REVIEW

Vitamin D Insufficiency

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Vitamin D deficiency, which classically manifests as bone disease (either rickets or osteomalacia), is characterized by impaired bone mineralization. More recently, the term *vitamin D insufficiency* has been used to describe low levels of serum 25-hydroxyvitamin D that may be associated with other disease outcomes. Reliance on a single cutoff value to define vitamin D deficiency or insufficiency is problematic because of the wide individual variability of the functional effects of vitamin D and interaction with calcium intakes. In adults, vitamin D supplementation reduces the risk of fractures and falls. The evidence for other purported beneficial effects of vitamin D is primarily based on observational studies. We selected studies with the strongest level of evidence for clinical decision making related to vitamin D and health outcomes from our personal libraries of the vitamin D literature and from a search of the PubMed database using the term *vitamin D* in combination with the following terms related to the potential nonskeletal benefits of vitamin D: *mortality*, *cardiovascular*, *diabetes mellitus*, *cancer*, *multiple sclerosis*, *allergy*, *asthma*, *infection*, *depression*, *psychiatric*, and *pain*. Conclusive demonstration of these benefits awaits the outcome of controlled clinical trials.

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BMD = bone mineral density; CI = confidence interval; 1,25(OH)$_2$D = 1,25-dihydroxyvitamin D; HR = hazard ratio; HRT = hormone replacement therapy; NHANES = National Health and Nutrition Examination Survey; 25(OH)D = 25-hydroxyvitamin D; OR = odds ratio; PTH = parathyroid hormone; RCT = randomized controlled trial; RR = relative risk; WHI = Women's Health Initiative

The past decade has seen renewed interest in the sunshine vitamin, vitamin D, because new data suggest that its benefits extend beyond healthy bones. Accompanying this renewed interest has been a proliferation of published studies related to the effects of vitamin D in many varying clinical conditions. This article discusses the definition of vitamin D insufficiency, identifies the sources of variation in vitamin D status, reviews the evidence for the clinical benefits of vitamin D, and recognizes indications for vitamin D testing.

Representative studies were selected to highlight some of the limitations of current knowledge related to vitamin D insufficiency and the clinical benefits of vitamin D. We selected studies with the strongest level of evidence for clinical decision making related to vitamin D and health outcomes from our personal libraries of the vitamin D literature and from a search of the PubMed database using the term *vitamin D* in combination with the following terms related to the potential nonskeletal benefits of vitamin D: *mortality*, *cardiovascular*, *diabetes mellitus*, *cancer*, *multiple sclerosis*, *allergy*, *asthma*, *infection*, *depression*, *psychiatric*, and *pain*. The level of evidence was assessed with the following hierarchy: meta-analyses of randomized controlled trials (RCTs), RCTs, nonrandomized intervention studies, meta-analyses of observational studies (cohort and case-control studies), and observational studies.[1]

The road to the discovery of vitamin D began with recognition of the childhood bone disease of rickets. The first formal medical treatise on rickets was published by Francis Glisson in 1650, when it was identified as a new disease that was more frequent in the rich than in the poor. During the industrial revolution of the 1800s, the prevalence of rickets increased dramatically, ranging from 40% to 60% among children in crowded and polluted urban areas. In 1822, Sniadecki was the first to recognize and report the association of rickets with a lack of sunlight exposure. By the mid-1800s, cod liver oil had been established as an effective treatment for rickets. The work of Mellanby and McCollum led to the discovery of vitamin D as the agent in cod liver oil that had antirachitic properties. This discovery eventually led to the fortification of milk and other foods with vitamin D in the 1930s, and as a result rickets all but disappeared in North America and Europe.

VITAMIN D METABOLISM

The terminology related to the biochemistry of vitamin D can be confusing. Vitamin D has 2 forms and several metabolites. The 2 forms are vitamin D_2 and vitamin D_3, also called *ergocalciferol* and *cholecalciferol*, respectively. Vitamin D_3 is produced in the skin in response to ultraviolet B radiation from sunlight or can be obtained from the diet (ie, animal sources such as deep sea fatty fish, egg yolks, or liver) or from supplements. Few foods naturally have substantial vitamin D content, and dietary vitamin D is obtained primarily through fortified foods or supplements. Vitamin D_2, which is found in some plants in the diet and is produced commercially by irradiation of yeast, is used for fortification and supplementation. Both vitamin D_2 and vitamin D_3 can be used for supplementation.

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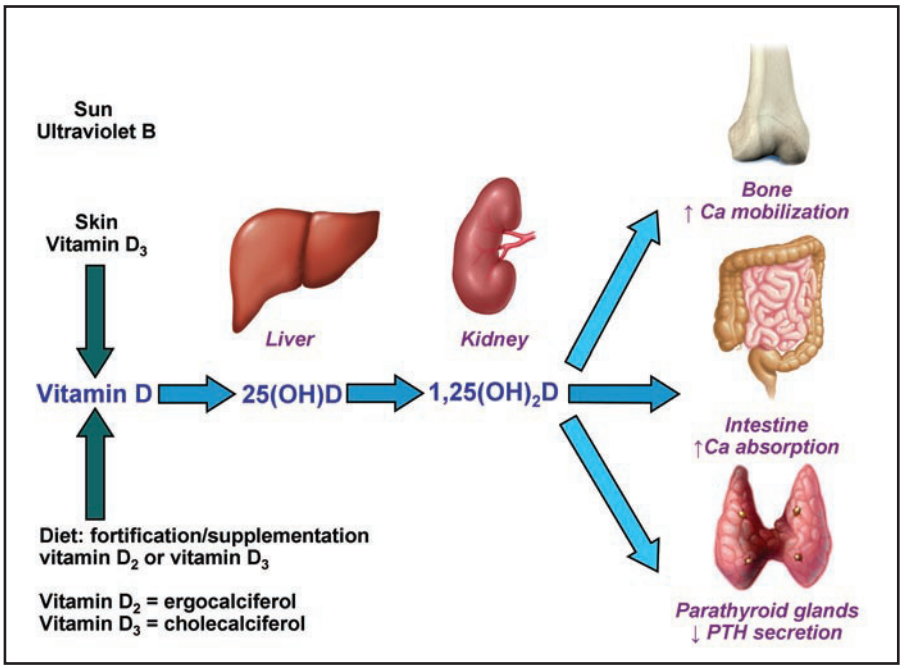

FIGURE. Vitamin D metabolism. Ca = calcium; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D; 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone.

