Review

Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—A review of recent evidence ☆

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ABSTRACT

Background: Optimal vitamin D intake and its status are important not only for bone and calcium-phosphate metabolism, but also for overall health and well-being. Vitamin D deficiency and insufficiency as a global health problem are likely to be a risk for wide spectrum of acute and chronic illnesses.
Methods: A review of randomized controlled trials, meta-analyses, and other evidence of vitamin D action on various health outcomes.
Results: Adequate vitamin D status seems to be protective against musculoskeletal disorders (muscle weakness, falls, fractures), infectious diseases, autoimmune diseases, cardiovascular disease, type 1 and type 2 diabetes mellitus, several types of cancer, neurocognitive dysfunction and mental illness, and other diseases, as well as infertility and adverse pregnancy and birth outcomes. Vitamin D deficiency/insufficiency is associated with all-cause mortality.
Conclusions: Adequate vitamin D supplementation and sensible sunlight exposure to reach optimal vitamin D status are among the front line factors of prophylaxis for the spectrum of disorders. Supplementation guidance and population strategies for the eradication of vitamin D deficiency must be included in the priorities of physicians, medical professionals and healthcare policy-makers.

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Contents

1. Introduction

Vitamin D was originally recognized as a vitamin needed in small amounts to affect the metabolism of calcium and phosphate. It has been known that rickets was caused by the lack of this important compound. After the role of vitamin D for calcium and phosphate metabolism was understood, rickets was almost eradicated at least in the modern world. The thousands of studies carried out to decipher the role of vitamin D in our body led to the finding of vitamin D receptors linked to chromosomes in almost every cell and tissue in the body, thus leading to its important effects on different organs. These effects and the facts that vitamin D is made in the skin by ultraviolet-B (UVB) irradiance followed by a thermal process, and that the active vitamin D circulates in the blood created a new understanding of vitamin D as a hormone.

2. Defining vitamin D deficiency and its consequences on skeletal health

Some debate remains as to what blood level 25-hydroxyvitamin D [25(OH)D] should be for an optimal skeletal health [1–4]. It has been suggested that rickets is not seen for serum 25(OH)D < 15 ng/mL [2]. Others have suggested that to maximize bone health in children and adults, the blood level of 25(OH)D should be at least 20 ng/mL [2]. Rickets is an overt manifestation of long-standing vitamin D deficiency that is also associated with an elevated serum alkaline phosphatase, elevated serum parathyroid hormone, 25(OH)D below 15 ng/mL, elevated 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], low or low normal serum phosphorus and either a normal or low serum level of calcium [6,7]. Rickets therefore should not be used as a bellwether for determining what the cut off should be for 25(OH)D to define vitamin D deficiency bone disease in infants and children. Classic skeletal abnormalities including valus and valgus deformities of the legs, widened epiphyseal plates at the ends of the long bones and costochondral junctions (rachitic rosary), Harrison's groove of the sternum, frontal bossing, widening of the fontanelles and softening of borders of the fontanelles (craniotabes) are not usually observed until the infant is at least 6 months of age and some do not appear until the infant begins to walk [6,7]. For adults, vitamin D deficiency bone disease is even more subtle since the epiphyseal plates are closed and the skeleton has enough calcium to prevent skeletal deformities such as bowed legs.

Vitamin D deficiency in both children and adults causes a decrease in the efficiency of intestinal calcium absorption that results in a transient decline in the serum ionized calcium concentration. This decline is instantly recognized by the calcium sensor in the parathyroid glands resulting in signal transduction to enhance the expression, production and secretion of parathyroid hormone [8]. PTH increases tubular reabsorption of calcium in the kidneys and stimulates the kidneys to produce 1,25(OH)$_2$D which in turn travels to the small intestine to interact with the vitamin D receptor (VDR) to enhance intestinal calcium absorption. PTH and 1,25(OH)$_2$D also travel to the skeleton to interact with osteoblasts to increase the expression of the receptor activator of

NFkB (RANK) ligand (RANKL). RANKL interacts with RANK on monocytes inducing them to become mature osteoclasts [1]. Osteoclasts release collagenases to destroy the matrix and HCl to dissolve the calcium hydroxyapatite releasing precious calcium into the circulation. This process results in a decrease in bone mass that can precipitate and exacerbate osteopenia (low bone mass) and osteoporosis in both children and adults. PTH also causes a decrease in phosphate reabsorption in the kidneys causing a lowering of the blood phosphorus level. This subtle effect results in an inadequate calcium X phosphorus product in the extracellular space causing a mineralization defect of newly laid down collagen matrix. In infants and young children there is also a disruption in chondrocyte maturation leading to a widening of the epiphyseal plates which is not seen in adults. Because infants and young children have very little mineral in their skeleton, as they begin to stand and walk the force of gravity on the standing infant results in the inward or outward bowing of the legs. In addition muscle tension can cause curvature in the arms. For adults the consequences of vitamin D deficiency on bone health result in a decrease in bone mineral density due to the increased bone resorption by PTH as well as the mineralization defect causing osteomalacia. Both diseases look the same on X-ray and bone densitometry.

Since PTH increases the mobilization of calcium from the skeleton and also causes a mineralization defect of newly produced collagen matrix any significant increase in PTH levels could be used as a surrogate biomarker for detecting vitamin D deficiency. Before 1998 it had been accepted based on the determination of a normal range, i.e. mean $\pm$ SD of a healthy group of children and adults that the normal range for 25(OH)D was 10–55 ng/mL. However it was demonstrated that healthy adults who received 50,000 IU of vitamin D$_2$ once a week for 8 weeks along with calcium supplementation and who had a blood level of 25(OH)D of between 11 and 19 ng/mL had on average a 35% decrease in their PTH level [9]. As a result vitamin D deficiency was redefined as a blood level of 25(OH)D < 20 ng/mL.

Using this new definition for vitamin D deficiency it was quickly realized that many more children and adults were vitamin D deficient than previously thought. This observation begged the question if the normal range for 25(OH)D was in error then so too must be the normal range for PTH which by most reference laboratories is still 15–80 pg/mL. It has been suggested that the upper limit for PTH, when determined in a population that has a 25(OH)D of at least 20 ng/mL, should be 40–50 pg/mL [10–12].

There have been several studies reporting on the blood levels of 25(OH)D in relationship to PTH levels. Most [13–15] but not all studies have suggested that PTH levels begin to plateau when the circulating levels of 25(OH)D are between 30 and 40 ng/mL [16]. In a study of over 1500 postmenopausal women there was a statistically significant decline in PTH levels until the serum level of 25(OH)D was greater than 30 ng/mL [15]. In fact using the upper limit of normal for PTH of 40 pg/mL (normal range 6–40 pg/mL) this study reported that women who had a blood level of 25(OH)D of 20 ng/mL had a 2-fold higher risk of having secondary hyperparathyroidism compared to women who had a blood level of 25(OH)D > 30 ng/mL.

The mineralization defect caused by vitamin D deficiency and insufficiency can be very subtle and often only determined by bone histology. Clinical manifestations of osteomalacia in adults include nonspecific throbbing aching bone pain and muscle weakness and muscle discomfort [17–19]. Priemel et al. [20] reported the presence of osteomalacia from bone biopsies of 675 otherwise presumed healthy adults, ages 20–70 years, in Germany who died prematurely due to an accident, often from a motor vehicle accident, and related these biopsy results with their serum level of 25(OH)D. Although there is no uniformly accepted osteoid volume cut-off for the histologic diagnosis of osteomalacia, the authors reported using a conservative threshold >2% osteoid volume/bone volume (OV/BV) for the diagnosis of osteomalacia, that over 25% of these otherwise presumed healthy adults had evidence of osteomalacia. Furthermore more than 36% had evidence of osteoidosis, i.e. unmineralized matrix within mineralized bone (this is presumed due to intermittent vitamin D deficiency likely in the winter). The authors concluded that since they did not observe any evidence of osteomalacia or osteoidosis in bone biopsies from adults who had a serum 25(OH)D > 30 ng/mL, to maximize bone health in adults a blood level of 25(OH)D > 30 ng/mL should be sustained throughout the year. There is no reason to believe that infants and children would not also benefit by maintaining a blood level of 25(OH)D > 30 ng/mL.

