Optimal Vitamin D Status: A Critical Analysis on the Basis of Evidence-Based Medicine

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Context: Public health authorities around the world recommend widely variable supplementation strategies for adults, whereas several professional organizations, including The Endocrine Society, recommend higher supplementation.

Methods: We analyzed published randomized controlled clinical trials to define the optimal intake or vitamin D status for bone and extraskeletal health.

Conclusions: The extraskeletal effects of vitamin D are plausible as based on preclinical data and observational studies. However, apart from the beneficial effects of 800 IU/d of vitamin D_3 for reduction of falls in the elderly, causality remains yet unproven in randomized controlled trials (RCTs). The greatest risk for cancer, infections, cardiovascular and metabolic diseases is associated with 25-hydroxyvitamin D (25OHD) levels below 20 ng/mL. There is ample evidence from RCTs that calcium and bone homeostasis, estimated from serum 1,25-dihydroxyvitamin D and PTH, calcium absorption, or bone mass, can be normalized by 25OHD levels above 20 ng/mL. Moreover, vitamin D supplementation (800 IU/d) in combination with calcium can reduce fracture incidence by about 20%. Such a dose will bring serum levels of 25OHD above 20 ng/mL in nearly all postmenopausal women. Based on calculations of the metabolic clearance of 25OHD, a daily intake of 500–700 IU of vitamin D_3 is sufficient to maintain serum 25OHD levels of 20 ng/mL. Therefore, the recommendations for a daily intake of 1500–2000 IU/d or serum 25OHD levels of 30 ng or higher for all adults or elderly subjects, as suggested by The Endocrine Society Task Force, are premature. Fortunately, ongoing RCTs will help to guide us to solve this important public health question. (***J Clin Endocrinol Metab*** **98: E1283–E1304, 2013)**

The essential role of vitamin D for bone was discovered at the beginning of the 20th century after an intensive search for the etiology of rickets. Vitamin D is mainly derived from photosynthesis in the skin after conversion of 7-dehydrocholesterol. Small amounts of vitamin D can also be obtained by nutritional intake of either vitamin D_3 or D_2, but most food items other than oily fish have low vitamin D content. It then took nearly the full 20th century to discover the complex metabolism of vitamin D into a hormone (1–6), 1,25-Dihydroxyvitamin D [1,25$(OH)_2$D], the ligand for the vitamin D receptor (VDR) (7, 8), or 24,25$(OH)_2$D, a ligand for a putative G protein-coupled membrane receptor (9), and more than 30 other metabolites (10). The essential role of 1,25$(OH)_2$D and VDR for bone and calcium metabolism was then further confirmed by studies of patients and animals with mutations in crucial genes of the vitamin D endocrine system (VDR or CYP27B1) (5, 6, 11–16). The nearly universal presence of VDR, the large number of tissues expressing CYP27B1 responsible for systemic or local production of the active

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Abbreviations: BMD, bone mineral density; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CL, confidence limit; COPD, chronic obstructive pulmonary disease; FGF23, fibroblast growth factor 23; HR, hazard ratio; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D; OR, odds ratio; RCT, randomized controlled trials; RR, relative risk; VDR, vitamin D receptor.

hormone, and the very large number of genes (about 3% of the mouse and human genome) under the control of $1,25(OH)_2D$ all point toward a broader role of the vitamin D endocrine system beyond the regulation of calcium and bone metabolism (5, 6, 17). Therefore, the attention of basic and clinical scientists as well as the lay press (18, 19) and the general population for vitamin D has increased nearly exponentially over the last decade (6, 17, 20–22). The essential question of how much vitamin D is needed for optimal bone and global health, however, remains unsolved (17, 20, 21, 23, 24). The diversity of opinions on this topic has created lively discussions because the conclusion based on randomized controlled trials (RCTs) differs from the conclusions based on the large body of observational studies. Others have extrapolated the potential benefits of a commonly available natural substance and recommend very high levels, as presumably was the case during the early evolution of human mankind. The controversy is reflected in the diversity of recommendations regarding vitamin D intake for healthy adults from European authorities (from nil to 5 μg/d; recommendations for Europe reviewed in Ref. 25), from the Institute of Medicine (IOM) in the United States (15–20 μg/d) (26, 27), from The Endocrine Society (20–50 μg/d) (28), and from several other societies and expert groups (see Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org, and references therein). A lobbying group, the Vitamin D Council (http://www.vitamindcouncil.org), recommends an intake of 100 μg or even more in order to achieve levels of well above 40 ng/mL.[1]

Recommendations for a single nutrient should preferably be based on results of well-performed preclinical and clinical research, whereby the results of well-designed randomized controlled clinical trials have a greater impact than observational studies. Long-term efficacy and safety data are especially needed when dealing with very large groups of healthy people (the general population and, by extrapolation, the whole world population). Therefore, we re-evaluated the data on optimal vitamin D status and thereby question the validity of the 2012 Endocrine Society guidelines (28) for vitamin D intake.

Historical Background: Does Evolution Tell Us How Much Vitamin D We Need or Tolerate?

Vitamin D is an old molecule that already existed in early unicellular organisms and may have played a role in the protection of DNA against the very abundant UV-B irradiation of phyto- or zooplankton species during the evolution of life more than 2 billion years ago (29–32). The vitamin D endocrine system became operational at the time of the transition of cartilaginous fish into bony fish. This system is thereafter fairly well conserved, even in primates. The early human ancestors in Africa certainly had plenty of exposure to UV-B and thus produced a fairly large amount of vitamin D in their skin. Serum 25-hydroxyvitamin D (25OHD) levels in humans living today in similar surroundings, such as the Masai and Hadzabe tribe, and in children and adults in South Africa and Gambia are about 30–45 ng/mL (see Table 1 and references therein) and are likely similar to the situation described as "the primitive intake model." Several authors therefore conclude that such levels are the desirable levels of 25OHD for optimal skeletal and extraskeletal health (31, 33–35). The intensity of UV-B light in Africa and the enormous capacity to synthesize vitamin D based on in vitro studies— up to 10 000 IU by 30 minutes of whole body exposure to direct sunlight (36, 37)— however, suggest that serum 25OHD levels are in fact surprisingly low in modern day Africans and Asian-Australians living with plentiful exposure to sunlight (Table 1). Therefore, it seems much more likely that there was, during most of the primate and human evolution and even well before that, a constant threat of overexposure to vitamin D rather than to vitamin D deficiency. This could explain why there are many mechanisms to protect against vitamin D excess and few against vitamin D deficiency (Supplemental Table 2). This is also reflected in the rarity of vitamin D toxicity in humans because only access to large pharmacological doses of vitamin D leads to vitamin D toxicity. During the evolution of primates, 2 further events argue for this hypothesis: New World monkeys living in the treetops of the South American forest developed several additional mechanisms to inactivate vitamin D activity by producing intracellular vitamin binding proteins that inhibit VDR action (38, 39). By contrast, the human evolution out of Africa exposed early humans to less UV-B exposure, and this selected for the simultaneous depigmentation of the skin whereby the constitutive melanin expression was replaced by low but adaptive melanin synthesis (40). This pigmentation is moreover dependent on exposure to either UV-B or $1,25(OH)_2D$ (by up-regulating gene and protein expression) so that the skin synthesis of vitamin D can be modified according to habitual UV-B exposure.

Sunlight, especially UV-B, is a well-known photocarcinogen (41–44). The trade-off between decreasing skin pigmentation to allow better vitamin D synthesis and the possible UV-B-induced skin carcinogen, however, did not create a problem during most of human evolution because

[1] Conversion factors: To convert 25OHD from ng/mL to mmol/L, multiply by 2.5; to convert vitamin D dosage, 800 IU = 20 μg.