Both forms of vitamin D undergo identical metabolism (Figure). Some evidence indicates that vitamin D_2 may be metabolized more rapidly than vitamin D_3,[2,3] but with regular daily intake they can be considered bioequivalent.[4,5] Both forms of vitamin D are converted to 25-hydroxyvitamin [25(OH)D] in the liver, and the serum level of 25(OH)D is measured to determine the adequacy of vitamin D status. In the kidney, 25(OH)D is hydroxylated to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], which is the only biologically active form of vitamin D. Acting principally on the duodenum, $1,25(OH)_2D$ increases calcium absorption. It also acts on bone cells, both osteoblasts and osteoclasts, to mobilize calcium.

The characteristics of $1,25(OH)_2D$ are those of a hormone, and consequently vitamin D is a prohormone rather than a true vitamin. The structure of $1,25(OH)_2D$ is similar to that of other steroid hormones. As long as sunlight exposure is adequate, $1,25(OH)_2D$ can be produced by the body without the requirement for ingestion in the diet. Like other hormones, $1,25(OH)_2D$ circulates at picogram concentrations that are 1000 times less than those of the precursor 25(OH)D. Based on the need for increased calcium absorption, the synthesis of $1,25(OH)_2D$ is tightly regulated and stimulated primarily by serum parathyroid hormone (PTH), as well as low serum calcium or phosphorus levels, and inhibited by circulating FGF23 produced by osteocytes.[6] Although produced in the kidney, $1,25(OH)_2D$ acts at a distance in the intestinal cell to increase calcium absorption or in the bone to stimulate differentiation and activation of osteoblasts and osteoclasts.[7]

ASSESSING VITAMIN D STATUS

Determination of vitamin D status is not based on measurement of serum $1,25(OH)_2D$ concentrations. Vitamin D status is assessed by measuring the prohormone 25(OH)D, which is an indicator of supply rather than function. The most stable and plentiful metabolite of vitamin D in human serum, 25(OH)D has a half-life of about 3 weeks, making it the most suitable indicator of vitamin D status. In the past, vitamin D deficiency was identified by the presence of bone disease, either rickets or osteomalacia. Bone disease caused by vitamin D deficiency is associated with serum 25(OH)D values below 10 ng/mL (to convert to nmol/L, multiply by 2.496). More recently, the term *vitamin D insufficiency* has been used to describe suboptimal levels of serum 25(OH)D that may be associated with other disease outcomes. Precisely defining vitamin D deficiency or insufficiency on the basis of 25(OH)D values is still a matter of much debate. A useful but rather simplistic classification of vitamin D status is shown in the Table. A cutoff value of 30 ng/mL is sometimes used for optimal vitamin status. On the basis of measured concentrations of 25(OH)D, many patients are given a diagnosis of vitamin

TABLE. **Classification of Vitamin D Status by 25(OH)D Concentration[a,b]**

25(OH)D concentration	Classification
≤10 ng/mL	Deficient
11-20 ng/mL	Insufficient
>20 ng/mL	Optimal

[a] 25(OH)D = 25-hydroxyvitamin D.
[b] To convert from ng/mL to nmol/L, multiply by 2.496.

D deficiency or insufficiency when most have no evidence of disease.

As discussed in detail in recent reviews,[8,9] investigators have considered various functional measures to assess the adequacy of vitamin D status. One functional definition of optimal vitamin D status is the 25(OH)D level that maximally suppresses PTH secretion, because the major stimulus for PTH secretion is a low level of serum ionized calcium. In adults, multiple cross-sectional examinations of the relationship between serum PTH and 25(OH)D levels demonstrate a plateau in suppression of PTH when the 25(OH)D level reaches approximately 30 ng/mL.[10] This is the rationale for selecting 30 ng/mL as the cutoff value for defining optimal vitamin D status. However, this definition represents an average value at a population level but does not account for the wide variation in the 25(OH)D level that represents adequacy at an individual level. Many patients have very low 25(OH)D values without evidence of increased production of PTH, and conversely, 25(OH)D levels greater than 30 ng/mL do not guarantee PTH suppression.[10] Another limitation of this definition is that, in children, an elevated PTH level does not indicate inadequate vitamin D status and has been associated with increased calcium absorption.[11] In puberty, the PTH concentration increases, which may stimulate increased periosteal bone formation and increased bone accrual. In fact, preliminary evidence suggests that, with adequate calcium intake, a high-normal PTH level and low-normal 25(OH)D level may result in greater bone size and mass during puberty.[12]

Another method used in some research studies for defining optimal vitamin D status is the 25(OH)D level at which there is no incremental increase in $1,25(OH)_2D$ levels after administration of vitamin D, because the level of $1,25(OH)_2D$ is adequate to meet demand.[13-15] Similar to the findings related to PTH in adults, an incremental increase in the level of $1,25(OH)_2D$ was observed after administration of vitamin D in children when values of 25(OH)D were less than 25 to 30 ng/mL.[5] In situations of very low calcium intakes, some evidence suggests that the demand for $1,25(OH)_2D$ may be greater.[14,16,17] Thus, vitamin D requirements may vary based on customary calcium intake.

Another functional measure of vitamin D status is the 25(OH)D level that results in maximal intestinal calcium absorption. By combining the results of 3 studies in adults, Heaney[18] concluded that optimal calcium absorption occurred at 25(OH)D levels of 32 ng/mL or greater. In contrast, another study found no association between 25(OH)D levels and calcium absorption in healthy women.[19] Fractional calcium absorption was high (>50%) in Nigerian children with presumed dietary calcium deficiency rickets and low dietary calcium intakes despite low normal serum 25(OH)D concentrations.[5,20] After vitamin D administration and a marked increase in 25(OH)D and $1,25(OH)_2D$ concentrations, fractional calcium absorption did not increase any further.[5] In these studies in children, fractional calcium absorption was not related to serum $1,25(OH)_2D$ levels either before or after vitamin D administration. In a study of adults attending an osteoporosis clinic, concentrations of $1,25(OH)_2D$ and intestinal calcium absorption did not appear to decline until 25(OH)D concentrations fell to 4 ng/mL or less, a level that is generally considered to be indicative of severe vitamin D deficiency.[21]

More recently, the criterion for optimal vitamin D status has moved away from being defined as the 25(OH)D concentration needed to achieve skeletal health to that which demonstrates optimal benefits on nonskeletal health outcomes. The evidence related to these outcomes will be considered later in this review.

SOURCES OF VARIATION IN VITAMIN D STATUS

Factors known to influence 25(OH)D levels include race, vitamin D intake, sun exposure, adiposity, age, and physical activity. Even when all the factors known to influence 25(OH)D concentrations are taken into account, most of the individual variation of 25(OH)D values is difficult to explain. Consequently, it is difficult to assess the risk of clinical or biochemical consequences of vitamin D insufficiency in a patient on the basis of concentrations of 25(OH)D alone. The duration of vitamin D insufficiency, the responsiveness of the vitamin D receptor, dietary calcium intake, and individual calcium requirements likely modify the clinical consequences of vitamin D deficiency or insufficiency based on levels of 25(OH)D.

