Dermatologic Therapy, Vol. 23, 2010, 23–30
Printed in the United States · All rights reserved

DERMATOLOGIC THERAPY
ISSN 1396-0296

Health effects of vitamin D

Heike Bischoff-Ferrari
Centre on Aging and Mobility, University of Zurich and Department of Rheumatology and Institute of Physical Medicine, University Hospital Zurich, Zurich, Switzerland

ABSTRACT: Increasing data suggest that many or most adults in the United States and Europe would benefit from vitamin D supplements. This review summarizes the benefits of vitamin D with the strongest evidence today from randomized controlled trials for fall and fracture prevention.

Beyond fall and fracture prevention, vitamin D may also reduce overall morbidity by multiple mechanisms. Prospective epidemiological studies supported by strong mechanistic evidence suggest a reduction of cardiovascular disease (incident hypertension and cardiovascular mortality) and colorectal cancer, extending to weaker evidence on immune-modulatory and anti-inflammatory benefits of vitamin D.

KEYWORDS: cardiovascular health, colorectal cancer, falls, fractures, vitamin D

Strongest evidence: vitamin D benefits on fall and fracture prevention

Vitamin D modulates fracture risk in two ways: by decreasing falls and increasing bone density. Two most recent meta-analyses of double-blind randomized controlled trials came to the conclusion that vitamin D reduces the risk of falls by 19% (1), the risk of hip fracture by 18%, and the risk of any nonvertebral fracture by 20% (2); however, this benefit was dose dependent. Fall prevention was only observed in trial of at least 700 international units [IU] vitamin D per day, and fracture prevention required a received dose (treatment–dose adherence) of more than 400 IU vitamin D per day. Antifall efficacy started with achieved 25-hydroxyvitamin D [25(OH)D] levels of at least 60 nmol/L (24 ng/mL) (1), and antifracture efficacy started with achieved 25(OH)D levels of at least 75 nmol/L (30 ng/mL) (2), and both end points improved further with higher-achieved 25(OH)D levels.

Address correspondence and reprint requests to: Heike Bischoff-Ferrari, MD, DrPH, Centre on Aging and Mobility, University of Zurich and Department of Rheumatology and Institute of Physical Medicine, University Hospital Zurich, Gloriastrasse 25, 8081 Zurich, Switzerland or email: Heike.Bischoff@usz.ch

Based on these evidence-based data derived from the general older population, vitamin D supplementation should be at least 700 to 1000 IU per day and taken with good adherence to cover the needs for both fall and fracture prevention.

Vitamin D: its role in muscle health

In humans, four lines of evidence support a role of vitamin D in muscle health. *First*, proximal muscle weakness is a prominent feature of the clinical syndrome of vitamin D deficiency (3). Vitamin D deficiency myopathy includes proximal muscle weakness, diffuse muscle pain, and gait impairments such as waddling way of walking (4). *Second*, vitamin D receptor (VDR) is expressed in human muscle tissue (5), and VDR activation may promote de novo protein synthesis in muscle (6). Suggesting a role of vitamin D in muscle development, mice lacking the VDR show a skeletal muscle phenotype with smaller and variable muscle fibers and persistence of immature muscle gene expression during adult life (7,8). These abnormalities persist after correction of systemic calcium metabolism by a rescue diet (8). *Third*, several observational studies suggest a positive association between 25(OH)D and muscle strength or lower extremity function in older persons (9,10). *Finally*, in a number of double-blind randomized controlled trials, vitamin

D supplementation increased muscle strength and balance (11,12), and reduced the risk of falling in both community-dwelling (12–14) and institutionalized individuals (11,15). Notably, a study by Glerup and colleagues suggest that vitamin D deficiency may cause muscular impairment even before adverse effects on bone occur (3).

A dose–response relationship between vitamin D status and muscle health was examined in The Third National Health and Nutrition Examination Survey (NHANES III) including 4100 ambulatory adults aged 60 years and older. Muscle function measured as the 8-foot walk test and the repeated sit-to-stand test was poorest in subjects with the lowest 25(OH)D (below 20 nmol/L) levels (10). Similar results were found in a Dutch cohort of older individuals (9). Notably, although from the smaller Dutch cohort a threshold of 50 nmol/L has been suggested for optimal function (9), a threshold beyond which function would not further improve was not identified in the larger NHANES III survey, even beyond the upper end of the reference range (>100 nmol/L) (10). In NHANES III, a similar benefit of higher 25(OH)D status was documented by gender, level of physical activity, and level of calcium intake.