Table 1. Serum 25OHD Concentrations in Different Populations With High Habitual Exposure to Natural Sunlight

First Author (Ref.)	Study Population	n	Age, y	Mean ± SD 25OHD, ng/mL
Native blacks in Africa				
Luxwolda (236)	Tanzanian tribes (Masai and Hadzabe), males and nonpregnant women	88	33 ± 10	43 ± 11
Poopedi (237)	South African children (Johannesburg)		10	
	Black	295		37 ± 13
	White	90		48 ± 14
Thacher (238)	Nigerian children (control)	123	42 mo (IQ range, 25–70)	21 ± 6
Prentice (239)	Gambian children			
	Community reference children	44	8–11	38 ± 8
Prentice (240)	Gambian people			
	Children	44	8–12	38 ± 8
	Young women	20	<45	33 ± 8
	Older women	152	45	36 ± 9
M'Buyamba-Kabangu (241)	Black males in Kinshasa (Congo)	33	31	26
Glew (242)	Nigeria Fulani men	22	48 ± 8	45% between 10 and 30 ng
	Nigeria Fulani women	29	56 ± 14	83% between 10 and 30 ng
Aspray (243)	Gambia, rural women	48	55–64	36 ± 9
Australasia				
Heere (244)	Pacific Islands Fidji			
	Indigenous Fijian	306	55–64	32 ± 14
	Indian Fijian	205	15–44	28 ± 12
Nessvi (245)	New Zealand men and women		18–85	
	European	123		23 ± 8
	Maori	128		21 ± 9
	Pacific	123		19 ± 9
	Asian	129		15 ± 9
Rockell (246)	New Zealand boys and girls		5–14	
	European and others	483		21 ± 20
	Maori	456		17 ± 17
	Pacific	646		14 ± 20
Rockell (247)	New Zealand men and women		≥15	
	European and others	2440		20 ± 15
	Maori	370		17 ± 12
	Pacific	136		15 ± 7
Vanlint (248)	Men and women: aboriginal Australians	58	39 ± 10	23 ± 9

Results for children with rickets, pregnant women, mothers at delivery, cord blood, and mothers postpartum were not presented.

UV-B-induced DNA damage only results in skin cancer after a long lag time so that the life expectancy of less than 50 years de facto prohibited widespread clinical consequences of skin cancer. In addition, $1,25(OH)_2D$ itself is able to partially protect against photodamage of DNA (45–47). Only during the last centuries when life expectancy increased in Western countries and later in other populations by about 1 year every 4 years, the long-term consequences of UV-B photodamage of the skin became clear. The major risks for people are greatest when non-Black people, especially at a young age, are exposed to higher UV-B doses in comparison with their skin phototype. That the evolution of human skin pigmentation was successful to overcome vitamin D deficiency is best demonstrated by the low frequency of rickets during human evolution; the disease only became a real problem fairly recently because of a lack of UV-B exposure for those living in cities with low exposure to sunlight and high exposure to air pollution or because of the voluntary decrease in exposure of the skin to sunlight for economic, cultural, or religious reasons. Conversely, people with increased skin pigmentation living at latitudes with low UV-B exposure are at increased risk of vitamin D deficiency due to decreased UV-B efficacy to produces vitamin D synthesis.

It is thus likely that the mean serum 25OHD level, as observed in humans living in surroundings similar to that of early human ancestors, may well reflect the maximal levels that evolution allowed in the presence of excess sunlight rather than represent the optimal vitamin D status. Therefore, only a closer look at the links between vitamin D status and bone or global health can define whether

there is a causal link and what threshold, if any, of vitamin D status generates the best effects.

25OHD Assays: Accuracy as a Major Limitation of Guidelines?

There is a very large consensus that serum 25OHD is by far the best marker of the vitamin D status. However, the measurement of serum concentrations of 25OHD is noticeably problematic, especially with regard to "drift-overtime" and accuracy (this is correct absolute concentration) despite substantial improvement of the precision of most assays (48–52). The older assays that were used in many of the RCTs were especially problematic because the difference between reported values and results obtained by repeat analysis of some samples by more accurate measurements were sometimes very high (50% or greater difference). Most presently available commercial and simplified assays still have problems with accuracy when compared with methods using extraction and RIA or with the "gold standard," liquid chromatography followed by tandem mass spectrometry (50, 51, 53). Therefore, routine platform assays for 25OHD may differ 20% above or below the values obtained with the gold standard assays (54). When interpreting RCT data, one should give priority to dose intake rather than to "achieved" serum 25OHD levels, especially when the assays are not calibrated to international standards such as DEQAS (www.deqas.org) or NIST (55) standardization. The accuracy of vitamin D intake by supplementation is much better, at least within 20% of the stated amount (eg, see Ref. 56).

Optimal Vitamin D Status for Calcium Homeostasis and Bone Health

Surrogate endpoints

The regulation of calcium homeostasis is based on the correct production and action of many hormones, but $1,25(OH)_2D$, PTH, fibroblast growth factor 23 (FGF23), and their feedback systems are by far the most important. Therefore, the (combined) measurement of these hormones can be used to evaluate the optimal vitamin D status, as reflected by serum 25OHD. This is just like the common clinical or research use of measurements of TSH, LH/FSH, or ACTH as markers for the concentration of thyroid, sex steroid, or glucocorticoid hormones.

Serum $1,25(OH)_2D$ as marker of vitamin D status

25OHD is only a precursor of the final hormone, $1,25(OH)_2D$, comparable with the thyroid hormone T_4 as a precursor of T_3, because both 25OHD and T_4 have a low affinity for their nuclear receptor but a high affinity for the serum binding protein (vitamin D binding protein and thyroxin binding globulin, respectively) (5). Therefore, the serum concentrations of free 25OHD or T_4 are not able to significantly activate their receptors. The rate-limiting concentration of 25OHD below which the production of $1,25(OH)_2D$ is compromised has been studied in many cross-sectional studies where both serum 25OHD and $1,25(OH)_2D$ have been measured, but only RCTs using vitamin D or 25OHD supplementation of otherwise healthy subjects with low or (low-)normal vitamin D status are most informative. A potential confounder of these studies, however, is that the increase in serum $1,25(OH)_2D$ may also be dependent on the prevailing calcium intake. Studies in elderly Belgian subjects revealed a poor vitamin D status as reflected by very low 25OHD and low $1,25(OH)_2D$ concentrations in comparison with healthy young blood donors (57). When supplemented with vitamin D, serum 25OHD increased from very low levels (5 ng/mL) to mean levels of 15 ng/mL, but serum $1,25(OH)_2D$ was perfectly normalized. Similar studies by Need et al (58) showed a plateau level of $1,25(OH)_2D$ once serum 25OHD exceeded 6 ng/mL. Increasing serum 25OHD levels from 22 to 40 ng/mL, however, by daily administration of 2000 IU of vitamin D_3 also did not increase serum $1,25(OH)_2D$ levels (59). Vitamin D and calcium supplementation in the placebo group of a RCT with alendronate, with a mean baseline 25OHD level of 15 ng/mL, similarly did not increase serum $1,25(OH)_2D$ levels (60). Other studies confirm this conclusion: vitamin D or 25OHD supplementation did not increase serum $1,25(OH)_2D$ in subjects with a mean 25OHD baseline level of 28 ng/mL (61). Because the CYP27B1 gene is also expressed and regulated differently in many tissues outside the kidney, it is not unlikely that such tissues have requirements for optimal serum 25OHD levels that differ from that in the kidneys.

Thus, the renal production of $1,25(OH)_2D$ is perfectly normal when the substrate concentration, 25OHD, exceeds 15 ng/mL, at least in the absence of chronic kidney disease (CKD). In case of CKD, however, a different precursor–end product relationship may be operative because CKD can limit the functional CYP27B1 availability and increase FGF23 concentrations, so that higher serum 25OHD may be needed to optimize the production of $1,25(OH)_2D$; a good example is the near doubling of serum $1,25(OH)_2D$ in nonhemodialized CKD patients who received 40 000 IU of D_3 per week for 8 weeks, thereby increasing their baseline 25OHD level of 16 to 67 ng/mL (62, 63).

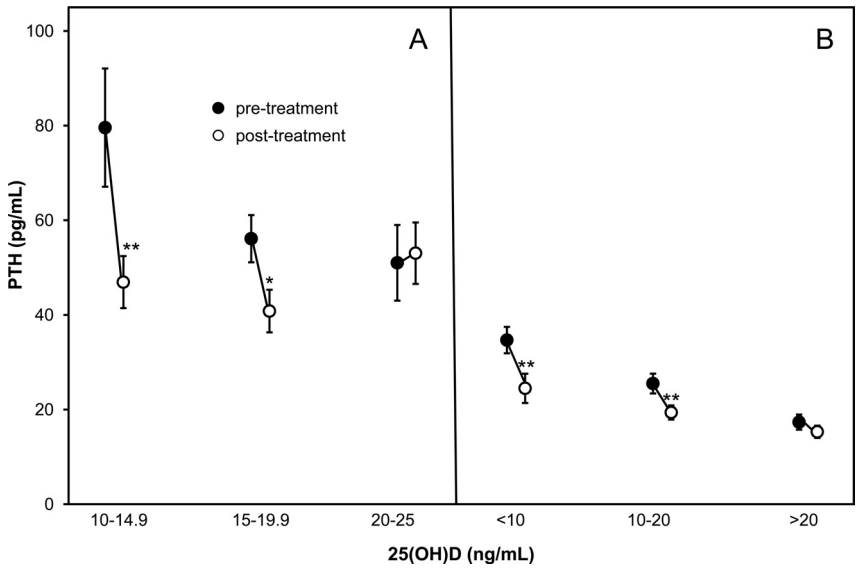

Figure 1. Changes in serum PTH concentrations (mean ± SE) in response to vitamin D supplementation of adults, according to baseline serum 25OHD concentrations. Adapted from Refs. 67 (A) and 68 (B). *, $P < .02$; **, $P < .001$.

