during pregnancy has been obtained from hospital or community-based studies from South Asia. Many other regions have yet to generate data. In the absence of local data, a clue to the burden of this condition during pregnancy should be obtained from existing studies performed on non-pregnant females in their reproductive age group. The old universal statement that vitamin D insufficiency during pregnancy is not an issue in developing countries as many of the women live at low lying latitude, is not necessarily correct. It is hard to predict the magnitude of this condition based on latitude, as the prevalence data is markedly heterogeneous between studies from similar latitudes. As an example, in a study of south-east Asian women of child-bearing age group (18–40 years) residing near the equator at Jakarta (6°S) and Kuala Lumpur (2°N), over 60% of participants had insufficient 25(OH)D <50 nmol/l (Green et al., 2008). A study from Fiji (18°S) showed that among females in the reproductive age group (15–44 years) mostly from rural communities, approximately half of the participants had 25(OH)D less than 80 nmol/l, with 11% of all women participants at a level below 50 nmol/l (Heere et al., 2010). The mean 25(OH)D from Fijian Indians from this study was 70 nmol/l, with 21% having 25(OH)D levels less than 50 nmol/l. In contrast, rural women from South India (latitude 13.4°N) demonstrated a mean of 47.5 nmol/l with 70% of them having 25(OH)D levels less than 50 nmol/l (Harinarayan et al., 2007). To add to such heterogeneity, observations in pregnant women from rural areas of The Gambia, West Africa (13°N) revealed that none of the pregnant females participating in a study had 25(OH)D value <50 nmol/l (Prentice et al., 2009a). What may explain such wide variation is the actual duration of sunshine exposure, as some have noticed that women spend significant time outdoors (Prentice et al., 2009a) while other studies have suggested factors such as pigmentation, dress, and deliberate avoidance of sunshine during peak solar energy time (Islam et al., 2002; Sahu et al., 2009a). All these studies only reinforce the notion that the cheapest abundant source of vitamin D (sunshine!) goes underutilized in many rural areas as well.

Predisposition to extremely low levels of vitamin D has been noted in winter despite the fact that some of these regions have abundant solar energy year round (Ergür et al., 2009; Maghbooli et al., 2007; Marwaha et al., 2011; Namgung et al., 1998; Sahu et al., 2009a). Foggy winters and the monsoon season are vulnerable periods that can limit the amount of UVB solar energy reaching the ground’s surface. Because calcium accretion occurs at maximal rates during the third trimester (Prentice, 2011), homebound pregnant women in their last trimester during winter are the most vulnerable population. Coexistent infection in mothers, prevalent in developing regions, also constitutes a risk. Observations from Tanzania (6°S) suggest that vitamin D status at 12–27 weeks of gestation in HIV-infected mothers affects mother-to-child transmission and child mortality. Approximately 39% of pregnant women (347 out of 885) had vitamin D levels <80 nmol/l. Infants born to mothers who had low levels of vitamin D had a 49% higher risk of dying or being HIV-infected at birth and were more likely to report cough during follow-up along with an increased risk of stunting and being underweight (Finkelstein et al., 2012; Mehta et al., 2009).
33. Vitamin D replacement in pregnant women in developing countries

In summary, many pregnant women from developing countries, even from rural regions, have insufficient 25(OH)D levels. Specific issues relevant to developing countries are low intake of calcium among pregnant women; early marriage and adolescent pregnancy with its adverse effect on bone health if calcium intake and vitamin D status are inadequate; higher incidence of low birth weight; predisposition to maternal and neonatal infection; and less contact made with health care delivery system (see Section 33.4). Despite the controversy about what constitutes adequate vitamin D levels during pregnancy, there are vast numbers of females residing in developing countries who would benefit from vitamin D supplementation.

33.3 Vitamin D supplementation

Some suggest that the daily dosage required by pregnant women may be considerably higher than the usual 400 IU available in most supplements or higher than the 600 IU recommended dietary allowance or actually be as high as 4,000 IU daily (Holick et al., 2011; Hollis and Wagner, 2011; Hollis et al., 2011). Others have questioned the utility of and need for more vitamin D than the usual 400 to 600 IU daily (Abrams, 2011; Rosen et al., 2012). While the debate goes on, it needs to be emphasized that pregnant women in developed countries take either vitamin D fortified foods or supplements that contain vitamin D, a scenario that does not exist in many developing parts of the world. Moreover, as noted in the preceding section, an appreciable proportion of pregnant women (in some places 50-90%) from developing countries do not have sufficient vitamin D levels of 50 nmol/l and many (in some places one third to a half) have vitamin D levels less than 25 nmol/l.

In the context of vitamin D supplementation, one of the limitations felt to be characteristic for low resource settings is a lack of diagnostic tools to check and monitor 25(OH)D levels as well as monitor any adverse effects caused by supplementation in pregnant women who spend many hours in the sun. Secondly, although the utilization of antenatal services has increased in recent years in developing nations, the rural population with poor educational status and living in poverty still remain neglected and have infrequent contact with the antenatal health care system (Barros et al., 2012; Pallikadavath et al., 2004; WHO, 2009). Compliance with daily supplementation can thus be challenging given such infrequent contact. There are some solutions to the above challenges: firstly, the lack of adverse effects in humans reported from interventional studies including trials using a daily dose of 4,000 IU provide some comfort for low resource settings without tools for monitoring. Secondly, to enhance compliance, a weekly or monthly pharmacological dose (stoss) therapy during each trimester is a practical alternative, with the latter having the added advantage of being combined with the limited antenatal health care visits.

Very few intervention studies have been conducted in hospital or community-based settings from developing countries.

Pregnant Asian immigrant women in the UK receiving ergocalciferol 1000 units/ day during the last trimester experienced a significant elevation of their vitamin D levels at term, with a
mean value of 168 nmol/l compared to baseline level of 20 nmol/l (Brooke et al., 1980). Of clinical importance is the observation that none of the infants born to mothers who took supplementation developed symptomatic hypercalcaemia. Though there was no significant effect on anthropometry immediately after birth, Brooke et al. (1981) later demonstrated improved post-natal growth at one year in infants whose mothers received vitamin D during pregnancy. Outwardly it may seem that 1000 units per day is good enough for pregnant Asians in view of the rise in 25(OH)D at term, but another study with a similar ethnic group showed that on giving calciferol 800 IU per day, 25(OH)D levels rose from 14.9 nmol/l to only 27.5 nmol/l (Datta et al., 2002). Detailed analyses suggest that the above-mentioned study that used ergocalciferol 1000 IU per day may have contained a higher dose than was indicated on the label, hence the anomalous prominent response in raising 25(OH)D levels (Hollis and Wagner, 2004). In contrast to no significant effect on birth anthropometry by daily supplementation (Brooke et al., 1980), a study carried out in India reported significant improvements in the anthropometry of newborns in women who received a pharmacological dose of 600,000 IU each in the seventh and eighth month of pregnancy, without any reports of hypercalcemia (Marya et al., 1988). Vitamin D was not reported from this study. The improvement in birth weight and maternal weight was speculated to be partly due to better maternal nutrition after the removal of ill-defined anorexia due to hypovitaminosis D (Brooke et al., 1980; Marya et al., 1988).

In a pilot intervention study carried out in a rural community setting in India (Sahu et al., 2009b), 84 women belonging to a poor socioeconomic class were compared after providing them with either education about sun exposure, 60,000 IU of cholecalciferol during the second trimester, or 120,000 IU each in the fifth and seventh month. In the group that received mega doses of 120,000 IU during the second and third trimester, there was a significant elevation in 25(OH)D levels post-delivery as compared to baseline. However, even with this regimen only 34% of women attained 25(OH)D levels more than 80 nmol/l. There was no documentation of hypervitaminosis D (more than 375 nmol/l) in any of the subjects. Education on sunlight exposure did not lead to any further behavioral change. Although the 25(OH)D levels at delivery may not reflect the dose effect of cholecalciferol if given three months before measurement (Ilahi et al., 2008), the regimen selected was a practical measure to coincide with the minimal once per trimester antenatal visit in rural areas.

As part of the next step of assessment, at the same latitude and using the same dose regimen, 97 pregnant females of lower middle and middle socioeconomic status were followed up in a hospital setting and observed for maternal 25(OH)D levels and newborn and infant anthropometry (Kalra et al., 2012). Maternal 25(OH)D levels at term were significantly higher in the group that received 120,000 IU of vitamin D during the second and third trimester, with 62% of these women having 25(OH)D more than 50 nmol/l at delivery. Significantly better anthropometry in the newborn was observed in those mothers who received either regimen, as compared to a control group. The better biophysical profile continued to remain till nine months of follow-up. These findings acquire further significance in the light of a study report in which it was observed that vitamin D status during late pregnancy correlates to whole body bone mineral content in children at age 9 years (Javaid et al., 2006).
Weekly dose has also been recently studied in Bangladesh. Non-pregnant and pregnant women between the 26th and 30th week of gestation were enrolled and observed with either a single dose 70,000 IU or a weekly dose of 35,000 IU in a preliminary dose finding and safety study (clinicaltrials.gov identifier: NCT00938600). Unpublished data suggest that using 35,000 IU per week in the third trimester showed no study drug related adverse event (Roth, 2011). At the time of writing this chapter, a trial subsequent to the dose-finding study is underway in Bangladesh and is studying the effect of 35,000 IU per week starting from the 26th to 29th week of gestation on the outcome of maternal and infant vitamin D status and neonatal immune function (clinicaltrials.gov identifier: NCT01126528).

Given the fact that there is a limited dietary source of vitamin D, a daily minimum dose is also a valuable alternative for pregnant women, if compliance allows, and it should be advocated at a bare minimum. In a nutrient intervention trial conducted in rural Nepal, 400 IU of vitamin D as a component of multiple micronutrient supplements was provided from the first trimester onwards. Although it was not a study designed to observe the effect of vitamin D supplementation, data from this trial suggest that the intake of 400 IU of vitamin D daily from the first trimester onwards is beneficial in modestly raising 25(OH)D levels with a change in mean value of 17.8 nmol/l from a baseline of 47.4±23.5 nmol/l (Christian et al., 2006). A recent report from an elegant randomized study conducted at latitude 32°N in the USA, has shown that taking 400 IU daily from the first trimester leads to changes in circulating 25(OH)D levels around delivery. However, it was only in pregnant women taking 2,000 or 4,000 IU daily that the vast majority (93% and 94%, respectively, compared to 57% in 400 IU daily) achieved 25(OH)D levels more than 80 nmol/l one month prior to or at delivery There was no difference observed between maternal and fetal outcome at time of delivery between the different dosage (Hollis et al., 2011).

What should be the best regimen for pregnant women in low resource settings? In the absence of large-scale data from such areas, the choice of treatment should be individualized for either daily, monthly or trimester specific therapy based on setting and anticipated compliance. There are only a few comparative studies between the daily or periodic pharmacological dose of vitamin D during pregnancy to help provide an answer. In a comparative study from the UK, daily supplementation of 800 IU daily and a single oral dose supplementation of 200,000 IU beginning from the 27th week of gestation showed comparable results, however only 30% of the women achieved vitamin D sufficiency (>50 nmol/l) at term (Yu et al., 2009). Similar findings were also reported, albeit in lactating women, who received a daily ergocalciferol dose of 2,000 IU daily or a monthly dose of 60,000 IU for three months, with both being effective. Only 35% in the daily group and 20% in the monthly group attained vitamin D sufficiency after three months of use (Saadi et al., 2007).

The debate will continue to run on what constitutes an efficacious and safe regimen of vitamin D supplementation (IOM, 2011; Roth, 2011), and whether some developing countries should focus on this aspect, especially if some regions have not revealed any significant burden of vitamin D deficiency (Prentice et al., 2009b). With the current knowledge on the widespread prevalence of vitamin D insufficiency especially in some parts of the world such as the Asian region, it does not seem prudent to withhold vitamin D supplementation in these areas. Even in regions
near the equator, the adequacy of vitamin D levels during pregnancy is not widespread (see section on magnitude above). At present, the use of intermittent high dose (stoss) therapy in low resource settings appears to be convenient, inexpensive, practical, and without any reports of hypercalcemia from the limited intervention studies available. It should be noted, however, that most of the high dose supplementation studies have been carried out during the second or third trimester (Kalra et al., 2012; Madelenat et al., 2001; Roth, 2011; Sahu et al., 2009b). Thus, pharmacological stoss therapy should not be attempted during the first trimester where safety has not been established. The use of parenteral cholecalciferol (available in doses of three to six lac units in some countries) should be avoided during pregnancy despite limited data on efficacy and safety in post-partum women (Mozaffari-Khosravi et al., 2012).

In summary, though debatable and subjective, based on current data from low resource settings, it appears that more than the recommended 400-600 IU of vitamin D daily is required during pregnancy to achieve satisfactory blood levels of 25(OH)D at term. In the absence of large-scale randomized data from safety and health outcome data in infants, in settings where vitamin D deficiency cannot be diagnosed, a minimum dose of 60,000 IU of vitamin D given once in the middle of the second and third trimester seems promising without generating excessive concerns about safety. As seen across some trials, this dose can be conservative and may lead to only modest elevation of vitamin D levels. Thus, providing an additional dose of 60,000 IU vitamin D during the second and third trimester apart from the above regimen, especially during the winter, may prove beneficial. In the absence of elaborate safety data during pregnancy, we suggest at least a month’s interval between each pharmacological dosing. Consideration should also be given to this higher dosing regimen if a pregnant woman presents with a clinical picture suggestive of osteomalacia. As an alternative to the high-dose therapy, if compliance can be ensured, daily supplementation of vitamin D with at least 600 to 1000 IU daily from the second trimester or a higher dose of 2,000 IU can be offered. However, daily supplements at this dose may be unavailable in many regions, and there may also be compliance problems. Lastly, it makes no sense to focus purely on vitamin D supplementation without reinforcing the importance of a calcium-rich diet or calcium supplements, the intake of which is very low among pregnant women in many rural areas.

3.4 Areas of uncertainty and progress

Though there has been an assurance that no cases of maternal hypervitaminosis D have emerged during vitamin D supplementation trials in pregnancy, there is still no long-term safety follow-up in children whose mothers received mega doses of vitamin D as opposed to a daily dose. There a few trials in progress at the time of writing this chapter, and detailed reports may shed some more light on the risk-benefit ratio of supplementation (Harvey et al., 2012; Roth, 2011). While the magnitude of VDD during pregnancy is being appreciated as per new studies, reports on the beneficial health outcome of pregnancy have been inconsistent (De-Regil et al., 2012; Specker, 2012). Nevertheless, this does not diminish the fact that in some regions of developing countries,
even in rural areas, there are appreciable numbers of pregnant women who suffer from vitamin D deficiency and would benefit from vitamin D replacement.