The upper range of normal for 25(OH)D in the 1990s by some reference laboratories was reported as 55 ng/mL. However this upper limit of normal varied depending on latitude and season which of course made no sense. A review of the literature has suggested that patients with classic biochemical abnormalities for vitamin D intoxication, i.e. hypercalcemia and hyperphosphatemia, are only seen when blood levels of the circulating levels of 25(OH)D > 200 ng/mL [21–23]. The only exception is in patients with granulomatous disorders who have a hypersensitivity to vitamin D because of the exuberant production of $1,25(OH)_2D$ by the macrophages within the granuloma that is released into the circulation [4]. As a result the upper limit by most reference laboratories is reported as a 25(OH)D of 100 ng/mL.

The Institute of Medicine (IOM) used a population model for its recommendations to satisfy 97.5% of the population for adequate 25(OH)D for maximum bone health. They conducted a thorough review of the literature and made its recommendation defining vitamin D deficiency for bone health as a 25(OH)D > 20 ng/mL. They unfortunately erroneously concluded from the Priemel et al.'s [20] study that 99% of the 675 accident victims had no evidence of osteomalacia when they had a blood level of 25(OH)D > 20 ng/mL. In fact the authors reported that 21% of otherwise healthy German adult men and women with a 25(OH)D of 21–29 ng/mL had evidence of osteomalacia or osteoidosis. The IOM also dismissed studies relating 25(OH)D levels with the plateauing of PTH because of significant scattering of the data in some of the studies and instead relied on the provocative study of Malabanan et al. [9]. who reported that PTH levels did not decrease in healthy adults who received calcium and vitamin D supplementation and who had a blood level of 25(OH)D > 20 ng/mL. The IOM also recommended that the upper limits (ULs) should be 1000 IU and 1500 IU of vitamin D for infants 0–6 months and 6–12 months respectively. For children 1–3 years and 4–8 years ULs of 2500 IU and 3000 IU per day were recommended and for children 9 years and older and all adults the UL was set at 4000 IU of vitamin D daily.

The Endocrine Society which used a medical model for their recommendations, i.e. to treat and prevent vitamin D deficiency, also concluded that vitamin D deficiency should be defined as 25(OH)D < 20 ng/mL. However based on the Priemel et al.'s [20], studies relating serum 25(OH)D with PTH levels as well as other evidence for reducing risk of fracture [24,25], improving muscle strength [25,26] and some chronic diseases not associated with calcium metabolism [4,5,27], vitamin D insufficiency be used as a term and defined as 25(OH)D of 21–29 ng/mL and therefore vitamin D sufficiency was defined as 25(OH)D > 29 ng/mL with a preferred range 40–60 ng/mL [4,5]. Both the IOM and the Endocrine Society concluded that the upper limit of 25(OH)D at 100 ng/mL was safe and would not cause vitamin D intoxication [2,4].

The IOM concluded that to prevent vitamin D deficiency, i.e. 25(OH)D < 20 ng/mL, children 0–1 year require 400 IU of vitamin D whereas all other children and adults up to the age of 70 years required 600 IU of vitamin D daily. For adults over the age of 70 years the recommendation was 800 IU of vitamin D daily. The Endocrine Society whose goal was to make recommendations to prevent and treat vitamin D deficiency and insufficiency recommended that instead of giving an exact amount, which is unrealistic in a clinical practice, a range was more reasonable. For children 0–1 year the recommendation was 400–1000 IU vitamin D daily. For children one year and older and all adults the recommendations were 600–1000 IU and 1500–2000 IU of vitamin D daily respectively [4]. Both the IOM and the Endocrine Society recommended that either vitamin D_2 or vitamin D_3 could be used since it was concluded that they have the same ability to raise and maintain circulating levels of 25(OH)D [2,4,28]. However, Heaney and colleagues pointed on vitamin D_3 as more potent compared to vitamin D_2 in humans [29]. Further, the Endocrine Society also recognized that because body fat can store and sequester vitamin D, obese children and adults may require as much as 2–3 times the recommended amount of vitamin D to both treat and prevent recurrent vitamin D deficiency compared to normal weight child and adult [4].

There is an additional variable that has added to the confusion as to what blood level of 25(OH)D should be attained in order to satisfy the body's requirement for vitamin D for skeletal health, i.e. the wide variability in assay methodologies for the 25(OH)D assay. Several studies have reported that even using the same assay by different laboratories resulted in different results [5,30,31]. A majority of assays use some form of antibody technology whereas the Center for Disease Control in the United States and several reference laboratories and research laboratories use liquid chromatography tandem mass spectroscopy which unlike the antibody assays can measure individually $25(OH)D_2$ and $25(OH)D_3$. Many of the antibody assays often have difficulty in recognizing $25(OH)D_2$ with equal efficiency as it does for $25(OH)D_3$ making them less valuable for determining the vitamin D status of the patients who are being treated with vitamin D_2.

3. Vitamin D and musculoskeletal system

The most widely known vitamin D actions are related to intestinal calcium absorption, serum calcium and phosphate homeostasis, and bone formation/resorption processes. Vitamin D deficiency induces defects of bone mineralization leading to clinical manifestations such as rickets in children and osteomalacia in adults. Vitamin D deficiency and insufficiency coincide with osteoporosis, a disorder characterized by low bone mineral density (BMD) and increased risk of fracture resulting in the majority of cases from impaired muscular function leading to falls.

The biological inter-relationship between bone and muscle tissues was proposed long ago [32]. It was postulated that bone strength is modeled by muscle contractions in a way to achieve a degree of biomechanical homeostasis avoiding the spontaneous fracture incidents [33]. It was postulated that the increases in muscle force drive the increase in bone strength reflecting the functional adaptation of bone to its function [34]. The functional relation between bone and muscle tissues seems to be strongly modulated by hormones, including GH–IGF-I axis, sex hormones and vitamin D. If muscle mass (force) is decreased, the decreased bone mass/BMD is expected because a decreased rate of muscle loading is applied on bone. If such a functional loop exists, increased risk of fracture could be, at least in part, a consequence of sarcopenia, a condition associated with gait and balance problems, and muscle weakness [35]. These functional impairments increase the tendency to fall, which, together with reduced bone mass and BMD and skeletal frailty, comprise the main risk factors for fracture [36].

Several randomized controlled trials (RCTs) and meta-analyses of RCTs investigated associations between vitamin D status and vitamin D supplementation with muscle function, physical performance, risk of falls and risk of fracture. A recent meta-analysis of RCTs evaluating vitamin D supplementation effects on muscles identified 16 RCTs that included 35,283 subjects [37]. Among them 15 RCTs were focused on adults aged 50 years and older and one RCT was performed in girls aged 10–17 years from Lebanon. The vitamin D doses as well as the duration of intervention varied widely. In 11 RCTs vitamin D_3 was administrated orally in average doses of 400–3700 IU/day given monthly, weekly or daily. In the remaining 5 RCTs vitamin D_2 has been given in a single intramuscular injection (up to 600,000 IU) or orally (up to single oral dose of 300,000 IU). Almost all RCTs, except one [38], showed a significant increase of 25(OH)D level after vitamin D supplementation compared to controls. Beneficial effects of vitamin D on muscle strength, body sway or physical performance were revealed only in seven of 16 studies. Although, positive effects on muscle were not consistently evidenced, as the vitamin D effects on muscle function were secondary endpoints, still, improved muscle strength [39–41], improved timed up-and-go test [41,42], improved the compiled measures of physical abilities [43,44], improved postural stability [41,42,44,45], or an increased 12-minute gait speed [42] was documented after vitamin D administration. Furthermore, an RCT performed in girls from Lebanon showed significantly higher muscle mass accrual (but not higher grip strength), after one year of vitamin D treatment compared to placebo treated counterparts, as estimated by lean body mass measures using dual-energy x-ray absorptiometry (DXA) [46]. Nonetheless, because of the discrepant effects of vitamin D on muscles in RCT setting, likely due to heterogeneity in most aspects of these studies (vitamin D dose, duration of treatment, different methods used for the evaluation of muscle strength/function/performance, etc.), drawing of a definite conclusion is difficult in light that more RCTs showed no effects. Indisputably, vitamin D has a direct effect on muscle cells through vitamin D receptor (VDR) in cell nuclei and a spectrum of indirect effects that all affect muscle tissue metabolism in a state of vitamin D deficiency. The same relates to bone tissue and its cells. It seems therefore, that vitamin D has a dual effect for musculoskeletal system: on bone mass/density/quality and on muscle mass/strength/function. In addition, adequate vitamin D status reduces the risk of falling in older individuals, most likely by improving neuromuscular functions.