Serum PTH as marker of vitamin D status

The parathyroid gland is the key tissue responsible for serum calcium homeostasis because it regulates the synthesis and secretion of PTH on the basis of calcium-sensing receptor signaling of serum ionized calcium. Moreover, selective deletion of the VDR or Cyp27b1 in the parathyroid gland clearly demonstrated that the vitamin D endocrine system also has direct effects on the parathyroid gland function (54, 64, 65). Therefore, it is no surprise that serum PTH has been extensively studied as a marker of vitamin D status. On the basis of a very large number of cross-sectional studies, an enormously broad range of "optimal" 25OHD levels has been defined, as reported in detail in the 2010 IOM report (27) and the subsequent discussion by Sai et al (66) and Rosen et al (20). From these reports a plateau level of serum PTH was observed when 25OHD was either as low as 12 ng/mL or up to more than 40 ng/mL. Some studies found no relation between 25OHD and PTH, whereas others found a linear relation without a threshold or plateau. There are, of course, many confounding factors in such cross-sectional studies, apart from the problems of assay methodology, such as kidney function, calcium intake, obesity, and race, and in most studies these factors were not available for correction. Therefore, the threshold of serum 25OHD below which serum PTH starts to increase is best defined by randomized vitamin D supplementation studies in (mildly) vitamin D-deficient subjects. Malabanan et al (67) observed an expected decrease in PTH when serum 25OHD was increased by administration of 50 000 IU of vitamin D_2 once weekly for 8 weeks, but such a decrease was only observed in subjects with a baseline 25OHD level of less than 20 ng/mL. Similarly, in the MORE study (68) on bisphosphonates, vitamin D and calcium supplementation in the control group decreased serum PTH only in subjects with a baseline level of less than 20 ng/mL (Figure 1). It thus seems that the parathyroid gland is "happy" with serum concentrations of 25OHD above 20 ng/mL.

Intestinal calcium absorption

Because the intestine is the primary and nonredundant target tissue for vitamin D action, it is fairly logical to use calcium absorption as the best target to assess the minimal or optimal vitamin D status. Unfortunately, however, measurement of active intestinal calcium absorption is complex, and several strategies have been developed. Moreover, there are other factors beyond the vitamin D status that influence calcium absorption, such as intake of calcium and phosphate (and other items influencing calcium solubility), age, estrogen status, body mass index (BMI), fat and protein intake, GH/IGF-I, and FGF23 (69–71). Calcium balance studies over a long period are very time-consuming but provide excellent data on total calcium requirements. They also allow good calculations of total net calcium absorption but do not reflect active, vitamin D-dependent calcium absorption. Therefore, the gold standard is the dual isotope calcium absorption, measuring simultaneously intestinal calcium uptake and endogenous calcium excretion in the gut. A careful comparison with calcium balance studies validated this method (72). The measurement of appearance of an orally given calcium isotope in serum is probably the best affordable compromise to estimate active calcium absorption in otherwise healthy individuals because it reflects the early phase and thus active uptake of the isotope, assuming probably correctly that the net calcium excretion in the gut is not affected by the vitamin D status. This method is well validated (73, 74). The measurement of small changes in total serum calcium after a high calcium load has the double handicap of relying on small changes in serum calcium (not so much higher than the variation coefficient of the measurement) and reflects the calcium influx after a high load of calcium, which is unlikely to reflect active calcium absorption (75). The relation between vitamin D status and intestinal calcium absorption has been extensively studied (20, 27, 75, 76). Most pharmacokinetic studies observed small changes in serum calcium after an acute calcium load and concluded that serum 25OHD levels of at least 32 ng/mL

were needed to optimize calcium absorption (75, 76). However, these studies have major limitations because they are based on a compilation of many studies in different subjects using slightly different study protocols and use of a methodology that reflects total and not active vitamin D-mediated absorption. Other cross-sectional studies using a more reliable single or double isotope methodology found a reasonable correlation of intestinal calcium absorption with serum levels of $1,25(OH)_2D$ but not with serum 25OHD, either in postmenopausal women or osteoporotic patients (74, 77, 78) or in young adolescents (79). Similarly, a marked decrease in serum 25OHD from late summer (49 ng/mL) to late winter (30 ng/mL) did not decrease intestinal calcium absorption (37). Therefore, we reanalyzed all published RCTs dealing with intestinal calcium absorption before and after vitamin D supplementation (Supplemental Table 3). A huge (600 000 IU) im dose of D_3 did not increase intestinal calcium absorption in Pakistani young adults with a baseline serum 25OHD level of 17 ng/mL and a level of 27 ng/mL 2 months later (80). After higher vitamin D supplementation, a small increase (74, 81) of a few percent contributes minimally to the overall calcium balance, especially in view of the large interindividual variation in calcium intake, calcium absorption, and other factors influencing calcium intake (70) (Supplemental Table 3). Intestinal calcium absorption was, however, decreased when serum 25OHD levels were really very low (below 4 ng/mL) (58), and in this study the absorption already reached a plateau when serum 25OHD was higher than 8 ng/mL. In such vitamin D-deficient subjects, a positive correlation between calcium absorption and 25OHD levels was found, but the correlation with serum $1,25(OH)_2D$ was still stronger. On the contrary, when the intake of vitamin D or 25OHD is truly pharmacological (more than 100 μg of 25OHD per day), intestinal calcium absorption increases together with increased serum and urinary calcium, reflecting the activity of 25OHD as VDR agonist because the same effect is also found in patients with CKD (82). Supplementation with 1000 IU of vitamin D_3 per day to adolescent girls with 25OHD baseline levels of 14 ng/mL also did not increase intestinal calcium absorption (83).

In conclusion, and giving more weight to intervention studies with reliable calcium absorption studies, no increase in intestinal calcium absorption can be observed after vitamin D supplementation when the baseline 25OHD level exceeds 10–15 ng/mL. High pharmacological doses of vitamin D or 25OHD, however, can mimic the effects of $1,25(OH)_2D$ probably by acting as a poor vitamin D agonist for VDR.

Bone histomorphometry

Bone histology and especially careful dynamic histomorphometry can reveal details of bone remodeling including impaired mineral deposition. Although increased osteoid surface and thickness are hallmarks of rickets or osteomalacia, their presence is, inversely, no proof of vitamin D deficiency because other mechanisms such as calcium or phosphate deficiency or even high bone turnover can generate similar histological abnormalities. Cross-sectional studies (n = 19) of bone biopsies in patients with hip fracture generally show low percentages of osteomalacia when osteoid thickness and osteoid volume are used as criteria, but higher percentages up to 37% when osteoid surface is used as the criterion (84). However, increased osteoid surface is a general finding in bone biopsies of patients with hyperparathyroidism, and secondary hyperparathyroidism is the first stage of vitamin D deficiency in the bone. There are no real RCTs with vitamin D supplementation using (dynamic) bone histomorphometry as the endpoint in humans. However, there is 1 recent, large (n = 675; mean age, 59 y) and meticulous cross-sectional study on German subjects who died suddenly and in whom serum 25OHD measurements and static bone histomorphometry were performed. The authors concluded that serum levels above 30 ng/mL avoid osteoid excess and therefore defined this as the lowest threshold for bone health (85). The IOM report and subsequent discussion by the IOM authors (26, 86) did not consider this as a valid conclusion, unlike The Endocrine Society Task Force authors (28, 87) and several other commentators (76, 88). The conclusion of the authors may well be premature for several reasons. First, the serum 25OHD levels are unlikely to represent the true vitamin D status in Germany because the mean levels are extremely low (about 8 ng/mL) and thus less than half that of other large-scale studies in the German population (89, 90). Because 25OHD was measured in postmortem samples, hemolysis, protein autolysis, or other matrix effects may have caused problems of accuracy. Secondly, the interpretation of the histology overestimates the prevalence of osteomalacia by choosing an upper reference limit for osteoid volume at 2%, whereas 5% is usually quoted (91). Using this threshold, less than 5% of the total population in the Priemel study has increased osteoid volume/bone volume, and no osteoid excess was observed if serum 25OHD was ≥20 ng/mL. In addition, normal osteoid thickness was observed in the vast majority of vitamin D-deficient subjects. Finally, the histomorphometry study was a cross-sectional study, not an intervention study, and therefore the causal relationship is still to be proven.