These associations between higher 25(OH)D status and better function observed in epidemiologic studies in the United States and Europe were confirmed by three recent double-blind randomized clinical trials (RCTs) with 800 IU vitamin D3 resulting in a 4–11% gain in lower extremity strength or function (11,12), and in an up to 28% improvement in body sway (12,14) in older adults aged 65+ years, within 2 to 12 months of treatment.

A dose-dependent benefit of vitamin D in regard to fall prevention was suggested by a 2004 meta-analysis (16) and a recent multidose double-blind RCT among 124 nursing home residents receiving 200, 400, 600, or 800 IU vitamin D compared to placebo over a 5-month period (15). Participants in the 800 IU group had a 72% lower rate of falls than those taking placebo or a lower dose of vitamin D (rate ratio = 0.28; 95% confidence interval (CI) = 0.11–0.75) (15). Including this trial, *a most recent meta-analysis of eight high-quality double-blind RCTs* (n = 2426) found significant heterogeneity by dose (low-dose: <700 IU/day versus higher dose: 700 to 1000 IU/day; p-value 0.02) and achieved 25(OH)D level (<60 nmol/L versus ≥60 nmol/L; p-value = 0.005) (1). *Higher-dose supplemental vitamin D* between 700 to 1000 IU per day reduced fall risk by 19% (pooled relative risk (RR) = 0.81; 95% CI, 0.71–0.92; n = 1921 from seven trials) versus a lower dose that did not decrease risk (pooled RR = 1.10, 95% CI, 0.89–1.35 from two trials). Additionally, achieved serum 25(OH)D concentrations less than 60 nmol/L did not reduce the risk of falling (pooled RR = 1.35, 95% CI, 0.98–1.84). Notably, at the higher dose, this meta-analysis documented a significant 38% reduction in the risk of falling with treatment duration of 2 to 5 months, and a sustained significant effect of 17% fall reduction with treatment duration of 12 to 36 months. Thus, benefits of vitamin D on fall prevention are rapid and sustained provided a high enough dose is provided. Notably, subgroup analyses for the prevention of falls at a dose of 700 to 1000 IU per day suggested a benefit in all subgroups of the older population, and possibly better fracture reduction with D3 compared to D2. Also, at the higher dose, fall reduction was not increased in one trial that combined vitamin D with a calcium supplement compared to the main effect of vitamin D (1).

Vitamin D: its role in bone health

A threshold for optimal 25(OH)D and hip bone mass density (BMD) has been addressed among 13,432 individuals of NHANES III including both younger (20–49 years) and older (50+ years) individuals with different ethnic racial background (17). In the regression plots, higher serum 25(OH)D levels were associated with higher BMD throughout the reference range of 22.5 to 94 nmol/L in all subgroups. In younger whites and younger Mexican Americans, higher 25(OH)D was associated with higher BMD even beyond 100 nmol/L.

Consistently, a 2009 meta-analysis of 12 double-blind RCTs for nonvertebral fractures (n = 42,279) and eight RCTs for hip fractures (n = 40,886) found that antifracture efficacy of vitamin D is dose dependent and increases significantly with a higher achieved level of 25(OH)D in the treatment group starting at 75 nmol/L (2). No fracture reduction was observed for a received dose of 400 IU or less per day, whereas a higher received dose (dose multiplied by adherence) of 482 to 770 IU supplemental vitamin D per day reduced nonvertebral fractures by 20% (pooled RR = 0.80; 95% CI, 0.72–0.89; n = 33,265 from nine trials) and hip fractures by 18% (pooled RR = 0.82; 95% CI, 0.69–0.97; n = 31,872 from five trials). Notably, subgroup analyses for the prevention of nonvertebral fractures with the higher received dose suggested a benefit in all subgroups of the older population, and possibly better fracture reduction with D3 compared to D2, whereas additional calcium did not further improve antifracture efficacy.