33.5 Conclusions

In conclusion, vitamin D deficiency in pregnant women is an under-recognized problem among many obstetricians and health care providers despite the high prevalence of this condition in some developing nations. The absence of food fortification programs, low calcium intake during pregnancy, issues of adolescent pregnancy, and limited utilization of antenatal care, makes pregnant women from developing countries more vulnerable and in need of attention and care in the context of vitamin D, calcium and skeletal health. Periodic vitamin D supplementation as opposed to daily supplementation during pregnancy, starting from the second trimester, seems a practical measure in settings with limited resources.

References

M.T. Sahu and M. Sahu


33. Vitamin D replacement in pregnant women in developing countries


IOM (Institute of Medicine), 2011. Dietary reference intakes for calcium and vitamin D. Committee to Review Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press, Institute of Medicine, Washington, DC, USA.


33. Vitamin D replacement in pregnant women in developing countries


Key facts

- Vitamin D metabolism adapts to the physiological state of pregnancy.
- Vitamin D inadequacy is highly prevalent during pregnancy despite the inclusion of vitamin D in prenatal vitamin supplements in many countries.
- Maternal vitamin D inadequacy may impact multiple maternal, fetal, and postnatal outcomes.
- There is no consensus on optimal vitamin D status during pregnancy or the intake necessary to achieve it.
- No adverse outcomes have been demonstrated in trials supplementing pregnant women with vitamin D doses up to 100 μg/d.

Summary points

- 25-hydroxyvitamin D is the predominant form of vitamin D to cross the placenta.
- Fetal vitamin D status is dependent on maternal status.
- Vitamin D inadequacy is highly prevalent among pregnant populations worldwide.
- While there is no consensus on defining optimal vitamin D status during pregnancy, inadequacy may adversely affect pregnancy outcomes and offspring health.
- There is inconsistent evidence that vitamin D inadequacy before and during pregnancy may predispose women for infertility, preeclampsia, gestational diabetes, bacterial vaginosis, spontaneous preterm birth, Cesarean section, periodontal disease, and human immunodeficiency virus progression and transmission.
- Gestational vitamin D deficiency may impact fetal skeletal development, intrauterine growth, immune maturation, and brain development.
- Poor vitamin D status in utero could place offspring at higher risk for development of asthma, type I diabetes, schizophrenia, and multiple sclerosis.
- Current vitamin D intake recommendations during pregnancy are 5-15 μg/d, though recent evidence suggests that higher intakes are necessary to achieve and maintain vitamin D adequacy.
- Supplementation with 25-100 μg/d vitamin D during pregnancy has not resulted in adverse pregnancy or birth outcomes.
34. Vitamin D in pregnancy

D. Dror
Western Human Nutrition Research Center, 430 W. Health Sciences Dr., Davis, CA 95616, USA;
dkdror@ucdavis.edu

Abstract

Pregnancy is a time of dynamic change in mother and fetus, with vitamin D playing a key role in the normal development and performance of multiple physiological systems. Maternal vitamin D metabolism is modified during pregnancy as evidenced by elevated circulating concentrations of vitamin D binding protein and 1,25-dihydroxyvitamin D. Maternal 25-hydroxyvitamin D (25(OH)D), which shows little change in pregnancy, is the main vitamin D metabolite to cross the placenta. Although there is no consensus on optimal 25(OH)D concentrations during pregnancy, suboptimal status is prevalent in populations of pregnant women around the world. Impairment of vitamin D status during pregnancy has been investigated with regards to multiple maternal outcomes including infertility, preeclampsia, gestational diabetes, bacterial vaginosis, spontaneous preterm birth, mode of delivery, periodontal disease, and human immunodeficiency virus progression and vertical transmission, though evidence for the majority of these outcomes is inconsistent. Gestational vitamin D status may influence skeletal development, intrauterine growth, immune maturation, and neural development of the fetus. Vitamin D inadequacy in utero may program for later life health outcomes, potentially increasing the risk of asthma, type I diabetes, schizophrenia, and multiple sclerosis in the offspring. Vitamin D intake recommendations during pregnancy in most countries range from 5-15 μg/d. However, in the absence of sun exposure such intakes may be insufficient to achieve adequate circulating 25(OH)D, and several expert committees have recommended vitamin D intakes from 25-100 μg/d during pregnancy. While adverse effects have not been reported in trials utilizing these doses, additional research is necessary to verify long-term safety to mother and offspring and to establish a clinical protocol for monitoring and maintaining vitamin D adequacy during pregnancy.

Keywords: mother, fetus, deficiency, 25(OH)D
Introduction

Despite increasing awareness of the multiple roles of vitamin D in pregnancy and fetal development, vitamin D inadequacy is prevalent during pregnancy in diverse populations around the world. While optimal vitamin D status during pregnancy and intake requirements to achieve it are widely debated, there is general consensus that inadequate maternal 25(OH)D concentration can adversely affect mother and offspring. This chapter discusses adaptations of vitamin D metabolism during pregnancy, evidence of the potential consequences of inadequacy, and considerations regarding vitamin D intake and supplementation.

Adaptations of vitamin D metabolism during pregnancy

The multifold physiological changes of pregnancy include an increase in circulating concentrations of DBP and 1,25(OH)₂D with little change in 25(OH)D, the main biomarker of vitamin D status (Papapetrou, 2010). DBP, the major binding protein for 25(OH)D and 1,25(OH)₂D, is abundant in circulation and binds 85% to 90% of both forms of vitamin D while albumin binds most of...
34. Vitamin D in pregnancy

the remaining 10-15% (Bikle et al., 1985, 1986). DBP increases in pregnancy, with an estimated elevation of 7% to 152% during the course of pregnancy beginning as early as 8-10 weeks gestation (Brannon and Picciano, 2011; Ritchie et al., 1998). The pregnancy-induced rise in DBP may be related to estrogen levels, supported by evidence of higher serum levels of DBP in women taking estrogen-containing contraceptives (Haddad and Walgate, 1976; Van Hoof et al., 2001) or treated with oral estrogen (Bikle et al., 1992; Dick et al., 1995).

Total serum 1,25(OH)\(_2\)D is elevated from early pregnancy and rises steadily to a peak concentration nearly double that of non-pregnant controls by term (Kovacs, 2008). The rise in circulating DBP during pregnancy accounts in part for elevated total 1,25(OH)\(_2\)D, with which it is significantly correlated (Bikle et al., 1984; Bouillon et al., 1981). Biologically active free serum 1,25(OH)\(_2\)D is comparable to pre-pregnant concentrations except in the final trimester, when investigators have found it variably to be normal or elevated (Bikle et al., 1984; Bouillon et al., 1981; Van Hoof et al., 2001). The fact that placental and decidual mRNA expression of 1-α-hydroxylase, the enzyme required to synthesize 1,25(OH)\(_2\)D from 25(OH)D, increases early in pregnancy while 1,25(OH)\(_2\)D peaks late in pregnancy suggests maternal rather than placental origin (Zehnder et al., 2002). However, it has recently been demonstrated in vitro that methylation of the placental CYP24A1 gene promoter directly down-regulates 24-hydroxylase mediated catabolism of 1,25(OH)\(_2\)D (Novakovic et al., 2009), possibly contributing to increased 1,25(OH)\(_2\)D concentrations observed during pregnancy. Cord blood 1,25(OH)\(_2\)D is not elevated during gestation, suggesting different physiological use between mother and fetus (Liu and Hewison, 2012).

The correlation between maternal and cord 25(OH)D, but not 1,25(OH)\(_2\)D, suggests that 25(OH)D is the predominant vitamin D metabolite to cross the placenta and that fetal status is dependent on maternal status (Brannon and Picciano, 2011). Concentrations of 25(OH)D do not increase significantly in pregnant women compared with non-pregnant controls (Papapetrou, 2010). In fact, early increases in decidual and placental 1-α-hydroxylase activity may lower maternal 25(OH)D concentrations (Lanham-New et al., 2011). While elevated concentrations of 1,25(OH)\(_2\)D increase catabolism of 25(OH)D in non-pregnant rats and humans, this regulation appears to be uncoupled during pregnancy as evidenced by a ratio of 1,25(OH)\(_2\)D/1,25(OH)D 2.5 times higher than in the non-pregnant state (Papapetrou, 2010).

34.3 Prevalence of vitamin D inadequacy during pregnancy

Though circulating 25(OH)D is considered the best biomarker of dietary exposure and endogenous synthesis, there is no consensus on definitions of status. The most recent United States Institute of Medicine recommendations, released in 2010, consider maternal 25(OH)D concentrations equal to or above 50 nmol/l during pregnancy to meet the needs of 97.5% of the population (Ross et al., 2010). However, 25(OH)D concentrations above 75-80 nmol/l are considered sufficient based on associations with PTH concentrations, calcium absorption, bone turnover markers, and bone mineral density (Dawson-Hughes et al., 2005; Holick, 2007; Hollis,
2005). The majority of recent prevalence estimates of vitamin D inadequacy during pregnancy have used serum 25(OH)D concentration below 50 nmol/l as a cut-off for deficiency and below 75-80 nmol/l as a cut-off for insufficiency. In the United States, Canada, Australia, Europe, the Middle East, and South Asia 26-98% of pregnant women at or near term are deficient and 66-100% are insufficient in vitamin D, with a trend of higher levels of deficiency and insufficiency in dark-skinned or shrouded women (Dror, 2011; Liu and Hewison, 2012).

### 34.4 Vitamin D and pregnancy outcomes

A number of maternal, fetal, and postnatal outcomes have been correlated with poor vitamin D status during pregnancy. Maternal pregnancy characteristics investigated in association with compromised vitamin D status include infertility, preeclampsia, GDM, mode of delivery, BV, periodontal disease, and HIV progression and vertical transmission, though evidence for many of these outcomes is inconclusive. In infants, attention has been devoted to the impact of the in utero vitamin D environment on bone mineralization and skeletal development, intrauterine growth, immune maturation, and brain development. There is some evidence suggesting long-term consequences of compromised vitamin D status during gestation, with increased offspring risk for later development of asthma, type I diabetes, schizophrenia, and MS.

### 34.5 Maternal outcomes

#### 34.5.1 Infertility

The role of vitamin D in maternal fertility is poorly understood, but experimental and ecological evidence suggest that vitamin D may be involved in successful implantation and maintenance of pregnancy. In VDD, VDR-ablated, or 1-α-hydroxylase null animal models, adult females had reduced fertility and smaller litter sizes (Kovacs, 2008). In humans, the VDR is expressed in the ovaries, pituitary gland, endometrium, and placenta (Lerchbaum and Obermayer-Pietsch, 2012) and conception rates peak in summer in northern countries with strong seasonal contrasts in luminosity (Rojansky et al., 1992). It has been speculated that the rise in 1,25(OH)₂D concentrations early in pregnancy is necessary for ensuring immunological adaptations, including dampening Th1 function and favoring Th2 domination for immune tolerance of implantation and pregnancy maintenance (Evans et al., 2004; Hypponen, 2011). However, studies measuring serum and follicular fluid concentrations of 25(OH)D and achievement of clinical in vitro fertilization are inconclusive, variably finding positive (Ozkan et al., 2010), negative (Anifandis et al., 2010), and null (Aleyasin et al., 2011) associations between vitamin D status and reproductive success.

#### 34.5.2 Preeclampsia

Preeclampsia, defined as hypertension and proteinuria after 20 weeks gestation, occurs in 3-5% of pregnancies worldwide and is the leading cause of maternal and fetal morbidity and mortality
34. Vitamin D in pregnancy

(Roberts and Lain, 2002). Higher incidence of preeclampsia during winter in non-tropical regions and during wet or humid periods in tropical climates (TePoel et al., 2011) as well as a higher incidence in dark-skinned compared with light-skinned women (Mostello et al., 2002) suggest a possible influence of sunlight and vitamin D status. Furthermore, it has been postulated that the adverse maternal response to the fetus that occurs in preeclampsia is mediated by Th1-type cytokines (Saito and Sakai, 2003), which are favored in vitamin D deficiency. There is consistent evidence of 1,25(OH)₂D suppression in preeclamptic compared with normal pregnancies (August et al., 1992; Halhali et al., 1995, 2000, 2004; Seely et al., 1992), supporting a disruption in immune tolerance mediated by altered vitamin D metabolism.

Several case-control studies have found significantly lower maternal 25(OH)D concentration in the second or third trimester of pregnancy in women who subsequently developed preeclampsia (Baker et al., 2010; Bodnar et al., 2007; Robinson et al., 2010). In a nested-case control study, maternal 25(OH)D concentration below 50 nmol/l at 15-20 weeks gestation was associated with a five-fold increase in the adjusted odds of severe preeclampsia diagnosis (adjusted OR 5.41, 95% CI 2.02 to 14.52) (Baker et al., 2010). In contrast, other studies found no association between first or second trimester 25(OH)D and later development of preeclampsia in women at low- or high risk based on clinical or biochemical factors (Powe et al., 2010; Shand et al., 2010; Yu et al., 2012). A single RCT in India involving supplementation of pregnant women with 30 μg vitamin D and 375 mg calcium daily starting at 20-24 weeks gestation found no difference in incidence of preeclampsia between the treatment and control groups (Marya et al., 1987).

34.5.3 Gestational diabetes

GDM is increasingly prevalent and has long-term health implications for both mother and offspring (Grundmann and von Versen-Hoynek, 2011). It has been postulated that 25(OH)D and 1,25(OH)₂D may directly or indirectly modulate pancreatic β-cell function and insulin sensitivity (McLeod et al., 2012; Takiishi et al., 2010), but confounders such as genetic polymorphisms and obesity can predispose women both for poor vitamin D status and GDM (Begum et al., 2011; Ramos-Lopez et al., 2008). Some studies have found significantly lower maternal 25(OH)D concentrations in women with GDM compared with pregnant controls (Lau et al., 2011; Maghbooli et al., 2008; Parlea et al., 2012; Sohelykhah et al., 2010; Zhang et al., 2008), while others have found no association between vitamin D status during pregnancy and GDM (Baker et al., 2012; Farrant et al., 2009). Plasma 25(OH)D concentration at 16 weeks gestation was significantly lower in women who subsequently developed GDM compared with matched controls following adjustment for multiple confounders in a nested case-control study, with a 12.5 nmol/l decrease in 25(OH)D associated with a 30% increase in odds of GDM (adjusted OR 1.29, 95% CI 1.05 to 1.60) (Zhang et al., 2008). In another study, 25(OH)D at mid-gestation was negatively correlated with fasting glucose, fasting insulin, and insulin resistance, but the odds of developing GDM was not significantly higher in women who had 25(OH)D concentrations below 50 nmol/l at mid-gestation compared to those with better vitamin D status (OR 1.92, 95% CI 0.89 to 4.17) (Clifton-Bligh et al., 2008). In the first study to measure vitamin D status and HbA₁c, an indicator of long-term blood glucose control, in women with GDM, third trimester 25(OH)D
was significantly inversely associated with HbA1c \( (P<0.001) \) and was an independent predictor of HbA1c in multivariate modeling, though no association was found between low vitamin D status and diagnosis of GDM (Lau et al., 2011).