Bone mineral density (BMD)

Several cross-sectional studies do not agree on the 25OHD threshold level needed to maximize BMD or content. In the analysis of the National Health and Nutrition Examination Survey (NHANES) data (92) and in the Longitudinal Aging Study Amsterdam (LASA) study (93), BMD decreased when serum 25OHD fell below 20 ng/mL, but there is discordance between both studies for the consequences of higher levels. In an overview of 11 intervention studies in elderly subjects (94), a modest increase (1–3%) in BMD was observed after supplementation with vitamin D (400–900 IU/d) with or without calcium, except in the Chapuy study of very vitamin D-deficient elderly subjects where a higher increase in BMD was observed. A higher dose of vitamin D, however, did not improve BMD values in less vitamin D-deficient subjects (95, 96). Similarly, supplementation with 25OHD (15 μg/d for 4 y) of postmenopausal women (97) did not increase BMD, despite an increase of serum 25OHD from mean levels of 24 to 48 ng/mL.

Therefore, it seems that BMD does not improve with vitamin D supplementation once baseline levels of 25OHD are above 20 ng/mL.

Evidence-based medicine proof of biological activity of vitamin D on fractures

A negative calcium balance, which is common in the elderly due to poor dietary calcium intake and vitamin D deficiency, leads to secondary hyperparathyroidism with osteoporosis and osteoporotic fractures. Meta-analyses comparing the effect of vitamin D alone with placebo showed that vitamin D in monotherapy is insufficient for fracture prevention (Supplemental Table 4). This is not surprising because the negative calcium balance in elderly individuals often results from low vitamin D and low serum calcium (98). For example, in a meta-analysis of 4 RCTs in 9083 patients by Boonen et al (99), 400–800 IU vitamin D per day did not significantly reduce the risk of hip fracture vs placebo (relative risk [RR], 1.10; 95% confidence interval [CI], 0.89, 1.36). This remained true when the 2 trials that used the higher dose of vitamin D (700–800 IU/d) were analyzed separately (RR, 1.04; 95% CI, 0.75, 1.46) (99). Also, in the meta-analysis of the DIPART group, a daily dose of 400–800 IU of vitamin D alone was no more effective than placebo in the prevention of any fractures (hazard ratio [HR], 1.01; 95% CI, 0.92, 1.12), hip fractures (HR, 1.09; 95% CI, 0.92, 1.29), or vertebral fractures (HR, 1.12; 95% CI, 0.70, 1.79) (100). Finally, the authors of a recent Cochrane Review also conclude that vitamin D alone is unlikely to be effective in preventing any fractures (RR, 1.01; 95% CI, 0.93, 1.09), hip fractures (RR, 1.15; 95% CI, 0.99, 1.33) or vertebral fractures (RR, 0.90; 95% CI, 0.42, 1.92) (101).

In contrast, several meta-analyses (Supplemental Table 4) have convincingly shown a reduction in fracture risk when vitamin D is combined with calcium and, compared with placebo, calcium alone or vitamin D alone. Compared with placebo, combined calcium and vitamin D supplementation reduced the risk of nonvertebral fractures by 12% (RR, 0.88; 95% CI, 0.78, 0.99) and the risk of hip fractures by 18% (RR, 0.82; 95% CI, 0.71, 0.94) in a meta-analysis of 6 RCTs in 45 509 patients by Boonen et al (99). Exclusion of the Women's Health Initiative (WHI) trial of Jackson et al (102), the only trial that used 400 IU of vitamin D instead of 700–800 IU, increased the risk reduction of hip fractures from 18 to 21% (RR, 0.79; 95% CI, 0.64, 0.97) (99). The meta-analysis of the DIPART group similarly showed that, contrary to vitamin D alone, combined calcium and vitamin D supplementation significantly reduced the risk of any fractures (HR, 0.92; 95% CI, 0.86, 0.99) and hip fractures (HR, 0.74; 95% CI, 0.60, 0.91) (100). Also in the aforementioned Cochrane Review, calcium plus vitamin D reduced the risk of hip fractures (RR, 0.84; 95% CI, 0.73, 0.96) (101). The meta-analysis of Tang et al (103) compared the antifracture efficacy of calcium and vitamin D with that of calcium alone. The combination of calcium plus vitamin D was associated with a 13% reduction in the risk of any fractures (RR, 0.87; 95% CI, 0.77, 0.97), but surprisingly, calcium alone was equally effective (RR, 0.90; 95% CI, 0.80, 1.00; $P = .63$) (103). Yet, in other meta-analyses, calcium in monotherapy was unable to reduce fracture risk (104). Subgroup analyses showed that the reduction in fracture risk was greatest with a calcium dose of 1200 mg or more and a vitamin D dose of 800 IU or more (103). However, some individual trials, including the WHI trial of Jackson et al (102) and the RECORD trial of Grant et al (105), failed to demonstrate a reduction in fracture risk despite supplementation with calcium and vitamin D. This suggests that the antifracture efficacy of calcium and vitamin D supplementation is determined by additional factors, like the dose of vitamin D, targeting of supplementation to persons with insufficiencies and persistent therapeutic compliance (98). Fracture prevention requires a relatively high dose of vitamin D supplementation. In a meta-analysis of the effect of vitamin D on the risk of fractures, Bischoff-Ferrari et al (106) showed that the risk reduction of hip fractures became statistically significant only after pooling those trials that used a "received" dose of vitamin D of more than 400 IU per day (482–770 IU; RR, 0.82; 95% CI, 0.69, 0.97). Also, the more recent per-patient meta-analysis of Bischoff-Ferrari et al (107) of 11 double-blind RCTs of oral vitamin D supplements with or

without calcium concluded that only individuals who took the largest amount of vitamin D experienced a reduced risk of hip fracture and any type of nonvertebral fracture. This analysis confirmed that only the highest "actual-intake" level of vitamin D (792–2000 IU; median dose of 800 IU/d) could reduce the risk of hip fractures (HR, 0.70; 95% CI, 0.58, 0.86) and nonvertebral fractures (HR, 0.86; 95% CI, 0.76, 0.96) (107). The U.S. Preventive Services Task Force also confirmed this conclusion (108). The extensive literature therefore can be summarized as follows. First, to reduce fracture risk, 700–800 IU or at least a dose in excess of 400 IU of vitamin D is required (99, 103, 107). Second, calcium and vitamin D supplementation needs to be targeted to persons with documented risk of calcium and/or vitamin D deficiency. Finally, poor compliance, which was estimated at only 40–60% in some of these trials (102, 105) may jeopardize the benefits. After discontinuation of supplementation therapy, bone turnover rate normalizes, and increases in BMD are lost (109, 110). Therefore, compliance and persistence with calcium and vitamin D supplementation is essential to obtain and keep therapeutic benefit (98). Most intervention studies have used vitamin D_3 rather than vitamin D_2, and therefore the therapeutic efficacy is better validated for vitamin D_3. High intermittent dosing of vitamin D (300 000 IU per injection or 500 000 IU orally every 12 mo) may not be safe for bone because 2 such studies revealed an increased risk of fractures (111, 112) and falls (111). Such high levels of 25OHD and $1,25(OH)_2D$ (which may be generated by overruling the normal feedback mechanisms as discussed above) may have surprising effects on bone because high levels of $1,25(OH)_2D$ are able to enhance bone resorption but may also directly inhibit mineralization by a coordinated regulation of genes involved in the mineralization process in mice (113). High serum $1,25(OH)_2D$ concentrations were also associated with increased bone resorption markers and with lower bone density in adult and elderly men (114).

In conclusion, strong scientific evidence supports the combined use of calcium and vitamin D supplementation in the prevention of osteoporotic fractures (Supplemental Table 4). These studies agreed on the minimal dose of vitamin D supplementation, but unfortunately they poorly documented the 25OHD levels before and after vitamin D supplementation, hampering definitive conclusions about optimal 25OHD levels for fracture prevention. A daily dose of 800 IU of vitamin D_3, however, generally increases the serum level of 25OHD above 20 ng/mL (50 nmol/L) (56, 115).

Optimal Vitamin D Status for General Health

Overwhelming data point to a role for the vitamin D endocrine system on general health, over and above calcium and bone metabolism (6, 17, 20, 22, 116–118). This conclusion is based on many in vitro genetic, molecular, and cellular data (6, 22) and is in line with the nearly universal expression of the VDR, the presence of the activating enzyme CYP27B1 in many tissues, and above all the large number of genes regulated by the vitamin D hormone. The extraskeletal effects are well demonstrated in animal models with either simple vitamin D deficiency (systemic or tissue selective), deletion of vitamin D metabolism (cyp27b1 null mice), or disruption of vitamin D action (deletion of VDR) (6, 22). A large number of observational studies in humans, whether cross-sectional or prospective, confirm the preclinical data on beneficial effects of the vitamin D endocrine system in many systems without a clear link to bone or calcium metabolism. The extraskeletal effects include effects on cell proliferation and differentiation (and thus cancer), the native and acquired immune system (and thus on infections and autoimmune diseases), the cardiovascular system (and thus on heart and vascular health), metabolic homeostasis (and thus on energy regulation and all aspects of the metabolic syndrome), reproduction, and the neurological system (including brain function).