To determine anti-falling efficacy of vitamin D supplementation in individuals 65 years and older, Bischoff-Ferrari and colleagues [24] did a meta-analysis including 8 double-blind RCTs that included 2426 subjects. Vitamin D2 or D3 was given in a daily dose ranging from 200 IU to 1000 IU. Treatment duration varied from 2 months to 36 months. A 13% reduction of risk of falling was demonstrated in vitamin D supplemented individuals compared with those receiving calcium or placebo. Again, a significant heterogeneity by dose and achieved 25(OH)D levels was observed. Supplementation in doses 700–1000 IU/day reduced the relative risk of falls by 19%, whereas supplementation with doses lower than 700 IU/day was not significant for the risk of fall. Moreover, at least 24 ng/mL (60 nmol/L) of 25(OH)D had to be achieved by subjects to significantly reduce a risk of falls (by 23%) and no effect on reduction of falling was revealed for 25(OH)D levels lower than 24 ng/mL (60 nmol/L). The aforementioned meta-analysis revealed that vitamin D doses of 700–1000 IU/day reduced falls by 19% or by up to 26% with vitamin D_3. The benefit might not depend on additional calcium supplementation, was significant within 2–5 months of treatment, and extended beyond 12 months of treatment [24].

The anti-fracture efficacy of oral vitamin D supplementation was documented mainly in elderly. In a 2009 year Bischoff-Ferrari and colleagues [26] published results of a meta-analysis of 12 double-blind RCTs for non-vertebral fractures including 42,279 subjects and 8 RCTs for hip fractures including 40,886 subjects. The pooled relative risk

(RR) was 0.86 (95% CI, 0.77–0.96) for the prevention of non-vertebral fractures and 0.91 (95% CI, 0.78–1.05) for the prevention of hip fractures. However, when trials using doses of 482–770 IU/day of vitamin D were considered (31,872 subjects), the risks for non-vertebral fractures and hip fractures were reduced by 20% and by 18%, respectively. It appeared that vitamin D doses of 400 IU/day and lower did not show any effect and fracture reduction [26]. However, some other studies, including meta-analyses, suggested that vitamin D may have no effect on total fractures [47] or may reduce hip fracture by 7 to 16%, if combined with calcium supplementation, regardless of the dose of vitamin D [48]. To address these discrepancies a pooled participant-level data from 11 RCTs of oral vitamin D supplementation (daily, weekly, or every 4 months), with or without calcium, as compared with placebo or calcium alone in persons 65 years and older was analyzed and published [49]. The analysis was designed to estimate the effects of vitamin D supplementation according to the actual intake of each participant, not the dose to which the participant was randomly assigned. The 31,022 subjects from 11 studies were included. Among 4383 participants with baseline measures of 25(OH)D level, 88% revealed levels lower than 30 ng/mL (75 nmol/L). It was shown that vitamin D supplementation in actual doses lower than 792 IU/day is not effective in fracture risk reduction. Both hip fracture risk and non-vertebral fracture risk were reduced respectively by 30% and 14% in subjects with actual intake level of 792–2000 IU/day. Further, subjects with baseline serum 25(OH)D levels of at least 24.4 ng/mL (61 nmol/L), compared to those showing less than 12 ng/mL (30 nmol/L), had a risk of hip fracture that was reduced by 37% and a risk of any nonvertebral fracture that was reduced by 31%. Consequently, at least 800 IU/day of vitamin D together with 25(OH)D serum level higher than 24 ng/mL (60 nmol/L) was highlighted as effective in fracture prevention.

Although RCT- based effects of vitamin D supplementation on specific muscle functions were not consistently supported, anti-fall and anti-fracture action of vitamin D administration of at least 800 IU/day with at least 24 ng/mL (60 nmol/L) of 25(OH)D serum levels appeared as beneficial for musculoskeletal machinery.

4. Vitamin D and immunity

Along the many important cells and tissues in which the vitamin D receptor (VDR) was found are the immune system agents: lymphocytes, monocytes and dendritic cells; therefore, the next step emerging, especially during the last decade, was to elucidate the role of vitamin D as a positive immunomodulator on the immune system. The impact of vitamin D deficiency in the pathogenesis of immunomediated diseases and the significant role of pharmacological doses of vitamin D in autoimmune diseases have been highlighted. So far, more than 30 positive effects of vitamin D on the immune system have been reported [50–54].

The close relationship with the production of the active metabolite of vitamin D upon exposure to UVB radiation has been well known for years. For instance, in the olden days, patients with tuberculosis were sent to a sanatorium to benefit from the exposure to the sun in combating the *Mycobacterium tuberculosis*. Only recently the beneficial effect of vitamin D on macrophages in phagocytizing the *M. tuberculosis* was detailed in RCT of either a single oral dose of 2.5 mg ergocalciferol or lactose placebo [55]. Vitamin D has roles in the maturation of macrophages, including the production of macrophage-specific surface antigens and the secretion of the lysosomal enzyme acid phosphatase and hydrogen peroxide. These features of antimicrobial function are impaired in the setting of vitamin D deficiency [1,56–58]. Thus, vitamin D has an important role in increasing the effects of innate immune processes while restraining the adaptive immune system, leading to improved outcomes in autoimmune diseases and possibly lowering risk of autoimmune disease [1,50].

Vitamin D has a part in shaping immune response by T and B cells. For instance, the number of vitamin D receptors on CD4 + T cells correlates with the degree of cell activation [59]. The addition of $1,25(OH)_2D_3$ to CD4 + T cells inhibits the proliferation of T-helper-1 cells and cytokine production [60]. Furthermore, suppression of T-helper-1 cytokines, such as interleukin (IL)-2, IL-12 and interferon γ, and increased production of T-helper-2 cytokines, such as IL-5 and IL-10, has been noted, suggesting that T-helper-2 cells are more dominant than T-helper-1 cells [60–62].

Vitamin D also modulates the responses of T-helper-17 cells, which are seminal to autoimmune reactions [63–66]. A nonhypercalcemic vitamin-D-receptor agonist, elocalcitol, was shown to decrease T-helper-1-type and T-helper-17-type cytokine secretion and to promote T-helper-2-type cytokine expression [67]. Interestingly, in 25-hydroxyvitamin D-1α-hydroxylase knockout mice, increased weight loss and colitis were associated with raised levels of IL-1 in the distal colon and of IL-17 in the proximal and distal colon [68]. This apparent relation suggests that similar effects might occur with vitamin D deficiency. Another study has shown that $1,25(OH)_2D_3$ increases the suppressive capacity of CD4+CD25 + immunoregulatory cells that originated from mouse draining lymph nodes [69]. CD4 + cells from the draining lymph nodes in the skin of mice treated with either $1,25(OH)_2D_3$ or UVB radiation had reduced capacity to proliferate to antigens presented in vitro, and these cells suppressed antigen-specific immune responses upon adoptive transfer into untreated mice. Autoantibody production was also suppressed by $1,25(OH)_2D_3$ [70].

The generation of bone marrow dendritic cells is not impaired by the elevated levels of $1,25(OH)_2D_3$, but maturation is slowed [71]. In vitro, $1,25(OH)_2D_3$ inhibits IL-12 secretion and differentiation of monocytes into dendritic cells, and it hinders the stimulatory effects that T cells have on their activity [71,72]. While $1,25(OH)_2D_3$ stimulates phagocytosis and the killing of bacteria by macrophages, it suppresses the antigen-presenting capacity of these cells, presenting dichotomic responses towards the innate and adoptive arms of the immune system [73]. Furthermore, $1,25(OH)_2D_3$ induces monocytic differentiation to macrophages and decreases the release of inflammatory cytokines and chemokines by these cells [74].

A review of the ways vitamin D supports the immune system is given in Lang et al. [75]. The role of the induction of cathelicidin and defensins in the innate immune response is discussed among other topics.