A single exposure to summer sun in a bathing suit for 20 minutes produces the equivalent of 15,000 to 20,000 IU of vitamin D_3. In a study of Hawaiian surfers with sun exposure of at least 15 hours per week for the preceding 3 months, 25(OH)D levels ranged from 11 up to 71 ng/mL, demonstrating wide individual variation.[22] Outdoor sun exposure and time spent outdoors are better predictors of serum 25(OH)D values than dietary vitamin D intake.[23]

The 25(OH)D level achieved with the same oral dose of vitamin D varies widely by individual.[23,24] The level of 25(OH)D that results in clinical consequences probably varies with calcium intake, race, age, body fat, and individual genetic factors, all of which may influence calcium homeostasis. Genetic variation represented by polymorphisms of certain genes in the vitamin D metabolic pathway explains some of the interindividual variability of 25(OH)D concentrations, particularly polymorphisms of the enzyme 7-dehydrocholesterol reductase in the skin, cytochrome P450 25-hydroxylase in the liver, and vitamin D–binding protein in the circulation.[25] The functional effect of a particular level of 25(OH)D depends on the uptake of 25(OH)D by target cells and the efficiency of 1α-hydroxylation to produce $1,25(OH)_2D$.

MEASUREMENT OF 25(OH)D LEVELS

Some controversy exists regarding the best method for measuring 25(OH)D levels. Radioimmunoassay has been the most common method reported in the literature and was the method used in some of the large-scale population studies of vitamin D, such as the National Health and Nutrition Examination Survey (NHANES) and the Women's Health Initiative (WHI).

The accuracy of measurement varies widely between individual laboratories and between different assay methods. In one study, identical serum samples were provided to 6 different laboratories, and the chemiluminescent assay tended to return higher values for 25(OH)D.[26] Competitive protein-binding assays are also known to generally yield higher 25(OH)D values. When serum samples were spiked with an additional 20 ng/mL of 25(OH)D, the increment in 25(OH)D level was less than 20 ng/mL in all the laboratories, except the one using high-performance liquid chromatography. Antibodies used in some radioimmunoassays do not detect both $25(OH)D_2$ and $25(OH)D_3$. The use of a standard cutoff value for adequate vitamin D status is problematic if applied to all laboratories and all methods. A single serum sample could be assessed as showing adequate vitamin D status in one laboratory and an insufficient level in another, with differences of up to 17 ng/mL.[26,27]

More recently, large medical laboratories have begun using liquid chromatography–tandem mass spectrometry, which identifies the 25-hydroxylated forms of both vitamin D_2 and D_3.[28] The total 25(OH)D, which is the sum of $25(OH)D_2$ and $25(OH)D_3$, is used to evaluate vitamin D status. Since 2003, there has been more than a 15-fold increase in the volume of 25(OH)D measurements at Mayo Clinic in Rochester, MN (Singh R., personal communication), reflecting the increasing attention clinicians are giving to vitamin D status.

CLINICAL MANIFESTATIONS OF VITAMIN D DEFICIENCY

Nutritional Rickets

The classical manifestation of vitamin D deficiency is nutritional rickets, which results from inadequate mineralization of growing bone. Consequently, rickets is a disease of children. Far from being eradicated, nutritional rickets continues to occur throughout the world, with reports from at least 60 countries in the past 20 years.[29] In a review of published cases of rickets in the United States, most occurred in children younger than 30 months.[30] The vast majority of cases in the United States occurred in African American infants who were fed with breast milk rather than formula. Florid rickets manifests with leg deformities; enlargement of the growth plates of the wrists, ankles, and costochondral junctions; and rib cage deformities. Subtle symptoms that should raise the clinical suspicion of rickets in children include bone pain in the legs, delayed age of standing or walking, frequent falling, and delayed growth. Hypocalcemic seizures in the first year of life may be the initial manifestation of rickets.

Radiography of the long bones at the knees and the wrists is necessary to confirm the diagnosis of rickets. Radiography demonstrates impaired mineralization of the growth plates, evident by widening of the growth plate and fraying of the margin of the metaphyses.[31] Biochemical features most consistently include hypophosphatemia and an elevated alkaline phosphatase level. As a result of vitamin D deficiency, serum concentrations of 25(OH)D are very low in patients with rickets, usually less than 5 ng/mL. However, concentrations of 25(OH)D may not be markedly reduced if rickets results from calcium deficiency or if the child has recently received vitamin D or sun exposure. In some tropical countries, where sun exposure is plentiful, calcium deficiency is more important than vitamin D deficiency as a cause of rickets.[32,33] However, even in the United States, only 22% of children with nutritional rickets had deficient levels of 25(OH)D, indicating that calcium deficiency as a cause of rickets needs to be considered domestically as well.[34]

Osteomalacia

Osteomalacia refers to the failure of organic osteoid formed by osteoblasts to become mineralized with calcium and phosphorus. Although histological osteomalacia is characteristic of rickets, the term *osteomalacia* is generally used to describe the bone disease caused by vitamin D deficiency in adults, who no longer have growing bones. The clinical manifestations of these 2 conditions are different.

Bone pain is a characteristic feature of osteomalacia, and it can be confused with arthritis or fibromyalgia. Bone pain

due to osteomalacia primarily affects the bones between the joints, whereas arthritis usually causes predominantly joint pain, and fibromyalgia causes more diffuse muscle and soft tissue pain; however, it can be difficult to distinguish between these disorders. Proximal muscle weakness and gait instability are often present. Because the growth plates have closed in adults, the radiographic features differ from those typical of rickets. Radiography may reveal pseudofractures of the pelvis, femurs, metatarsals, or lateral margins of the scapulae. The biochemical features of osteomalacia are similar to those of rickets, with increased serum alkaline phosphatase and PTH values, and low calcium, phosphorus, and 25(OH)D values in most cases. A review of all the archived cases of bone biopsy–proven osteomalacia seen by the Bone Histomorphometry Laboratory at Mayo Clinic concluded that radiographic examination as well as serum calcium, phosphorus, and alkaline phosphatase assays are adequate screening tests in patients who have a clinical presentation suggestive of osteomalacia, but that 25(OH)D values may be normal.[35]

In a cross-sectional study of iliac bone biopsy specimens obtained at autopsy, an excess accumulation of osteoid, which corresponds with histological osteomalacia, was found only in patients with 25(OH)D values less than 25 ng/mL.[36] However, even patients with very low values of 25(OH)D did not consistently have evidence of osteomalacia.

