

MANAGEMENT OF ENDOCRINE DISEASE

Therapeutics of vitamin D

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Abstract

Objective: The central role of vitamin D in bone health is well recognized. However, controversies regarding its clinical application remain. We therefore aimed to review the definition of hypovitaminosis D, the skeletal and extra-skeletal effects of vitamin D and the available therapeutic modalities.

Design: Narrative and systematic literature review.

Methods: An international working group that reviewed the current evidence linking bone and extra-skeletal health and vitamin D therapy to identify knowledge gaps for future research.

Results: Findings from observational studies and randomized controlled trials (RCTs) in vitamin D deficiency are discordant, with findings of RCTs being largely negative. This may be due to reverse causality with the illness itself contributing to low vitamin D levels. The results of many RCTs have also been inconsistent. However, overall evidence from RCTs shows vitamin D reduces fractures (when administered with calcium) in the institutionalized elderly. Although controversial, vitamin D reduces acute respiratory tract infections (if not given as bolus monthly or annual doses) and may reduce falls in those with the lowest serum 25-hydroxyvitamin D (25OHD) levels. However, despite large ongoing RCTs with 21 000–26 000 participants not recruiting based on baseline 25OHD levels, they will contain a large subset of participants with vitamin D deficiency and are adequately powered to meet their primary end-points.

Conclusions: The effects of long-term vitamin D supplementation on non-skeletal outcomes, such as type 2 diabetes mellitus (T2DM), cancer and cardiovascular disease (CVD) and the optimal dose and serum 25OHD level that balances extra-skeletal benefits (T2DM) vs risks (e.g. CVD), may soon be determined by data from large RCTs.

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Introduction

The central role of vitamin D in bone health is well recognized. However, a series of controversies regarding its clinical application remain. They primarily concern how to define hypovitaminosis D, the skeletal and extra-

skeletal effects of vitamin D and the different therapeutic modalities available. To identify the controversial issues, summarize the present state of knowledge and formulate some research questions to answer these controversies,

a meeting was held in June 2017, during which the various aspects of vitamin D measurement, its effects and therapeutic applications were reviewed and discussed by international experts in the field. In this paper, we address these issues.

Methods

This commentary reflects the discussion of a working group that reviewed the current evidence linking bone health and vitamin D therapy. It is based on both narrative and systematic literature reviews, focusing on the most robust clinical evidence, which constituted the search criteria in PubMed.

Vitamin D as a therapeutic agent and its administration

Questions relating to areas of agreement:

- Is vitamin D₃ superior to D₂? – Pharmacokinetics, clinical data.
- What is the role for 25-hydroxyvitamin D in replacement and its availability?
- Is there a place for active vitamin D metabolites in treating vitamin deficiency?
- Is daily dosing of vitamin D preferred to larger weekly, monthly or annual doses?
- Does the baseline level of serum 25-hydroxyvitamin D influence the choice and frequency of dose?
- Does BMI or race influence the dose or mode of administration?
- What is the role of fortification in increasing vitamin D?
- How much is too much vitamin D?
- What adverse outcomes are important?

Type and source of vitamin D: metabolites, active forms and analogues

Skin is the major source of cholecalciferol in humans, which is formed after exposure to the ultraviolet B (UVB) wavelength of sunlight. A number of personal and environmental factors reduce the skin production of vitamin D, such as low UVB availability, which is dependent, in part, on latitude, season, time of day and extent of air pollution (1, 2, 3). Even so, in regions of high UVB availability, a substantial prevalence of hypovitaminosis D has been reported (4). On the other hand, in countries where UVB availability is generally low,

such as in Scandinavian countries, the use of cod liver oil and food fortification with vitamin D leads to higher levels of 25-hydroxyvitamin D than would be expected (5, 6). Methods to increase vitamin D intake across a given population, including bio-fortification consisting of adding vitamin D to livestock feeds or UVB-irradiation of mushrooms and baker's yeast are under evaluation (7, 8).

For the treatment of hypovitaminosis D, either sun exposure or increased intake of vitamin D-fortified foods may not be sufficient. In these settings, vitamin D supplements should be considered, with various guidelines suggesting either vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol) (9). Vitamin D₃ seems to be preferred by many experts because of its greater potency (10, 11).

Interestingly, a meta-analysis of double-blind RCTs document that focused on a dose of vitamin D >480IU/day the pooled effect of vitamin D₃ on fractures was significantly lower by 23%, while the pooled effect on fractures was not significant with vitamin D₂ (12). When vitamin D₂ and D₃ are considered together, a Cochrane analysis showed a significant decrease in incidence of hip fractures and non-vertebral fractures in patients treated with vitamin D plus calcium vs no treatment (13).