### 34.5.4 Bacterial vaginosis

BV, a prevalent vaginal infection associated with adverse pregnancy outcomes including preterm delivery, is consistently inversely associated with vitamin D status during pregnancy. In early gestation, mean serum 25(OH)D concentration was significantly lower in women with BV compared with controls with normal vaginal flora \( (P<0.001) \) (Bodnar et al., 2009). Later studies have corroborated an inverse association between 25(OH)D and BV in pregnant women and adolescents (Davis et al., 2010; Dunlop et al., 2011; Hensel et al., 2011), with a higher prevalence of BV in dark-skinned than Caucasian pregnant women supporting potential involvement of vitamin D.

### 34.5.5 Length of gestation/spontaneous preterm birth

SPB occurs prior to 37 weeks gestation, with numerous pathophysiological causes including intrauterine infections and inflammation. It has been postulated that the immunomodulatory and anti-inflammatory properties of vitamin D may be protective against SPB (Grundmann and von Versen-Hoynck, 2011). In a single observational study, length of gestation was 0.7 weeks shorter in a small subset of women (7%) with 25(OH)D below 28 nmol/l at 28-32 weeks gestation (95% CI -1.3 to -0.1) (Morley et al., 2006). However, vitamin D status or supplementation was not associated with length of gestation or risk of SPB in later studies (Baker et al., 2011; Dror et al., 2011; Hollis et al., 2011; Mehta et al., 2009; Shand et al., 2010; Yu et al., 2009).

### 34.5.6 Mode of delivery

Historically, severe vitamin D deficiency and rickets were known to cause pelvic deformities, increasing the risk of obstructed labor (Urrutia and Thorp, 2012). In a study conducted in the United States, women with 25(OH)D concentrations below 37.5 nmol/l following delivery were nearly four times more likely to have required a Cesarean section than women with better vitamin D status (adjusted OR 3.84, 95% CI 1.71 to 8.62) (Merewood et al., 2009). However, other studies in Australia, Pakistan, the United States and the United Kingdom have failed to corroborate this finding (Bowyer et al., 2009; Brunvand et al., 1998; Dror et al., 2011; Savvidou et al., 2012).

### 34.5.7 Other

Several other conditions have been investigated with regards to vitamin D status during pregnancy, including periodontal disease and HIV progression and vertical transmission. Serum 25(OH)D at 14-26 weeks gestation was significantly lower in pregnant women with clinically moderate to severe periodontal disease compared with periodontally healthy controls \( (P<0.001) \) (Boggess et al., 2011). A longitudinal study following 884 HIV-infected Tanzanian women for approximately
6 years found that women in the highest quintile of serum 25(OH)D at 12-27 weeks gestation had a 42% lower risk of all-cause mortality compared with women in the lowest quintile (relative risk 0.58, 95% CI 0.40 to 0.84) (Mehta et al., 2010). Serum 25(OH)D below 80 nmol/l at mid-gestation was associated with greater maternal HIV disease progression (RR 1.25, 95% CI 1.05 to 1.50) and a higher risk of maternal to child transmission by 6 weeks (RR 1.50, 95% CI 1.02 to 2.20) and 24 months (RR 1.46, 95% CI 1.11 to 1.91) postpartum (Mehta et al., 2009, 2010).

### 34.6 Fetal and neonatal outcomes

#### 34.6.1 Skeletal development

Despite a recognized association between poor maternal vitamin D status and early development of infantile rickets, the role of vitamin D in fetal skeletal development and bone mineralization is uncertain. In South Korea, where vitamin D supplementation is uncommon, investigators reported significantly lower mean cord serum 25(OH)D concentrations and total body BMC in winter-born compared with summer-born neonates (Namgung et al., 1998). In a prospective cohort study using high-resolution three-dimensional ultrasound imaging, maternal serum 25(OH)D was inversely correlated with fetal femoral distal metaphyseal CSA (r=-0.16, 95% CI -0.25 to -0.06) and splaying index (metaphyseal CSA/femur length, r=-0.17, 95% CI -0.26 to -0.07) at 19 weeks gestation, with similar results at 34 weeks gestation. Fetal femoral splaying is analogous to that seen in childhood rickets, suggesting that the effects of vitamin D deficiency on bone development may initiate early in gestation (Mahon et al., 2010). Tibial BMC and CSA measured by peripheral quantitative computed tomography were significantly higher in Finnish newborns whose mothers’ serum 25(OH)D in the first trimester of pregnancy was above median (46.2 nmol/l, P=0.01 and P=0.02) (Viljakainen et al., 2010). By 14 months, tibial CSA remained significantly higher in the group of mothers with better vitamin D status during pregnancy (P=0.004), but there was no residual difference in BMC between groups (Viljakainen et al., 2011). In contrast, studies in Turkey, The Gambia, and the United Kingdom found no significant association between maternal or cord 25(OH)D and unadjusted neonatal BMC or bone area, though the time of measurement, range of vitamin D status, and methods of assessing bone parameters differed between studies (Akcakus et al., 2006; Congdon et al., 1983; Prentice et al., 2009; Weiler et al., 2005).

#### 34.6.2 Intrauterine growth

While fetal growth is maximal in the third trimester, the growth trajectory is determined earlier in pregnancy by factors possibly including vitamin D (Bodnar et al., 2010). A large multiethicic cohort study in the Netherlands reported that compared with infants of mothers whose serum 25(OH)D at 12-14 weeks gestation was greater than 50 nmol/l, those born to mothers with serum 25(OH)D below 29.9 nmol/l had significantly lower birth weights (-114.4 g, 95% CI=-151.2 to -77.6) and were more likely to be SGA (OR 2.4, 95% CI 1.9 to 3.2) (Leffelaar et al., 2010). Women in the lowest quartile of serum 25(OH)D during early pregnancy were more likely to give birth
to SGA offspring after adjustment for maternal age, height, and education in another Dutch population-based study (adjusted OR 1.57, 95% CI 1.03 to 2.39) (Van den Berg et al., 2012). Knee-heel, but not crown-heel, length was significantly shorter at birth in infants of a small subset of mothers (7%) with serum 25(OH)D below 28 nmol/l at 28-32 weeks gestation after adjustment for gestational length (-2.7 mm, 95% CI -5.4 to -0.1) and in winter-born vs. summer-born infants (-2.1 mm, 95% CI -3.8 to -0.5) (Morley et al., 2006). In contrast, a number of observational studies have found no association between third trimester or perinatal maternal vitamin D status and birth weight, length, or head circumference (Akcakus et al., 2006; Bowyer et al., 2009; Dror et al., 2011; Farrant et al., 2009; Gale et al., 2008; Prentice et al., 2009; Yu et al., 2009), while one found an inverse association between maternal 25(OH)D concentration at term and birth weight (Weiler et al., 2005).

A nested case-control study of nulliparous pregnant women in the United States demonstrated a significant association between early pregnancy serum 25(OH)D and risk of SGA amongst white but not black women. White mothers with serum 25(OH)D below 37.5 nmol/l had a significantly greater odds of an SGA infant than those with serum 25(OH)D 37.5-75 nmol/l (OR 7.5, 95% CI 1.8 to 31.9) (Bodnar et al., 2010). Similar results were found in the United Kingdom, where there was a significantly higher incidence of maternal serum 25(OH)D below the 10th percentile at 11-13 weeks gestation in Caucasian women ($P=0.0002$) but not in African women who delivered SGA neonates compared with those whose neonates were appropriate for gestational age (Ertl et al., 2012). Single nucleotide polymorphisms in the VDR gene may contribute to disparities in fetal growth (Bodnar et al., 2010; Morley et al., 2009; Swamy et al., 2011).

A recent Cochrane analysis including five RCTs found no effect of supplementation with 20-30 μg/d vitamin D during the third trimester of pregnancy on birth weight or birth length (De-Regil et al., 2012). Likelihood of low birth weight (<2,500 g) was lower in the supplemented groups with borderline significance (RR 0.48, 95% CI 0.23 to 1.01), and head circumference was greater in the supplemented groups (mean difference 0.43 cm, 95% CI 0.06 to 0.79) (De-Regil et al., 2012). In an RCT in India published after the Cochrane review, either a single oral dose of 1,500 μg vitamin D during the second trimester or two doses of 3,000 μg during the second trimester and at 28 weeks gestation resulted in significantly greater birth weight, length and head circumference compared with unsupplemented controls ($P<0.01$ for weight, $P<0.001$ for length and head circumference), with significant differences in anthropometric indices remaining between treatment and control groups at 3, 6, and 9 months (Kalra et al., 2012).

### 34.6.3 Immune maturation

The gestational vitamin D environment may influence fetal immune development and later propensity for allergy. A multicenter trial in Finland and France found significantly higher expression of tolerogenic ILT3 and ILT4 in cord blood of infants born to mothers taking vitamin D supplements during pregnancy ($P=0.012$ and $P<0.001$) (Rochat et al., 2010). A weak but significant positive correlation between cord plasma 25(OH)D and cord blood mononuclear cell release of interferon-γ, a cytokine playing a key role in Th1 cell development, was shown upon
stimulation with lipopolysaccharide \((r=0.11, P=0.01)\) (Chi et al., 2011). This finding suggests that prenatal vitamin D status could influence immune development and predisposition for allergy, though it must be interpreted cautiously given multiple correlation tests performed. Inverse associations between cord 25(OH)D and respiratory and general infections in neonates have been found in several recent studies (Belderbos et al., 2011; Camargo et al., 2011; Morales et al., 2012).

### 34.6.4 Brain development

Vitamin D directly regulates development of neuronal and non-neuronal cells, with disruption of these processes potentially impairing cell viability, cell signaling or connectivity (Eyles et al., 2011). A number of studies on gestational VDD and brain development have been conducted in rats, which like humans, express 1-α-hydroxylase and nuclear VDR in the brain (Levenson and Figueiroa, 2008). The brain morphology of rat pups born to VDD dams is altered, consistent with enhanced cell proliferation and decreased neuronal differentiation and apoptosis (Cui et al., 2007; Eyles et al., 2003; Ko et al., 2004). Although similar results are not available from humans, there is ample evidence for a role of vitamin D in brain development and function (McCann and Ames, 2008). A recent study evaluating cognitive development longitudinally in children whose mothers’ serum 25(OH)D was measured at 18 weeks gestation found a significant inverse trend between quartiles of maternal vitamin D status and language impairment at age 5 and 10 years \((P<0.05)\), but not between vitamin D status and behavioral or emotional problems at any age (Whitehouse et al., 2012).

### 34.7 Developmental programming for later life outcomes

The ‘fetal origins’ hypothesis that prenatal environmental factors influence disease risk in childhood and adulthood has stimulated speculation about the long-term implications of vitamin D status during gestation (Barker et al., 2002; Lucas et al., 2008). Epigenetic mechanisms that lead to persistent changes in structure and function of the endocrine system are hypothesized to account for lasting impacts of the intrauterine environment (Grundmann and von Versen-Hoynck, 2011).

#### 34.7.1 Childhood asthma and wheezing

Vitamin D is a factor in numerous biological pathways affected by asthma, including smooth muscle contraction, airway inflammation, and immune cell function (Litonjua and Weiss, 2007). Several prospective birth cohort studies demonstrated that higher maternal intake of vitamin D from foods and supplements during pregnancy was associated with lower risk of wheezing (Camargo et al., 2007; Devereux et al., 2007; Miyake et al., 2010) or asthma (Erkkola et al., 2009) in children aged 16 months to five years. Only two prospective studies have measured maternal or cord 25(OH)D in association with wheezing and asthma. In a New Zealand birth cohort, cord blood 25(OH)D was inversely associated with risk of wheezing by 15 months, three years, and five years of age after adjustment for confounders \((P<0.05\) for all) but was not associated with
incident asthma by 5 years (Camargo et al., 2011). In Spain, no association was found between early pregnancy 25(OH)D and risk of wheezing or asthma in offspring up to six years of age (Morales et al., 2012).

### 34.7.2 Type I diabetes

While the destruction of insulin-secreting pancreatic beta cells characterizing type I diabetes may be initiated before birth (Deluca and Cantorna, 2001), evidence of an association between maternal vitamin D intake from food and supplements or maternal vitamin D status during pregnancy and risk of type I diabetes in the offspring is inconsistent. In a United States birth cohort followed for an average of four years, recalled maternal intake of vitamin D from food but not supplements during the third trimester of pregnancy was associated with a lower risk of pancreatic islet autoimmunity, a preclinical stage of type I diabetes, after adjustment for HLA genotype, family history of type 1 diabetes, presence of GDM, and ethnicity (adjusted hazard ratio 0.37, 95% CI 0.17 to 0.78; vitamin D intake standard deviation (SD) = 155.6 IU) (Fronczak et al., 2003). In Sweden, daily consumption of prenatal supplements containing at least 5 μg vitamin D during pregnancy was associated with reduced diabetes-related autoimmunity in offspring at age one (adjusted OR 0.71, 95% CI 0.52 to 0.96) but not two and a half years (Brekke and Ludvigsson, 2007). However, in a Finnish cohort of children with a high-risk HLA genotype for type I diabetes, maternal vitamin D intake from food and/or supplements during pregnancy was not associated with risk of advanced beta cell autoimmunity or clinical type I diabetes in the offspring (Marjamaki et al., 2010).

Few studies have measured maternal vitamin D status during pregnancy and type I diabetes incidence in the offspring. A nested case-control study in Norway found a more than two-fold odds of type I diabetes in offspring of women in the lowest compared with the highest quartile of serum 25(OH)D late in pregnancy (adjusted OR 2.39, 95% CI 1.07 to 5.31) (Sorensen et al., 2012). In a nested case-control study in Finland, however, maternal 25(OH)D in early pregnancy did not differ between mothers whose children later developed type 1 diabetes and mothers of non-diabetic ‘healthy’ children of the same age (Miettinen et al., 2012).

### 34.7.3 Schizophrenia

Enlarged lateral ventricles and reduced cortical thickness of the brain and low plasma concentrations of nerve growth factor and synapsin II are common pathological features in rat pups born to VDD dams and in humans with schizophrenia (Harrison, 1999; Levenson and Figueiroa, 2008). In multiple studies, incidence of schizophrenia is higher in those born in winter or spring (Torrey et al., 1997), at higher latitudes (Saha et al., 2006), and to dark-skinned migrants (Cantor-Graae and Selten, 2005), factors also implicated in hypovitaminosis D (Holick, 1995). In the first study to examine neonatal vitamin D status and schizophrenia risk directly, neonates in the lowest three quintiles of 25(OH)D based on analysis of dried blood samples had a two-fold risk of developing schizophrenia later in life compared with neonates in the fourth quintile (RR 2.0-2.1 for each quintile, 95% CI: 1.3 to 3.5). However, the risk was nearly equally elevated in
neonates in the highest quintile of 25(OH)D (RR 1.71, 95% CI 1.04 to 2.8) (McGrath et al., 2010). The authors speculated that single nucleotide polymorphisms in vitamin D-related genes could result in the U-shaped relationship between gestational vitamin D status and schizophrenia risk.