POTENTIAL BENEFITS OF VITAMIN D

Apart from the deficiency diseases of rickets and osteomalacia, recent evidence suggests other skeletal and nonskeletal benefits of vitamin D. In evaluating the evidence, it is important to recognize the limitations inherent in the study design and methodology. Important issues that apply to vitamin D research include the following:

(1) Was the study design observational, which can only demonstrate associations and is subject to confounding, or was it an RCT that generally balances unmeasured confounding variables?

(2) How was the intake of vitamin D measured? Was the serum 25(OH)D value considered a proxy measure of vitamin D intake?

(3) What outcome was measured to assess the benefit of vitamin D? Was it the achieved 25(OH)D level or a specific clinical outcome that matters to the patient? Was assessment of the outcome the primary aim of the study?

(4) Is 25(OH)D the most appropriate biomarker of vitamin D status in all situations?

In the following section, representative studies of the available evidence related to the skeletal and nonskeletal effects of vitamin D are reviewed.

Skeletal Benefits

Bone Density. In addition to the treatment and prevention of vitamin D–deficiency rickets in children,[37] vitamin D has been associated with other beneficial skeletal effects. A retrospective cohort study of pubertal girls demonstrated increased bone mineral density (BMD) of the femoral neck, but not of the spine or radius, among those who received supplemental vitamin D in infancy.[38] Evidence of a positive association between BMD and serum 25(OH)D concentrations in adolescents is fair, but the evidence for a positive association in infants is inconsistent.[39] Serum 25(OH)D concentration was related to hip BMD in community-dwelling women and men aged at least 20 years who participated in the US NHANES III survey.[40] Higher calcium intake was significantly associated with higher BMD only for women with 25(OH)D values less than 20 ng/mL. One of the limitations of a cross-sectional study like the NHANES survey is that it can demonstrate only associations, not cause-and-effect relationships. Another confounding factor may be associated with low vitamin D intake and low bone density. For example, healthier people who exercise more outside in the sun may have greater bone density because of their exercise and higher 25(OH)D levels because of sun exposure. The WHI calcium and vitamin D supplementation trial showed that hip bone density was 1.06% higher in women receiving calcium and vitamin D supplementation vs placebo at 9 years, but that their lumbar spine and total body bone density did not differ significantly from those receiving placebo during this interval.[41]

Fractures and Falls. On the basis of RCTs, the strongest evidence for the benefit of vitamin D relates to the prevention of fractures and falls. In a meta-analysis of 12 RCTs, a reduced nonvertebral fracture risk was demonstrated only for doses of vitamin D greater than 400 IU/d (relative risk [RR], 0.80; 95% confidence level [CI], 0.72-0.89).[42] Similarly, a meta-analysis of 8 RCTs demonstrated that vitamin D reduced the risk of falls (RR, 0.78; 95% CI, 0.64-0.94), but only if the dose was 700 IU/d or greater and the 25(OH)D concentration was at least 25 ng/mL.[43] The benefit of vitamin D could have been limited to those with unrecognized osteomalacia, which is associated with proximal muscle weakness and gait instability. These high-quality studies provide clear evidence that a minimum dose of 800 IU/d of vitamin D will reduce the risk of falls and fractures in older adults. However, a recent RCT of a 500,000 IU annual dose of vitamin D in women of advanced age increased the median 25(OH)D concentration from 20 ng/mL to 48 ng/mL one month later but resulted in an increased risk of falls and fractures in the group receiving this regimen.[44]

Nonskeletal Benefits

Interest in the nonskeletal effects of vitamin D has been increasing since the discovery of vitamin D receptors and the 1α-hydroxylase enzyme in multiple tissues, including cells of the pancreas, immune system, macrophages, vascular endothelium, stomach, epidermis, colon, and placenta.[6] In these tissues, 25(OH)D can be converted to $1,25(OH)_2D$ locally, without altering serum $1,25(OH)_2D$ concentrations. Through these paracrine effects, $1,25(OH)_2D$ influences the expression of genes in local tissues. However, the evidence for the nonskeletal benefits of vitamin D is not as strong as the evidence for the skeletal effects.

Lower Mortality Rate. In a prospective observational study of adults older than 65 years participating in NHANES III, the risk of death was 45% lower in those with 25(OH)D values greater than 40 ng/mL compared with those with values less than 10 ng/mL (hazard ratio [HR], 0.55; 95% CI, 0.34-0.88).[45] However, this may simply reflect the fact that people with underlying illness or immobility (who are more likely to die) tend to have lower 25(OH)D levels, in part as a result of having spent less time outdoors or of having less adequate nutrition. Because vitamin D is sequestered in adipose tissue, obesity is also associated with lower 25(OH)D levels. However, observational studies cannot prove whether low 25(OH)D status is the cause of greater mortality or just a marker of other underlying risk factors.

In contrast, a meta-analysis of 18 RCTs of vitamin D supplementation in postmenopausal women of advanced age, with dosages ranging from 300 to 2000 IU/d, reported a 7% lower risk of death in those receiving a vitamin D supplement (RR, 0.93; 95% CI, 0.87-0.99).[46] This highlights the difference often found between RCTs and observational studies. The effect sizes found in observational studies are often attenuated or absent in RCTs.[47] The situation with vitamin D is analogous to that of hormone replacement therapy (HRT) in postmenopausal women. The beneficial effects of HRT were demonstrated for multiple health outcomes in observational studies,[48-50] but the WHI RCT in older postmenopausal women failed to confirm the beneficial effects of HRT on dementia and cardiovascular disease.[51,52] In the observational trials, healthier women were more likely to use estrogen replacement and had fewer adverse health outcomes, indicating a "healthy user" bias. Only an RCT definitively demonstrated that the risks of first-time use of HRT outweighed the benefits in women older than 60 years.

Despite the slight reduction in mortality associated with vitamin D supplementation, the primary aim of the RCTs included in the meta-analysis was not to assess mortality. Not all trials of vitamin D reported mortality outcomes, so those trials could not be included in the meta-analysis. Trials that showed a mortality effect would be more likely to report this outcome, leading to a high likelihood of reporting bias that could render the slight mortality reduction statistically insignificant.

Lower Cardiovascular Mortality. The reduced mortality in the aforementioned observational study mirrored in large part the reduced cardiovascular mortality in those with 25(OH)D values greater than 40 ng/mL compared with those with values less than 10 ng/mL (HR, 0.42; 95% CI, 0.21-0.85).[45] In another observational cohort study, patients who had angiography and 25(OH)D measurements were followed up for 8 years. Those from the highest 25(OH)D quartile (median, 28 ng/mL) had a lower mortality (HR, 0.45; 95% CI, 0.32-0.64) than those from the lowest quartile (median, 8 ng/mL).[53] Although these observational studies do not demonstrate that low 25(OH)D values accelerate cardiovascular mortality, low 25(OH)D concentrations were associated with serum markers of inflammation that are indicators of cardiac risk.