Regarding vitamin D metabolites in cases of age- or disease-related declines in hepatic and renal function (14), 25-hydroxyvitamin D (calcidiol) can be used, when available. However, calcidiol is not available in the United States. In patients with advanced liver disease in whom the hepatic hydroxylation of vitamin D is impaired, calcidiol would be a logical form because it bypasses that hydroxylation step in the liver (14). It is also a logical form of vitamin D to use in the setting of glucocorticoid-induced inhibition of hepatic 25-hydroxylase. (15). Since glucocorticoids may inhibit not only the hepatic 25-hydroxylation of vitamin D, but also the subsequent 1-hydroxylation step in the kidney, to form active calcitriol, the use of calcitriol has been proposed as a way of bypassing both hydroxylation steps for the treatment of glucocorticoid-induced osteoporosis (GIOP). Indeed, in some clinical studies, active vitamin D analogues were shown to be more effective than native vitamin D₃ in terms of bone mineral density (BMD) improvement and reduction of fracture risk in GIOP (16). However, in other studies of GIOP, the effects of native vitamin D₃ were comparable with those achieved by active vitamin D analogues (17). Moreover, calcidiol has been proposed for the treatment of hypovitaminosis D in obese patients due to its greater hydrophilic properties, in comparison with vitamin D itself, resulting in a smaller volume of

distribution and potentially more rapid and effective normalization of serum 25-hydroxyvitamin D values (14). Calcidiol may also be the most preferred form of vitamin D in cases of fat malabsorption due to a variety of diseases or following bariatric surgery (14). This discussion of advantages of calcidiol over vitamin D itself refers to establishment of vitamin D sufficiency. It does not imply that calcidiol is more effective than vitamin D₃ in improving BMD or in decreasing fracture risk, because this point has not been established (13, 14).

Evidence of anti-fracture effectiveness, in fact, is weaker for other vitamin D analogues as compared with vitamin D₃, while the risk of side effects is higher (13). Calcitriol (1,25(OH)₂ vitamin D) for example, had no statistically significant effect on hip fracture, non-vertebral fractures or new vertebral deformities (13, 18). Two trials suggested that vertebral fractures may be prevented by 1 α -hydroxycalciferol (alfacalcidol). This analogue, a synthetic active vitamin D compound, hydroxylated at position 1, can undergo rapid 25-hydroxylation in the liver and thus becomes fully active. While promising, these results were greatly influenced, and perhaps biased, by a quasi-randomized study design (13).

The use of calcitriol or alfacalcidol is recommended only in patients with renal insufficiency and secondary hyperparathyroidism in whom renal 1 α -hydroxylation of vitamin D is impaired (14). However, even in this clinical setting, there is some evidence that native vitamin D has the capability to normalize serum parathyroid hormone values, at least when the hyperparathyroidism is not severe (19). Use of the native vitamin D preparation minimizes the risk of hypercalcaemia and hypercalciuria frequently associated with the use of active vitamin D analogues. Interestingly, vitamin D₃ has also been shown to improve some extra-skeletal end-points, such as proteinuria and anaemia (20).

While food fortification practices are modifying the epidemiological surveys of vitamin D status throughout the world, it is unrealistic to expect that such measures alone will satisfy recommended daily vitamin D requirements across populations. Therefore, the use of vitamin D supplements is often required to ensure normal vitamin D status. The current evidence supports the use of vitamin D₃ as a first-line approach for treatment and prevention of hypovitaminosis D, whereas metabolites and active analogues of vitamin D may be used in some specific clinical settings.

To ensure vitamin D adequacy, a daily vitamin D supplement of 400 IU should be provided to all infants and a slightly higher dose (600 IU) (for small children; usually up to the age of 4 years). Similarly, in view of the high

prevalence of vitamin D deficiency in older individuals, a daily intake of 600–800 IU is recommended (21). At both ends of the life span, an adequate intake of calcium is also necessary.

Mode of administration and dose

Supplementation with vitamin D increases serum 25-hydroxyvitamin D levels, but the increment depends upon both body weight and the baseline serum 25-hydroxyvitamin D concentration. Body weight affects the serum 25-hydroxyvitamin D response to both loading and maintenance doses of vitamin D, with larger doses being required. Lower baseline serum 25-hydroxyvitamin D levels are associated with larger increases in serum 25-hydroxyvitamin D for a given dose of vitamin D.

How body weight affects dosing and serum 25-hydroxyvitamin D level is important to understand, given high prevalence of obesity and reported associations between low vitamin D status and weight-dependent conditions, such as type 2 diabetes mellitus. Compared with people with normal body weight, overweight or obese people have lower 25-hydroxyvitamin D levels and require longer exposure to UVB or higher doses of oral vitamin D supplementation to achieve desired 25-hydroxyvitamin D levels (22, 23). It has been postulated that adiposity accounts for the lower 25-hydroxyvitamin D levels seen in overweight/obese individuals due to sequestration of this fat-soluble vitamin D in excess adipose tissue. However, it appears that the difference in 25-hydroxyvitamin D levels is due to extravascular body size (fat and non-fat), although visceral adiposity may contribute more to the inverse association between weight and serum 25-hydroxyvitamin D levels (22, 23). Weight loss results in higher serum 25-hydroxyvitamin D levels (23).