### 34.7.4 Multiple sclerosis

Childhood sunlight exposure, vitamin D intake from food and supplements, and serum 25(OH)D are inversely associated with risk of MS, an autoimmune disease (Kampman et al., 2007; Munger et al., 2004, 2006; Van der Mei et al., 2003). Season of birth patterns and latitudinal gradients in MS distribution are evident, with MS patients more likely to be born in the spring (Staples et al., 2010; Willer et al., 2005) and a higher prevalence of disease further from the equator (Simpson et al., 2011). A higher concordance rate of MS among dizygotic twins than non-twin siblings suggests a gestational component of susceptibility (Ebers, 2008). In a large cohort study, mothers of female nurses followed prospectively for more than 20 years completed a questionnaire inquiring about their experiences and diet during pregnancy with their nurse daughters. Risk of MS in the offspring was significantly lower in nurses whose mothers were in the highest quintile of dietary vitamin D intake compared with the lowest (adjusted RR 0.57, 95% CI 0.35 to 0.91). Furthermore, predicted 25(OH)D of the pregnant mothers based on dietary and lifestyle factors was inversely associated with risk of MS in their daughters ($P=0.002$ for trend) (Mirzaei et al., 2011).

### 34.8 Recommended intakes and monitoring of vitamin D status in pregnancy

Vitamin D intake recommendations during pregnancy are available from many countries globally. As of 2008, recommendations for vitamin D intake in Western and Eastern Europe ranged from 5-11.3 µg/d, with a median and mode of 10 µg/d (Doets et al., 2008). The most recent World Health Organization/Food and Agriculture Organization guidelines, published in 2004, recommend an intake of 5 µg/d vitamin D during pregnancy (WHO/FAO, 2004). The governments of Australia and New Zealand likewise recommend 5 µg/d vitamin D during pregnancy (Council, 2006). In the United States and Canada, the estimated average requirement and recommended dietary allowance for vitamin D during pregnancy were recently set at 10 µg/d and 15 µg/d, respectively (Ross et al., 2010).

In the majority of countries recommendations for vitamin D intake during pregnancy exceed those for non-pregnant individuals, while in Australia, Austria, Bulgaria, Canada, Germany, Iceland, New Zealand, Romania, Switzerland, and the United States recommendations during pregnancy are equivalent to general recommendations. The dietary reference intakes for vitamin D in the United States and Canada were determined on the basis of an integrated bone health indicator and a simulated dose-response curve considering 25(OH)D ≥40 or 50 nmol/l to meet the needs of 50% and 97.5% of individuals, respectively, assuming minimal sun exposure (Ross et al., 2010). Recommended intakes during pregnancy parallel those for the general population due to insufficiency of evidence on the association of serum 25(OH)D with maternal bone mineral
density or fetal calcium homeostasis and skeletal outcomes. In Australia and New Zealand, the placental transfer of vitamin D and its metabolites was considered ‘too small to affect the mother’s vitamin D requirement’ (NMHRC, 2006).

Recent literature suggests that skeletal craniotabes in newborns are associated with in utero VDD (Yorifuji et al., 2008) and that adverse changes to fetal bone morphology may be seen as early as the second trimester of gestation (Mahon et al., 2010). As reviewed in the present chapter, gestational VDD may have lasting effects on multiple other physiological systems in the offspring. Furthermore, the recommended target range of serum 25(OH)D in non-pregnant adults is variably considered to be 80-250 nmol/l (Mulligan et al., 2010), considerably higher than the concentrations on which most recommended intakes were based. A review of recent scientific evidence concluded that circulating maternal 25(OH)D during pregnancy should be 100-150 nmol/l and that intakes of 50 µg/d are necessary to achieve this concentration (Holli and Wagner, 2011). The United States Endocrine Task Force on Vitamin D reported in 2011 that 15 µg/d may be insufficient to correct VDD in pregnant women and recommends intakes of 37.5-50 µg/d (Holick et al., 2011), while the Canadian Pediatric Society recommends 50 µg/d to maintain vitamin D sufficiency (First Nations, 2007). Due to continuous changes in scientific knowledge, revisions to European and other recommendations are expected (Doets et al., 2008).

Most prenatal vitamins contain 10 µg vitamin D as a result of prior and current guidelines (Holli et al., 2011), though studies have found an unacceptable prevalence of vitamin D deficiency and insufficiency among pregnant women consuming this dose (Dror et al., 2011; Li et al., 2011). Current prenatal care in most countries does not include monitoring of maternal vitamin D status. In particular for women with risk factors for deficiency, including location of residence far from the equator, winter pregnancy, limited sun exposure and/or regular use of sunscreen, dark skin, obesity, extensive clothing cover, or malabsorptive disorders, determination of maternal 25(OH)D in early and mid-pregnancy should be considered as a preventative measure in clinical care (Mulligan et al., 2010).

### 34.9 Supplementation and toxicity in pregnancy

Historically, vitamin D supplementation during pregnancy was considered a risk factor for supravalvular aortic stenosis, a congenital heart defect, in infants (Garcia et al., 1964). This condition was later attributed to Williams Syndrome, a rare genetic disorder characterized by dysmorphic facial features, multiorgan involvement including supravalvular aortic stenosis, and an exaggerated response of circulating 25(OH)D to exogenous vitamin D (Dror and Allen, 2010). Since the 1980s, trials involving regular supplementation with 10-30 µg/d or a single or double dose of 1,500-15,000 µg during the second half of pregnancy have demonstrated no adverse effects (Brooke et al., 1980; Cockburn et al., 1980; Delvin et al., 1986; Kalra et al., 2012; Mallet et al., 1986; Marya et al., 1981; Yu et al., 2009). In the first RCT to include higher doses of continued supplementation, women receiving 100 µg/d vitamin D starting at 12-16 weeks gestation experienced no adverse effects and achieved significantly higher maternal serum 25(OH)D one
month before delivery and at delivery compared with those receiving 50 or 10 μg/d (Hollis et al., 2011). In addition, cord blood 25(OH)D varied significantly by supplement group, with neonates of mothers receiving 100 μg/d achieving the highest levels of circulating 25(OH)D ($P<0.0001$). A biphasic relationship was demonstrated between circulating 25(OH)D and 1,25(OH)$_2$D, with circulating levels of 25(OH)D >100 nmol/l required to support maximum 1,25(OH)$_2$D output.

The tolerable upper limit for vitamin D in the United States and Canada is set at 100 μg/d for pregnant and non-pregnant individuals, based on evidence of hypercalcemia at intakes above 250 μg/d in non-pregnant adults and lack of data supporting a different tolerable upper limit specific to pregnancy (Ross et al., 2010). Intake of 100 μg/d is estimated to result in circulating 25(OH)D concentrations of approximately 150 nmol/l (Heaney et al., 2003). In the RCT involving supplementation of pregnant women with 100 μg/d vitamin D, evidence of toxicity was not observed despite circulating 25(OH)D exceeding 225 nmol/l in three cases (Hollis et al., 2011). Supplementation was initiated after 12 weeks gestation, such that additional research is necessary to ascertain safety of this dose prior to the twelfth week. Most experts agree that supplementation with 25-50 μg/d vitamin D during pregnancy is safe (ACOG, 2011).

### 34.10 Conclusion

The prevalence of vitamin D deficiency during pregnancy is concerning in light of potential implications on multiple maternal, fetal, and postnatal health consequences. While recommendations for vitamin D intake during pregnancy have been revised or are undergoing revision in many countries to account for recent evidence of elevated needs during pregnancy, in most cases existing recommendations are insufficient to raise circulating 25(OH)D to acceptable concentrations. Vitamin D supplementation studies during pregnancy have demonstrated short-term safety and efficacy, though additional research is necessary to verify long-term safety and to establish a clinical protocol for monitoring and maintaining vitamin D adequacy during pregnancy.

### References


D. Dror


34. Vitamin D in pregnancy


Key facts

- Low serum 25(OH)vitamin D concentrations are linked to an increased risk of many chronic diseases in observational studies.
- Results of observational studies and randomized clinical trials of nutrients and supplements in relation to chronic disease outcomes have often been divergent.
- Correlation does not prove causation in associational studies due to the potential for confounding and other biases.
- Previous randomized trials of supplemental vitamin D have generally tested low doses of vitamin D and have not included prespecified nonskeletal outcomes.
- Large-scale randomized trials of moderate- or high-dose vitamin D are needed to evaluate whether supplementation can reduce the risk of cancer, cardiovascular disease, and other nonskeletal health outcomes.

Summary points

- It is not yet known whether supplemental vitamin D plays a role in preventing cancer and cardiovascular disease (CVD).
- The ongoing VITamin D and OmegA-3 Trial (VITAL) is a 5-year, randomized, double-blind, placebo-controlled clinical trial among 20,000 US men and woman testing whether supplemental vitamin D (2,000 IU/day) is effective for the primary prevention of cancer and CVD.
- VITAL is the first – and, to date, only – large-scale trial of supplemental vitamin D at a dose sufficient to produce meaningful changes in vitamin D blood levels and designed to assess cancer and CVD as the primary outcomes under study.
- In ancillary studies, VITAL is also examining effects of vitamin D on risk of diabetes, hypertension; cognitive decline; depression; fractures; falls; respiratory disorders; infections; anemia; autoimmune disorders, and other outcomes.
- The results of VITAL are expected to shape clinical and public health guidelines regarding the use of supplemental vitamin D for the primary prevention of cancer, CVD, and other health conditions.
35. Design and rationale of the VITamin D and OmegA-3 Trial (VITAL)

S.S. Bassuk¹ and J.E. Manson¹,²
¹Division of Preventive Medicine, Department of Medicine, Brigham and Women’s Hospital, 
Harvard Medical School, 900 Commonwealth Avenue East, Boston, MA 02215, USA; 
²Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, 
MA 02115, USA; jmanson@rics.bwh.harvard.edu

Abstract

Although data from laboratory studies, observational research, and secondary prevention 
trials suggest that vitamin D may protect against the development of cancer and CVD, primary 
prevention trials with adequate dosing in general populations are needed to test this hypothesis. 
The VITamin D and OmegA-3 Trial (VITAL) is an ongoing randomized, double-blind, placebo-
controlled, 2×2 factorial trial of 5 years of supplemental vitamin D (vitamin D3 [cholecalciferol], 
2,000 IU/day) and marine omega-3 fatty acids (Omacor® fish oil, EPA + DHA, 1 g/day) in the 
primary prevention of cancer and CVD in a racially/ethnically diverse population of 20,000 
US men aged ≥50 and women aged ≥55. Blood samples will be collected from at least 16,000 
participants at baseline, with follow-up blood collection in about 6,000 participants. Annual 
questionnaires assess compliance with treatment, use of non-study supplements, occurrence 
of endpoints, and risk factors for cancer and CVD. Self-reported endpoints are confirmed by 
medical record review by physicians blinded to treatment assignment, and deaths are ascertained 
through national registries and other sources. Ancillary investigations are assessing whether the 
study agents affect risk for diabetes and glucose intolerance; hypertension; cognitive decline; 
depression; osteoporosis and fracture; physical disability and falls; asthma and other respiratory 
diseases; infections; kidney disease; anemia; eye problems such as macular degeneration and 
dry eye syndrome; and autoimmune disorders such as rheumatoid arthritis, systemic lupus 
erthematosus, and thyroid diseases. The results of the trial are expected to inform clinical and 
public health guidelines regarding the use of vitamin D for the primary prevention of cancer, 
CVD, and other chronic diseases.

Keywords: cancer, cardiovascular disease, cholecalciferol, docosahexaenoic acid, eicosapentaenoic 
acid, fish oil, marine omega-3 fatty acids, primary prevention, randomized controlled trial, 
vitamin D
35.1 Introduction

Whether vitamin D plays a role in preventing cancer (Manson et al., 2011) or CVD (Shapses and Manson, 2011) is unclear. Data from laboratory investigations (Deeb et al., 2007; Krishnan and Feldman, 2011; Manson and Bassuk, 2009), ecologic studies (Grant, 2002, 2003; Mizoue, 2004; Zittermann et al., 2005), epidemiologic studies (Abbas et al., 2008; Anderson et al., 2010; Bertone-Johnson et al., 2005; Dobnig et al., 2008; Freedman et al., 2007; Giovannucci et al., 2008; Giovannucci et al., 2006; Gorham et al., 2005, 2007; Lowe et al., 2005; Pilz et al., 2008; Wang et al., 2008), and secondary analyses of small randomized trials (Lappe and Heaney, 2008; Lappe et al., 2007; Prince et al., 2008; Trivedi et al., 2003) suggest that vitamin D might protect against the development of cancer and CVD. Plausible biological mechanisms support this hypothesis (Deeb et al., 2007; Krishnan and Feldman, 2011; Manson and Bassuk, 2009). As shown in Figure 35.1, vascular smooth muscle cells, endothelial cells, cardiomyocytes, and immune-system cells all produce 1α-hydroxylase – which converts 25(OH)D to 1,25(OH) 2D, the natural ligand of the vitamin D receptor – or express the vitamin D receptor. 1,25(OH) 2D appears to inhibit vascular smooth muscle cell proliferation and vascular calcification, control volume homeostasis and blood pressure via regulation of the renin-angiotensin-aldosterone system, exert anti-inflammatory effects, and improve insulin sensitivity and secretion (Bassuk and Manson, 2009). Through binding to VDR, 1,25(OH) 2D has also been shown to promote cell differentiation, inhibit cancer-cell proliferation, and exhibit proapototic and antiangiogenic effects (Manson et al., 2011). The Women's Health Initiative calcium-vitamin D trial, conducted among more than 36,000 postmenopausal participants, found that women randomized to daily calcium (1000 mg) plus low-dose vitamin D3 (400 IU) for a mean of 7 years did not have a lower risk for developing cancer or CVD compared to women randomized to placebo (Jackson et al., 2006; Wactawski-Wende et al., 2006), but the intervention’s effect on 25(OH)D blood levels was small (Giovannucci et al., 2006). However, large randomized trials of supplemental vitamin D at doses expected to produce biologically significant changes in 25(OH)D levels or designed to assess cancer or CVD events as primary prespecified endpoints are lacking. Given that vitamin D...
insufficiency is common in the United States (e.g. a national study found that ~30% of US adults aged ≥20 had 25(OH)D levels <50 nmol/l (<20 ng/ml) and >70% had 25(OH)D levels <80 nmol/l (<32 ng/ml) (Yetley, 2008)), clarifying the possible role of vitamin D supplementation for cancer and CVD prevention is of critical public health importance.