Given this understanding of the mechanisms whereby vitamin D reduces the risk of infection, it is worthwhile to look at some of the evidence that vitamin D reduces the risk of infectious diseases. There are several bacterial infections that vitamin D protects against besides tuberculosis. One that has been studied many years is the prevention of dental caries due to the action of oral bacteria. Studies in the 1930s–1950s found that young people living in sunnier locations in the United States had fewer dental caries than those living in less sunny locations [76]. A recent review of randomized controlled trials of vitamin D reported that vitamin D reduced the risk of dental caries by about 50% [76].

The effect of vitamin D in reducing risk of acute respiratory infections has been the focus of several recent studies. A study in Connecticut found levels "of 38 ng/mL or more were associated with a significant ($p < 0.0001$) two-fold reduction in the risk of developing acute respiratory tract infections and with a marked reduction in the percentages of days ill." [77].

In a supplementation study in Sweden involving 140 patients with frequent respiratory tract infections (RTIs) using 4000 IU/day vitamin D3, those on the supplementation arm increased their serum 25(OH)D level to 53 ng/mL while those in the placebo arm had 25(OH)D levels of 27 ng/mL [78]. Those taking vitamin D3 had a 23% (95% CI, 1–40%) reduction in RTIs and a 50% reduction in the number of days using antibiotics. There is mounting evidence that vitamin D reduces the risk of sepsis [79].

While the effects of vitamin D have been found mostly for bacterial infections, some have also been reported for viral infections such as influenza, HIV, and hepatitis C [75]. There is also strong evidence that vitamin D helps protect against the autoimmune disease, multiple sclerosis. Epstein Barr virus is an important risk factor for this disease. A recent paper presented the hypothesis that CD8 + T cell deficiency contributed along with the other factors [80].

Thus it seems that vitamin D may be instrumental in the immune system homeostasis, and in preventing autoimmune diseases [81–84] and lowering risk of infections [75–79]. The routine prescription of vitamin D in these conditions is highly recommended [85].

5. Vitamin D and cardiovascular disease

The cardiovascular system is a target tissue for vitamin D since VDRs as well as vitamin D metabolizing enzymes are expressed in arterial vessels, heart and almost all cells and tissues with relevance for the pathogenesis of cardiovascular diseases [86,87]. Associations of cardiovascular diseases and its risk factors with season and latitude lead to the hypothesis that vitamin D deficiency might be a causal cardiovascular risk factor [86,88]. Subsequent experimental animal and cell culture studies demonstrated a variety of effects by which VDR activation exerts cardiovascular protective actions: e.g. anti-atherosclerotic effects, renin suppression or prevention of myocardial damage [86,87,89,90]. While these data strongly support that VDR activation may protect against cardiovascular diseases, the question arises whether the vitamin D effects observed in vitro are of relevance in vivo, i.e. in the clinical setting. Systematic reviews and meta-analyses of observational studies confirm that low levels of 25(OH)D are associated with cardiovascular risk factors (e.g. diabetes mellitus, dyslipidemia and arterial hypertension) and predict cardiovascular events including strokes [87,90–94].

In a meta-analysis including 65,994 participants and 6123 cardiovascular disease cases, the risk of cardiovascular events significantly increased with decreasing 25(OH)D levels below 24 ng/mL (60 nmol/L) [92]. A major problem with the interpretation of these findings is that vitamin D deficiency is closely associated with obesity and physical (outdoor) activities [87,95]. Hence, it is challenging to clarify whether vitamin D deficiency is the cause or only the consequence of cardiovascular diseases. It must however be underlined that the majority of the existing literature shows that vitamin D deficient individuals are at increased cardiovascular risk even after adjustments for common cardiovascular risk factors [87,90–94]. Data from randomized controlled trials (RCTs) on vitamin D supplementation and cardiovascular risk are however relatively sparse and less clear [87,90]. RCTs on vitamin D effects on cardiovascular risk factors produced mixed results and we can at present not draw final conclusions regarding this. While a few RCTs showed beneficial effects of vitamin D supplementation on glucose metabolism, the majority of the studies did not report significant results [87,90,96].

For arterial hypertension, meta-analyses of RCTs have shown either no or some slight, yet statistically significant, reductions in systolic blood pressure [87,90]. Further RCTs are therefore needed and it should be considered that according to recent studies, potential cardiovascular benefits of vitamin D supplementation (e.g. antihypertensive effects) seem to be restricted to patients with both low 25(OH)D levels and at a certain cardiovascular risk (e.g. arterial hypertension) [87,97].

Regarding cardiovascular events there are no vitamin D RCTs published that were specifically designed to address this issue [87,90]. Data from RCTs reporting on cardiovascular events as secondary outcomes did not show any significant vitamin D effect, but there exists a wide consensus that these RCTs had several limitations (e.g. low vitamin D doses, poor compliance or concomitant calcium supplementation) so that further RCTs are definitely needed to clarify whether vitamin D can reduce the incidence of cardiovascular diseases [87,90]. Some large RCTs on vitamin D supplementation and cardiovascular risk are currently ongoing and their results can be expected in the years 2017 to 2020 [98,99].

6. Vitamin D and cancer

There have been several papers reporting results of randomized controlled trials (RCTs) of vitamin D or vitamin D plus calcium on risk of cancer incidence [100–105]. The Women's Health Initiative (WHI) used 400 IU/day vitamin D_3 plus 1500 mg/day calcium and did not find statistically significant benefits in general [101,104]. The reasons for the failure of the WHI to find beneficial effects of vitamin D include the low dose of vitamin D_3, poor compliance, estimated at 70%, and failure to measure for serum 25(OH)D level after supplementation and control for other sources of vitamin D.

On the other hand, an analysis of the subset of participants in the WHI study who had not taken personal vitamin D or calcium supplements prior to enrolling in the study found for total cancer, hazard ratio (HR) = 0.86 [95% confidence interval, 0.78–0.96], p = 0.007; total breast cancer, HR = 0.82 (0.70–0.97), p = 0.02; and invasive breast cancer, HR = 0.80 (0.66–0.96), p = 0.02 [103]. There were no statistically significant findings for women who were taking personal calcium or vitamin D prior to enrolling in the study.

Another RCT using 1100 IU/day vitamin D_3 and 1450 mg/day calcium was conducted on post-menopausal women living in Nebraska [102]. It found a relative risk (RR) for all-cancer incidence = 0.40 (95% confidence interval, 0.20–0.82), p = 0.01 for those taking vitamin D plus calcium, and 0.53 (0.27–1.03), p = 0.06 for those taking only calcium. The effect of vitamin D alone is likely the ratio of the two RRs, which is 0.75. A second analysis for all-cancer incidence between the ends of the first and fourth years significantly reduced risk: RR = 0.23 (0.09–0.60), p < 0.005, and for those taking only calcium, RR 0.59 (0.21–1.21), p = 0.15. The ratio of these two RRs is 0.39. Serum 25(OH)D levels changed from 29 ng/mL at baseline to 38 ng/mL at the end of the first year for those taking vitamin D, with no change for those taking calcium. The primary problem with this study was that there were only 50 cancer cases during the four years and 35 after the first year.

The results from the aforementioned two RCTs can be compared to the odds ratio for breast cancer incidence vs. serum 25(OH)D level derived from five case–control studies in which serum 25(OH)D level was determined near the time of cancer diagnosis [106,107]. For those not taking personal calcium or vitamin D prior to enrolling in the WHI, it can be assumed that the serum 25(OH)D level at time of enrollment was at the dividing line between the lowest two quartiles, 12.4 ng/mL, and that compliance was 70%, resulting in 280 IU/day vitamin D_3 intake. Based on data showing changes in serum 25(OH)D level from oral vitamin D intake vs. initial serum 25(OH)D level in study by Garland and colleagues [108], this corresponds to an increase of serum 25(OH)D level of 3.1 ng/mL. Using the breast cancer incidence vs. serum 25(OH)D level relation from case–control studies given in [106,107] this corresponds to a 16% reduction in breast cancer incidence that is close to the values reported by Bolland and colleagues [103].

Doing a similar analysis for the Lappe et al.'s [102] study based on the changes in serum 25(OH)D level finds an estimate from the breast cancer graph in Grant [107,108] of 18%. This value compares well to the 25% reduced risk for the full four-year study, but not to the result omitting the first year, 61%.