Loading doses The idea of a loading dose is logical, because this could theoretically establish vitamin D adequacy rather quickly and more conveniently. While these are worthy points, it should be noted that at very high loading doses of vitamin D the efficiency of conversion of vitamin D into 25-hydroxyvitamin D is much lower than when more physiologic doses are used.

The pool of 25-hydroxyvitamin D is estimated at about 160 or 240 μ g for a subject with serum 25-hydroxyvitamin D of 20 (50 nmol/L) or 30 ng/mL (75 nmol/L), respectively. To replace that pool of 25-hydroxyvitamin D with oral vitamin D, one should take into account the percentage of intestinal absorption (~70%) and the conversion efficiency

of vitamin D into 25-hydroxyvitamin D. Thus, a total loading dose of 700–1000 µg or 30–40 000 IU of vitamin D₃ should be sufficient to replace near total absence of vitamin D to normal 25-hydroxyvitamin D concentrations (20–30 ng/mL; 50–75 nmol/L). This conclusion is reached on the basis of pharmacokinetic data, but has not been convincingly demonstrated in clinical trials focused on this question.

King *et al.* (24) found that obese patients had a blunted serum 25-hydroxyvitamin D increment compared with non-obese patients in response to a single oral vitamin D₃ dose of 300 000 IU after both 6 and 52 weeks. Earlier, van Groningen *et al.* (25) treated severely vitamin D-deficient subjects with one of several loading regimens: (1) 25 000 IU q 2 weeks for 8 weeks (total of 100 000 IU), (2) 25 000 IU q week for 6 weeks (total 150 000 IU) or (3) 25 000 IU q week for 8 weeks (total of 200 000 IU). Serum 25-hydroxyvitamin D was measured at baseline and 10 days after the final dose (the time needed to convert 90–100% of the D₃ dose to 25-hydroxyvitamin D). The three loading regimens caused a dose-related increase in serum 25-hydroxyvitamin D resulting in mean 25-hydroxyvitamin D levels of 48.3 ± 13.0, 63.6 ± 27.5 and 89.7 ± 26.9 nmol/L, respectively. The change in 25-hydroxyvitamin D was significantly related to the dose per kg body weight and is described by the following formula:

$$\Delta 25\text{-Hydroxyvitamin D} = 0.025 \times (\text{dose per kg body weight})$$

However, they recommended that the formula should not be used in subjects with body weights >125 kg (25). No adverse effects in any subject was reported.

The impact of bolus dosing on fall risk is controversial. The question is important given recent evidence that (1) a single large annual oral dose of 500 000 IU of D₃ increased the risk of falling (26) and (2) in seniors who fell in the last year, monthly oral dosing with 60 000 IU (equivalent to 2000 IU/day) caused more falls than monthly oral dosing with 24 000 IU (equivalent to 800 IU/day) (66.9 vs 47.9% fallers, respectively) (27).

Do oral and intramuscular administrations of vitamin D result in equivalent increments in serum 25-hydroxyvitamin D? Romagnoli *et al.* (28) compared serum 25-hydroxyvitamin D increments after administration of either single oral or intramuscular doses of 300 000 IU of D₂ or D₃ in 32 elderly nursing home residents. The starting serum 25-hydroxyvitamin D levels in the four groups were low, ranging from 7.3 to

13.3 ng/mL. The 1-month increments in serum 25-hydroxyvitamin D after treatment with the D₃ form were 47.8 ± 7.3 ng/mL by the oral route and only 15.9 ± 11.3 ng/mL by the intramuscular route. Smaller increments, but similar differences in the two routes of administration were observed in the groups treated with D₂ (15.9 ± 11.3 and 5.0 ± 4.4 ng/mL, respectively). By 60 days however, increments and levels achieved by the two routes were similar. Thus, for loading purposes, the intramuscular route is less effective because of the 2-month lag time in matching the 25-hydroxyvitamin D increment seen with oral administration. For maintenance purposes, more long-term information is needed in order to determine the appropriate dosing frequency.

Maintenance doses The serum 25-hydroxyvitamin D responses to maintenance doses of vitamin D are also influenced by body weight. Gallagher *et al.* treated 163 healthy postmenopausal women for 1 year with one of eight doses of vitamin D₃ ranging from 0 (placebo) to 4800 IU/day (22). The mean starting serum 25-hydroxyvitamin D level was 39 nmol/L. Obese women (BMI ≥30) had 25-hydroxyvitamin D levels that were 17.8 nmol/L lower than normal weight women (BMI <25), with overweight women falling in between.