In August 2007, a review and meta-analysis commissioned by the US Department of Health and Human Services addressed the effect of vitamin D supplementation on all fractures in postmenopausal women and men ages 50 years and older (18). The pooled results for all fractures included 10 double-blinded and three open design trials (n = 58,712) and did not support a significant reduction of fractures with vitamin D (pooled odds ratio (OR) = 0.90; 95% CI 0.81–1.02). The report suggested that the benefit of vitamin D may depend on additional calcium and may be primarily seen in institutionalized individuals, which is consistent with the meta-analysis of Boonen et al. performed in the same year (19). However, in both reports, heterogeneity by dose may have been missed due to the inclusion of open design trials plus a dose evaluation that did not incorporate adherence. Biologically, the exclusion of heterogeneity by dose seems implausible even if a formal test of heterogeneity is not statistically significant.

Also, in 2007, Tang and colleagues suggested in their meta-analysis that together with calcium supplementation, a daily intake of 800 IU vitamin D or more increases total fracture reduction by 3% compared to daily doses of vitamin D less than 800 IU. However, with their focus on calcium, the authors excluded four high-quality trials of vitamin D alone compared to placebo (20–23).

Adding calcium to vitamin D for bone health

The pooled RR reduction was 21% with or without additional calcium for the higher dose of vitamin D based on the 2009 meta-analysis. Previous meta-analyses may have missed this finding due to their analyses including all doses of vitamin D. Physiologically, the calcium-sparing effect of vitamin D may explain why there was no additional benefit of calcium supplementation at a higher dose of vitamin D in the 2009 meta-analysis (24,25).

The calcium-sparing effect of vitamin D is supported by two recent epidemiologic studies suggesting that both PTH suppression (25) and hip bone density (26) may only depend on a higher calcium intake if serum 25(OH)D levels are very low.

Thus, as calcium absorption is improved with higher serum 25(OH)D levels (25,27), future studies may need to evaluate whether current calcium intake recommendations may require downward adjustment, especially with higher doses of vitamin D (27). If dietary calcium is a threshold nutrient, as suggested by Dr. Heaney (19), then the threshold for optimal calcium absorption may be at a lower calcium intake when vitamin D supplementation is adequate.

Cardiovascular disease

The development of mice lacking the receptor for vitamin D (VDR) provided insight into the global physiologic role of vitamin D. Mice lacking the VDR have impaired mineralization of bone, have small and variable muscle fibers, suffer from hypertension, and die of congestive heart disease (29).

Data from epidemiologic studies on the RR of incident hypertension (30), cardiovascular (31), and all-cause (31) mortality show an inverse association between higher 25(OH)D levels and lower disease risk. For all end points, the desirable median serum 25(OH)D level associated with the lowest risk was approximately 100 nmol/L in the different prospective cohort studies.

Epidemiologic findings are supported by two small RCTs in hypertensive subjects (32) and community-dwelling women aged 65 years and older (33). In one trial, within 2 months, supplementation with vitamin D (800 IU/day) plus calcium (1200 mg/day) decreased systolic blood pressure (SBP) by 13 mmHg (p = 0.02), diastolic blood pressure (DBP) by 6 mmHg (p = 0.10) and heart rate by 4 beats/minute (p = 0.02) compared to calcium alone (1200 mg/day) (33). Similarly, in a randomized controlled trial by Krause and colleagues, ultraviolet-B (UVB) irradiation significantly lowered SBP by 6 mmHg [–14;–1] and DBP by 6 mmHg [–12;–2] within 6 weeks as compared to ultraviolet-A (UVA) irradiation (32). 25(OH)D levels increased in the UVB group from 58 to 151 nmol/L, whereas there was no increase in the UVA group.

Mechanistically, Li and colleagues found that renin and angiotensin II expression was increased in mice lacking the VDR, leading to vasoconstriction (34). In humans, the stimulation of the renin–angiotensin system has been associated with hypertension, myocardial infarction, and stroke (35,36). Furthermore, several studies suggest a potential anti-atherosclerotic activity of vitamin D. The VDR is present in vascular smooth muscle (37,38), and in vitro studies found that 1,25-dihydroxyvitamin D antagonizes the mitogenic effect of epidermal growth factor on mesangial cell growth (39,40). Epidemiologically, a potential anti-arteriosclerotic effect of vitamin D is supported by recent findings within NHANES 2001 to 2004, where across quartiles of 25(OH)D, from lowest to highest, the prevalence of peripheral arterial disease was 8.1, 5.4, 4.9, and 3.7% (p trend <0.001) (41).