Because the preclinical data are typically based on studies whereby the vitamin D endocrine system is totally eliminated or when cells or tissues are exposed to a supraphysiological concentration of the active hormone or its analogs, the major remaining questions are whether these observations can be translated to humans, and what degree of vitamin D deficiency or excess can generate these extraskeletal effects in humans (Figure 2). Moreover, it is plausible (but yet to be proven) that different target tissues require different prevailing 25OHD concentrations. A good comparison is that of iodine deficiency (another nutritional precursor for a nuclear receptor ligand) because mild to severe deficiency can cause variable degrees of goiter, hypothyroidism, or several types of irreversible (intrauterine or postnatal) brain damage. Although preclinical and observational studies make assumptions on a detrimental role of vitamin D deficiency on health and a potential benefit of supplementation of vitamin D in disease plausible, only well-performed large-scale clinical intervention studies can solve the question of causality and dosages or the optimal vitamin D status. Therefore, we will focus the discussion on such RCTs.

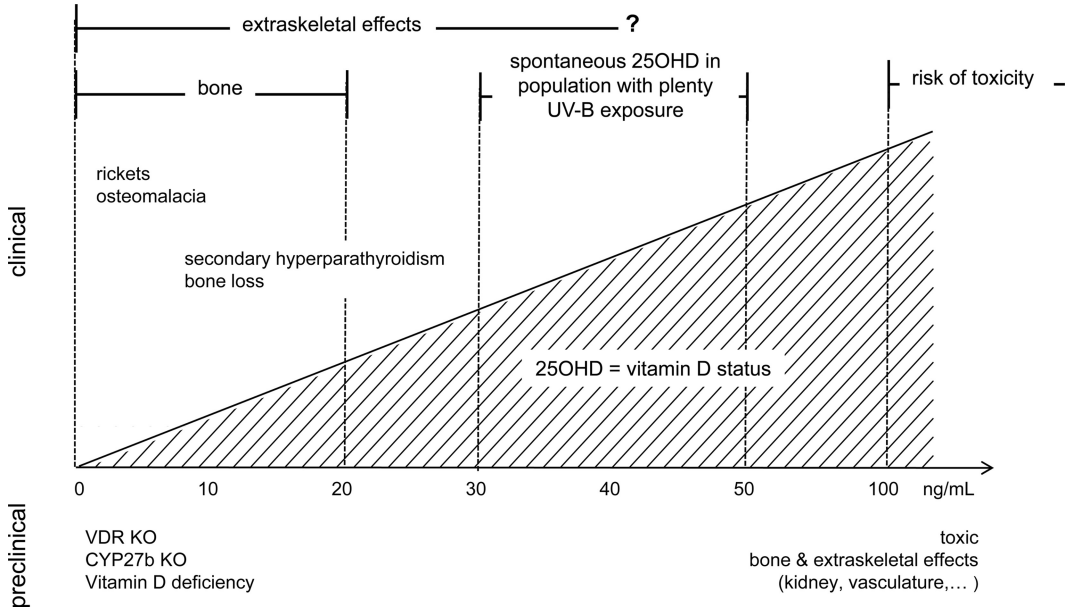

Figure 2. Possible relationship between vitamin D status and skeletal and extraskeletal health effects.

Vitamin D Status and Cancer

The antiproliferative effect of $1,25(OH)_2D$ on cancer cells has been known since 1981 (119, 120) and has been confirmed in most normal and cancerous cells with inhibition of the cell cycle at the G_1 stage. More malignant cells may lose their sensitivity to $1,25(OH)_2D$ by a variety of mechanisms such as loss of VDR expression or loss of postreceptor signaling. By contrast, overexpression of the catabolizing enzyme, CYP24A1, is fairly frequent in malignant cells and was even described as an oncogene in breast cancer (reviewed in Refs. 6 and 121–123). The inhibition of cell cycle progression can be explained by the effects of $1,25(OH)_2D$ on a very large number of genes that are coherently regulated and include effects on E2F transcription factors, Rb phosphorylation, cyclin-dependent kinase activity, c-myc expression, TGF-β, and prostaglandin signaling (reviewed in Refs. 6, 122, and 123). Moreover, $1,25(OH)_2D$ can inhibit angiogenesis, induce apoptosis, and decrease inflammation, invasion, and metastasis. VDR-deficient mice do not spontaneously develop more cancers but are more prone to a variety of cancers when exposed to oncogenes, loss of antioncogenes, or exposure to chemocarcinogens or UV-B light (22, 124). There is abundant literature on the relation between UV-B exposure or vitamin D status and a large number of cancers. The World Health Organization-sponsored International Agency for Research on Cancer report (125) concluded that a low vitamin D status was associated with a higher risk of bowel cancer, but the data for other cancers was inconsistent. Essentially, this is also the conclusion of the IOM report (27) and the U.K. Consensus Vitamin D Position Statement from a large number of U.K. scientific organizations, including Cancer UK (126). The association between a low vitamin D status and colon cancer is, however, fairly consistent as reported in several meta-analyses (108, 127–130). Several meta-analyses (127, 130, 131) of epidemiological studies indeed found a significantly increased risk of colorectal cancer in subjects with the lowest vs the highest quintile of serum 25OHD, and more careful analysis revealed that serum 25OHD levels below 20 ng/mL conveyed the greatest risks. Serum 25OHD levels above 30 ng/mL did not provide further risk reduction in an analysis of more than 10 cohort studies (132). In some studies, high 25OHD levels (>40 ng/mL) were associated with increased risk for pancreatic carcinoma (133). No clear relation, however, between vitamin D status and prostate cancer incidence or aggressive type of cancer could be found (134), although more recent data suggest an inverse relationship (135). There are unfortunately only a few RCTs that address the question of vitamin D status and cancer (reviewed by Refs. 108 and 136), indicating that the 3 existing studies could not detect a significant effect on cancer incidence after vitamin D supplementation (400–1100 IU/d for up to 8 y of follow-up). Larger ongoing studies such as the VITAL study (137) or other large-scale trials (138) are needed to provide more decisive conclusions. The overall conclusion at present is therefore that the link between vitamin D status and cancer is plausible, but causality is not proven. Therefore, supplementation cannot be recommended for the sole purpose of primary or secondary prevention of cancer. 25OHD levels below 20 ng/mL convey the greatest association with cancer, but levels above 40 ng/mL are also associated with an increased risk for cancer, although

less strongly than for low vitamin D status (22, 122, 126, 128, 139).

Vitamin D Status and the Immune System

All cells of the immune system express VDR, whether at the basal state (such as antigen-presenting or dendritic cells) or when activated by immune signals (such as T lymphocytes). A large number of immune-related genes are coherently controlled by $1,25(OH)_2D$ (140). The native immune system, largely controlled by macrophages, is stimulated by $1,25(OH)_2D$, whereas the acquired immune system (especially T lymphocytes and B lymphocytes) is suppressed, largely due to its effects on the antigen-presenting cells and their signaling to the T lymphocyte population (Th1, Th17, and Th2 cells) (for review, see Refs. 141 and 142). The generation of T regulator cells is enhanced via direct and indirect effects (140).

$1,25(OH)_2D$ is known to inhibit multiplication of *Mycobacterium tuberculosis* in vitro (143, 144), and further details about its mechanism of action have been revealed more recently (142, 145, 146). A low vitamin D status and clinical rickets are associated with an increased risk for all types of infections, especially pulmonary infections and tuberculosis (147–152). Intervention studies are equivocal because some studies showed positive (albeit transient) effects on viral respiratory infections in Japanese children (153) or on sputum clearance of patients with active tuberculosis (154). Larger RCTs, however, could not confirm a positive effect of vitamin D therapy of patients with active tuberculosis with good (155) or poor (156) vitamin D status at baseline. In chronic obstructive pulmonary disease (COPD) patients, treatment with high doses of vitamin D could decrease neither the incidence nor the time to exacerbation, except in patients with the most severe vitamin D deficiency at baseline (25OHD level below 10 ng/mL) (157). A more recent study on vitamin D-deficient children in Kabul could not demonstrate a beneficial effect of vitamin D supplementation (at the equivalent of 3000 IU/d) on the incidence of pneumonia (158), but a similar study in Mongolian children with severe vitamin D deficiency revealed a nearly 50% lower rate of viral infections even by a low-dose (300 IU/d) vitamin D supplementation (159). A Cochrane database analysis concluded that there are as yet insufficient data to define a role for vitamin D in the prevention or treatment of tuberculosis (160).