35.2 Methods

35.2.1 Overview

To test whether supplemental vitamin D and another promising nutritional intervention – the marine omega-3 fatty acids EPA and DHA, which are found in fish and fish oil – are effective for the primary prevention of cancer and CVD, our research group is conducting the VITamin D and OmegA-3 Trial.
OmegA-3 TrialL (VITAL), a randomized, double-blind, placebo-controlled clinical trial among 20,000 US men aged ≥50 and women aged ≥55 without a history of cancer or CVD at baseline. African Americans (blacks) are being oversampled. In a 2×2 factorial design, participants are being randomly assigned to a mean of 5 years of treatment with vitamin D3 (cholecalciferol; 2,000 IU/day) and marine omega-3 fatty acids (Omacor® fish oil, EPA + DHA, 1 g/day) supplements (or placebos). The 2×2 factorial design permits a cost-effective assessment of the independent (as well as synergistic) effects of the interventions. With the exception of some ancillary studies and the in-clinic visit component in a subcohort, the trial is being conducted by mail, which also adds to its cost effectiveness. Baseline blood samples will be collected in at least 80% of participants (n=16,000), with follow-up samples collected in ~6,000 participants. Annual questionnaires assess compliance with treatment (plasma biomarker measures will also assess compliance in a random sample of participants), use of non-study supplements, occurrence of endpoints, and risk factors for cancer and CVD. Self-reported endpoints are confirmed by medical record review by study physicians blinded to treatment assignment, and deaths are ascertained through national registries and other sources. Figure 35.2 provides an overview of the study design. Because this is a textbook on vitamin D, we focus on the vitamin D component of the trial. We refer readers interested in the omega-3 fatty acid component of the trial to our recent design paper (Manson et al., 2012), from which this chapter has been adapted.

VITAL and its ancillary studies are funded by the US National Institutes of Health. The study pills and placebos are being donated by Pharmavite LLC of Northridge, California (vitamin D3) and Pronova BioPharma of Norway (Omacor® fish oil). (Both study pills are packaged together in monthly calendar packs [2 pills per day] to assist with compliance in pill taking; packaging is done at GPSI, Inc., in New York, with support from Pronova.) VITAL has received approval from the Institutional Review Board of Partners Healthcare/Brigham and Women’s Hospital.

Figure 35.2. The VI{T}amin D and OmegA-3 TrialL (VITAL) design. Reprinted from Manson et al. (2012), with permission from Elsevier.
The study agents have received Investigational New Drug Approval from the US Food and Drug Administration. An independent Data and Safety Monitoring Board meets yearly to review the trial's progress and the unblinded data on endpoints and possible adverse effects in order to advise continuation, modification, or early termination of the trial. VITAL is registered at clinicaltrials.gov (NCT01169259), and the study website is www.vitalstudy.org.

### 35.2.2 Study aims

The primary aims of VITAL are to test whether supplemental vitamin D and/or marine omega-3 fatty acids lower the risk for total cancer and major CVD events, a composite endpoint of MI, stroke, and cardiovascular mortality. The secondary aims are to test whether each study agent reduces the risk for site-specific cancers, including incident colorectal cancer, breast cancer (in women), and prostate cancer (in men); total cancer mortality; an expanded composite cardiovascular endpoint of MI, stroke, cardiovascular mortality, and coronary revascularization (CABG or PCI); and the individual components of the primary cardiovascular endpoint. The tertiary aims are to explore whether the study agents have synergistic or additive effects on the risk for total cancer, major CVD events, and the secondary endpoints, and to explore whether the effect of the study agents on cancer and CVD risk varies by baseline blood levels of these nutrients, and – for vitamin D – by race and body mass index. Studying the effect of supplemental vitamin D in blacks is critical because this group is at higher risk for vitamin D deficiency than whites – dark skin synthesizes less vitamin D in response to solar radiation and US blacks tend to have lower intakes of dietary and supplemental vitamin D (Harris, 2006; Moore et al., 2005) – and also at higher risk for certain cardiovascular events (e.g. stroke) (Harris, 2011) and cancers (e.g. prostate cancer) (American Cancer Society, 2009), as well as mortality from CVD (Harris, 2011) and cancer (American Cancer Society, 2009). Similarly, studying the effect of vitamin D supplementation in people who are obese is important, as the prevalence of obesity in the US is high (Flegal et al., 2012) and obese individuals are at elevated risk for low vitamin D status (in part because of decreased bioavailability of this fat-soluble vitamin (Harris and Dawson-Hughes, 2007; Martins et al., 2007)) as well as for CVD and certain cancers (Bassuk and Manson, 2008).

### 35.2.3 Vitamin D intervention

Our review of the literature suggests that testing a vitamin D dose of 2,000 IU/day best balances efficacy and safety concerns. With respect to efficacy, we aim to achieve a sufficient difference in vitamin D status between the treatment and placebo groups to detect reductions in the primary endpoints of cancer and CVD. In 2008, when VITAL was designed, the RDAs were 400 IU/day for US adults aged 50-70 and 600 IU/day for adults aged >70 (Institute of Medicine Food and Nutrition Board, 1999). In 2011, these RDAs increased to 600 IU/day and 800 IU/day, respectively (Institute of Medicine, 2011). These RDAs correspond to a serum 25(OH)D level of 50 nmol/l and are sufficient to maintain bone health in ≥97.5% of the US and Canadian population (Institute of Medicine, 2011). Although the evidence is not conclusive, some data suggest that higher vitamin D intakes may yield additional health benefits. For example, in a review of studies of serum 25(OH)D in relation to various outcomes, including colorectal cancer, falls, fractures,
physical functioning, and dental health, Bischoff-Ferrari et al. (2006) found that advantageous 25(OH)D levels began at 75 nmol/l, and optimal levels were between 90-100 nmol/l. An older individual generally requires an oral vitamin D intake of at least 800-1000 IU/day to achieve a serum 25(OH)D of 75 nmol/l (Dawson-Hughes et al., 2005). Among the Women’s Health Initiative cohort (a population similar to that of VITAL), 400 IU/day of vitamin D was estimated to have raised median plasma 25(OH)D only modestly – from 42.3 to 54.1 nmol/l (Giovannucci et al., 2006; Wactawski-Wende et al., 2006). A study by Aloia et al. (2008) found a nonlinear dose-response relation between serum 25(OH)D and vitamin D intake; the rate of increase in 25(OH)D level was less at higher levels of intake. Taken together, the latter two findings suggest that 2,000 IU/day of vitamin D would be required to reach the postulated optimal level of 90-100 nmol/l in participants assigned to active vitamin D. Given this dose, the expected difference in achieved 25(OH)D levels between the active treatment and placebo groups is ~50 nmol/l.

With respect to safety, because (a) individuals who report supplemental vitamin D intakes of more than 800 IU/day are not permitted to enroll in VITAL and (b) average dietary vitamin D intake in the US is ~200 IU/day (Harris, 2007), few if any participants randomized to active vitamin D will be consuming a total vitamin D dose of more than 3,000 IU/day, which is below the safety limit of 4,000 IU/day set by US and European authorities (Hathcock et al., 2007; Institute of Medicine, 2011). Moreover, because nonstudy intakes of vitamin D at RDA levels are allowed, participants randomized to placebo are not expected to develop deficiency. To minimize risk for potential side effects of supplemental vitamin D, which include gastrointestinal symptoms, hypercalcemia, and kidney stones, participants are asked to limit calcium intake to 1,200 mg/day from all supplemental sources, and persons with a history of hypercalcemia or sarcoidosis (or other chronic granulomatous diseases) are not permitted to enroll in the trial. A random subsample of participants will be monitored for kidney dysfunction and abnormal blood levels of calcium and parathyroid hormone.

We decided against including calcium as a component of the intervention, for several reasons. First, a separate assessment of the effects of vitamin D, calcium, and calcium-plus-vitamin D would entail a much larger and more expensive trial than one of vitamin D alone. Second, the widespread use of supplemental calcium by older women would greatly reduce the pool of eligible females. Third, calcium plus vitamin D was associated with a statistically significant 17% increase in kidney stone risk in the Women’s Health Initiative (Jackson et al., 2006). Fourth, calcium supplements may more quickly increase blood calcium levels than dietary calcium, which could lead to greater calcium deposition in coronary arteries and an increased risk for coronary heart disease (Reid and Bolland, 2012), although this remains unproven (Wang et al., 2012). Fifth, high calcium or milk intakes have been associated with an increased prostate cancer risk in some studies (Ahn et al., 2007; Park et al., 2007; Stacewicz-Sapuntzakis et al., 2008).

35.2.4 Study population

The randomized study population will consist of 20,000 apparently healthy adults, half of whom are men aged ≥50 and half of whom are women aged ≥55. These are ages at which chronic disease
35. Design and rationale of the VITamin D and OmegA-3 Trial

rates start rising substantially. Recruitment for the study is currently occurring throughout the United States, and blacks are being oversampled (the goal is a study population that is 25% black). Because this is a primary prevention trial, eligible individuals cannot have a history of cancer (except non-melanoma skin cancer), MI, stroke, transient ischemic attack, angina pectoris, or coronary revascularization (CABG or PCI). In addition, they must agree to limit consumption of supplemental vitamin D to 800 IU/day or less and supplemental calcium to 1,200 mg/day or less from all supplemental sources combined and also avoid taking fish oil supplements while participating in the trial. For safety reasons, individuals with the following conditions cannot participate: renal failure or dialysis, hypercalcemia, hypo- or hyperparathyroidism, severe liver disease (cirrhosis), or sarcoidosis or other granulomatous diseases such as chronic active tuberculosis or Wegener’s granulomatosis; allergy to soy (an ingredient in the vitamin D placebo pill) or fish/shellfish (for the marine omega-3 fatty acid intervention); or other serious illness that would preclude participation. Individuals who satisfy these requirements, sign the informed consent form, and demonstrate good compliance in pill taking – defined as taking ≥2/3 of the study pills during the run-in period – are eligible for randomization.

35.2.5 Recruitment, run-in, randomization, follow-up, and endpoint determination procedures

Our primary recruitment strategy is to mail invitational letters to individuals on commercially available membership lists of professional and other organizations, subscription lists of magazines that appeal to people likely to be eligible for the trial, and lists of residents within certain geographic regions (e.g. cities with large black populations and regions within driving distance of Boston (see ‘Ancillary studies and the Clinical and Translational Science Center subcohort’ later in the chapter)). We are also recruiting potential participants via direct appeals in articles and advertisements in print media. We have also invited participants in one of our previously completed trials, the Women’s Health Study (Ridker et al., 2005), to join VITAL. Finally, we are employing targeted recruitment efforts in the black community, including neighborhood-based presentations, the creation and distribution of specialized information about the study supplements and the burden of cancer and CVD in blacks, and other initiatives, to achieve our goal of a study population that is 25% black.

Potential participants receive the following materials by postal mail: (1) an invitational letter that explains the rationale for VITAL and outlines what participation entails; (2) an informed consent form; (3) brief questionnaires containing items on demographics; medical history; allergy to fish or soy; current use of supplements containing vitamin D or fish oil; current use of other supplements or medications; dietary intake of vitamin D and consumption of fish; cancer and vascular risk factors; and (4) pre-paid envelopes for returning study forms. Questionnaire responses are evaluated to determine respondents’ willingness and eligibility to enter the run-in phase of the trial. Our goal is to identify 40,000 individuals to participate in this phase.

During the run-in phase, eligible individuals take one placebo vitamin D pill and one placebo fish oil pill per day for 3 months. The run-in is helpful in selecting excellent compliers for long-
term follow-up, which increases the trial’s power (Lang et al., 1991). Because some effects of the interventions on the endpoints of interest (particularly cancer) may be chronic, it is not appropriate to use active agent in the run-in and then randomize to placebo. A placebo run-in also maximizes the likelihood of detecting side effects during the trial, should such side effects exist. If the active agents were used during the run-in, potential participants may drop out not only because of poor compliance but also because of side effects, and the true rate of the latter would be underestimated among those ultimately randomized. Individuals are randomized into the trial only if, during the run-in, they demonstrate good compliance in pill taking, defined as taking ≥2/3 of the study pills; report no new history of cancer or cardiovascular endpoint under study, hypercalcemia, sarcoidosis, or other serious illness; and remain willing to follow the guidelines on non-study use of supplemental vitamin D, calcium, and fish oil. We estimate that half of the 40,000 individuals who enter the run-in (n=20,000) will be randomized to a mean of 5 years of treatment with vitamin D and/or marine omega-3 fatty acid supplements (or their placebos).

Randomized participants receive follow-up questionnaires at six months and one year after randomization and annually thereafter. The questionnaires include items on compliance with randomized treatments, use of nonstudy supplemental vitamin D and marine omega-3 fatty acids, development of study endpoints and other illnesses, cancer and cardiovascular risk factors, and potential side effects of the study agents. Non-responders receive two additional requests by mail and are then telephoned to collect study data. At a minimum, vital status is ascertained. At 6-month intervals between the annual follow-ups, participants receive a short questionnaire on the development of new primary endpoints (cancer, MI and stroke), pill-taking compliance, and address changes.

Participants who report an endpoint of interest are asked to sign a medical release for relevant hospital and physician records. Using a defined protocol, study physicians blinded to the randomized treatment assignment review the records to confirm or disconfirm the endpoint. Cancer diagnoses are confirmed with histologic or cytologic data or strong clinical evidence accompanied by radiologic evidence or laboratory markers (Fritz et al., 2000). MI is confirmed using Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Task Force for the Redefinition of Myocardial Infarction criteria (Thygesen et al., 2007). Stroke is confirmed according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (Adams et al., 1993). Cardiovascular mortality is confirmed by convincing evidence of a cardiovascular event from all available sources, including death certificates, hospital records, autopsy reports, and, for deaths outside the hospital, observer accounts.

If a death is reported by a family member, copies of the medical records and death certificate are requested from the next of kin. Alternatively, a copy of the death certificate is obtained from the vital records bureau in the state where the death occurred. Study physicians review the records to assign a cause of death. If records are not available, the National Death Index Plus is searched to
obtain an International Classification of Disease-coded cause of death based on death-certificate information.

### 35.2.6 Blood samples

Fasting blood samples are collected at baseline (i.e. during the run-in) from as many participants as are willing to provide them. We expect that 80% of the 20,000 participants who are ultimately randomized will provide a baseline sample (n=16,000). Follow-up fasting blood samples will also be collected at years 1, 2, and/or 4 from a random subset of ~6,000 participants who provide baseline samples. The procedure for collecting the baseline sample is as follows: during the run-in, individuals are mailed a blood collection kit, including a freezer pack and overnight courier air bill, and are given the option of having their blood drawn by their own healthcare provider, at a local blood-drawing facility, or at home by a national company that provides phlebotomy services. Individuals are asked to ship the blood sample in the freezer pack to our laboratory within 24 hours of the blood draw. Upon receipt, the samples are centrifuged to separate plasma, serum, red blood cells, and buffy coat, which are stored in nitrogen freezers (-170 °C) within 30-36 hours of the blood draw. Identical procedures will be used for the follow-up blood collection.