Colorectal cancer

$1,25(OH)_2D$ enhances the differentiation of normal cells and inhibits proliferation of most malignant cells, although escape mechanisms have been described (i.e., loss of the VDR in cancer cells) (42). Colon cancer cells express the VDR (43) and $1,25(OH)_2D$ promotes their differentiation (44).

Consistently, most epidemiologic studies that have found a lower risk of adenoma or colorectal cancer associated with higher 25(OH)D levels (45–52) with few exceptions (53). Furthermore, when the relationships between colorectal cancer and dietary or supplementary vitamin D have been investigated in cohort studies of men (54,55) and women (47,48,56–58) or both sexes (59,60), and in case-control studies (61–68), the majority of studies suggested inverse associations of vitamin D intake with incidence of colon or rectal cancer, or both (54–57,60,62,64,66,67). Most importantly, all the studies of colorectal cancer incidence that accounted for supplementary vitamin D reported an inverse association (55–57,60,67–69). Finally, after supplementation with vitamin D, circulating 25(OH)D levels are inversely associated with the size of the proliferative compartment in the colorectal mucosa in humans (70), and both $1,25(OH)_2D$ and 25(OH)D reduce proliferation and increase differentiation in vitro for colorectal cancer cells (71–74).

A recent meta-analysis of observational studies that reported risk of colorectal cancer by quintiles of 25(OH)D documented a significant dose–response relationship with a lower risk among individuals with higher 25(OH)D serum concentrations (p trend <0.0001) (75). According to the pooled analysis, individuals with serum 25(OH)D of approximately 92.5 nmol/L (median of the top quintile) had a 50% lower risk of colorectal cancer than those with serum <15 nmol/L in the lowest quintile (75). Thus, consistent with an earlier review (76) and supported by a 2004 National Institute of Health-sponsored symposium on the role of vitamin D in cancer chemoprevention and treatment (28,77,78), optimal colorectal cancer prevention may be associated with serum 25(OH)D concentrations close to 100 nmol/L.

Lending further support to the vitamin D-cancer hypothesis, an increment of 25 nmol/L in predicted 25(OH)D level was associated with a 17% reduction in total cancer incidence, a 29% reduction in total cancer mortality, and a 45% reduction in digestive system cancer mortality in a comprehensive prospective analysis from a large male US cohort (Health Professionals Follow-Up Study) (79). Finally, the present author has evidence today from one moderately sized trial in 1179 community-dwelling postmenopausal women aged 56 years and older randomly assigned to receive 1100 IU vitamin D per day plus 400–1500 mg supplemental calcium per day, calcium alone, or placebo. Over 4 years, women in the vitamin D plus calcium and calcium alone groups had a 60% lower risk of incident cancer ($p = 0.01$) (80). In the same trial, achieved serum 25(OH)D concentration was a significant and independent predictor of cancer risk (80).

Immune-modulation/ Anti-inflammation

There is an emerging recognition of the role of vitamin D in the immune response to infectious agents, such as tuberculous bacteria (81) or viral and bacterial infectious of the respiratory tract (82,83). The VDR has a wide presence in cells of the immune system, including B and T lymphocytes, neutrophils, and antigen-presenting cells, such as macrophages (84). For tuberculosis, recent data show a link between toll-like receptors and vitamin D-mediated innate immunity, and suggest that lower 25(OH)D levels in black individuals may contribute to their susceptibility to microbial infection (85).

Regarding potential immune-modulatory benefits of vitamin D, a low 25(OH)D status has been repeatedly associated with a higher risk for multiple sclerosis. A large prospective nested case-control study among more than 7 million US military personnel revealed that among whites (but not blacks), low 25(OH)D levels, especially before the age of 20 years, was a strong risk factor for later occurrence of multiple sclerosis (OR for a 50 nmol/L increase of 25(OH)D = 0.59, confidence limits 0.36–0.97) (86).

Similarly, epidemiologic data suggest that vitamin D intake in early life may reduce the risk of type 1 diabetes in later life (87–89). Risk reduction was 26% with cod liver oil (87), 33% with general vitamin D supplementation (88), and 78% with 2000 IU per day vitamin D supplementation (89). These observations point to the importance of preventing vitamin D deficiency in early childhood. Whether a higher intake of vitamin D during pregnancy affects the diabetes risk of the offspring is less clear; however, in one study, lower levels of anti-islet cell antibodies were found in children of

mothers with a higher food-derived intake of vitamin D during the third trimester of pregnancy (90).