Recently, concern has been expressed that vitamin D could potentially accelerate vascular disease. In a study of African Americans with type 2 diabetes mellitus, 25(OH)D levels correlated with increased calcified plaque in the aorta and carotids, but not in the coronary arteries.[54] Vascular disease associated with chronic kidney disease, especially that associated with very low bone turnover, may also be accelerated with supplementation with standard doses of vitamin D. Furthermore, concern has been raised recently that other disorders characterized by vascular inflammation, such as diabetes mellitus, rheumatoid arthritis, or systemic lupus erythematosus, may not benefit from standard recommended doses of vitamin D supplementation.

Vitamin D may affect other cardiovascular and metabolic disease risks. In an observational study of adolescents in NHANES III, those with the lowest 25(OH)D values (<15 ng/mL) had more than a 2-fold greater odds of having an elevated blood pressure compared with the group of adolescents with higher 25(OH)D levels (>26 ng/mL) (odds ratio [OR], 2.4; 95% CI, 1.3-4.2).[55]

The NHANES III data in adults indicated that those with 25(OH)D levels of less than 21 ng/mL had an increased risk of hypertension, diabetes, obesity, and high triglyceride levels—all metabolic manifestations associated with increased cardiovascular mortality.[56] Although obesity is associated with lower serum 25(OH)D levels because of the sequestration of vitamin D in adipose tissue, it is likely not the consequence of low 25(OH)D levels. Additionally, the 25(OH)D level may be a marker of other factors associated with obesity, such as physical inactivity and reduced outdoor sun exposure.

Reduced Risk of Diabetes Mellitus. A meta-analysis of 5 observational studies of vitamin D supplementation

in childhood reported a nearly 30% reduction in the risk of type 1 diabetes in children who had ever received vitamin D supplements (OR, 0.71; 95% CI, 0.60-0.84).[57] Unfortunately, most studies had no information about vitamin D dosage or adherence. Because these were observational studies, and vitamin D was not randomly assigned to children, it is possible that characteristics of families who provided supplemental vitamin D to their children contributed to the decreased risk of type 1 diabetes in children receiving supplements.

Vitamin D receptors are present in pancreatic β cells, and vitamin D may augment insulin secretion and insulin sensitivity. Adolescents in NHANES III with serum 25(OH)D levels of less than 15 ng/mL were more likely to have elevated blood glucose levels than those with the highest 25(OH)D values (>26 ng/mL) (OR, 2.5; 95% CI, 1.0-6.4).[55] The observational Nurses Health Study found that vitamin D supplementation and calcium supplementation were both associated with a reduction in risk of type 2 diabetes.[58] Current data related to vitamin D and the risk of type 2 diabetes are limited by inadequate adjustment for confounding variables, post hoc analyses, and inability to identify the separate effects of calcium and vitamin D.[59] Because milk is the major source of both vitamin D and calcium in the diet, it is difficult to identify the independent effects of dietary calcium and vitamin D on the basis of intake or 25(OH)D levels. Skim milk intake is also inversely associated with obesity, which could account for an association between the intake of dietary calcium and vitamin D and a reduced risk of type 2 diabetes.

Reduced Risk of Cancer. Vitamin D is known to promote cellular differentiation, inhibit cellular proliferation, and reduce the growth of certain tumors in laboratory animals. A meta-analysis of case-control studies of those with and without colon cancer found that, for each 20 ng/mL increase in serum 25(OH)D levels, the odds of colon cancer were reduced by more than 40% (OR, 0.57; 95% CI, 0.43-0.76).[60] Other studies have shown that dietary calcium intake is also associated with reduced colon cancer risk and adenoma formation.[61,62] Because milk intake is a major determinant of serum 25(OH)D levels, it is difficult to separate the effect of vitamin D from that of calcium intake.

In the case of colon cancer, one large RCT was performed to evaluate the effect of combined supplementation with calcium and vitamin D on the risk of colon cancer. In the WHI trial, supplementation with calcium and vitamin D had no significant effect on the risk of colorectal cancer during 8 years of follow-up.[63] Several limitations of this study may have contributed to this lack of effect. Colorectal cancer is a long latency disease, and 8 years may not have been sufficient time to observe the effect of calcium and vitamin D. Another criticism is that the relatively low dose of 400 IU of vitamin D may have not been protective or sufficient to increase serum 25(OH)D levels adequately. Concentrations of 25(OH)D were measured at baseline but not during follow-up. Declining adherence over time would have further reduced the effective doses of calcium and vitamin D.

Breast cancer has also been associated with vitamin D insufficiency. A meta-analysis combining 7 observational studies reported a lower risk of breast cancer among women in the highest compared with the lowest quartile of 25(OH)D values (OR, 0.55; 95% CI, 0.38-0.80).[64] As with colon cancer, calcium intake was also associated with a reduced risk of breast cancer. Because obesity is associated with an increased risk of breast cancer and low 25(OH)D levels, it is a confounding factor in the association between breast cancer risk and vitamin D.

As with colon cancer, the WHI RCT of a combined regimen of calcium and vitamin D showed no benefit of supplementation on the risk of breast cancer, again highlighting the different conclusions of observational studies and RCTs.[65] The limitations of the breast cancer study are similar to those of the study focused on colon cancer. This study demonstrated the potential confounding effects of physical activity and obesity. Baseline 25(OH)D levels were greater among women with lower body mass index and more recreational physical activity. When controlling for body mass index and physical activity, serum 25(OH)D concentration was not associated with breast cancer risk.

In a meta-analysis of 11 observational studies, prostate cancer was not associated with serum 25(OH)D levels.[66] The evidence regarding an association between pancreatic cancer and 25(OH)D levels is conflicting.[67] A multinational cohort study found no protective association between greater 25(OH)D values and gastric, esophageal, endometrial, ovarian, kidney, non-Hodgkin lymphoma, and pancreatic cancers.[68] Drake et al[69] recently showed that event-free survival and overall survival were reduced in vitamin D–insufficient patients newly diagnosed as having diffuse large B-cell lymphoma and T-cell lymphoma during 34.8 months of follow-up.

To date, studies have not shown impressive effects of vitamin D treatment on malignancies.[70]

Reduced Risk of Multiple Sclerosis. The incidence of multiple sclerosis increases with increasing latitude, corresponding with reduced ultraviolet B sun exposure and lower serum levels of 25(OH)D. A case-control study demonstrated that the odds of having multiple sclerosis were lower in the group with the highest 25(OH)D levels.[71] However, the association was found only in white patients [OR, 0.59; 95% CI, 0.36-0.97 for a 20 ng/mL increase in 25(OH)D], not in African American patients.