The link between vitamin D and autoimmune diseases is suggested in genetic studies, where polymorphisms in genes involved in the vitamin D metabolism are linked to autoimmune diseases like type 1 diabetes and multiple sclerosis (161). In vitro data and animal models confirm a potential role for the system because vitamin D deficiency leads to a higher prevalence of autoimmune disease, whereas treatment with high doses of vitamin D, $1,25(OH)_2D$, or its analogs prevents autoimmune disease (141). In humans, epidemiological studies confirm such associations, but intervention studies until now fail to show preventive effects (162).

Effect of Vitamin D on Muscle Strength, Balance, and Falls

Vitamin D deficiency is associated with muscle weakness and falls. Vitamin D can influence muscle strength through genomic and nongenomic pathways (163, 164). The VDR was demonstrated in human muscle tissue by immunohistochemistry with a decreasing number of receptors with aging (165, 166). Recently, however, other investigators using highly specific VDR antibodies did not find the receptor in adult muscle tissue (167). The active vitamin D metabolite, $1,25(OH)_2D$, influences muscle fiber proliferation and differentiation. It may influence calcium influx and phosphate transport. Part of the effect on muscle may result from receptors in the nervous system influencing the metabolism of neurotransmitters or stimulating neural growth factor and thus indirectly influencing muscle activity (163). Skeletal muscle development is abnormal in VDR null mice with a smaller size of muscle fibers than in the wild-type mouse (168). A clever in vivo study recently used ^{32}P nuclear magnetic resonance spectroscopy of skeletal muscle of severely deficient patients (169) and revealed that vitamin D supplementation improved mitochondrial ATP (or energy) production after modest exercise. A critical analysis revealed a wide range of potential mechanisms, including decreased intracellular calcium storage (170).

Vitamin D status was associated with walking performance and chair stand test in the NHANES (171). Similarly, a positive association was observed in the LASA between serum 25OHD and physical performance score, assessed by a walking test, 5 chair stands, and the tandem stand (172). Optimal physical performance was obtained when serum 25OHD was between 20 and 24 ng/mL (50 and 60 nmol/L). A low serum 25OHD also predicted a decrease in physical performance over the next 3 years (172). In the LASA study, vitamin D status also predicted loss of muscle mass and fall risk (173, 174).

The effect of vitamin D supplementation on muscle strength and balance has been studied in several RCTs (Table 2). Vitamin D supplementation (400 IU/d) improved body sway by 9% in ambulatory women 70 years

Table 2. RCTs of Vitamin D Supplementation, With or Without Calcium, on Fall Incidence

First Author (Ref.)	No. of Patients	Study Type	Vitamin D Dose	Calcium, mg/d	25OHD, ng/mL		Outcome, No. of Fallers
					Baseline	Post-Treatment	
Graafmans (179)	330	db	400 IU/d	—	11	21	NS
Pfeifer (175)	148	db	800 IU/d	1200	11	26	−40%
Latham (177)	243	db	300 000 IU	—	16	24	NS
Harwood (180)[a]	150	o	800 IU/d	1000	12	20	−52%
Flicker (181)	625	db	1000 IU/d	600		<16	−27%
Bischoff-Ferrari (182)	445	db	700 IU/d	500	28	41	−46% (women)
Law (183)	223	o	100 000 IU/3 mo	—	19	32	NS
Broe (184)[b]	124	db	800 IU/d	—	21	30	−72%
Prince (185)	302	db	1000 IU/d	1000	18	24	−19%
Pfeifer (186)	242	db	800 IU/d	1000	22	33	−27%
Kärkkäinen (187)	1645	o	800 IU/d	1000	20	30	NS[c]
Sanders (111)	2256	db	500 000 IU/y	—	20	48[d]	+15%

Abbreviations: db, double blind; o, open; NS, no significant change; —, no calcium supplementation.

[a] Four arms in Harwood study: 1) vitamin D_2, 300 000 IU injection once; 2) vitamin D_2, 300 000 injection + calcium 500 mg twice daily; 3) vitamin D_3, 400 IU and calcium 500 mg twice daily; 4) no treatment.

[b] Five arms in Broe study: 200, 400, 600, and 800 IU/d, or placebo; significant decrease of participants with falls in the 800-IU group only.

[c] 30% less multiple falls.

[d] After 1 month, 48 ng/mL; after 3 months, 36 ng/mL.

of age and older (175). Vitamin D in a single dose of 300 000 IU did not improve physical performance in frail older persons compared with placebo, even in those who were vitamin D deficient (serum 25OHD < 12 ng/mL at the onset) (176). A vitamin D_3 dose of 8400 IU/wk did not improve sway, measured on a sway platform, in healthy older persons with a low serum 25OHD at baseline, in comparison with placebo (177). However, subgroup analysis of those with a high sway at baseline showed an improvement after this high vitamin D dose. A meta-analysis of sway and the timed up and go test showed a significant improvement of these tests compared to placebo, but an improvement of lower extremity strength did not occur (178).

At least 12 clinical trials with vitamin D vs placebo have been performed with the incidence of falls as the primary outcome (111, 175, 177, 179–187). Seven studies, 6 of these combining vitamin D and calcium vs double placebo, showed a significant decrease of the incidence of falls ranging from −19% to −70%. Four studies had a neutral outcome, and 1 study from Australia showed an increased fall incidence after supplementation. In the latter study comparing vitamin D 500 000 IU once yearly vs placebo, the fall incidence was about 15% higher in the vitamin D group than in the placebo group (111). Increased fall incidence was mainly visible in the first 3 months after the vitamin D dose, and this was associated with a high serum 25OHD (>50 ng/mL). The studies in which the fall risk decreased used vitamin D doses between 800 and 1000 IU/d. A recent clinical trial comparing 800 and 2000 IU/d on complications in patients with hip fracture showed that 2000 IU was not more effective than 800 IU/d concerning fall prevention (96). Another recent study in malnourished older adults with vitamin D and protein supplementation showed a decrease of fall risk (188).

Recently, several meta-analyses on this subject have been published (189–191). The Cochrane Review did not show a significant effect of vitamin D supplementation on the number of fallers, although the rate of falls decreased in nursing home residents (189). The other 2 meta-analyses showed a decrease in the number of fallers after vitamin D supplementation (190, 191). In conclusion, mechanistic studies suggest effects of vitamin D on muscle tissue, and epidemiological studies show strong relationships between vitamin D status, physical performance, and falls. RCTs demonstrate that vitamin D supplementation in target groups with low vitamin D status can improve physical performance and decrease fall risk. This is also the recommendation from the U.S. Services Task Force for the prevention of falls in community-dwelling older adults (192).

Vitamin D Status and the Cardiovascular System

An extensive 2011 review concluded that 25OHD levels below 25 ng/mL are consistently associated with increased risks of cardiovascular endpoints (193), and several other very long-term prospective studies confirm an association between low vitamin D status and cardiovascular events or stroke (194–197). However, intervention studies are still inconclusive (20, 193, 198). Indeed, the most recent extensive meta-analyses concluded that there is a significant association between low 25OHD levels and cardio-

vascular risks and events, but that levels above 25 ng/mL do not further improve the potential benefit (193, 198). The most recent RCTs of vitamin D supplementation could also not demonstrate beneficial effects on cardiovascular risk factors or events (199–202). Even for a well-studied endpoint such as hypertension, the same conclusion holds, ie, strong association data but inconsistent results from RCTs (203–205). Patients with CKD, known to be associated with deficiency of both 25OHD and $1,25(OH)_2D$, carry a high risk of cardiovascular mortality. These risks can be improved by treatment with vitamin D metabolite or analogs (206), but too much vitamin D is also a major risk for vascular calcifications in these patients (207, 208). This is another example of a possible U-shaped relationship between vitamin D status and major outcome parameters.

Vitamin D Status and Metabolism

There are overwhelming data on an association between low vitamin D status and obesity and most if not all aspects of the metabolic syndrome (6, 20, 209–214). The question is, however, whether this association is causative or due to reverse causation, or whether it just originates by association with common lifestyle factors. Vitamin D supplementation has not yet (215–218) convincingly shown a positive effect on evolution of body weight or glucose control except for minor changes in insulin sensitivity (219). Data from vdr- or cyp27b1-deficient mice as well as from mice with transgenic overexpression of VDR in adipocytes, however, clearly revealed a major energy phenotype with low fat mass, resistance to diet-induced insulin resistance, and obesity all due to increased thermogenesis in mitochondria (220). The animal data thus indicate that severe vitamin D deficiency protects against obesity, whereas in humans low vitamin D status is associated with obesity and metabolic syndrome. Further studies are clearly needed to clarify this dilemma and to help guide clinical decision making for treatment or not of low vitamin D status in human obesity.