Dose frequency – daily, weekly, monthly

Ish-Shalom *et al.* (29) reported serial 25-hydroxyvitamin D levels over 2 months of treatment with the same cumulative dose of D₃ (equivalent to 1500 IU per day) but given daily, weekly or monthly. The mean levels over the 2-month period were similar although there was slightly more variability with the monthly dosing. Vieth demonstrated that it takes about 3 months for serum 25-hydroxyvitamin D levels to reach steady state after a change in vitamin D₃ intake (30), but there is no reason to expect that the conclusion would have been different had the intervention in the Ish-Shalom study continued for the same duration as the study of Vieth. Binkley *et al.* (31) compared 25-hydroxyvitamin D levels in 64 healthy older adults treated with 1600 IU daily and 50 000 IU per month over a 1-year period. Similar serum 25-hydroxyvitamin D levels were achieved with the two dosing frequencies, consistent with the findings of Ish-Shalom.

However, a recent meta-analysis comparing vitamin D₂ and D₃ supplementation in raising serum 25-hydroxyvitamin D status showed that vitamin D₃ was more efficacious at raising serum 25-hydroxyvitamin D

concentrations than was vitamin D₂. Additionally, when the frequency of dosage administration was compared, there was a significant response to vitamin D₃ when given as a bolus dose compared with administration of vitamin D₂, but only a non-significant trend was present for daily supplementation (32).

Dietary fat and vitamin D absorption

Vitamin D is a fat-soluble vitamin. One study showed the increase in serum 25-hydroxyvitamin D seen 12h after taking a 50 000IU vitamin D₃ supplement is about 30% greater when the supplement is taken with a meal containing fat (33), suggesting vitamin D supplements be taken with a meal containing some fat, whenever practicable.

Variables that influence dosing and levels

Race: How race affects dosing and the blood 25-hydroxyvitamin D level is important to understand given reported associations of low vitamin D status and chronic conditions, such as type 2 diabetes, which are prevalent in certain races (e.g. blacks). Compared to Caucasians, blacks have lower total 25-hydroxyvitamin D levels attributed primarily to lower cutaneous biosynthesis. Although absorption and metabolism of oral vitamin D supplementation appears to be the same between blacks and Caucasians, (34) dietary vitamin D intake may be a stronger determinant of 25-hydroxyvitamin D in blacks than Caucasians. Despite a lower total 25-hydroxyvitamin D level, blacks appear to have a more efficient calcium economy and superior bone mass and lower risk of fractures. Race may also modulate the increased risk of chronic disease (e.g., CVDs, diabetes) associated with low 25-hydroxyvitamin D levels (35, 36).

Despite abundant, year-long sunshine in the Middle East, vitamin D deficiency and inadequacy is highly prevalent due, in part, to traditional clothing covering most of the body and the lack of foods rich in, or fortified with, vitamin D. It is highest in women of child-bearing age in the Kingdom of Saudi Arabia at 86.4% and in elderly Egyptians at 77.2% (37). A recent multicentre survey of vitamin D status in China using the LC-MS/MS method showed that 55.9% of the population had serum 25-hydroxyvitamin D levels <20 ng/mL (<50 nmol/L). Serum 25-hydroxyvitamin D levels were lower in women than in men, in younger (age 18–39 years) than older (>59 years) individuals

and in certain regions. These results emphasize the importance of factors other than latitude (38).

Extra-skeletal effects

Low vitamin D status, assessed by circulating 25-hydroxyvitamin D levels, has been associated with many acute or chronic medical conditions. One of the most consistent associations is with type 2 diabetes mellitus (T2DM). Longitudinal cohort studies, summarized in recent meta-analyses, have estimated an approximately 40% risk reduction for incident diabetes in the highest vs the lowest category of 25-hydroxyvitamin D level (39). However, the observational nature of these studies precludes a definitive assessment of cause and effect because reverse causation or residual confounding cannot be excluded. Confounding is especially problematic in this area because blood 25-hydroxyvitamin D level is an excellent marker of good health.

The largest trial of vitamin D supplementation for prevention of T2DM is the Tromso study from Norway (511 white adults with pre-diabetes received 20 000 units/week (~2900 units/day) vitamin D₃ or placebo; follow-up ~3.3 years for incident diabetes) (40). The risk of diabetes was lower in the vitamin D-supplemented group vs placebo throughout the study; however, the difference was not statistically significant (HR 0.90; 95% CI 0.69, 1.18). The vitamin D and type 2 diabetes (D2d) study, an ongoing large trial that tests the efficacy of 4000 units/day of vitamin D on prevention of T2DM in people at high risk, is expected to answer this question.