It is difficult to exclude the possibility that other confounding exposures associated with increasing latitude and greater indoor activity during winter months contribute to the risk of multiple sclerosis. Little evidence supports a therapeutic role for vitamin D in the treatment of multiple sclerosis.[72]

Reduced Risk of Allergy and Asthma. Several lines of evidence demonstrate the effects of vitamin D on proinflammatory cytokines, regulatory T cells, and immune responses, with conflicting interpretation of the effects of vitamin D on allergic diseases.[73,74] In a cross-sectional study of Costa Rican children, low 25(OH)D levels were associated with elevated IgE and eosinophil counts, as well as with increased asthma-related hospitalizations and use of anti-inflammatory medication.[75] However, an association does not prove causation, and alternative explanations can account for this association. For example, children with more severe asthma may spend more time indoors and have less sun exposure.

Low maternal vitamin D intake in pregnancy has been associated with an increased likelihood of childhood wheezing at ages 3 and 5 years.[76,77] In contrast, maternal 25(OH)D levels of greater than 30 ng/mL in pregnancy have been associated with childhood eczema at age 9 months and asthma at age 9 years.[78] Vitamin D supplementation in infancy has been associated with increased atopy and allergic rhinitis in adulthood.[79] Increasing 25(OH)D levels were associated with increasing risk of allergic rhinitis among adults in NHANES III.[80] The conflicting data indicate the need for RCTs to demonstrate the effect of vitamin D on the prevention and control of allergic diseases.

Reduced Risk of Infection. Vitamin D is required for the expression of cathelicidin by macrophages, which is involved in bacterial killing.[81] A meta-analysis of 7 observational studies noted a reduced risk of active tuberculosis in those with the highest vs the lowest values of 25(OH)D (OR, 0.68; 95% CI, 0.43-0.93).[82] However, an RCT in a West African population with baseline mean 25(OH)D values of 31 ng/mL showed no effect of 100,000 IU of supplemental vitamin D given at the beginning and at 3 and 8 months of tuberculosis treatment on the rate of sputum conversion or resolution of markers of clinical severity.[83] However, this dose of vitamin D may have been insufficient because the increase in 25(OH)D concentration during treatment did not differ between the supplement and placebo groups.

In observational data from NHANES III, persons with 25(OH)D values lower than 10 ng/mL were more likely to have had a recent upper respiratory tract infection than those with higher 25(OH)D values in all 4 seasons of the year.[84] This association was even stronger in those with asthma or chronic obstructive pulmonary disease. Whether this association is explained by the fact that people who remain indoors are more likely to catch colds remains unclear.

A case-control study reported that mean 25(OH)D values were lower in children with bronchiolitis or pneumonia admitted to the pediatric intensive care unit than in healthy control children or in children with pneumonia admitted to the general pediatric ward.[85]

Reduced Risk of Mental Illness. A cohort of Finnish children who received supplemental vitamin D in their first year of life had a lower risk of developing schizophrenia.[86] However, the significance of this association is unclear because it was unrelated to adherence to vitamin D supplementation, was only evident in males, and was not found with any other mental illness.

To examine the effect of vitamin D on depression, overweight and obese patients were randomized to receive 20,000 or 40,000 IU of vitamin D or placebo weekly for 1 year.[87] At baseline, those with 25(OH)D concentrations lower than 16 ng/mL had greater Beck Depression Inventory scores, indicating that they were more depressed, than those with higher 25(OH)D levels. The 2 groups receiving vitamin D supplementation had significant improvement in their scores, whereas the placebo group did not.

Less Musculoskeletal Pain. A small descriptive study reported that most patients (93%) with persistent musculoskeletal pain had 25(OH)D values of 20 ng/mL or less.[88] In one RCT, patients with diffuse musculoskeletal pain or osteoarthritis and 25(OH)D values lower than 20 ng/mL were randomized to receive vitamin D or placebo for 3 months.[89] Those given vitamin D had no improvement in their pain compared with baseline or compared with placebo-treated patients. In another double-blind RCT, primary care patients with 25(OH)D levels of 10 to 25 ng/mL were randomized to receive 50,000 IU of vitamin D or placebo weekly for 8 weeks.[90] The treated group showed significantly greater improvement in fibromyalgia assessment scores than the placebo group. Patients with 25(OH)D values lower than 10 ng/mL were treated in an unblinded fashion with 50,000 IU of vitamin D weekly for 8 weeks but had no symptom improvement.

Reduced Risk of Renal Disease. In a subgroup analysis of the NHANES III data set, low 25(OH)D values were associated with a greater risk of kidney failure in African American but not in white participants.[91] However, the opposite trend was observed in whites.

INDICATIONS FOR VITAMIN D TESTING

Measurement of serum 25(OH)D levels is indicated in select circumstances. If clinical symptoms of rickets in children or osteomalacia in adults are present, measurement of

25(OH)D levels will confirm vitamin D deficiency. Such testing would be appropriate in adults or children with bone pain, elevated serum alkaline phosphatase or PTH levels, and low serum calcium or phosphorus levels. Persons of advanced age, those with osteoporosis, or those at increased risk of falls or fractures may also benefit from measurement of 25(OH)D levels. However, one could argue that providing at-risk groups with routine supplementation of adequate doses of vitamin D may make testing for vitamin D insufficiency unnecessary. When to test and how to treat adults with vitamin D deficiency have recently been reviewed in this journal.[8] No evidence shows benefit for screening 25(OH)D levels in the asymptomatic population.

CONCLUSION

Critically evaluating the evidence regarding the purported benefit of vitamin D on a multitude of health outcomes is difficult. The bulk of current data is based on observational, epidemiological studies, which are useful for generating hypotheses but not for proving causality. It is particularly difficult to tease out the effects of confounding variables that relate both to health outcomes and to vitamin D status, such as physical activity, milk intake, and adiposity. Few of the observational associations have been confirmed by RCTs, and many of the interventional studies of vitamin D also included calcium supplementation. Future clinical trials, including a National Institutes of Health–funded 5-year 20,000-participant prospective RCT comparing the effect of supplementation with 2000 IU/d of vitamin D_3 or placebo, will help clarify the benefits and risks of vitamin D supplementation in many of the disorders discussed in this review.[92]

On the basis of the current data, it seems prudent for persons older than 60 years to take a vitamin D supplement of 800 to 2000 IU/d to reduce the risk of falls and fractures. These recommendations are consistent with the recently released report of the Institute of Medicine, which recommended that healthy adults take 600 IU/d to maintain skeletal health and also concluded that information about the health benefits beyond bone health could not be considered reliable.[93] Dark-skinned infants who are exclusively breast-fed are at greater risk of rickets and should receive 400 IU/d of supplemental vitamin D. Vitamin D supplementation in these ranges is likely to prevent bone loss, may improve bone density, may reduce fractures, and appears to reduce falls. Although vitamin D intoxication has been associated only with intakes of 50,000 to 1 million IU/d over the course of months or years,[7] the potential risks of kidney stones, vascular disease, and fractures with high-dose vitamin D regimens are unclear. Until more data from RCTs are available, a healthy dose of skepticism should be maintained regarding the other health claims for vitamin D.