Vitamin D Status and Mortality

If the vitamin D status would have indeed so many extraskeletal effects on cancer, immune function, cardiovascular and metabolic risks, then a link with mortality would be expected. Cross-sectional data showed a significantly increased mortality risk for subjects with low 25OHD levels (reviewed in Refs. 6, 196, 197, and 221) with some suggestions for a U-shaped curve so that higher 25OHD levels would also convey a higher mortality risk. A recent long-term follow-up study, however, concluded that lower levels of 25OHD were associated with a better longevity in offspring of Dutch nonagenarians, and these lower 25OHD levels were associated with a lower frequency of the "most active" polymorphisms of CYP2R1, the major 25-hydroxylase (222). Intervention studies used mortality only as a secondary endpoint, and several meta-analyses have yielded conflicting results because the confidence limits (CLs) included 1 or were close to 1 (223) (odds ratio [OR] = 0.92; CL, 0.86–0.99) (198) (OR = 0.96; CL, 0.93–1.00). The most recent meta-analysis (224) analyzing 8 RCTs in more than 70 000 subjects found a modest decrease in overall mortality risk (OR = 0.94) by combined supplementation with calcium and vitamin D, but not with vitamin D alone.

Vitamin D Status and Extraskeletal Health: Conclusions

The vitamin D endocrine system is likely to have many extraskeletal effects, at least based on a very large body of preclinical data generated in cellular or animal models of severe vitamin D deficiency or exposure to supraphysiological concentrations of the vitamin D hormone. This hypothesis is further supported by many observational studies in men linking poor vitamin status with nearly all major diseases of mankind. Intervention studies, however, are equivocal. Careful meta-analysis of published RCTs using vitamin D (with or without calcium) supplementation generated beneficial effects on risk of falls and maybe on overall mortality risk of elderly subjects. The doses of vitamin D used in these studies (around 800 IU/d) were probably able to raise serum 25OHD levels above 20 ng/mL in nearly all subjects but are unlikely to increase such levels above 30 ng/mL (56). Effects on cancer, immune diseases, and infections or cardiovascular and metabolic risks or events are not firmly established so that optimal serum 25OHD levels for such extraskeletal effects cannot be defined.

As long as RCTs have not formally proved causality, there is still a possible scenario of reverse causation or that the associations just reflect lifestyle or environmental effects. It also remains possible that the link between vitamin D status and extraskeletal outcome is only operative at the extreme levels of very low or very high vitamin D status (Figure 2). Some experts have argued for higher vitamin D intake in view of the natural origin and high safety profile of vitamin D and the possibility that some extraskeletal endpoints may require higher vitamin D status than for bone health (17, 24, 76, 225). However, some cross-sectional studies found a U-shaped relationship between serum 25OHD levels and

cancer, falls and fractures, cardiovascular events or mortality, as discussed above (111, 133, 207, 208, 221, 222, 226). In addition, the frequency of subtle mutations of CYP24A1, capable of induction of severe hypercalcemia and hypercalciuria when exposed to extra vitamin D intake (54, 227, 228), in the general population is yet unknown and could be problematic when high-dose vitamin D therapy would be generalized. For these reasons, the principle of "primum non nocere" should restrict the use of higher than recommended dosages of vitamin D for the general population, pending further proof of efficacy and safety in RCTs.

Vitamin D Requirements Based on Metabolism of Vitamin D

The vitamin D requirements of subjects with low exposure to UV-B light are similar to substitution therapy for hormone deficiencies such as hypothyroidism or adrenal insufficiency, whereby the daily endogenous production rate can be used to define the replacement needs. The metabolic clearance of 25OHD has been measured by using radiolabeled vitamin D and found to vary between 10 and 40 days (229–231), with increased clearance in case of

Table 3. Overview of RCTs Using a Daily Dose of Vitamin D ≥ 2000 IU/d (or Equivalent)

First Author, Year (Ref.)	n	Mean Age, y	Vitamin D, IU/d	Months	Endpoint	Result	25OHD, ng/mL Baseline	25OHD, ng/mL After R/
Mikati, 2006 (249)	184	13	2000	12	BMD	±	11	28
Zittermann, 2009 (250)	82	47	3332	12	Weight	ns	26	45
Jorde, 2010 (202)	150	46	6000	12	Glucose, high BMI	ns	24	56
Aloia, 2010 (78)	78	20–80	4000	4	Bone turnover markers	ns	26	45
von Hurst, 2010 (219)	106	41	4000	6	Insulin sensitivity	+	8	30
von Hurst, 2010 (251)	81	41	4000	6	Bone turnover markers	±	8	30
Bischoff-Ferrari, 2010 (96)	173 hip fractures	84	2000	12	Falls	−	13	45
Martineau, 2011 (156)	126	30	10 000	1.5	TB	ns	8	40
Grimnes, 2011 (218)	49	51	6000	6	Insulin sensitivity	ns	17	58
Grimnes, 2012 (252)	149	63	6500	12	BMD	ns	28	46
Mozaffari-Khosravi, 2012 (253)	45 gestational DM	30	3333 (300 000 bolus)	3	Insulin resistance	+	10	25
Rastelli, 2011 (254)	60 breast cancer with aromatase inhibitor-induced musculoskeletal symptoms	60	7142 (2 mo or 4 mo), then 1666 (4 mo or 2 mo)	6	Musculoskeletal pain at 2 mo Musculoskeletal pain at 6 mo	+ ns	23	30 (6 mo)
Bock, 2011 (255)	59	32	4666	3	Regulatory T cells β-cell function	+ ns	25.5	?
Stein, 2011 (256)	23 relapsing-remitting MS	34	12 000	6	Lesions on brain MRI	ns	24	48
Lehouck, 2012 (157)	182	68	3300	12	COPD exacerbation	ns	20	52
Coussens, 2012 (257)	95 antimicrobial TB therapy	30	10 000	1.5	Sputum smear conversion Resolution inflammatory response	+ +	8	40
Beilfuss, 2012 (258)	332 28 < BMI > 47	50	2857 or 5714	12	IL-6 C-reactive protein TNF-α Insulin resistance	+ − ns ns	22	40
Osunkwo, 2012 (259)	46 sickle cell disease with chronic pain	13	5714–14 285	6 wk	Pain days Physical activity QoL scores	+ +	20	sign ↑
Rossini, 2012 (260)	36	76	6666 (600 000 bolus)	3	Bone turnover markers	− (d 3) ns (d 90)	22	67 (d 3) 35 (d 90)
Alvarez, 2012 (261)	46 CKD stage 2–3	62	7142 (12 wk), then 3571 (40 wk)	12	Blood pressure Serum FGF-23	ns ns	27	43 (12 wk) 40 (12 mo)
Grossmann, 2012 (262)	30 cystic fibrosis	25	2976 (250 000 bolus)	12 wk	IL-6 TNF-α	+ trend +	31	58 (1 wk) 37 (12 wk)
Schreuder, 2012 (263)	84	42	3570 (150 000 bolus)	6 wk	Musculoskeletal pain	+	8	25
Davidson, 2012 (264)	109 prediabetes	52	12 695	12	Insulin secretion and sensitivity Development of DM	ns ns	22	70
Hornikx, 2012 (265)	50 COPD patients in revalidation program	68	3300	3	Inspiratory muscle strength Maximal oxygen uptake Quadriceps strength 6-min walking distance	+ + ns ns	15	51
Kjærgaard, 2012 (266)	230	53	5714	6	Depressive symptoms	ns	19	59
Khoo, 2012 (267)	16 HIV infection	33	3571	2	Expression of chemokine receptors on regulatory T cells	±	12	55
Tellioglu, 2012 (268)	66	75	6666 (im or oral) (600 000 bolus)	3	Quadriceps muscle strength SPPB score	+ +	12 15	52 (im) 43 (oral)

Abbreviations: TB, tuberculosis; DM, diabetes mellitus; MS, multiple sclerosis; MRI, magnetic resonance imaging; QoL, quality of life; SPPB, short physical performance battery; +, improvement in health outcome/surrogate marker; −, deterioration in health outcome/surrogate marker; ns, no significant change; R/, after vitamin D supplementation. Overview of RCTs with a daily dose (or equivalent) of ≥2000 IU of vitamin D that reported a health or surrogate endpoint for health other than 25OHD level. Update is based on literature search in Pubmed, November 2012.