In relation to T2DM, higher 25-hydroxyvitamin D level is monotonically associated with a lower diabetes risk, suggesting no apparent threshold for benefit (39). However, for non-diabetes outcomes, several observational studies have reported a J-shaped association curve between 25-hydroxyvitamin D levels and important outcomes (e.g., cardiovascular outcomes, mortality) (41, 42).

Risks of vitamin D overdose

Vitamin D contributes to calcium homeostasis by facilitating intestinal calcium absorption and bone resorption. Overdosing can result from endogenous production of the active metabolite, calcitriol, through 1- α hydroxylation in abnormal macrophages encountered in sarcoidosis or other granulomatous diseases. Another cause of endogenous vitamin D excess is its release from fat tissue storage after rapid loss of fat mass. However, the most frequent cause of vitamin D overdosing is exogenous,

with extremely high doses and consequent production of very high levels of serum 25-hydroxyvitamin D. Clinical expression of vitamin D toxicity includes hypercalcaemia and/or hypercalcaemia, with the consequences of calcium-containing mineral deposits in soft tissues (43, 44). Because of the long biological half-life of 25-hydroxyvitamin D, the hypercalcaemia/hypercalcaemia can last for several weeks after vitamin D discontinuation.

A recently recognized other potential feature of excessive exogenous vitamin D dosing is the increased risk of fracture and falls observed when large, 'bolus' doses of vitamin D are employed (26, 27, 45). The adverse consequences of this form of excessive vitamin D is not accompanied by changes in serum or urine calcium concentrations. The relationship between vitamin D and a hard outcome like mortality is biphasic, with an increased mortality observed under both vitamin D insufficiency and excess.

Specific therapeutic areas relating to vitamin D

Vitamin D in primary hyperparathyroidism and hypoparathyroidism

Primary hyperparathyroidism and hypoparathyroidism, classical disorders of parathyroid hormone excess and deficiency, respectively, illustrate well the vital role that vitamin D plays in general calcium homeostasis and in the therapeutics of these two disorders. Under normal circumstances, PTH and vitamin D regulate each other's actions: PTH stimulates renal 1- α hydroxylase activity and, thus, facilitates the production of 1,25-dihydroxyvitamin D. In primary hyperparathyroidism, this enzyme activity is facilitated further leading to levels of active vitamin D that are typically in the upper normal range or frankly elevated (46). In hypoparathyroidism, the absence of PTH leads to relative inactivity of this enzyme, an effect exacerbated by the inhibitory actions of hyperphosphataemia. In hypoparathyroidism, the resulting sluggish renal enzymatic conversion system leads to a deficiency of active vitamin D, despite levels of 25-hydroxyvitamin D that may be normal.

The role of vitamin D metabolism in primary hyperparathyroidism is further illustrated by the actions of active vitamin D to control PTH. In primary hyperparathyroidism, vitamin D deficiency fuels the processes associated with excessive secretion of PTH, as illustrated by both higher levels of PTH among patients with primary hyperparathyroidism who are also vitamin D deficient, as well as by histomorphometric and clinical manifestations of the disease that are typically worsened in

the setting of coexistent vitamin D deficiency (47, 48, 49, 50, 51, 52, 53). Some of the most severe clinical examples of primary hyperparathyroidism occur in countries like India, where vitamin D deficiency is common (54, 55). In countries, like China, where vitamin D deficiency has become less common, the clinical manifestations of primary hyperparathyroidism have become less severe. In Western Europe and North America, where vitamin D deficiency is regarded to be generally mild when present, primary hyperparathyroidism most often presents as an asymptomatic disease (56). Therapeutic directives related to abnormal vitamin D metabolism in primary hyperparathyroidism and hypoparathyroidism stem from these physiological and pathophysiological considerations.

Primary hyperparathyroidism

In primary hyperparathyroidism, it is important to ensure vitamin D sufficiency in order to control the component of PTH overactivity due to vitamin D deficiency. Without addressing the controversy over what is the best definition of a normal 25-hydroxyvitamin D level, it seems prudent, with some evidence to back it up, that levels should be aimed at >30 ng/mL (75 nmol/L) in primary hyperparathyroidism (57, 58). Treating a hypercalcaemic condition with vitamin D, a calcaemic molecule, leads to caution in choosing the amount of supplemental vitamin D, which should begin with modest amounts. Limited experience with higher doses argues for the more prudent approach of 1000 IU daily. The serum calcium should be monitored within a few weeks of instituting or changing the dosage of vitamin D. Vitamin D supplementation results in small increases in spinal BMD in primary hyperparathyroidism (59). There is no rationale for using active vitamin D metabolites in primary hyperparathyroidism.