High doses of $1,25(OH)_2D$ prevent insulitis and the onset of type 1 diabetes in VDR(-I-) mice (91) and non-obese diabetic-prone mice (NOD) (92), whereas dietary correction of hypocalemia alone is not preventative. Furthermore, in NOD mice, vitamin D deficiency appears to accelerate type 1 diabetes (93), which mechanistically, may be explained by altered T-lymphocyte response (94).

Furthermore, laboratory studies among humans and large epidemiologic studies support a benefit of higher serum 25(OH)D concentrations on insulin sensitivity. In one study of 126 healthy adults, there was a positive correlation between 25(OH)D serum concentrations and insulin sensitivity as measured with hyperglycemic clamp (r = 0.25) (95). Additionally, in a study of 142 Dutch men aged 70 to 88 years, 25(OH)D serum concentrations were inversely correlated with serum insulin levels (r = –0.18 to –0.23) and glucose concentrations (r = –0.26) during an oral glucose tolerance test (96). In the NHANES III survey, including adults aged 20 years and older, for 25(OH)D levels of 81 nmol/L or higher compared to 43.9 nmol/L or lower, the OR for diabetes (fasting glucose > or = 7.0 mmol/L) was 0.25 (95% CI 0.11–0.60) among whites and 0.17 (95% CI 0.08–0.37) among Mexican Americans, without a difference observed among African-Americans (97). Furthermore, in the same cohort, serum 25(OH)D concentrations were inversely associated with insulin resistance (HOMA-IR), with best levels observed in the top quartile of 25(OH)D of 81 nmol/L or higher (98).

In intervention studies, vitamin D supplementation increased insulin secretion among a small group of 10 patients with type II diabetes (99), whereas in another study of 35 type II diabetes patients, there was no change in insulin secretion with $1,25(OH)_2D$ treatment (100). In a larger 3-year randomized controlled trial among 445 individuals aged 65 years and older treated with either 700 IU vitamin D3 plus 500 mg calcium or placebo, there was significant effect modification by baseline fasting glucose (101). Among participants with impaired fasting glucose at baseline, those who took combined calcium–vitamin D supplements had a lower rise in fasting blood glucose at 3 years compared with those on placebo (p = 0.04) and a lower increase in HOMA-insulin resistance (p = 0.03). As the present study combined vitamin D with calcium, the effect may have been caused by either substances, vitamin D or calcium (102).

Summary

Based on evidence from randomized-controlled trials, vitamin D supplementation reduces both falls and nonvertebral fractures, including those at the hip. However, this benefit is dose dependent. According to two 2009 meta-analysis of double-blind RCTs, no fall reduction was observed for a dose of less than 700 IU per day, whereas a higher dose of 700 to 1000 IU supplemental vitamin D per day reduced falls by 19% (1). Similarly, no fracture reduction was observed for a received dose of 400 IU or less per day, whereas a higher received dose of 482 to 770 IU supplemental vitamin D per day reduced nonvertebral fractures by 20% and hip fractures by 18%. Consistently, fall prevention and nonvertebral fracture prevention increased significantly with higher achieved 25(OH)D levels in the 2009 meta-analyses. Fall prevention occurred with 25(OH)D levels of at least 60 nmol/L up to 95 (1), whereas 75 to 112 nmol/L were required for nonvertebral fracture prevention (2). Given the absence of data beyond this beneficial range, these recent meta-analyses do not preclude the possibility that higher doses or higher achieved 25(OH)D concentrations would have been even more efficacious in reducing falls and nonvertebral fractures.

Vitamin D benefits on cardiovascular health and colorectal cancer are supported by promising data from large cohort studies, mechanistic studies, and small- to moderate-sized RCTs, which demonstrated a significant reduction for blood pressure (32,33) and incident cancer risk (80). Further evidence is being collected in large RCTs.

Anti-inflammatory and immune-modulatory benefits of vitamin D, including benefits on other cancers, are promising and point to a similar desirable range of 75 to 100 nmol/L as documented for fall and fracture reduction. More evidence from randomized controlled trials is needed to establish causality for these endpoints.

References

1. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and alpha-hydroxylated vitamin D: a meta-analysis of randomized controlled trials BMJ 2009; **339**: b3692.
2. 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**: 551–561.
3. Glerup H, Mikkelsen K, Poulsen L, et al. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. Calcif Tissue Int 2000; **66**: 419–424.