calcium deficiency or hyperparathyroidism. More recently, such studies have been repeated in U.K. and Gambian healthy subjects using deuterium-labeled 25OHD, and the mean half-life of 25OHD was 15 days in both groups (231). The plasma pool of 25OHD is very similar to that of vitamin D binding protein and is close to the plasma or extracellular volume (232), ie, about 6 to 9 L in a normal adult. Therefore, the total amount of circulating

Table 4. Overview of Ongoing RCTs With High Vitamin D Doses

Principal Investigator	n (Total)	Age Range, y	Vitamin D (IU/d)	Months	Primary Endpoint	Study Acronym	Estimated Completion Date
Borchhardt	200 (kidney transplant recipients, 25OHD < 20 ng/mL)	18–85	6800 IU, placebo	12	Glomerular filtration rate, no. of acute rejection episodes, no. of infections, CRP levels, courses of calcium levels	VITA-D	May 2014
Aloia	250 (African American women, ambulatory, 25OHD 8–21 ng/mL)	60+	60, 90, 120, 150 μg, placebo	48	Bone density loss, markers of bone turnover, serum PTH, physical performance	NIHD	August 2015
Hammami	300 (type II diabetics)	18–60	5000 IU, 2000 IU, placebo	6	Area under the curve of HA1C		December 2012
Tuomainen	18 000	60+ (men), 65+ (women)	3200 IU, 1600 IU, placebo	60	CVD, cancer	FIND	December 2019
Craxi	200 (NAS)	18+	20 000 IU/wk	22	1) Improvement in NAS by at least 2 points spread across at least 2 of the NAS components or post-treatment NAS of 3 points or less; 2) at least 1 point improvement in the score for ballooning degeneration; and 3) no worsening of the fibrosis score.		September 2014
Ghada El Hajj Fuleihan	250 (BMI ≥ 25 kg/m^2)	65–95	Calcium, vitaminD (500 IU/d), vitamin D_3 (20 000 IU/wk) vs calcium, vitaminD (500 IU/d), placebo	12	McAuley index of insulin resistance, BMD hip, bone turnover markers	GEHF-VitD	January 2015
Hammami	500 (prediabetics, 25OHD 4–12 ng/mL)	18–60	5000 IU, placebo	24	Incidence of DM		December 2014
Maguire	400	1–5	2000 IU, 400 IU	4–9	No. of laboratory confirmed upper respiratory tract infections	DO IT Trial	September 2013
Manson and Buring	20 000	50+ (men), 55+ (women)	2000 IU, fish oil, placebo (factorial design)	60	Cancer, CVD	VITAL	June 2016
Mauger	400 (asthma, 25OHD < 30 ng/mL)	18+	100 000 IU loading dose, 4000 IU/d, ciclesonide vs placebo + ciclesonide		Treatment failure with regard to asthma control	VIDA	August 2013
Tangpricha	280 (cystic fibrosis)	16+	Bolus dose of 250 000 IU, maintenance dose of 50 000 IU vitamin D every other week to be initiated 3 mo after bolus dose	12	Composite measure of deaths + rehospitalization	DISC	April 2014
Testori	878 (melanoma)	18–75	100 000 IU/50 d, placebo	36	Disease-free survival and overall survival	MelaViD	January 2019
Takacs	300 (chronic lymphoid leukemia, 25OHD 10–30 ng/mL)	18+	180 000 IU/mo, placebo	60	Overall survival, blood lymphocyte count	D-HEM	January 2015
Takacs	300 (chronic heart failure, 25OHD 10–30 ng/mL)	18+	180 000 IU/mo in months 0, 1, 2, 3, 6, placebo	24	Survival rate		September 2012
Yusuf and Pais	5500 (INTERHEART ≥ 10)	55+ (men), 60+ (women)	Polycap DS, aspirin, 60 000 IU D_3/mo (factorial design)	60–120	Composite of major CVD, composite of CV events, fractures	TIPS-3	January 2019

Abbreviations: CRP, C-reactive protein; CV, cardiovascular; CVD, CV disease; DM, diabetes mellitus; HA1C, glycosylated hemoglobin; NAS, nonalcoholic steatohepatitis. Overview of ongoing RCTs recruiting >100 people, using a vitamin D supplement >200 IU/d, and evaluating a biologically relevant primary endpoint. Data were selected from www.clinicaltrials.gov on the basis of the following criteria: vitamin D | Open Studies | Exclude Unknown | Interventional Studies | Phase 3; and then further selected for studies using >2000 IU/d (or equivalent), involving >100 persons and using a major health criterion as the primary endpoint.

25OHD is about 180 μg in adult subjects with a serum 25OHD concentration of 20 ng/mL. Therefore, the daily replacement need for a half-life of 15 days is about 6 μg. The conversion rate of vitamin D_3 into 25OHD is not well known but is probably high, based on isotope studies (233) or on a study on supplementation with 25OHD in comparison with vitamin D, whereby a 2-fold higher serum 25OHD concentration was observed after a daily dose of 20 μg of 25OHD instead of vitamin D (59). If the conversion efficacy would be 2-to-1 or 3-to-1, then the daily replacement need would be 12–18 μg of vitamin D to maintain a serum level of 20 ng/mL. This is remarkably similar to the dose of vitamin D needed to obtain such in the serum level in postmenopausal Caucasian or Afro-American women in a dose-response study (56, 74). To maintain a serum 25OHD level of 30 ng/mL, a daily replacement need of 18–27 μg would be necessary, again in line with the dose-response curve of vitamin D supplementation (56, 74). Similar conclusions were reached by studying vitamin D supplementation in healthy elderly Canadian or Irish men and women (234, 235).

Conclusions

Using RCTs as the main guideline, we can conclude that serum levels of 25OHD above 20 ng/mL are sufficient to normalize calcium and bone homeostasis as measured by surrogate endpoints such as $1,25(OH)_2D$, PTH, calcium absorption, or bone mass. Such levels can be obtained in nearly all postmenopausal women by a daily intake of 600–800 IU of vitamin D_3. The effects of vitamin D supplementation on surrogate endpoints may, however, be confounded by calcium intake. Several meta-analyses of vitamin D supplementation for fracture prevention, however, also concluded that a vitamin D supplement of 800 IU/d in combination with calcium can reduce the fracture incidence by about 20%. Careful dose-response studies of vitamin D supplementation suggest that such a dose would increase serum 25OHD to above 20 ng/mL in nearly all postmenopausal women of either Caucasian or African American origin. Finally, calculation of the replacement dose of vitamin D needed to compensate for the normal metabolic clearance of 25OHD suggests that a daily intake of 12–18 μg (500–700 IU) of vitamin D_3 is sufficient to maintain a serum 25OHD level of 20 ng/mL. There is thus consistent evidence for recommending a daily intake of 600–800 IU of vitamin D_3 to avoid vitamin D deficiency at all ages and to optimize bone health in adult or elderly subjects. The requirements of vitamin D for extraskeletal health are not finally defined. A modest reduction of falls in elderly subjects was observed when supplemented by 800-1000 IU/d of vitamin D_3. For all other potential target tissues, a causal relationship is still unclear, despite a wealth of data supporting a plausible hypothesis for many extraskeletal beneficial effects of vitamin D. Therefore, according to the currently available RCTs, no conclusion can be drawn with regard to optimal vitamin D intake or 25OHD levels for such actions. However, based on association studies, the greatest risk of several major diseases such as colon cancer, infections, cardiovascular and metabolic diseases is, however, found in subjects with 25OHD levels below 20 ng/mL. Assuring serum 25OHD levels above 20 ng/mL in nearly all adults is a formidable task because the mean level of serum 25OHD in adult subjects worldwide is about 20 ng/mL. Moreover, the increased use of automated less accurate assays for 25OHD is a matter of concern because it may lead to an overestimation of vitamin D deficiency. Whether higher levels of 25OHD requiring higher vitamin D intake or higher sun exposure would be beneficial for bone or general health or for patients with established diseases potentially related to vitamin D status requires adequate RCTs. Moreover, there are some hints from observational and intervention studies that a high vitamin D status may carry an increased risk of fractures, falls, cancer, cardiovascular events, and even mortality. The presently published RCTs using 2000 IU of vitamin D per day or more do not show clear benefit over a lower dose (Table 3). Therefore, the recommendations for a daily intake of 1500–2000 IU or serum 25OHD levels of 30 ng/mL or higher for all adults or elderly subjects, as suggested by The Endocrine Society Task Force, are premature. Fortunately, there are very many small and large ongoing RCTs, as summarized in Table 4. Hopefully, these studies will guide us to solve important public health questions concerning the long-term efficacy and safety of vitamin D supplementation of the general population.

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This manuscript is dedicated to the memory of Steven Boonen who, sadly, died during the meeting of the European Calcified Tissue Society in Lisbon on May 20, 2013.

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