The baseline blood samples will permit an assessment of whether treatment effects are modified by baseline blood levels of 25(OH)D (for vitamin D) and EPA+DHA (for the marine omega-3 fatty acids). The follow-up blood samples will allow an assessment of pill-taking compliance (see also next paragraph); changes in biomarkers in response to the intervention; and, in the placebo group, the effect of changing trends in background intakes of vitamin D and marine omega-3 fatty acids. In addition, changes in blood calcium and parathyroid hormone levels will be measured to assess possible hypercalcemia, a potential side effect of high vitamin D intake. The blood samples will also be used to study whether the interventions affect biomarkers related to lipids, glucose tolerance, inflammation, endothelial dysfunction, thrombosis, insulin, and insulin-like growth factor pathways. Finally, the samples will allow for future biochemical and genetic studies.

### 35.2.7 Assessment of compliance

The primary measure of compliance with pill-taking is participants’ responses to questionnaire items on adherence. Our experience with large trials similar to VITAL indicates that although most participants make an effort to take their pills, those who fail to do so will admit this. Thus, blood levels and self-reported adherence data have been strongly correlated in our past trials (Satterfield et al., 1990). However, to obtain an objective measure of compliance, we will periodically visit with limited advance notice a small group of randomly sampled local participants to draw a blood sample for determination of 25(OH)D and EPA+DHA levels. The distribution of these values will be compared between the active treatment and placebo groups, and compared with the questionnaire data on adherence, as a check on the validity of the latter. In addition, as described in the previous paragraph, follow-up blood samples collected from ~6,000 participants will allow another assessment of pill-taking compliance.
35.2.8 Assessment of background intake of vitamin D

Measuring participants’ background intakes of vitamin D, marine omega-3 fatty acids, and other nutrients at baseline and during the course of the trial is necessary to determine whether the effects of the study agents depend on these variables. For example, participants with low baseline vitamin D intakes may benefit more from the vitamin D intervention than those with high baseline intakes. At baseline, two years, and at the end of the trial, participants will be asked to answer a semi-quantitative food frequency questionnaire, which accurately categorizes an individual’s intake of various nutrients, including vitamin D and marine omega-3 fatty acids (Feskanich et al., 1993, 2003; Hunter et al., 1992; Jacques et al., 1993; Rimm et al., 1992; Salvini et al., 1989). Respondents estimate their average intake over the past year of various foods, beverages, and supplements that contain vitamin D, marine omega-3 fatty acids, and other nutrients. Additional questions on use of non-study supplements or drugs containing vitamin D or marine omega-3 fatty acids are asked at baseline, six months, and on yearly follow-up questionnaires. Nutrient intakes from food alone, supplements alone, and the two sources combined will be analyzed to determine whether the effects of the study agents vary according to these variables.

35.2.9 Data analysis and statistical power

The main analyses will compare the main effects of intention-to-treat with vitamin D and with marine omega-3 fatty acids on the primary cancer and CVD endpoints of interest, using a Cox proportional hazards model to allow for variable follow-up lengths (Cox, 1972).

To compute statistical power, the following assumptions were made: (1) a 2×2 factorial trial in 10,000 men aged ≥50 and 10,000 women aged ≥55 at baseline; (2) independent and equal allocation of participants to each treatment (achieved by randomization); (3) an age distribution based on that observed at baseline in our past trials for men aged ≥50 and women aged ≥55, but limited to 30% in the youngest age groups (50-59 years in men and 55-64 in women); (4) age-specific event rates based on the observed rates in the first 5 years of follow-up in our trials with similarly aged populations; (5) a target of 25% blacks, with a corresponding increase in rates of CVD (Hozawa et al., 2007) and cancer (Surveillance Research Program of the National Cancer Institute); (6) a trial follow-up period of 5 years, with little loss to follow-up as achieved in our past trials; and (7) compliance (80%) similar to that in published trials upon which our estimated RR reductions are based. Power was calculated for a two-sided test with a significance level of 0.05. Assuming that only vitamin D is effective (i.e. that omega-3s have no effect), with 5 years of treatment and follow-up there will be 91% power to detect an observed RR of 0.85 for the primary endpoint of total cancer incidence and 92% power to detect an observed RR of 0.80 for the primary composite endpoint of MI, stroke, and cardiovascular mortality (99% power for the expanded CVD outcome that includes coronary revascularizations). For site-specific cancers and individual CVD outcomes, the trial will be powered to detect 25-30% reductions in risk. If the vitamin D and the omega-3 interventions interact, power will be affected to the extent of the interaction. Should the interventions act synergistically, power would increase. Should the agents interact in a subadditive fashion, power would be reduced.
35. Design and rationale of the VITamin D and OmegA-3 Trial

35.2.10 Ancillary studies and the Clinical and Translational Science Center subcohort

VITAL is expected to shed light on the effect of vitamin D on health outcomes other than cancer and CVD through well-integrated ancillary studies. These ancillary studies will assess whether vitamin D can prevent diabetes and glucose intolerance; hypertension; cognitive decline; depression; osteoporosis and fracture; physical disability and falls; asthma and other respiratory diseases; infections; kidney disease; anemia; eye problems such as macular degeneration and dry eye disease; chronic knee pain symptoms; and autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and thyroid diseases.

In addition, a subcohort of 1000 participants who live within driving distance of the CTSC at Brigham and Women’s Hospital in Boston, Massachusetts are having a detailed health assessment at the CTSC site at or near the end of the run-in period (prior to randomization) and will have a repeat assessment two years later. The two health assessments will use the same protocol to gather basic clinical data as well as data on variables related to ancillary study aims (e.g. glucose tolerance testing, physical performance batteries, lung function exams, cognitive assessments, and structured interviews to diagnose depression). Non-invasive imaging techniques are also employed to collect data for other ancillary studies, including dual energy X-ray absorptiometry to measure bone density and body composition; mammography to assess breast tissue density; and Doppler echocardiography to assess left ventricular function. The results of the latter two investigations may help to clarify mechanisms by which vitamin D possibly protects against breast cancer and CVD, respectively.

At the CTSC visits, blood samples are drawn not only for glucose tolerance testing but also for assays of 25(OH)D and EPA+DHA levels. The timing of the second visit will be matched by month to the first visit to minimize variability in seasonal sun exposure, a major contributor to within-person variation in 25(OH)D. The CTSC visits represent an important opportunity for in-person contact with some participants, allowing for in-depth phenotyping and face-to-face validation of the remote assessment methods used in the main trial and ancillary studies. For example, in-person cognitive testing at the CTSC visit will be used to validate telephone-based assessments in the cognitive function ancillary study, and in-person structured diagnostic interviews for clinical depression at the CTSC visit will be used to validate clinical depression cases identified by screening checklists in the depression ancillary study.

The CTSC subcohort is expected to mirror the diverse racial/ethnic composition of the overall VITAL study population. Members of the subcohort are being randomized equally into the four treatment groups created by the factorial design (i.e. 250 participants per treatment group).

35.2.11 Strengths and limitations

Among the many strengths of VITAL is the fact that it is testing two promising nutritional agents (vitamin D and marine omega-3 fatty acids) for the prevention of two major diseases (cancer and CVD) in a racially/ethnically diverse population. The trial is using a mail-based, large simple trial
design, at great cost efficiency. The study includes the collection and storage of baseline blood samples in most of the cohort to allow assessment of effect modification by baseline 25(OH)D and EPA+DHA levels, and the collection and storage of follow-up samples in a large subgroup of participants to allow assessment of pill-taking compliance; changes in biomarkers in response to treatment; and, in the placebo group, the effect of changing background intakes of vitamin D and marine omega-3 fatty acids. The trial has excellent power to detect small to moderate effects of the study agents on the primary endpoints of interest and will also advance our knowledge of the agents’ effects on other health outcomes through well-integrated ancillary studies. Among the study’s limitations is the fact that it is examining only a single dose of each agent, precluding an assessment of potential dose-response relationships. However, the chosen dose was selected after an extensive review of available data, with careful consideration given to both efficacy and safety. Latent effects, especially for cancer endpoints, may produce artifactually null results. However, accumulating data suggest that vitamin D may act at later stages of carcinogenesis (Deeb et al., 2007; Lappe et al., 2007; Welsh, 2007), suggesting that salutary effects of treatment could emerge within 5 years. Finally, because the study population is older, the findings may not apply to younger individuals. However, older populations have higher rates of disease, allowing the trial to be completed in a timely fashion, which is important for cost efficiency.

35.3 Conclusion

There has been much recent interest among both medical professionals and the lay public about possible nonskeletal health benefits of vitamin D. Many healthcare providers now routinely include vitamin D blood tests as part of lab work and recommend vitamin D supplements to patients. Sales of such supplements have dramatically increased in recent years (Parker-Pope, 2010). However, the evidence to support such enthusiasm for vitamin D is far from compelling. In a 2011 report, the Institute of Medicine comprehensively reviewed the literature on this vitamin in relation to multiple health outcomes and concluded that although there is strong evidence that vitamin D – at doses of 600 to 800 IU/day – confers bone benefits, available data are insufficient to determine whether higher vitamin D protects against the development of nonskeletal diseases, including cancer and CVD (Institute of Medicine, 2011; Ross et al., 2011). To address this knowledge gap, the Institute of Medicine called for additional studies, including large randomized controlled trials such as VITAL, to determine whether high-dose vitamin D supplements confer nonskeletal benefits and whether such supplements pose any health risks. Randomized trials of other single-agent nutritional interventions, including certain antioxidant vitamins, selenium, B-vitamins, and calcium, have disproved some purported health benefits for these supplements and even uncovered harmful effects that may have otherwise gone unrecognized (Bjelakovic et al., 2007; Bolland et al., 2010; Byers, 2010; Kris-Etherton et al., 2004; Lee et al., 2005). Indeed, some data suggest adverse effects on CVD (Freedman et al., 2007; Wang et al., 2008), pancreatic cancer (Stolzenberg-Solomon et al., 2009), all-cause mortality (Melamed et al., 2008), and even fracture (Sanders et al., 2010) at very high 25(OH)D levels. The results of VITAL are expected to shape clinical and public health guidelines regarding the use of supplemental vitamin D for the primary prevention of cancer, CVD, and other health conditions.
35. Design and rationale of the VITamin D and OmegA-3 Trial

Acknowledgments

VITAL is supported by grant U01 CA138962, which includes support from the National Cancer Institute; National Heart, Lung and Blood Institute; Office of Dietary Supplements; National Institute of Neurological Disorders and Stroke; and the National Center for Complementary and Alternative Medicine. The ancillary studies are supported by grants from several other NIH institutes. Members of the Data and Safety Monitoring Board include Lawrence S. Cohen, Theodore Colton, Mark A. Espeland, I. Craig Henderson, Alice H. Lichtenstein, Rebecca A. Silliman, and Nanette K. Wenger (chair), and Josephine Boyington, Cindy D. Davis, Rebecca B. Costello, Lawrence Fine, and Peter Greenwald (ex-officio members).

References


35. Design and rationale of the VITamin D and OmegA-3 Trial


35. Design and rationale of the VITamin D and OmegA-3 Trial


Index
Index

A
aBMD 85
ABPA 254, 262
absorption 19
accelerated aging 307
Acinar cells 394
acute lower respiratory infection – See: ALRI
acute rejection – See: AR
adaptive immunity
  – suppression 430
adenocarcinomas 464
adipocytes 504
adiponectin 513
  – gene 569
adipose tissue 513
adiposity 154, 512, 532, 610
adolescent
  – girls 86, 652
  – pregnancy 652
adverse
  – effects supplementation 122, 680
  – events 610
  – pregnancy 533
aetiology of melanoma 425
age
  – effects 70
  – specific dietary intake 283
aggressive periodontitis 358
aging
  – accelerated 307
  – skin 148
  – telomeric 180
albuminuria 202
alcohol 392
alfacalcidol 74, 198
allelic variations in vitamin D receptor 326
allergic
  – asthma 228
  – bronchopulmonary aspergillosis – See: ABPA
  – response 375
allergy
  – food ~ 215
  – predisposition 677
ALRI 289, 638
altitude 215
alveolar
  – bone 355, 365
  – bone resorption 242
  – crest heights 245
  – ridge resorption 243
  – type II pneumocytes 285
American Academy of Pediatrics
  – recommendations 619
AMP-activated protein kinase 513
analogs 198, 381
  – efficacy 259
  – noncalcemic 401
ancillary studies 701
angiogenesis 186, 332, 399
ankylosing spondylitis patients 490
anorexia 658
antenatal services
  – in developing countries 657
  – in rural areas 658
anthropometry 658
  – in newborn infants 650, 658
anti-angiogenic effects 428
anticancer immune response 423
anticarcinogenic effects 397, 468
anti-convulsant effect 597
antifracture efficacy 108
antigen 230
  – presenting cell – See: APC
  – specific antibody 490
anti-inflammatory
  – effects 356
  – properties 246
antimicrobial
  – activity 247, 375
  – peptides 261, 286
antioxidants 426
antiproliferative
  – effect 397, 427
  – properties 185
antirachitic
Index

– activity 633, 642
– deficient foods 16
– metabolites 641

antiretroviral
– drugs 299, 305
– treatment 299

antiseizure
– drugs 589
– medications and bone metabolism 593

antitumor activity 401

APC 476, 492

apoptosis 186, 397, 420, 445, 468, 511

AR 573

areal bone mineral density – See: aBMD

arteriosclerosis in renal patients 570

asthma 232, 287, 677
– allergic 228

atmospheric pollution 15, 651

atrial fibrillation 558

attachment apparatus of teeth 355

autoimmune
– disease 139, 377, 475
– encephalomyelitis 478
– thyroiditis 380

autoimmunity 140

autophagy 309

B

Bacillus Calmette Guerin vaccination – See: BCG vaccination

bacterial vaginosis – See: BV

Barrett’s esophagus 466, 468

baseline blood sample 699

B cells 377, 490

BCG vaccination 330

BD 488

Behcet’s disease – See: BD

beta cells – See: pancreatic β-cells

bile 502

biomarker 277

birth
– status 636
– weight 675

bisphosphonates 255

BMC 86
– in neonates 675

BMD 108, 142, 157, 255
– in epilepsy patients 591
– in formula-fed infants 639
– in HIV patients 307

BMI 302, 427

body mass index – See: BMI

bone
– as a critical endocrine organ 534
– cross-sectional area 118
– density scans 598
– development 616
– geometry 86
– mineral content – See: BMC
– mineral density – See: BMD; See also: aBMD (areal BMD); See also: vBMD (volumetric BMD)
– mineralization 635
– reabsorbing osteoclasts 379
– resorption 358

bone health 526, 529, 695
– breastfeeding infants 637
– guidelines 277
– indicator 679
– infants 610
– post-menopausal 633
– reduced 425
– supplementation 637

bone loss
– after transplantation 566, 568
– cortical 42

bone mass 88
– accrual 116
– mandibular 245
– peak 84

bone size
– and diabetic risk 533
– and osteocalcin 534

bone turnover 85, 109
– in epilepsy patients 592
– markers 118, 157
Index

brain 677
breast cancer 24, 184
breastfed infants 636, 637
  – supplementation 21, 638, 639
breastfeeding 620
  – bone health 637
  – supplementation of mothers 640
breastmilk 19, 280, 633
  – calcium 633
bronchiolitis 287, 613
BV 674