While some researchers have cautioned that serum 25(OH)D levels measured near the time of cancer diagnosis could be affected by the disease state (reverse causality), graphs of risk with respect to follow-up time find linear relations for breast, colorectal and prostate cancer [106] and all-cause mortality rate [109]. In addition, there does not seem to be evidence that the existence of cancer affects serum 25(OH)D level.

Most of the evidence that vitamin D reduces the risk of cancer comes from ecological, observational, and laboratory studies [107]. The ecological studies are based on geographical variations of cancer incidence and/or mortality rate with respect to indices for solar UVB doses. The single-country studies are quite consistent for a number of countries, and no factor other than vitamin D production has been proposed to explain the UVB-cancer link. The observational studies show significant inverse correlations between serum 25(OH)D level and incidence rates for case–control studies for breast cancer and both case–control and cohort studies for colon cancer [106]. Breast cancer can develop so rapidly that for long follow-up times, the 25(OH)D level at time of enrollment is not a good index.

Most vitamin D-cancer RCTs to date were not well designed and conducted [110]. The guidelines for proper vitamin D RCTs were outlined in a recent paper by Lappe and Heaney [111]. Perhaps the most important guideline is that the design of the study should start with an estimated serum 25(OH)D level-health outcome relation. Such relationships can be derived from observational studies [107,108]. However, care should be taken to assess the effect of follow-up time after blood draw on health outcome since the longer the follow-up period, the lower will be the observed beneficial effect as shown for breast and colorectal cancer [106] and all-cause mortality rate [109]. Next is to enroll people in the study with serum 25(OH)D levels near where the beneficial effect begins to rise. Next, the vitamin D dose should be large enough to raise serum 25(OH)D levels to near where beneficial effects no longer increase rapidly. Next, serum 25(OH)D levels should be measured perhaps a year after the study begins. Doing so finds the change in 25(OH)D level as well as identifies those who are not compliant with the study protocol. It also permits some control over other sources of vitamin D. It would also be useful to examine vitamin D without calcium. Since vitamin D RCTs are expensive and research funds are limited, many ongoing and proposed studies are not able to incorporate all of the guidelines. Thus, it may be a number of years until RCTs demonstrate that vitamin D reduces the risk of cancer to the satisfaction of health policy makers who rely on "evidence-based medicine" [112].

7. Vitamin D and mortality

Large epidemiological studies have by the majority shown that individuals with low 25(OH)D levels are at significantly increased risk of mortality [87,113–116]. This has been underscored by the meta-analyses of studies in general populations and in patients with chronic kidney diseases [113,114]. Studies in specific populations such as nursing home residents or patients with liver diseases confirmed that low 25(OH)D levels indicate an increased mortality risk [115,116]. Regarding detailed data on optimal 25(OH)D levels, it has been shown in the general population that the relationship between 25(OH)D and mortality seems to be non-linear with the lowest mortality risk at 25(OH)D levels ranging from 30 to 35 ng/mL (75 to 87.5 nmol/L) [113]. Mortality risk shows a steep increase at very low 25(OH)D levels, but it should also be considered that some study results suggest a U-shaped relationship of 25(OH)D and mortality [87,113]. In this context, it must also be stressed that in a large meta-analysis there was no significantly increased mortality risk with 25(OH)D levels up to 45 ng/mL (112.5 nmol/L) [113]. Higher 25(OH)D levels are not proven to be harmful but are just of largely unknown clinical significance with regard to mortality [113]. In line with these observational studies, it has been shown in the meta-analyses of RCTs that vitamin D3 treatment was associated with reduced mortality [117,118]. In detail, a Cochrane meta-analysis including 74,789 individuals showed that vitamin D3 reduces mortality by 6%, corresponding to 161 individuals treated with vitamin D3 to save one additional life [117]. Similar results were reported by a further individual patient level meta-analysis [118]. These results were mainly derived from elderly women. It should also be considered that vitamin D was frequently given with calcium supplementation, which raises some still unresolved questions on the separate or joint effects of vitamin D and calcium [117,118]. In a meta-analysis of vitamin D

or vitamin D plus calcium, a significantly reduced risk of all-cause mortality rate was found for vitamin D plus calcium [odds ratio = 0.95 (0.91–1.00)] but not for vitamin D alone [odds ratio = 0.98 (0.91–1.06)] [118]. The amount of vitamin D used in the trials in general may have been too low for optimal results. Thus, it is reasonable to expect that RCTs including both vitamin D and calcium would represent the effects of each compound independently. However, there could also be some synergism, such as calcium intake possibly affecting serum 25(OH)D levels [119]. Calcium has been found associated with the reduced risk of cancer in many observational studies such as those related to mineral-rich (hard) water [120].

While it will therefore be a challenge for future research to identify the optimal way of vitamin D treatment, it must also be emphasized that a mortality reduction evidenced by meta-analyses of RCTs is usually a striking argument for a widespread clinical use of such a treatment.

8. Vitamin D and fertility

There are several lines of evidence suggesting an important role of vitamin D in human fertility. The vitamin D receptor (VDR) is distributed across various human tissues including ovaries, endometrium, placenta, testis, spermatozoa and the pituitary gland suggesting an active role of vitamin D in those tissues. Further, data from animal studies suggest an important role of vitamin D in female and male fertility (reviewed in [121]).

In women, most studies focussed on the possible association of vitamin D with polycystic ovary syndrome (PCOS) and with in vitro fertilization (IVF) success. There is accumulating evidence from cross-sectional studies suggesting that vitamin D deficiency might be involved in the pathogenesis of insulin resistance and the metabolic syndrome in PCOS [121,122], whether vitamin D is also related to endocrine parameters and fertility in PCOS is less clear. The evidence on the effects of vitamin D supplementation in PCOS women is sparse. There are, however, some small intervention trials showing promising results. In a small-scale intervention study including 13 premenopausal women with chronic anovulation and hyperandrogenism, vitamin D repletion with 50,000 IU ergocalciferol weekly or biweekly combined with calcium administration of 1500 mg calcium daily resulted in the normalization of menstrual cycles in 7 women and 2 became pregnant [123]. Another study in 12 overweight and vitamin D deficient PCOS women showed reductions in total testosterone and androstenedione levels following 3-month supplementation with a daily dose of 3533 IU (increased to 8533 IU after the first five participants) and 530 mg calcium daily [124]. In contrast, in a pilot study among 13 obese women with PCOS, the administration of the single dose of 300,000 IU of vitamin D3 orally did not significantly change BMI or the levels of DHEAS, total testosterone, free testosterone, and androstenedione levels but had a beneficial impact on insulin resistance assessed by HOMA-index [125]. Similarly, in a small study including 15 obese PCOS women treated with 1 μg alphacalcidol daily over 3 months, vitamin D treatment improved insulin secretion and had a beneficial effect on lipid status [126]. Another study including 57 women who received 50,000 IU vitamin D3 weekly over 24 weeks, vitamin D supplementation resulted in improved glucose metabolism as well in an improvement of menstrual frequency [127]. To date there is no RCT to evaluate the effects of vitamin D treatment on endocrine and metabolic parameters in PCOS women. Considering the association of vitamin D deficiency with insulin resistance and type 2 diabetes in PCOS as well as in other cohorts, further research including large RCTs is highly warranted in this high risk cohort.

Studies investigating the association of vitamin D status with IVF outcome revealed inconsistent results. A recent study in 91 anovulatory, infertile women with PCOS who underwent clomiphene citrate stimulation suggests that vitamin D deficiency (<10 ng/mL) is an independent predictive parameter of clomiphene citrate stimulation outcome,

in terms of follicle development and pregnancy [128]. In a study among 84 infertile women undergoing IVF, women with higher levels of 25(OH)D in serum and follicular fluid were significantly more likely to achieve clinical pregnancy following IVF [129]. Another study in 101 women reported that women with a sufficient vitamin D status (25(OH)D > 30 ng/mL in follicular fluid) had a lower quality of embryos and were less likely to achieve clinical pregnancy when compared to women with lower vitamin D levels [130]. To date, there is no intervention trial investigating the effect of vitamin D supplementation in women undergoing IVF and present data are insufficient to accurately evaluate the effects of vitamin D in women undergoing IVF.