Points:

- The three phenotypes of primary hyperparathyroidism each is influenced by vitamin D. In symptomatic hyperparathyroidism (PHPT) with low 25-hydroxyvitamin D, there will be very high PTH and worse disease. Asymptomatic PHPT is associated classically with low 25-hydroxyvitamin D and among those with very low 25-hydroxyvitamin D, PTH levels are higher with some evidence that bone disease might be worse. In normocalcemic PHPT, the diagnosis requires vitamin D sufficiency to rule out secondary hyperparathyroidism.

- Treatment is aimed at maintaining sufficient vitamin D levels and reducing or eliminating a role for a secondary hyperparathyroidism to add to the primary hyperparathyroid state. Use of vitamin D should be careful and judicious (60, 61, 62).
- Guidelines for ascertaining vitamin D sufficiency in PHPT are available (57, 58, 62).

Hypoparathyroidism

In hypoparathyroidism, vitamin D is a mainstay of conventional therapy. In contrast to primary hyperparathyroidism, in which active vitamin D is not recommended, this metabolite is a key component of virtually all therapeutic regimens. Nevertheless, high levels of 25-hydroxyvitamin D can engage with the vitamin D receptor and therefore act as an active form of vitamin D. Thus, in settings where active vitamin D is not available as a therapeutic, vitamin D, in high doses, can be effective. Therapeutic, daily doses of 1,25-dihydroxyvitamin D (calcitriol) vary widely among patients from as little as 0.25 µg to as much as 4 µg. If the 1-α analogue is available, as it is in Europe, the dosing regimen is approximately twice the amount of active vitamin D because of differences in potency. Active vitamin D is typically given in divided doses. While parental forms of vitamin D, cholecalciferol or ergocalciferol, would not seem to be a rational approach, due to the relative inactivity of the renal 1α-hydroxylase in hypoparathyroidism, many clinicians will include a parental form of vitamin D as part of the therapeutic regimen. The rationale for this approach is based on two points. First, there is some conversion of 25-hydroxyvitamin D (which is produced unimpaired in the liver of these patients) to active vitamin D in hypoparathyroidism. Second, since the liver appears not to be hampered in its ability to metabolize parental vitamin D to 25-hydroxyvitamin D and it further converts that metabolite to others that may have both conventional and unconventional targets, it seems reasonable to aim for a level of 25-hydroxyvitamin D that is within the normal range. High levels of active vitamin D in hypoparathyroidism cause concern about hypercalciuria, always an issue in this disease. The approach to this therapeutic dilemma has two different outcomes: one emphasizes oral calcium dosing and the other active vitamin D as the primary conventional modality. With the advent of rhPTH(1–84) as a registered therapy for hypoparathyroidism, both by the FDA and the EMA, it is possible to reduce the need for supplemental active vitamin D to physiological equivalents or, in a

substantial number of patients, to eliminate its need altogether (63).

Points:

- Vitamin D metabolism is abnormal in hypoparathyroidism because PTH is an important facilitator of vitamin D activation and it is deficient or absent in this disease.
- The low turnover state is defined, in part, by the combination of low vitamin D and PTH.
- Densitometric observations of increased bone mass are due to the low turnover state.
- With conventional therapy, amounts of active and parent vitamin D required to maintain asymptomatic serum calcium levels can be very high. Such chronically high intake of parent and active vitamin D presents a threat of target organ and ectopic calcium-phosphate deposition.
- The target for serum calcium levels, measured after an overnight fast, should be in the low normal range to avoid hypercalciuria and calcium-phosphate deposition.
- Treatment with rhPTH(1–84) substantially reduces the need for active vitamin D in many patients and in some cases eliminates any need for active vitamin D.

Bariatric surgery

Bariatric surgery procedures for obesity (64) can be narrowly classified in three groups: restrictive (laparoscopic-adjusted gastric bending and sleeve gastrectomy), malabsorptive (Roux-en-Y gastric bypass and biliopancreatic diversion) or a combination of the two (65). These procedures have the potential to interfere with the achievement of optimal vitamin D status, independent of the threshold suggested to define it (66). Indeed, both the reduction of absorptive area of the small intestine and/or the modified delivery of pancreatic secretions and bile salts lead to decreased vitamin D absorption. It is also noteworthy that obese patients are often vitamin D insufficient under basal conditions due to a number of factors including sequestration of vitamin D by adipose tissue and reduced mobility with limited sun exposure (see discussion above) (67).