C
calcidiol – See: hydroxyvitamin D
calciﬁediol – See: hydroxyvitamin D
calcitriol – See: dihydroxyvitamin D
calcitroic acid 450
calcium 74
  – absorption 17, 103, 260, 453, 567
  – accretion 656
  – balance 122
  – breast milk 633
  – cord blood 652
  – CVD risk of – supplementation 181
  – deposition 696
  – during lactation 632
  – epilepsy patients 590
  – homeostasis 102, 127, 163, 261, 354, 475, 512, 637
  – intestinal absorption 567
  – intracellular levels 511
  – optimal absorption 103
  – -rich diet 660
  – supplementation 109, 123, 124, 141, 245
  – transport 446
calculus 358
calmodulin 106
CAMP 286
cancer 183, 209, 692, 695
  – breast 24, 184
  – colon 161
  – colorectum 24, 183
  – esophageal 464
  – geographical variation 209, 393
  – in renal TR 571
  – nonmelanoma skin – See: NMSC
  – pancreatic 184, 402
  – prostate 184, 444, 572
  – risk reduction 211, 571
  – sex specific impact 184
  – skin 183
  – skin prevention 399
  – supplementation 693
  – treatment 395
  – upper gastrointestinal 467
carbamazepine 593
cardiac myocytes 548
cardiomyopathy 615
cardioprotective role 182
cardiovascular
  – benefits 23
  – disease – See: CVD
carrier in vitamin D supplementation 259
cathelicidin 247, 328, 356, 375, 572
  – antimicrobial peptide – See: CAMP
CD4 count 302
cell growth
  – inhibition 398
  – prostate cells 449
central nervous system – See: CNS
cerebral palsy 597
CF 254
  – European – bone mineralisation
    guidelines 258
  – long term complications 254
  – supplementation 255
  – transmembrane regulator – See: CFTR
CFTR 262
chemokine 286
cholecystectomy 393
chondrocyte metabolism 379
chromosome 20 323
chronic
  – allograft deterioration 573
  – immune diseases 226
Index

– inflammation 429
– kidney disease – See: CKD
circulating monocytes 489
CKD 182
clinical attachment 248
CNS
– demyelination 234
– tissue 476
coagulation 310
cod liver oil 14, 396, 432, 531
cognitive function 42
colon
– cancer 161
– tumor 399
colorectal cancer 24, 183
compliance 156, 699
complier selection 697
congestive heart failure 615
contact hypersensitivity 233
contemporary lifestyle 634
controlled environments 59
cord blood 288, 609, 636
– calcium level 652
– of newborns 488
cortical bone
– loss 42
– mass 593
C-reactive protein – See: CRP
criteria for causality 216
Crohn's disease 213, 379, 489
– supplementation 379
CRP 379, 381, 479
– levels 429
crushed bones 15
cumulative intake 279
cutaneous synthesis 281
– during lactation 634
cutaneous tuberculosis 422
CVD 180, 214, 528, 548, 692, 695
– confirmation of ~ diagnose 698
– mortality 555
– risk factors 570
– risk in TR 570
– risk reduction 555
– supplementation 693
cyclosporine A 574
CYP24A1 478
CYP27B1 449, 478, 570
cystic fibrosis – See: CF
cytochrome P450 99, 305, 445, 447, 592
– inhibitors 452
– mutations in ~ enzymes 445
cytokine 42, 142, 212, 243, 261, 286, 320,
328, 422, 429, 478, 479, 615, 673
cytosolic receptor 298
D
daily reference intake – See: DRI
DAS of RA 141
DBC – See: VDBC
DC 262, 287, 476, 573
deficiency – See: VDD
7-dehydrocholesterol 99, 148, 227
dehydrocholesterol 322
demyelination 475
– of central nervous system 234
dendritic cell – See: DC
dental caries 211, 361
– sunlight exposure 362
dental plaque 355
dentin 365
dentition 611
dermal synthesis 16
diabetes mellitus 23, 229, 232, 263, 393
– due to corticosteroid and tacrolimus
treatment 569
– gestational – See: GDM
– prevalence 527
– risk reduction 532
– type 1 213, 530, 615, 638, 678
– type 1, incidence 527
– type 1, latitude and risk 615
– type 1, reduced risk 530
– type 2 503, 531
– type 2, global epidemic 528
– UVB exposure 530
Index

dialysis patients 555
dietary
  – guidelines esophageal cancer 469
  – requirements 277
  – supplements 362
1,25-dihydroxyvitamin D 16, 17, 98
  – analog 452
  – during pregnancy 671
  – functions 397
  – in cord blood 671
  – suppression 673
24,25-dihydroxyvitamin D 320
disease
  – activity score – See: DAS
  – remission 489
DNA
  – damage 228
  – repair 233
domestic animals 124
dose 149
  – daily ~ 257
  – during pregnancy 657, 659, 660
  – for breastfeeding infants 638
  – for fall prevention 76
  – -response 150
  – to balance efficacy and safety 695
doxercalciferol 199
draining lymph nodes 230
DRI 59
drugs
  – antiretroviral 299, 305
  – antiseizure 589
  – immunosuppressive 567
dual energy x-ray absorptiometry – See: DXA
DXA 86, 87, 701
elastosis 211
dergery 101
  – patients 105
  – women 74
enamel 365
  – hypoplasia 363
endocrine
  – pancreas 535
  – system 99
endogenous
  – insulin secretion 507
  – synthesis 526
endothelial dysfunction in renal patients
  – 570
enzyme-inducing medications 592
epidermal growth factor receptor – See: EGFR
epigenetic
  – alterations 395
  – control 452
  – fetal programming 284
  – mechanisms 677
epilepsy 588
  – BMD 591
  – bone turnover 592
  – calcium intake 590
  – etiology 588
  – fracture risk 590
  – obesity 591
  – sunlight exposure 592
  – supplementation 597
  – treatment-resistant 590
epiphyses calcification 15
epithelial
  – barriers 261
  – cells 254
erythema 227, 229
esophageal cancer 464
  – adenocarcinoma 466
  – dietary guidelines 469
  – incidence 465
  – protective factors 464
  – racial disparities 465
- risk 464, 467
- squamous cell carcinoma 466
esophageal tissue 468
esoteric tests 557
Esslinger Fitness Index 88
estimated average intake – See: EAR
estriol 632
estradiol 632
estrogen 671
ethnic backgrounds 57
ethnicity 119, 531
excision 418
exogenous consumption 526
experimental autoimmune encephalomyelitis
- See: EAE
extra-osseous calcification 197
extra-renal tissues 502, 567

F
factorial design 694
falecalcitriol 200
falling 70, 105
- definition 68
- fear of ~ 69
- medical costs 68
- occurrence 108
- prevention 69, 72
- reduction 108, 157
- related fractures 42
- risk factors 69
- surrogate markers for risk 71
fast twitch muscle fibers 71
fat
- accumulation of vitamin D 161
- -soluble A 15
- -soluble vitamin 532
- stores 507
fatty fish 56
femur bone lengths 534
fetal
- bone mass 118
- femoral splaying 675
- programming 650
- skeletal development 675
fetus 280
FFA 510
fibrogenesis 574
fibromyalgia 380
food
- allergy 215
- frequency questionnaire 700
- sensitivity of infants 614
- sources 90
foodstuffs 148
formula 635
- fortification 620
formula-fed infants
- BMD 639
fortification 60, 276
- baby foods 282
- dairy 507
- formula 620
- milk 54, 56, 124
FOXP3 expression 231
fractures 42, 68, 108, 157, 163, 255
- epilepsy patients 590
- renal patients 197
- risk 177
- risk reduction 178
frailty 45
free fatty acids – See: FFA
free radicals 333
frequency of delivery 155

G
GC protein 126
GDM 529, 533, 673
gene mutations 394
genetically modified animals 125
genomics 433
- increased risk 614
- pathway 99
genotype 125, 127
gestational
- diabetes mellitus – See: GDM
- supplementation 289
gingiva 355
Index

gingival
   – epithelial cells 357
   – inflammation 244
   – recession in sheep 360
gingivitis 355

girls
   – adolescent 86, 652
   – premenarcheal 123
   – prepubertal 638

glucose
   – and pancreatic cancer 402
   – disposal 512
   – hepatic output 505
   – homeostasis 506, 512
   – lowering 263
   – supplementation 508
   – tolerance 505
GLUT4 504, 513
glycaemia 569
glycemic status 532
graft
   – rejection 380
   – survival in animal models 573
group-specific component protein – See: GC protein
growth consequences 611

H
HAART 308
harmful effects 429
HbA1c 673
health assessment 701
hepatic conversion 154
high-dose therapy 75, 660
highly active antiretroviral therapy – See: HAART
hip fractures 68, 108
HIV 674
   – glomerular filtration rate in patients 308
   – mother-to-child transmission 308, 613
   – related factors 302
   – supplementation 310
   – transmission during gestation 656
   – untreated infected pregnant women 308
HLA 323
homebound pregnant women 656
homeostasis 89
   – calcium 102, 127, 163, 261, 354, 475, 512, 637
   – glucose 506, 512
   – immune 478
   – mineral 98
   – phosphorus 176
   – T cell 476
human leukocyte antigen – See: HLA
1α-hydroxylase 180, 195, 692
hydroxylation 126, 444, 447
25-hydroxyvitamin D 16, 17
   – catabolism induced by immunosuppressive drugs 567
   – cord blood 280, 671
   – deficiency 70
   – during pregnancy 671
   – half-life 149, 150
   – HIV infected persons 299, 309
   – insufficiency 70
   – levels and symptoms 102
   – recommended serum level in CF population 258
   – response to vitamin D intake 25
   – serum concentration 54
   – status in neonates 638
   – target levels 150
   – toxicity 75
   – upper safety limit 177
   – usefulness of monitoring 76
hypercalcaemia 41, 103, 162, 232, 310, 381, 401, 454, 476, 558, 658, 681, 699
hypercalciuria 162, 401, 641
hyperglycaemia 511, 533
hyperinsulinemia 505, 511
hyperlipidemia 553
hyperparathyroidism 44, 105, 163, 551, 566, 568
hyperphosphatemia 195
hypertension 23, 553
hypervitaminosis D 89, 640, 658
  – and teeth 363
  – maternal 660
hypocalcaemia 530, 609, 615
hypophosphatemia 363
hypovitaminosis D 19, 84
  – and CVD risk 552
  – clinical signs 102
  – myopathy 105
  – wintertime 24

I
IBD 373, 379
IGF-1 124, 127, 612
IgG 262
immigrants 651
immune
  – cells 374
  – dysregulation in MS 475
  – homeostasis 478
  – regulation 354
immune response 309, 356, 475, 477
  – tuberculosis 247
immune system 139, 612
  – in lungs 285
immunoglobulin G – See: IgG
immunological
  – adaptations 672
  – responses to UV-induced damage 425
immunomodulation 138, 246, 373, 492, 566
  – after transplantation 574
immunosuppression 421, 567
  – by UV exposure 228, 420
  – therapy 226
  – treatments 566
immunotherapy 429
impaired mobility 591
India 61
infant
  – growth 121
  – health 632
  – infections 614
  – plasma concentration 617
  – prematurely born 616
infants
  – supplementation 61, 121, 610
infections 422
  – in transplantation recipients 572
infectious diseases 211
inflammation 142, 186, 310, 400, 429
  – chronic 429
  – gingival 244
  – markers 479
  – markers in neonatal immune cells 613
  – soluble markers 491
  – synovial 379
  – vascular 551
inflammatory
  – bowel disease – See: IBD
  – process in tuberculosis 333
  – skin conditions 226
influenza A 289
innate immunity 422
  – receptors 375
  – system 286, 492
  – system response 326, 450, 613
insufficiency
  – at birth 612
  – in CF patients 256
  – in HIV infected persons 299
insulin
  – effect of parathyroid hormone on sensitivity 512
  – endogenous ~ secretion 507
  – insensitivity 507, 528
  – -like growth factor 1 – See: IGF-1
  – receptor 504, 505, 511
  – resistance 503, 508, 569
  – -responsive tissues 504, 510
  – secretion 511, 530
  – sensitivity 503
  – skeletal muscle ~ resistance 509
  – supplementation 508
intake
  – daily reference ~ – See: DRI
  – during pregnancy 679
Index

- frequency during lactation  633
- tolerable limit  58, 640
- upper level for mothers  636
intestinal
  - malabsorption  260
  - vitamin D receptor expression  423
intramuscular injection  509
intrauterine
  - bone growth  119
  - nutritional deficits  117
Islets of Langerhans  394

J
Jordan  61

K
keratinocytes  422
ketoacidosis  528
ketogenic diet  597
kidney  444, 446, 611
  - disease  24, 552
  - disease, chronic – See: CKD
killer cell  428
knee cartilage loss  142
kyphosis  255

L
lactating mothers  633
lamotrigine  596
lanolin  73
latent effects  702
latitude  139, 226, 656
  - and diabetes type 1  530, 615
leukocyte telomere  180
linear growth  128
lipoprotein particles  552
liver  444, 446
  - meat  56
low birth weight  616
low-cost therapy  248
lower respiratory tract infection  288
low income countries  651
lungs
  - function in rats  285
  - production of vitamin D in ~  261
lymphocytes  429, 475, 478, 489
lymphoid tissue  491