There is ample evidence showing that calcium is important in the male reproductive tract, where it is essential for spermatogenesis, sperm motility, hyperactivation and acrosome reaction [131]. However, the role of vitamin D, which is known as an important regulator of calcium metabolism, in semen quality and spermatogenesis is less clear and was the focus of several studies conducted in the last years. A study in 300 men from the general population suggests that men with vitamin D deficiency (<10 ng/mL) had a lower proportion of motile, progressive motile and morphologically normal spermatozoa compared with men with sufficient vitamin D status (>30 ng/mL) [132]. Further, $1,25(OH)_2D_3$ increased intracellular calcium concentration in human spermatozoa in vitro through VDR-mediated calcium release from an intracellular calcium storage, increased sperm motility and induced the acrosome reaction in vitro [132]. In contrast, another study investigating the association of vitamin D status with semen quality in 307 young healthy men found a trend towards an association of high vitamin D levels with lower total sperm count and percentage of normal morphology sperm [133]. However, those trends totally disappeared in the multivariate model. Besides those observational studies, to date there are no data from randomized controlled trials investigating the effects of vitamin D treatment on semen quality. However, the discovery that $1,25(OH)_2D_3$ influences sperm function may be useful for the development of novel therapeutic approaches to the treatment of male reproductive disorders.

Low levels of vitamin D and androgens are both associated with increased mortality in men [134,135].

Recent data from the LURIC study including 2069 men referred for coronary angiography suggest that the coexistence of vitamin D and testosterone deficiency in men is associated with particularly adverse consequences for cardiovascular health [136]. While the presence of either a vitamin D deficiency or a testosterone deficiency is associated with an approximately 1.5-fold increased risk of death, the simultaneous presence of a deficiency of both hormones is linked with a 2.5-fold increased risk of dying, compared to men with a sufficient vitamin D and testosterone level. Moreover, the low testosterone levels were associated with increased mortality only in men who had a vitamin D deficiency, whereas men with a sufficient vitamin D level did not show any significant correlation between androgen deficiency and increased mortality.

Further data from the LURIC study suggest that, androgen levels and 25(OH)D level were independently associated and revealed a concordant seasonal variation [137]. The relationship between testosterone and vitamin D has recently been confirmed in two additional studies (European Male Aging Study and the Health Professionals Follow-up Study) [138,139]. Moreover, previous data indicate that vitamin D therapy might increase testosterone levels [140]. In men undergoing a weight reduction program who received either 83 μg (3332 IU) vitamin D daily for 1 year (n = 31) or placebo (n = 23), a significant increase in testosterone levels was observed in the vitamin D supplemented group with no significant change in the placebo group. In view of the clinical significance of low testosterone and 25(OH)D levels we want to stress that further studies are needed to investigate the impact of vitamin D supplementation on androgen status in men and to evaluate the effect of testosterone replacement in men with respect to vitamin D status.

9. Vitamin D during pregnancy and early infancy

At no other time during the lifecycle is vitamin D status more important than during pregnancy as the mother is the sole source of vitamin D substrate for her developing fetus. While vitamin D status during pregnancy varies around the globe as a function of maternal sunlight exposure, degree of skin pigmentation, latitude, lifestyle, body mass index (BMI) and the intake of oral vitamin D supplements, it is clear that those with darker pigmentation and limited sunlight exposure are at greatest risk for deficiency [141–146]. What is well established is that if a woman is deficient during her pregnancy, her fetus also will be deficient during gestation [141,147]. Whether such variation in vitamin D status can be associated with worse pregnancy outcomes still remains an open question and is the subject of this section of the paper.

With the exception of pregnancy, vitamin D metabolism is relatively constant throughout life. Yet, during pregnancy, vitamin D metabolism differs substantially, a point that until recently has gone largely unappreciated. Metabolism of vitamin D during pregnancy does not diverge from the nonpregnant state in the conversion of vitamin D to 25(OH)D, following first- and zero-order enzyme kinetics [141,148]. It is with the conversion of 25(OH)D to 1,25-dihydroxyvitamin D $(1,25(OH)_2D)$ that differences appear: known for decades that during pregnancy $1,25[OH]_2D$ levels become extremely elevated [149–151], this increase in circulating $1,25(OH)_2D$ has been attributed to an increase in the serum vitamin D-binding protein (DBP) that would regulate the amount of "free" $1,25(OH)_2D$ available in the circulation [150]. Yet, while DBP rises some 46–103% during pregnancy depending on the assay employed [152], it does not account for the nearly three- to fourfold increase in circulating $1,25(OH)_2D$ noted in a recent study by Hollis et al. [141]. Some insight into this process comes from a classic paper published in 1984 by Bikle et al. [151], who clearly demonstrated that free $1,25(OH)_2D$ levels are increased during pregnancy despite the significant increase in DBP levels.

Vitamin D metabolism is greatly altered during pregnancy, and pregnancy itself is the primary driver for these extraordinary circulating $1,25(OH)_2D$ levels. From the Hollis et al.'s data, it is evident that the production of $1,25(OH)_2D$ is independent of the classic regulators of calcium, phosphorus, and PTH [141]. The dramatic rise in maternal circulating $1,25(OH)_2D$ level following conception is remarkable for many reasons: by 12 weeks of gestation, maternal circulating $1,25(OH)_2D$ is already triple those of a nonpregnant female [141,153]. Going forward through gestation, $1,25(OH)_2D$ continues to rise as a function of substrate—25(OH)D—availability. This substrate dependence of $1,25(OH)_2D$ on 25(OH)D for optimal production is never observed in normal human physiology driven by classic calcium homeostasis. There is an independence during pregnancy between $1,25(OH)_2D$ level and calcium metabolism such that the pregnant women in attaining supraphysiologic levels of $1,25(OH)_2D$ (relative to the nonpregnant state), sometimes exceeding 700 pmol/L [141,153], never exhibit hypercalciuria or hypercalcemia [141]. Additional data from Hollis et al. [141] demonstrated that a circulating 25(OH)D level of approximately 40 ng/mL (100 nmol/L) is required to optimize the production of $1,25(OH)_2D$ during pregnancy through renal and/or placental production of the hormone [141], a relationship that is also noted in the neonate but at no other time during the lifecycle [141,154].

There are four contending theories of how this occurs during pregnancy [153]: (1) higher placental conversion rates of 25(OH)D to $1,25(OH)_2D$ by placental 1-α-hydroxylase; (2) uncoupling of renal 1-α-hydroxylase from feedback control and for reasons other than maintaining calcium homeostasis (a distinct possibility as women with nonfunctional renal 1-α-hydroxylase and normal placental function fail to increase circulating $1,25(OH)_2D$ during pregnancy [155]); (3) there is methylation of the catabolic CYP24A1 placental gene [156]; and (4) increased calcitonin during pregnancy may be a contributor to this process in that it rises during pregnancy, is known to stimulate the renal 1-α-hydroxylase gene independently of calcium levels, and protects by opposing hypercalcemia [157–159]. Suggested as a possible stimulator of 1-α-hydroxylase during pregnancy [160], prolactin is less likely to play a major role in $1,25(OH)_2D$ metabolism during pregnancy as prolactin increases significantly during lactation and yet there is not a perpetuation of the high pregnancy $1,25(OH)_2D$ levels [161]. Clearly, vitamin D metabolism during pregnancy is unique in human physiology; but what is its purpose?

Studies conducted in the 1980's and 1990's found associations between maternal deficiency and abnormal fetal growth, dentition and maternal health, yet the robustness of these findings was held in question as the studies were plagued by small sample sizes and the amount of vitamin D given was often low with few differences noted between women who had received placebo and those who had received treatment—typically 400 IU vitamin D/day [162–166]. Prior studies did not establish the optimal vitamin D requirements and blood levels during pregnancy. In addition, the effects of vitamin D during pregnancy were thought, until recently, to be limited to calcium and bone metabolism and that the daily requirements were met by casual outdoor sunlight exposure and a prenatal vitamin containing 400 IU. This way of thinking has persisted into the 21st century and was reiterated by the Institute of Medicine in their 2010 report [167]. There is evidence, however, that a woman's vitamin D requirements are not 400 IU/day, but rather, 4000 IU/day [141,146]. As discussed earlier, the optimal conversion of 25(OH)D to the active form the hormone—$1,25(OH)_2D$—is not attained until 25(OH)D level is at least 100 nmol/L (40 ng/mL), most easily achieved with 4000 IU/day dosing [141]. Yet, it is unclear why $1,25(OH)_2D$ rises more than two and half times nonpregnant levels with virtually no change in calcium levels, although early data suggest a role in maintaining immune homeostasis that is essential during pregnancy for the health of mother and fetus. Various health effects of vitamin D deficiency during pregnancy continue to be reported; notably with increased risk of preeclampsia [168,169], infection [146,170], preterm labor and preterm birth [146], cesarean section [171], and gestational diabetes [172]. What do these seemingly diverse groups of disease states and events have to do with vitamin D? What is the plausible mechanism of action that links them to vitamin D?