4. Schott GD, Wills MR. Muscle weakness in osteomalacia. Lancet 1976; **1**: 626–629.
5. Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res 2004; **19**: 265–269.
6. Sorensen OH, Lund B, Saltin B, et al. Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. Clin Sci (Colch) 1979; **56**: 157–161.
7. Bouillon R, Bischoff-Ferrari H, Willett W. Vitamin D and health: perspectives from mice and man. J Bone Miner Res 2008; **28**: 28.
8. Endo I, Inoue D, Mitsui T, et al. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology 2003; **144**: 5138–5144. Epub 2003 Aug 13.
9. Wicherts IS, van Schoor NM, Boeke AJ, et al. Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab 2007; **6**: 6.
10. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged >=60 y. Am J Clin Nutr 2004; **80**: 752–758.
11. Bischoff HA, Stahelin HB, Dick W, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. J Bone Miner Res 2003; **18**: 343–351.
12. Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H. Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. Osteoporos Int 2008; **16**: 16.
13. Bischoff-Ferrari HA, Orav EJ, Dawson-Hughes B. Effect of cholecalciferol plus calcium on falling in ambulatory older men and women: a 3-year randomized controlled trial. Arch Intern Med 2006; **166**: 424–430.
14. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C. Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. J Bone Miner Res 2000; **15**: 1113–1118.
15. Broe KE, Chen TC, Weinberg J, Bischoff-Ferrari HA, Holick MF, Kiel DP. A higher dose of vitamin d reduces the risk of falls in nursing home residents: a randomized, multiple-dose study. J Am Geriatr Soc 2007; **55**: 234–239.
16. Bischoff-Ferrari HA, Dawson-Hughes B, Willett CW, et al. Effect of vitamin D on falls: a meta-analysis. JAMA 2004; **291**: 1999–2006.
17. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin d levels and bone mineral density: a population-based study of younger and older adults. Am J Med 2004; **116**: 634–639.
18. Cranny A, Horsley T, O'Donnell S, Puil L. Effectiveness and safety of vitamin D in relation to bone health. http://www.ahrqgov/clinic/tp/vitadtphtm. Accessed 2007.
19. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: evidence from a comparative meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 2007; **30**: 30.
20. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 2003; **326**: 469.
21. Lyons RA, Johansen A, Brophy S, et al. Preventing fractures among older people living in institutional care: a pragmatic randomised double blind placebo controlled trial of vitamin D supplementation. Osteoporos Int 2007; **18**: 811–818.
22. Lips P, Graafmans WC, Ooms ME, Bezemer PD, Bouter LM. Vitamin D supplementation and fracture incidence in elderly persons. A randomized, placebo-controlled clinical trial. Ann Intern Med 1996; **124**: 400–406.
23. Meyer HE, Smedshaug GB, Kvaavik E, Falch JA, Tverdal A, Pedersen JI. Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomized controlled trial. J Bone Miner Res 2002; **17**: 709–715.
24. Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. J Clin Endocrinol Metab 1997; **82**: 4111–4116.
25. 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**: 2336–2341.
26. 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**: 935–942.
27. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 2003; **22**: 142–146.
28. Heaney RP. The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 2005; **15**: 15.
29. Bouillon R, Bischoff-Ferrari H, Willett W. Vitamin D and health: perspectives from mice and man. J Bone Miner Res 2008; **23**: 974–979.
30. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007; **19**: 19.
31. 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; **22**: 22.
32. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet 1998; **352**: 709–710.
33. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab 2001; **86**: 1633–1637.
34. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002; **110**: 229–238.
35. Laragh JH. Renin-angiotensin-aldosterone system for blood pressure and electrolyte homeostasis and its involvement in hypertension, in congestive heart failure and in associated cardiovascular damage (myocardial infarction and stroke). J Hum Hypertens 1995; **9**: 385–390.
36. Fujita T. Symposium on the etiology of hypertension – summarizing studies in 20th century. 5. Renin-angiotensin system and hypertension. Intern Med 2001; **40**: 156–158.