From studies attempting to establish the optimal dosing regimen for vitamin D repletion after bariatric surgery, it is difficult to draw firm conclusions, mainly because of different doses and vitamin D preparations employed and the different assays utilized for measuring 25-hydroxyvitamin D. The impact of various surgical

procedures is also different. Therefore, information derived from patients undergoing sleeve gastrectomy cannot be simply translated to other surgical procedures. In this context, the Roux-en-Y gastric bypass seems to carry the highest risk of vitamin D deficiency post surgery because it bypasses the small intestine, the primary site of vitamin D absorption (68). In this respect, the intramuscular route of vitamin D administration (69, 70) may be effective, especially in obese individuals undergoing malabsorptive procedures. Oral 25-hydroxyvitamin D, when available, may be a good solution to restore vitamin D deficiency in case of severe fat (and vitamin D) malabsorption. Apart from vitamin D deficiency, intestinal calcium absorption may be severely impaired even after restoration of normal vitamin D status (71).

There is a need for studies defining unanswered research questions in obese patients undergoing bariatric procedures (see below). These surgical interventions also represent a threat for skeletal health with an increased fracture risk (72).

Chronic kidney disease

Almost all healthy adults experience an age-related decline in renal function. Chronic kidney disease (CKD) is one of the most common problems encountered in internal medicine. Most patients have grade 1, 2 or 3 CKD with estimated glomerular filtration rates (eGFR) of ≥ 90 , 60–89 and 30–59 mL/min, respectively. However, as of about 15 years ago, approximately 300 000 Americans have more severe kidney disease defined as grade 4 CKD (15–29 mL/min) and about 450 000 had grade 5 CKD (<15 mL/min) (73, 74). CKD-Metabolic Bone Disorder (MBD) occurs in grade 4 and 5 CKD and is characterized by bone (renal osteodystrophy), soft tissue (calcifications) and mineral (calcium, phosphate, fibroblast growth factor-23, calcitriol, sclerostin, Dickkopf-1) abnormalities. Even worse, other pathological end-points of CKD-MBD include increased cardiovascular risk, mortality and fractures.

Recent Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (75) recommend different forms of vitamin D depending upon the level of renal impairment. In patients with CKD, grade 3, vitamin D₃ supplements should be used to correct deficiency, if present. The use of calcitriol and active vitamin D analogues should be reserved for use in patients with CKD Grades 4 or 5 with severe and progressive secondary hyperparathyroidism. The guidelines suggest that patients with levels of intact PTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable

factors, including hyperphosphataemia, hypocalcaemia, high phosphate intake and vitamin D deficiency. Importantly, however, the guidelines state the optimal level of PTH to achieve with PTH-lowering therapy is as yet unknown. In patients with CKD grade 5 (dialysis-dependent), and requiring PTH-lowering therapy, the guidelines recommend the use of calcimimetics, calcitriol or vitamin D analogues, or their combination to maintain intact PTH levels of approximately 2–9 times the upper normal limit for the assay.

Recent European Calcified Tissue Society and European Renal Association of Nephrology Dialysis and Transplantation guidelines (76) suggest there is still value in using the actual PTH concentration to differentiate between high and low turnover states in renal osteodystrophy. In cases with either lower PTH levels, or low or intermediate levels of a bone formation marker, bone-specific alkaline phosphatase (BSAP), a bone biopsy may be required to exclude causes of low turnover renal osteodystrophy, such as adynamic bone disease and osteomalacia. If osteomalacia is seen on bone biopsy, active vitamin D is recommended.

Idiopathic infantile hypercalcaemia

Idiopathic infantile hypercalcaemia (IIH) is a rare disorder caused by genetic mutations of *CYP24A1* (25OHD₃-24-hydroxylase) (77) and *SLC34A1* (NaPi-IIa cotransporter) (78). In the case of mutations of *CYP24A1*, the hypercalcaemia is due to the loss of the *CYP24A1* enzyme responsible for the multistep catabolism of the side chain of the active form of vitamin D, 1,25(OH)₂D₃, and its precursor, 25-hydroxyvitamin D₃, which leads to elevations of both of these vitamin D metabolites in the blood (77, 79). The genotyping of these two genes is critical for determining the correct diagnosis in IIH, but is expensive. In this regard, the careful measurement of the main serum catabolite, 24,25-dihydroxyvitamin D₃ (24,25(OH)₂D₃), can be useful as a screening tool for IIH where it would be anticipated to be either very low or absent. In fact, rapid liquid-chromatography tandem mass spectroscopy (LC-MM/MS) and non-LC-MM/MS assays have detected a small residual amount of 24,25(OH)₂D₃ (80). A ratio of 25-hydroxyvitamin D: 24,25(OH)₂D₃ of >80 (normal 5–25) identifies IIH due to a loss-of-function *CYP24A1* mutation (81). In most affected patients, this ratio is 100–150. Recently, the residual 24,25(OH)₂D₃ activity has been confirmed by extended chromatography, however, another component of this peak, 25,26(OH)₂D₃ was also resolved (82). In the future,

it is expected that measurement of serum 24,25(OH)₂D₃ will be more widely adopted and the development of a reference method for 24,25(OH)₂D₃ (83) will be critical to allow for interlaboratory comparisons.