The answers to these questions surround vitamin D's non-calcium effects on immune modulation. As discussed earlier in this paper, during the past two decades there has been mounting evidence that vitamin D plays a role not only in calcium metabolism but also in the modulation of both innate and adaptive immunity [173,174]. Differences in immune function on the basis of vitamin D status, however, have not yet been shown; rather, only differences in disease state risks. Specific changes during pregnancy in immune modulation, innate and adaptive function, as they relate to specific disease states are being evaluated now in more recent ongoing trials.

To begin to answer the question of what constitutes vitamin D sufficiency during pregnancy and health-related effects of that sufficiency, two recent randomized clinical trials conducted by Hollis, and Wagner et al. were published [141,146]. The largest was an NICHD-sponsored randomized controlled trial of vitamin D supplementation beginning at 12–16 weeks of gestation where healthy women were randomized to one of three treatment groups—400, 2000, and 4000 IU vitamin D_3/day [141]. In the second clinical trial sponsored by the Thrasher Research Fund, women were randomized at 12–16 weeks of gestation to either 2000 or 4000 IU vitamin D_3/day [146]. Vitamin D status and health characteristics were recorded for both studies. Both studies showed improved vitamin D status throughout pregnancy and fewer comorbidities of pregnancy in the 4000 IU group [141,146]. When analyzed by 25(OH)D level, improved vitamin D status was associated with better pregnancy outcomes [141,146].

Given that both the NICHD and Thrasher Research Fund studies were conducted concurrently by the same study team using a common data dictionary, the datasets were combined to increase sample size and to collectively address the following questions: (1) what are the potential health effects of vitamin D supplementation during pregnancy, and (2) what are the implications of vitamin D deficiency on the mother and her fetus? These findings recently published were as follows: there were no differences in maternal baseline 25(OH)D, but there were consistent differences in maternal and cord blood 25(OH)D levels achieved with higher rates of sufficiency using various cutpoints in the 4000 IU group and 2000 IU group compared to the control group (p-values generally <0.0001), an effect that persisted even after controlling for race and study. When the four main comorbidities of pregnancy were combined, for every 10 ng/mL increase in maternal 25(OH)D at delivery, the odds ratio was reduced to 0.84 (p = 0.006).

Extending the findings of pregnancy to lactation, it is evident that if 400 IU/day is inadequate in attaining vitamin D sufficiency during pregnancy, then during lactation when a woman is transferring ~20% of her vitamin D daily in her milk, her requirements will be higher than during pregnancy [153]. Two pilot studies by Hollis and Wagner provided evidence that when a mother is replete in vitamin D, her milk is replete and therefore, her recipient infant also will be replete [175,176]. Preliminary findings from a larger, two-site NICHD trial involving more than 300 women from the same group support the earlier findings [177]: women randomized to 6400 IU vitamin D_3/day while their infants received placebo had superior vitamin D status compared to women taking 400 IU/day. Those infants of mothers in the 400 IU group also received 400 IU vitamin D_3/day and their total circulating 25(OH)D levels were similar to the infants in the 6400 IU whose sole source of vitamin D was their mothers. Both groups had similar serum calcium and urinary calcium/creatinine profiles with no increased toxicity in the 6400 IU group as deemed by the Data Safety and Monitoring Committee.

What do these collective studies tell us about vitamin D requirements during pregnancy and lactation? It is clear that higher doses are necessary to recapitulate the action of sunlight and that the doses given up to 4000 IU/day in the pregnant woman and 6400 IU/day in the lactating woman are safe and effective in achieving sufficiency and improving health not only in the mother but also in the developing fetus, and later—in her breastfeeding infant. Public health policies must be enacted to ensure that women are being counseled appropriately regarding options during these important times in the lifecycle.

10. Vitamin D and dementia

Alzheimer's disease and other forms of dementia are debilitating and costly to families and societies, and yet no proven intervention currently exists to prevent, delay or treat dementia. The incidence and prevalence of dementia are strongly age-associated, and as a result the number of people with dementia is projected to increase from 24 million in 2005 to 81 million in 2040 due to our aging worldwide population [178]. Much of this increase will be in developing countries, such as India and China, and it is therefore crucial to identify cost-effective interventions to combat dementia [178]. Currently available treatments for dementia are not disease modifying, and do not result in symptomatic benefits for all patients. The allure of vitamin D is that it may confer genuine protection in the elderly population against Alzheimer's disease, other forms of neurodegeneration, and vascular pathologies including stroke. Vitamin D receptors and 1α-hydroxylase are widespread in brain regions important for cognitive functions including the hippocampus [179], and deficiency is associated with vascular neuropathology [180]. Several neuroprotective mechanisms have been suggested, including increased phagocytosis of amyloid plaques [181], regulation of neurotrophins [182], and alterations in calcium homeostasis [183]. This raises the question whether circulating vitamin D levels are related to cognitive function or dementia status in humans.

Early small clinical studies provided somewhat limited evidence relating to serum 25(OH)D levels in relation to dementia status or cognitive impairment. The first large cross-sectional population-based study in 2007 using data from the Third National Health and Nutrition Examination Survey (NHANES III) did not support the hypothesis that adequate circulating vitamin D may play a neuroprotective role—indeed those with the highest levels of serum 25(OH)D were more likely to be impaired on a limited test of delayed verbal memory [184]. However, this data was reanalyzed using the full range of cognitive tests available to produce a more robust measure of cognition and found that low vitamin D levels were strongly associated with increased odds of cognitive impairment [185]. To address this uncertainty in 2009 the association between serum 25(OH)D levels and cognitive impairment was investigated in 1766 older adults from the Health Survey for England (HSE), a nationally representative population-based study [186]. Odds ratios (95% confidence intervals [CIs]) for cognitive impairment in participants who were severely 25(OH)D deficient (<10 ng/mL), deficient (≥10 ng/mL and <20 ng/mL), and insufficient (≥20 ng/mL and <30 ng/mL) compared with participants with sufficient 25(OH)D (≥30 ng/mL) were 2.7 (1.5–5.0), 1.37 (0.8–2.3), and 0.9 (0.5–1.6) after adjustment for age, sex, education, ethnicity, season of testing, and additional risk factors for cognitive impairment. Interest in the association between vitamin D and dementia has subsequently burgeoned, and a large number of clinical and population-based studies have followed.

Several systematic reviews and meta-analyses have now been conducted to make sense of this complex and evolving body of literature. It is clear that despite mixed early findings a consistent picture has since emerged. Balion and colleagues recently published a meta-analysis establishing that serum 25(OH)D levels were lower in Alzheimer's disease patients than cognitively healthy controls, though significant heterogeneity in the differences was observed on the basis of assay used [187]. Participants with serum 25(OH)D levels < 20 ng/mL also scored 1.2 points (95% CI 0.5–1.9) lower on the widely used Mini-Mental State Examination test of cognitive function than those with higher levels of circulating vitamin D [187]. Similarly, Etgen and colleagues' meta-analysis suggests that the odds of cognitive impairment are significantly higher (OR = 2.4, 95% CI 1.8 to 3.2) in participants with low 25(OH)D levels (a cut-point of <10 ng/mL in most studies incorporated) [188].