REFERENCES

1. Harbour R, Miller J. A new system for grading recommendations in evidence based guidelines. *BMJ*. 2001;323(7308):334-336.
2. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab*. 2004;89(11):5387-5391.
3. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am J Clin Nutr*. 1998;68(4):854-858.
4. Holick MF, Biancuzzo RM, Chen TC, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab*. 2008;93(3):677-681.
5. Thacher TD, Obadofin MO, O'Brien KO, Abrams SA. The effect of vitamin D2 and vitamin D3 on intestinal calcium absorption in Nigerian children with rickets. *J Clin Endocrinol Metab*. 2009;94(9):3314-3321.
6. Plum LA, DeLuca HF. The functional metabolism and molecular biology of vitamin D action. In: Holick MF, ed. *Vitamin D: Physiology, Molecular Biology, and Clinical Applications*. 2nd ed. New York, NY: Humana Press; 2010:61-97.
7. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-281.
8. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc*. 2010;85(8):752-757.
9. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. *Am J Clin Nutr*. 2008;88(2):500S-506S.
10. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int*. 1997;7(5):439-443.
11. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO. Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. *J Clin Endocrinol Metab*. 2005;90(10):5576-5581.
12. Tylavsky FA, Ryder KM, Li R, et al. Preliminary findings: 25(OH)D levels and PTH are indicators of rapid bone accrual in pubertal children. *J Am Coll Nutr*. 2007;26(5):462-470.
13. Peacock M, Selby PL, Francis RM, Brown WB, Hordon L. Vitamin D deficiency, insufficiency, sufficiency and intoxication: What do they mean? In: Norman AW, Schaefer K, Grigoleit H-G, Herrath DV, eds. *Vitamin D: Chemical, Biochemical and Clinical Update*. Berlin, Germany: Walter de Gruyter; 1985:569-570.
14. Thacher TD, Fischer PR, Isichei CO, Pettifor JM. Early response to vitamin D2 in children with calcium deficiency rickets. *J Pediatr*. 2006;149(6):840-844.
15. Docio S, Riancho JA, Perez A, Olmos JM, Amado JA, Gonzalez-Macias J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies? *J Bone Miner Res*. 1998;13(4):544-548.
16. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. *JAMA*. 2005;294(18):2336-2341.
17. Thacher TD, Fischer PR, Obadofin MO, Levine MA, Singh RJ, Pettifor JM. Comparison of metabolism of vitamins D(2) and D(3) in children with nutritional rickets. *J Bone Miner Res*. 2010;25(9):1988-1995.
18. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. *Am J Clin Nutr*. 2004;80(6 suppl):1706S-1709S.
19. Aloia JF, Chen DG, Yeh JK, Chen H. Serum vitamin D metabolites and intestinal calcium absorption efficiency in women. *Am J Clin Nutr*. 2010;92(4):835-840.
20. Graff M, Thacher TD, Fischer PR, et al. Calcium absorption in Nigerian children with rickets. *Am J Clin Nutr*. 2004;80(5):1415-1421.
21. Need AG, O'Loughlin PD, Morris HA, Coates PS, Horowitz M, Nordin BE. Vitamin D metabolites and calcium absorption in severe vitamin D deficiency. *J Bone Miner Res*. 2008;23(11):1859-1863.
22. Hollis BW, Wagner CL, Drezner MK, Binkley NC. Circulating vitamin D3 and 25-hydroxyvitamin D in humans: an important tool to define adequate nutritional vitamin D status. *J Steroid Biochem Mol Biol*. 2007;103(3-5):631-634.

23. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr*. 2003;77(1):204-210.

24. Aloia JF, Patel M, Dimaano R, et al. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr*. 2008;87(6):1952-1958.

25. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet*. 2010;19(13):2739-2745.

26. Binkley N, Krueger D, Cowgill CS, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab*. 2004;89(7):3152-3157.

27. Granado-Lorencio F, Mosteiro JS, Herrero-Barbudo C, Navarro ED, Blanco-Navarro I, Perez-Sacristan B. 25-OH-vitamin D assay variation and subject management in clinical practice. *Clin Biochem*. 2010;43(4-5):531-533.

28. Singh RJ. Quantitation of 25-OH-vitamin D (25OHD) using liquid tandem mass spectrometry (LC-MS-MS). *Methods Mol Biol*. 2010;603:509-517.

29. Thacher TD, Fischer PR, Strand MA, Pettifor JM. Nutritional rickets around the world: causes and future directions. *Ann Trop Paediatr*. 2006;26(1):1-16.

30. Weisberg P, Scanlon KS, Li R, Cogswell ME. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. *Am J Clin Nutr*. 2004;80(6 suppl):1697S-1705S.

31. Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. Radiographic scoring method for the assessment of the severity of nutritional rickets. *J Trop Pediatr*. 2000;46(3):132-139.

32. Fischer PR, Rahman A, Cimma JP, et al. Nutritional rickets without vitamin D deficiency in Bangladesh. *J Trop Pediatr*. 1999;45(5):291-293.

33. Thacher TD, Fischer PR, Pettifor JM, et al. A comparison of calcium, vitamin D, or both for nutritional rickets in Nigerian children. *N Engl J Med*. 1999;341(8):563-568.

34. DeLucia MC, Mitnick ME, Carpenter TO. Nutritional rickets with normal circulating 25-hydroxyvitamin D: a call for reexamining the role of dietary calcium intake in North American infants. *J Clin Endocrinol Metab*. 2003;88(8):3539-3545.

35. Bingham CT, Fitzpatrick LA. Noninvasive testing in the diagnosis of osteomalacia. *Am J Med*. 1993;95(5):519-523.

36. Priemel M, von Domarus C, Klatte TO, et al. Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. *J Bone Miner Res*. 2010;25(2):305-312.

37. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008;122(5):1142-1152.

38. Zamora SA, Rizzoli R, Belli DC, Slosman DO, Bonjour JP. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. *J Clin Endocrinol Metab*. 1999;84(12):4541-4544.