37. Koh E, Morimoto S, Fukuo K, et al. 1,25-Dihydroxyvitamin D3 binds specifically to rat vascular smooth muscle cells and stimulates their proliferation in vitro. Life Sci 1988; **42**: 215–223.
38. Merke J, Hofmann W, Goldschmidt D, Ritz E. Demonstration of 1,25(OH)2 vitamin D3 receptors and actions in vascular smooth muscle cells in vitro. Calcif Tissue Int 1987; **41**: 112–114.
39. Hariharan S, Hong SY, Hsu A, MacCarthy EP, Gartside PS, Ool BS. Effect of 1,25-dihydroxyvitamin D3 on mesangial cell proliferation. J Lab Clin Med 1991; **117**: 423–429.
40. Mitsuhashi T, Morris RC, Jr., Ives HE. 1,25-dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. J Clin Invest 1991; **87**: 1889–1895.
41. Melamed ML, Muntner P, Michos ED, et al. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease. Results from NHANES 2001 to 2004. Arterioscler Thromb Vasc Biol 2008; **16**: 16.
42. Bouillon R, Eelen G, Verlinden L, Mathieu C, Carmeliet G, Verstuyf A. Vitamin D and cancer. J Steroid Biochem Mol Biol 2006; **102**: 156–162.
43. Kallay E, Bises G, Bajna E, et al. Colon-specific regulation of vitamin D hydroxylases – a possible approach for tumor prevention. Carcinogenesis 2005; **19**: 19.
44. Holt PR, Arber N, Halmos B, et al. Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. Cancer Epidemiol Biomarkers Prev 2002; **11**: 113–119.
45. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet 1989; **2**: 1176–1178.
46. Tangrea J, Helzlsouer K, Pietinen P, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control 1997; **8**: 615–625.
47. Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin d metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004; **13**: 1502–1508.
48. Levine AJ, Harper JM, Ervin CM, et al. Serum 25-hydroxyvitamin D, dietary calcium in take, and distal colorectal adenoma risk. Nutr Cancer 2001; **39**: 35–41.
49. Peters U, McGlynn KA, Chatterjee N, et al. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2001; **10**: 1267–1274.
50. Platz EA, Hankinson SE, Hollis BW, et al. Plasma 1,25-dihydroxy-and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. Cancer Epidemiol Biomarkers Prev 2000; **9**: 1059–1065.
51. Grau MV, Baron JA, Sandler RS, et al. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trail. J Natl Cancer Inst 2003; **95**: 1765–1771.
52. Slattery ML, Neuhausen SL, Hoffman M, et al. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. Int J Cancer 2004; **111**: 750–756.
53. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). Cancer Causes Control 1995; **6**: 235–239.
54. Garland C, Shekelle RB, Barrett-Conner E, Criqui MH, Rossof AH, Paul O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. Lancet 1985; **1**: 307–309.
55. Kearney J, Giovannucci E, Rimm EB, et al. Calcium, vitamin D and dairy foods and the occurrence of colon cancer in men. Am J Epidemiol 1996; **143**: 907–917.
56. Bostick RM, Potter JD, Sellers TA, McKenszie DR, Kushi H, Folsom AR. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer in older women. Am J Epidemiol 1993; **137**: 1302–1317.
57. Martinez ME, Giovannucci EL, Colditz GA, et al. Calcium, vitamin D, and the occurrence of colorectal cancer among women. J Natl Cancer Inst 1996; **88**: 1375–1382.
58. Lin J, Zhang SM, Cook NR, Manson JE, Lee IM, Buring JE. Intakes of calcium and vitamin D and risk of colorectal cancer in women. Am J Epidemiol 2005; **161**: 755–764.
59. Jarvinen R, Knekt P, Hakulinen T, Aromaa A. Prospective study on milk products, calcium and cancers of the colon and rectum. Eur J Clin Nutr 2001; **55**: 1000–1007.
60. McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the cancer prevention study II nutrition cohort (United States). Cancer Causes Control 2003; **14**: 1–12.
61. Heilbrun LK, Nomura A, Hankin JH, Stemmermann GN. Dietary vitamin D and calcium and risk of colorectal cancer (letter). Lancet 1985; **1**: 925.
62. Benito E, Stiggelbout A, Bosch FX, et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int J Cancer 1991; **49**: 161–167.
63. Peters RK, Pike MC, Garabrandt D, Mack TM. Diet and colon cancer in Los Angeles County, California. Cancer Causes Control 1992; **3**: 457–473.
64. Ferraroni M, La Vecchia C, D'Avanzo B, Negri E, Franceschi S, Decarli A. Selected micronutrient intake and the risk of colorectal cancer. Br J Cancer 1994; **70**: 1150–1155.
65. Boutron MC, Faivre J, Marteau P, Couillault C, Senesse P, Quipourt V. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case-control study. Br J Cancer 1996; **74**: 145–151.
66. Pritchard RS, Anthony BJ, Gerhardsson de Verdier M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. Cancer Epidemiol Biomarkers Prev 1996; **5**: 897–900.
67. Marcus PM, Newcomb PA. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. Int J Epidemiol 1998; **27**: 788–793.
68. Kampman E, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposures, dairy products and colon cancer risk (United States). Cancer Causes Control 2000; **11**: 459–466.
69. Zheng W, Anderson KE, Kushi LH, et al. A prospective cohort study of intake of calcium, vitamin D, and other micronutrients in relation to incidence of rectal cancer among postmenopausal women. Cancer Epidemiol Biomarkers Prev 1998; **7**: 221–225.
70. Holt PR, Arber N, Halmos B, et al. Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. Cancer Epidemiol Biomarkers Prev 2002; **11**: 113–119.
71. Meggouh F, Lointier P, Saez S. Sex steroid and 1,25-dihydroxyvitamin D3 receptors in human colorectal adenocarcinoma and normal mucosa. Cancer Res 1991; **51**: 1227–1233.
72. Giuliano AR, Franceschi RT, Wood RJ. Characterization of the vitamin D receptor from the Caco-2 human colon carcinoma cell line: effect of cellular differentiation. Arch Biochem Biophys 1991; **285**: 261–269.