In 2010 using the InCHIANTI study of 858 Italians we established that severely deficient (<10 ng/mL) elders had increased risk of cognitive decline over 6 years (relative risk = 1.6, CI 1.2 to 2.0) compared to those with sufficient levels (≥30 ng/mL) [189]. Slinin and colleagues also observed a similar association in the Health ABC study in the US across quartiles of serum 25(OH)D, though the trend across groups became non-significant in their fully adjusted model (OR for lowest versus highest quartile = 1.4, 95% CI 0.9 to 2.2) [190]. It should be noted that the cut-point for the lowest quartile in their cohort was relatively high (<20 ng/mL) and their strategy for adjustment for covariates differed from ours. Two more recent studies by Annweiler and colleagues using the EPIDOS cohort in France showed that low 25(OH)D (<10 ng/mL) in elderly women at baseline predicted the onset of non-Alzheimer's dementia over 7 years [191], and those in the highest quintile of baseline dietary vitamin D intake had a reduced risk of developing Alzheimer's disease [192]. Using the Study of Osteoporotic Fractures Slinin and colleagues found that those with baseline vitamin D levels of <10 ng/mL had an increased risk of global cognitive decline compared to those with ≥30 ng/mL (OR = 1.6, 95%, CI 1.1 to 2.2) [193]. Similarly, Breitling and colleagues using the ESTHER study showed that the highest quintile of vitamin D was associated with significantly lower levels of cognitive decline, with a stronger effect observed in women than in men [194].

Controversy remains regarding the appropriate adjustment for co-variates in observational studies of vitamin D in relation to cognitive decline or dementia. For example, the sources of vitamin D itself (sunlight exposure, dietary intake, fortification and supplementation) are not likely to be confounders, and adjustment for these variables or proxy measures such as time spent outdoors or latitude is likely to represent over adjustment. Even adjustment for age is not without controversy, as human skin is known to become less efficient at vitamin D production with age. Age is therefore related to the synthesis of vitamin D and is not just a proxy measure for possible unmeasured confounding.

Ultimately randomized controlled trials are needed to establish whether vitamin D supplementation has clinical relevance in this context and can be used to prevent, delay or treat dementia. At this point no large well-designed randomized controlled trials have been conducted, and the causal relationship between vitamin D and dementia remains uncertain and caution should be exercised. Existing trials on vitamin D and cognitive decline have produced inconclusive results and had a number of important drawbacks including small sample sizes (<100) [195,196] and the use of low doses of vitamin D (<520 IU/day) with a combination of other nutrients [195,197], making interpretation difficult. However, several large trials are currently underway which will provide important new information. The DOHealth trial is being conducted in around 2000 participants aged 70 years and older across eight European cities. Vitamin D3 supplements (2000 IU/day) are one of the three interventions incorporated and cognitive outcomes will be measured over 3 years. Another key trial is the VITAL study in the US that aims to recruit around 20,000 middle aged and older adults. Again one of the interventions investigated will be vitamin D3 supplements (2000 IU/day), although cognitive outcomes over 4.5 years will only be assessed in a subsample of around 10% of participants. Neither trial targets older adults who are known to have low levels of vitamin D and early cognitive changes indicating that they are at high risk for dementia. If these trials do not produce promising results we may be left wondering if a more targeted approach or a different dose of vitamin D supplementation might be more effective.

11. Conclusion

It is now recognized that vitamin D deficiency and insufficiency are a global health problem [1,5,198–201]. A multitude of studies have suggested that vitamin D deficiency and insufficiency not only have negative consequences on bone health but are also likely to be a risk for many acute and chronic illnesses including infectious diseases, autoimmune diseases, cardiovascular disease, type 1 and type 2 diabetes mellitus, several types of cancer, neurocognitive dysfunction and mental illness, and other diseases, as well as infertility and adverse pregnancy and birth outcomes [24,26,37,49,55,75–79,85,90–94,100–105,109,117,118, 136,141,146,186,187,202,203].

It is interesting that healthy black children in South Africa have blood levels of 25(OH)D of 49 ± 4 ng/mL [204] similar to adult Maasai herders of 47 ± 10 ng/mL [205]. It is well documented that blood levels of 25(OH)D are maximum at the end of the summer and are at their nadir at the end of the winter even in Denmark [206]. Physiologically it makes no sense to have wide swings in the circulating levels of 25(OH)D. This is the reason why a three-part strategy to maintain circulating levels of 25(OH)D of at least 30 ng/mL should be encouraged. Sensible sun exposure, which remains the major source of vitamin D for most children and adults [1,207], along with including foods that naturally contain or are fortified with vitamin D [1], and taking a daily supplement of vitamin D should be able to sustain blood levels of 25(OH)D in a range similar to our hunter-gatherer forefathers, i.e. 25(OH)D ~40–50 ng/mL. Since there is no downside to increasing children's and adults' vitamin D status (with the exception of patients with granulomatous disorders) it is reasonable to attain and maintain a circulating level of 25(OH)D of 40–60 ng/mL as recommended by the Endocrine Society Experts or even slightly lower (30–50 ng/mL)

as recommended in "Practical guidelines for supplementation of vitamin D and treatment of deficits in Central Europe: Recommended vitamin D intakes in general population and groups being at risk of vitamin D deficiency" [208], not only for optimal bone health but also for overall health and well-being.

Take-home messages

- Vitamin D deficiency is a global health problem for children and adults. Vitamin D deficiency is associated with rickets and growth retardation in children and osteoporosis and osteomalacia in adults. Vitamin D deficiency has also been linked to many acute and chronic illnesses including some cancers, autoimmune diseases, cardiovascular disease, type 1 and type 2 diabetes mellitus, infectious diseases and neurocognitive dysfunction and other diseases, as well as infertility and adverse pregnancy and birth outcomes.
- A three-part strategy should be implemented to combat the vitamin D deficiency pandemic which includes:
  ➢ Eating foods that naturally contain vitamin D,
  ➢ Encouraging food fortification with vitamin D in countries that do not practice this fortification and,
  ➢ Providing guidelines for both vitamin D supplementation of general population and for sensible sun exposure as a reliable source of vitamin D.
- Anti-fall and anti-fracture action of vitamin D administration of at least 800 IU/day with at least 24 ng/mL (60 nmol/L) of 25(OH)D serum levels appeared effective and beneficial for musculoskeletal machinery.
- Vitamin D may be instrumental in the immune system homeostasis, and in preventing autoimmune diseases and lowering risk of infections.
- Vitamin D deficient individuals are at increased cardiovascular risk even after adjustments for common cardiovascular risk factors.
- Risk for breast and colorectal cancer decreases as serum 25(OH)D level increases to 30–40 ng/mL (75–100 nmol/L).
- All-cause mortality risk in general population seems to be the lowest at 25(OH)D levels ranging from 30 to 45 ng/mL (75 to 112.5 nmol/L).
- Vitamin D supplementation up to 4000 IU/day in pregnant woman is safe and effective in achieving sufficiency and improving health not only in the mother but also in the developing fetus, every 10 ng/mL increase in maternal 25(OH)D at delivery reduces the risk of four main comorbidities of pregnancy by 16%.
- It is reasonable to attain and maintain a circulating level of 25(OH)D of 30–60 ng/mL as recommended by the Endocrine Society or even slightly lower (30–50 ng/mL) as recommended in "Practical guidelines for supplementation of vitamin D and treatment of deficits in Central Europe", not only for optimal bone health but also for overall health and well-being.

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Autoantibodies against Class II-associated invariant chain are biomarkers of early spondyloarthritis

Spondyloarthritis (SpA) is a common musculoskeletal inflammatory disease occurring with heterogeneous clinical features. It typically affects the spine but also peripheral joints, sometimes with no evidence of axial involvement. At present, the diagnosis of SpA in the early phase of the disease is difficult, due to the lack of specific biomarkers and the delay in radiographic signs of spinal or joint damage. The aim of recently published study by Baerlecken et al. (Ann Rheum Dis 2013; May 17. Epub ahead of print PMID:23687263) was the identification of new autoantibodies as diagnostic biomarkers of SpA. Serum samples from a large cohort of patients affected with axial or peripheral SpA, and from a large number of healthy or disease controls, including patients with psoriatic arthritis, rheumatoid arthritis, systemic lupus erythematosus, HIV infection, and blood donors, were tested for autoantibodies towards new autoantigens. A double methodological approach was employed. Protein array technology, based on cDNA of fetal brain tissue, was used as a screening test in a number of patient and control sera; subsequently results were confirmed by ELISA using as autoantigens Class II-associated invariant chain CD74 recombinant protein, or a peptide representing the extracellular domain of CD74, namely CLIP. The authors identified IgG antibodies against either complete CD74 protein or CLIP domain in a large proportion of patients with SpA, in particular in HLA-B27 negative patients in the early disease stage. In conclusion, autoantibodies towards CD74 have been proposed as diagnostic tools for early diagnosis of SpA.
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