M
macrophages  329, 331, 375
mandibular bone mass  245
MART-10  452
mast cells  231
maternal
  - bone density loss  633
  - bone mineral resorption  632
  - fertility  672
  - hypervitaminosis D  660
  - intake  643
  - supplements  620
  - vitamin D status  128
matrix metalloproteinases – See: MMPs
maxacalcitol  199
MCTD  490
mDC  375
MDR  321, 325
melanin  20
melanocytes  420
melanoma
  - aetiology  425
  - cumulative hazard ratio  421
  - genetic predisposition  419
  - risk  424
  - sunburn  419
mesenchymal stem cell  127
metabolic
  - consumption  155
  - syndrome  427, 506
metalloproteinases  243
metastasis  399
microsomal enzymes  445
microvascular complications  533
milk fortification program  54
mineral
  - homeostasis  98
  - metabolism  195
mitochondrial
  – biogenesis 513
  – enzymes 445
mitogen activated protein kinase 450
MMPs 331
mobility 163
mole 418
  – atypical ~ syndrome phenotype 419
monocytes 613
mood 24
mortality 41
  – after transplantation 575
  – risk for rickets 178
  – risk reduction 179
MS 24, 212, 232, 381, 679
  – primary progressive ~ 475
  – relapse 476, 478
  – risk factor 228
  – secondary progressive ~ 475
  – supplementation 476
multidrug-resistant – See: MDR
multiple
  – primary tumours 424
  – sclerosis – See: MS
murine
  – colitis model 376
  – models 229
muscle 504
  – biopsy 104
  – function 157
  – insulin-responsive tissue 510
  – pain 509
  – strength 23, 88
  – weakness 45, 70, 104, 108
muscle cell 98
  – differentiation 106
  – proliferation 106
  – striated 106
muscle fibers
  – composition 105
  – fast twitch (type II) 71
musculoskeletal
  – health 91
  – outcomes 158
  – pain 104
  – symptoms 124
mushrooms 56
mycobacteria
  – immunity to ~ 330
  – infections 329
Mycobacterium tuberculosis 332, 572
myeloid
  – cells 477
  – dendritic cells – See: mDC
myoblast cytoskeleton 446
myogenic differentiation 510
N
native form 201
necrotic cell populations 399
neoadjuvant therapy 468
neonatal
  – immune function 659
  – markers of inflammation 613
  – period 609
  – supplementation 620
neonate anthropometry 650, 658
nephrolithiasis 41, 47
nephroprotective role 575
neurological function 72
nitric oxide 214
NMSC 209
NNRTI 305
NOD 329
non-calcemic
  – benefits 611
  – functions 138
non-genomic effects 107, 423
non-melanoma skin cancer – See: NMSC
non-nucleosidic reverse transcriptase inhibitor – See: NNRTI
non-skeletal benefits 22
non-specific pain 23
non-specific rheumatic diseases 105
normoglycemia 504
NRTI 307
nucleoside reverse transcriptase inhibitor –  
See: NRTI
nucleotide-binding oligomerization domain  
– See: NOD
nulliparous women  642

O
OA  142
obese females  488
obesity  19, 243, 299, 393, 507, 514, 598, 695
  – in epilepsy patients  591
obstructed labor  674
omega-3 fatty acids  556
oncogene  451
optimal level  142
ossification  117
ostearthritis – See: OA
osteoblasts  535
osteocalcin  513, 534
osteoclastic progenitors  44
osteocytes  127
osteoid seams  44
osteomalacia  14, 54, 117, 527, 652
  – in successive pregnancies  652
  – muscle biopsy in ~ patients  104
osteopenia of prematurity  617
osteoporosis  109, 244, 534
  – after transplantation  568
  – definition of ~  84
oxcarbazepine  595
oxidative
  – injuries  334
  – stress  334, 420

P
paediatric renal transplantation recipients  568
pale-skinned people  419
pancreas  535
pancreatic
  – adenocarcinoma – See: PCA
  – cell destruction  528
  – insufficiency  254
  – islet autoimmunity  678
pancreatic cancer  184
  – risk  402
  – sunlight  402
  – treatment  395
pancreatic β-cells  504, 505, 507, 510, 528, 673
  – dysfunction  528, 551
pancreatitis  393
parathyroid
  – gland  195, 201
  – hormone – See: PTH
  – levels  430
  – suppression  17
parental resistance to supplementation  609
parenteral cholecalciferol  660
paricalcitol  199
PBMC  488, 491
PCA  392
peaks
  – bone mass  84
  – infection rates  214
pelvic opening  208, 652
perinatal pulmonary maturation  285
periodontitis  674
  – aggressive  358
  – definition  357
  – during pregnancy  244
  – prevalence  242
periosteal apposition  117
peripheral
  – blood mononuclear cells – See: PBMC
  – tissue  200
PGs  333
phagocytes  324
phagocytosis  246, 329
pharmacological dose therapy  657
phenobarbital  593
phosphate
  – binders  196
  – uptake  107
phosphorus homeostasis  176
photoadaptation  421
photoprotective 422
phototherapy 257
physical
  – activity levels 148
  – performance 46
pigmentary genotypes 418
pigmentation 208, 651
  – dark 637
  – pale skin 419
pigmented skin 651
PIs 305
placental
  – CYP24A1 gene promoter 671
  – infection modulation 613
  – transfer 680
plaque stability 552
plasma concentration 617
pleiotropic
  – functions 276
  – steroid hormone 138
pneumonia 287, 614
polycystic ovary syndrome 509
polymorphisms 323
  – in toll-like receptors 328
  – in VDR gene 126
polytherapy 592
post-menopausal
  – bone health 633
  – women 18, 104, 162, 421, 557
post-natal growth 658
post-transplant bone loss 566, 568
postural instability 71
precursor lesions 418
preeclampsia 25, 120, 533, 672
  – supplementation 673
pregnancy 120, 609
  – adolescent 652
  – adverse 533
  – hormones 529
  – periodontitis 244
  – supplementation 609, 658, 680
  – untreated HIV-infected women 308
pregnant women 278, 529, 534, 650
  – oral health 245
premalignant lesions 394
premenarcheal girls 123
prepubertal girls 638
preterm infants 616
  – rachitic respiratory distress 617
primary prevention trial 697
primidone 593
proliferation 445, 476
  – of T cells 324
prostaglandins – See: PGs
prostate
  – cancer 184, 444, 572
  – cell growth 449
  – cells 447, 450, 451
  – tissue 448
prostatic hypertrophy 449
protease inhibitors – See: PIs
proteinuria 194, 574
psoriasis 25, 453
psoriatic arthritis patients 490
psychomotor function 108
PTH 87, 102, 119, 196, 259, 372, 451, 511,
  512, 549, 568, 574, 609, 617
  – insulin sensitivity 512
  – levels 260
  – secretion 453
  – suppression 198
public health programs 63
pulmonary
  – disease 254
  – infections 284
purdah (use of veils) 651
putative risk factor 476
pyrazinamide 334

R
RA 138, 214, 378, 490
  – patients 140
rachitic respiratory distress 617
racial backgrounds 57
  – esophageal cancer 465
RANKL 230
RAS 202, 551, 574
RDA 89, 696
reactive
  – nitrogen intermediates – See: RNI
  – oxygen species – See: ROS
receptor
  – activator of NFkB ligand – See: RANKL
  – agonists 430
recommended dietary allowance – See: RDA
recruitment strategy 697
reflux esophagitis 466
relapsing remitting multiple sclerosis – See: RRMS
remineralization 363
renal
  – cells 450
  – failure 330
  – osteodystrophy 197
  – reabsorption of calcium 632
  – stones 161, 162, 179, 430
renal patients
  – arteriosclerosis 570
  – bone fracture 197
  – endothelial dysfunction 570
  – supplementation 575
renal transplantation recipients 567
  – cancer 571
  – paediatric 568
  – skin cancer 571
  – supplementation 575
renin-angiotensin system – See: RAS
respiratory
  – epithelial cells 286
  – muscle weakness 288
  – tract infections 284
  – viral infections 213
rheumatoid arthritis – See: RA
ricketes 14, 117, 527
  – and antiseizure drugs 589
  – resurgence 18, 610, 618, 634
  – risk factors 635
risk factors 279
  – during fetal period and lactation 636
RNI 334
ROS 333
RRMS 475
S
safety limit 696
sarcopenia 42, 45, 71
schizophrenia 678
screening 619
secosteroid hormone 276
seizures 588
selective receptor modulator concept 101
serum
  – biomarkers 21
  – hydroxyvitamin D 40, 101, 506, 526
  – insulin 507
  – levels 40, 432
  – minimal level 430
  – parathyroid hormone 502
  – triglycerides 508
sex specific impact of cancer 184
SGA 120, 676
side effects 696
signaling pathways 505
Simplified Acute Physiology Score II 179
single-nucleotide polymorphism – See: SNP
skeletal
  – bone resorption 163
  – craniotabes in newborns 680
  – mineralization 45
  – muscle insulin resistance 509
  – pain 104
skin 40
  – aging 148
  – cancer 183
  – cancer in renal transplantation recipients 571
  – cancer, nonmelanoma – See: NMSC
  – cancer prevention 399
  – cancer risk 418
  – colour 426, 431
  – pale 419
  – pigmentation 208, 651
Index

– type 281
– UVB irradiation 227
SLE 378, 379, 490, 492
small for gestational age – See: SGA
smoking 321
SNP 326, 423, 427, 614
solar elastosis 426
spaying index 118
SPB 674
spontaneous preterm birth – See: SPB
squamous
– cell carcinomas 464
– dysplasia 467
standardized measuring method 60
statistical power 700
striated muscle cells 106
stunted growth 116, 612
sufficiency
– definition 656
sunburn
– melanoma risk 419
sun exposure 14, 226, 256, 656
– children 280
– decreased cutaneous penetration 380
– dental caries 362
– epilepsy patients 592
– pancreatic cancer 402
– regular 422
– seasonal variety 701
– tuberculosis treatment 331
sunscreen 20, 634
supplementation 57, 75, 109, 123, 141, 151, 245, 429, 432
– adverse effects 122
– and cardiovascular health 570
– birth weight/length 676
– developing countries 657
– during lactation 633
– efficacy 156
– form 155
– individualisation 257
– nursing homes 59
– responsiveness 123
– seasonal effects 154
– teeth defects 362
– to both mother and infant 642
– viral response 572
supra-physiologic dosages 570
supravalvular aortic stenosis 634
surfactants 286
surrogate marker of health 143
survival of CKD patients 201
synovial inflammation 379
systemic lupus erythematosus – See: SLE
T
t cell 376, 573
– homeostasis 476
– response 478
teeth 15
– attachment apparatus 355
– composition 354
telomerase activity 180, 399
telomeric aging 180
temperate climates 322
tender joint count 141
tenofovir 307
teriparatide 360
testosterone 72
tetanus toxoid vaccination 490
thymus receptor binding assay 448
thyroid disease patients 490
tibian length 123
tilt of the earth 20
tissue
– diseases 373
– epidermal 376
– inhibitor 488
– mixed connective disease 488
– non-traditional target 98
– vitamin D receptor expression 101
TLRs 326
tobacco smoke 392
toddlers
– outdoor exposure 281
tolerable upper intake level – See: UL
Index

toll-like receptors – See: TLRs
tomography  86, 118
tooth
  – development  362
  – loss  243, 360, 363
topiramate  596
toxicity  21, 103, 381
  – concerns  640
  – symptoms  59
TR  566
  – cancer  571
  – CVD  570
  – infections  572
transcription-independent effects  423
transplantation  263
  – recipients – See: TR
  – supplementation  567
Treg cells  380
tuberculosis  331
  – cutaneous  422
  – immune response  247
  – inflammatory process  333
  – seasonal variations  322
  – sunlight  331
tuberculous meningitis  332
tumor  426
  – colon  399
  – multiple primary –s  424
  – suppressor  450

U
UC  213
UL  162
ulcerative colitis – See: UC
unhealthy children  89
unwanted systemic effects  373
upper gastrointestinal cancers  467
up-regulation
  – of genes  448
  – of p27 proteins  398
uremic patients  508
UVB
  – exposure  15, 120
  – exposure of face only  639
  – index for men  210
  – radiation  183, 322
  – risk of diabetes type 1  530
  – treatment  260
UV light  16
  – wavelength  227, 233

V
vaccination
  – BCG  330
  – reduced efficacy  231
  – tetanus toxoid  490
valproate  594
vascular
  – calcification  182, 197, 202
  – endothelial growth factor – See: VEGF
  – function  552
  – inflammation  551
  – tissue  548
vBMD  44
VDBP  194, 200, 396, 670
  – during pregnancy  671
VDD  650
  – definition  549
  – predisposition  557
  – prevalence  40, 550
  – risk groups  40
  – secondary  652
  – vulnerable subgroups  54
VDR  71, 99, 100, 139, 180, 200, 261, 298,
  356, 372, 374, 397, 423, 444, 454,
  468, 477, 503, 510, 570, 611, 672, 692
  – agonists  573
  – alkylating derivative  401
  – deficient mice  125, 139
  – immuno-staining  286
  – muscle cells  106
  – polymorphism  247, 324, 332, 361, 468,
    531, 534, 676
  – polymorphism and muscle function  105
  – skin cancer  424
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>tuberculosis patients</td>
<td>324</td>
</tr>
<tr>
<td>vegetarian diet</td>
<td>330</td>
</tr>
<tr>
<td>VEGF</td>
<td>332</td>
</tr>
<tr>
<td>veils</td>
<td>651</td>
</tr>
<tr>
<td>Viosterol</td>
<td>16</td>
</tr>
<tr>
<td>viral infections</td>
<td>287, 422</td>
</tr>
<tr>
<td>vitamin D binding protein</td>
<td>See: VDBP</td>
</tr>
<tr>
<td>vitamin D receptor</td>
<td>See: VDR</td>
</tr>
<tr>
<td>vitiligo</td>
<td>429</td>
</tr>
<tr>
<td>volumetric bone mineral density</td>
<td>See: vBMD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheezing</td>
<td>288</td>
</tr>
<tr>
<td>window-glass</td>
<td>20</td>
</tr>
<tr>
<td>winter</td>
<td>656</td>
</tr>
<tr>
<td>wintertime hypovitaminosis D</td>
<td>24</td>
</tr>
<tr>
<td>women of child-bearing age</td>
<td>608</td>
</tr>
</tbody>
</table>
About the editor

Ronald Ross Watson, PhD, attended the University of Idaho but graduated from Brigham Young University in Provo, Utah, with a degree in chemistry in 1966. He earned his PhD in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained 2 years of postdoctoral research experience in immunology and nutrition.

From 1973 to 1974 Dr. Watson was assistant professor of immunology and performed research at the University of Mississippi Medical Center in Jackson. He was assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982 Dr. Watson joined the faculty at the University of Arizona Health Sciences Center in the Department of Family and Community Medicine of the School of Medicine. He is currently professor of health promotion sciences in the Mel and Enid Zuckerman Arizona College of Public Health.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and alcoholism research societies. Among his patents he has one on a dietary supplement; passion fruit peel extract, with more pending. He continues to do research in animals and in clinical trials on dietary supplements and health. For 30 years he was funded by the Wallace Research Foundation to study dietary supplements in health promotion. Dr. Watson has edited more than 100 books on nutrition, dietary supplements and over-the-counter agents, and drugs of abuse as scientific reference books. He has published more than 500 research and review articles.

Acknowledgments

The work of Dr. Watson’s editorial assistant, Bethany L. Stevens and Wageningen’s project manager, Mike Jacobs in communicating with authors and working on the manuscripts was critical to the successful completion of the book. It is very much appreciated. Support for Ms. Stevens’ and Dr. Watson’s work was graciously provided by the Natural Health Research Institute (www.naturalhealthresearch.org). It is an independent, non-profit organization that supports science-based research on natural health and wellness, and which is committed to informing about scientific evidence on the usefulness and cost-effectiveness of diet, supplements and a healthy lifestyle to improve health and wellness, and reduce disease. Finally the work of librarians of the Arizona Health Science Library, Mari Stoddard and Nguyen Nga Thi, was vital and very helpful in identifying key researchers who participated in the book.