39. Cranney A, Horsley T, O'Donnell S, et al. Effectiveness and safety of vitamin D in relation to bone health. Evidence Report/Technology Assessment No 158. Published August 2007. AHRQ Publication No 07-E013. Rockville, MD: Agency for Healthcare Research and Quality. http://www.ahrq.gov/downloads/pub/evidence/pdf/vitamind/vitad.pdf. Accessed November 8, 2010.

40. Bischoff-Ferrari HA, Kiel DP, Dawson-Hughes B, et al. Dietary calcium and serum 25-hydroxyvitamin D status in relation to BMD among U.S. adults. *J Bone Miner Res*. 2009;24(5):935-942.

41. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med*. 2006;354(7):669-683.

42. Bischoff-Ferrari HA, Willett WC, Wong JB, et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2009;169(6):551-561.

43. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ*. 2009;339:b3692.

44. Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA*. 2010;303(18):1815-1822.

45. Ginde AA, Scragg R, Schwartz RS, Camargo CA Jr. Prospective study of serum 25-hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older U.S. adults. *J Am Geriatr Soc*. 2009;57(9):1595-1603.

46. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2007;167(16):1730-1737.

47. Lawlor DA, Davey Smith G, Kundu D, Bruckdorfer KR, Ebrahim S. Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? *Lancet*. 2004;363(9422):1724-1727.

48. Grady D, Rubin SM, Petitti DB, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med*. 1992;117(12):1016-1037.

49. Grodstein F, Manson JE, Stampfer MJ. Postmenopausal hormone use and secondary prevention of coronary events in the nurses' health study: a prospective, observational study. *Ann Intern Med*. 2001;135(1):1-8.

50. Kawas C, Resnick S, Morrison A, et al. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology*. 1997;48(6):1517-1521.

51. Shumaker SA, Legault C, Rapp SR, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA*. 2003;289(20):2651-2662.

52. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288(3):321-333.

53. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008;168(12):1340-1349.

54. Freedman BI, Wagenknecht LE, Hairston KG, et al. Vitamin D, adiposity, and calcified atherosclerotic plaque in African-Americans. *J Clin Endocrinol Metab*. 2010;95(3):1076-1083.

55. Reis JP, von Muhlen D, Miller ER III, Michos ED, Appel LJ. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. *Pediatrics*. 2009;124(3):e371-e379.

56. Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2007;167(11):1159-1165.

57. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child*. 2008;93(6):512-517.

58. Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care*. 2006;29(3):650-656.

59. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92(6):2017-2029.

60. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. *Aliment Pharmacol Ther*. 2009;30(2):113-125.

61. Jenab M, Bueno-de-Mesquita HB, Ferrari P, et al. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: a nested case-control study. *BMJ*. 2010;340:b5500.

62. Pufulete M. Intake of dairy products and risk of colorectal neoplasia. *Nutr Res Rev*. 2008;21(1):56-67.

63. Wactawski-Wende J, Kotchen JM, Anderson GL, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med*. 2006;354(7):684-696.

64. Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. *Breast Cancer Res Treat*. 2010;121(2):469-477.

65. Chlebowski RT, Johnson KC, Kooperberg C, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Inst*. 2008;100(22):1581-1591.

66. Gandini S, Boniol M, Haukka J, et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma [published online ahead of print July 21, 2010]. *Int J Cancer*. doi: 10.1002/ijc.25439.

67. Stolzenberg-Solomon RZ. Vitamin D and pancreatic cancer. *Ann Epidemiol*. 2009;19(2):89-95.

68. Helzlsouer KJ; VDPP Steering Committee. Overview of the cohort consortium vitamin D pooling project of rarer cancers. *Am J Epidemiol*. 2010;172(1):4-9.

69. Drake MT, Maurer MJ, Link BK, et al. Vitamin D Insufficiency and Prognosis in Non-Hodgkin's Lymphoma. *J Clin Oncol*. 2010;28(27): 4191-4198.

70. Krishnan AV, Trump DL, Johnson CS, Feldman D. The role of vitamin D in cancer prevention and treatment. *Endocrinol Metab Clin North Am*. 2010;39(2):401-418.

71. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA*. 2006;296(23):2832-2838.

72. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. *Lancet Neurol*. 2010;9(6):599-612.

73. Litonjua AA. Childhood asthma may be a consequence of vitamin D deficiency. *Curr Opin Allergy Clin Immunol*. 2009;9(3):202-207.

74. Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? *Allergy*. 1999;54(7):757-759.

75. Brehm JM, Celedon JC, Soto-Quiros ME, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med*. 2009;179(9):765-771.

76. Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr*. 2007;85(3):788-795.

77. Devereux G, Litonjua AA, Turner SW, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr*. 2007;85(3): 853-859.

78. Gale CR, Robinson SM, Harvey NC, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr*. 2008;62(1):68-77.

79. Hypponen E, Sovio U, Wjst M, et al. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. *Ann N Y Acad Sci*. 2004;1037:84-95.

80. Wjst M, Hypponen E. Vitamin D serum levels and allergic rhinitis. *Allergy*. 2007;62(9):1085-1086.

81. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006;311(5768):1770-1773.

82. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol*. 2008;37(1):113-119.

83. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med*. 2009;179(9):843-850.

84. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2009;169(4):384-390.

85. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatr Pulmonol*. 2009;44(10):981-988.

86. McGrath J, Saari K, Hakko H, et al. Vitamin D supplementation during the first year of life and risk of schizophrenia: a Finnish birth cohort study. *Schizophr Res*. 2004;67(2-3):237-245.

87. Jorde R, Sneve M, Figenschau Y, Svartberg J, Waterloo K. Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. *J Intern Med*. 2008;264(6): 599-609.

88. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc*. 2003;78(12):1463-1470.

89. Warner AE, Arnspiger SA. Diffuse musculoskeletal pain is not associated with low vitamin D levels or improved by treatment with vitamin D. *J Clin Rheumatol*. 2008;14(1):12-16.

90. Arvold DS, Odean MJ, Dornfeld MP, et al. Correlation of symptoms with vitamin D deficiency and symptom response to cholecalciferol treatment: a randomized controlled trial. *Endocr Pract*. 2009;15(3):203-212.

91. Melamed ML, Astor B, Michos ED, Hostetter TH, Powe NR, Muntner P. 25-hydroxyvitamin D levels, race, and the progression of kidney disease. *J Am Soc Nephrol*. 2009;20(12):2631-2639.

92. Manson JE, Buring JE. Brigham and Women's Hospital. Vitamin D and Omega-3 Trial (VITAL). In: ClinicalTrials.gov [Internet]. Bethesda, MD: National Library of Medicine (US). 2010-[cited 2010 Dec 2]. Available from http://clinicaltrials.gov/show/NCT01169259. NLM Identifier: NCT01169259.

93. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC, November 30, 2010. http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx. Accessed November 30, 2010.