73. Vandewalle B, Adenis A, Hornez L, Revillion F, Lefebvre J. 1,25-dihydroxyvitamin D3 receptors in normal and malignant human colorectal tissues. Cancer Lett 1994; **86**: 67–73.
74. Zhao X, Feldman D. Regulation of vitamin D receptor abundance and responsiveness during differentiation of HT-29 human colon cancer cells. Endocrinology 1993; **132**: 1808–1814.
75. Gorham ED, Garland CF, Garland FC, et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. Am J Prev Med 2007; **32**: 210–216.
76. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; **84**: 18–28.
77. National Institutes of Health. Vitamin D and cancer. Proceedings of the vitamin D workshop meeting. November 17–19, 2004. J Steroid Biochem Mol Biol 2005; **97**: 1–2. Epub 2005 Oct 20.
78. Bouillon R, Moody T, Sporn M, Barrett JC, Norman AW. NIH deltanoids meeting on Vitamin D and cancer. Conclusion and strategic options. J Steroid Biochem Mol Biol 2005; **97**: 3–5. Epub 2005 Jul 25.
79. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006; **98**: 451–459.
80. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007; **85**: 1586–1591.
81. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; **23**: 23.
82. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. Epidemiol Infect 2006; **134**: 1129–1140.
83. Laaksi I, Ruohola JP, Tuohimaa P, et al. An association of serum vitamin D concentrations <40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 2007; **86**: 714–717.
84. Adorini L, Penna G, Giarratana N, et al. Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. J Steroid Biochem Mol Biol 2004; **89–90**: 437–441.
85. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; **311**: 1770–1773.
86. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006; **296**: 2832–2838.
87. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Am J Clin Nutr 2003; **78**: 1128–1134.
88. The EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. Diabetologia 1999; **283**: 51–54.
89. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001; **358**: 1500–1503.
90. Fronczak CM, Baron AE, Chase HP, et al. In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care 2003; **26**: 3237–3242.
91. Gysemans C, van Etten E, Overbergh L, et al. Unaltered diabetes presentation in NOD mice lacking the vitamin D receptor. Diabetes 2008; **57**: 269–275.
92. Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M, Bouillon R. 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. Diabetes 1992; **41**: 1491–1495.
93. Giulietti A, Gysemans C, Stoffels K, et al. Vitamin D deficiency in early life accelerates Type 1 diabetes in non-obese diabetic mice. Diabetologia 2004; **47**: 451–462.
94. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L. A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 2002; **51**: 1367–1374.
95. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004; **79**: 820–825.
96. Baynes KC, Boucher BJ, Feskens EJ, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia 1997; **40**: 344–347.
97. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 2004; **27**: 2813–2818.
98. Chonchol M, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. Kidney Int 2007; **71**: 134–139.
99. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract 2003; **57**: 258–261.
100. Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1994; **59**: 1083–1087.
101. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007; **30**: 980–986.
102. 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**: 650–656.