Nutritional rickets

Nutritional rickets (NR), secondary to vitamin D deficiency and/or dietary calcium deficiency, remains a significant global, public health problem despite the availability of supplementation. A recent international consensus document defined NR and its diagnostic criteria and described the clinical management of rickets and osteomalacia (84). Risk factors, particularly in mothers and infants, were ranked, and specific prevention recommendations including food fortification and supplementation were made.

The diagnosis of NR is made on the basis of history, physical examination and biochemical testing and is confirmed by radiographs. Vitamin D deficiency was defined as a serum 25-hydroxyvitamin D <12 ng/mL, while a dietary calcium intake of <300 mg/day was considered deficient for children over 12 months of age and increases the risk of rickets interdependent of serum 25-hydroxyvitamin D levels. Children with radiographically confirmed rickets have an increased risk of fracture, while those with vitamin D deficiency alone do not. Treatment modalities can be summarized as follows:

1. Vitamin D supplementation to prevent NR:
 - 400 IU/day (10 µg) is adequate to prevent rickets and is recommended for all infants from birth to 12 months of age, independent of their mode of feeding.
 - Beyond 12 months of age, all children and adults need to meet their nutritional requirement for vitamin D through diet and/or supplementation, which is at least 600 IU/day (15 µg), as recommended by the Institute of Medicine (IOM).
2. Dose of vitamin D and calcium to treat NR:
 - The minimal recommended dose of vitamin D is 2000 IU/day (50 µg) for a minimum of 3 months.
 - Oral calcium, 500 mg/day, either as dietary intake or supplement should be routinely used in conjunction with vitamin D in the treatment regardless of age or weight.
3. Route of administration and duration of therapy:
 - Oral treatment, which more rapidly restores 25-hydroxyvitamin D levels than IM treatment, is recommended.

- For daily treatment, both vitamin D₂ and D₃ are equally effective.
- When single large doses are used, D₃ appears to be preferable compared to D₂ because the former has a longer half-life.
- Vitamin D treatment is recommended for a minimum of 12 weeks, recognizing that some children may require longer treatment duration.

Identification and treatment of risk factors are also important to prevent NR. Importantly, dietary practices and nutrient intakes among mothers associated with NR in infants should be corrected. Maternal vitamin D deficiency should be avoided by ensuring that women of child-bearing age meet intakes of 600 IU/day recommended by the IOM. Pregnant women should receive 600 IU/day of vitamin D, preferably as a combined preparation with other recommended micronutrients such as iron and folic acid. Early feeding, supplementation and nutrient intake should also be addressed in infants. In addition to an intake of 400 IU/day of vitamin D, complementary foods introduced no later than 26 weeks should include sources rich in calcium. Subsequently, an intake of at least 500 mg/day of elemental calcium must be ensured during childhood and adolescence.

Public health approaches to prevent NR include universally supplementing all infants with vitamin D from birth to 12 months of age, independent of their mode of feeding. Beyond 12 months, all groups at risk and pregnant women should be supplemented. Vitamin D supplements should be incorporated into childhood primary health care programmes along with other essential micronutrients and immunizations, and into antenatal care programmes along with other recommended micronutrients. Fortification of staple foods with vitamin D and calcium, as appropriate, based on dietary patterns should be a priority, and supported by legislation and appropriately monitored.

Treatment of hereditary vitamin D-dependent rickets (HVDDR) and hereditary calcitriol-resistant rickets

Two different hereditary deficiencies in vitamin D action were described in the 1960s and 1970s and were named pseudo vitamin D deficiency type I and II or vitamin D-dependent rickets type I and II (85). The first term emphasizes the fact that the clinical, radiological, histological and most of the biochemical features of both diseases are identical to vitamin D deficiency with no history or biochemical evidence of such deficiency, with the exception of an extremely rare form to be

discussed below. The term vitamin D-dependent rickets reflects the response to treatment of the type I patients, but it is a misnomer for the type II disease, where more than 50% of the patients do not respond to treatment with any dose of vitamin D or its 1- α hydroxylated active metabolites. Based on our current understanding of vitamin D metabolism, action and the elucidation of the molecular and genetic defects in most of these patients, it is suggested to define patients with a defect in the synthesis of 1,25-dihydroxyvitamin D (calcitriol) as hereditary vitamin D-dependent rickets (HVDDR), type I and II, respectively, and patients with a defect in the response of target tissues to calcitriol as hereditary calcitriol-resistant rickets (HCRR) (86).

Both of these diseases are very rare forms of hypocalcaemic rickets, transmitted as autosomal recessive: the patients are homozygotes and the parents obligate heterozygotes for the mutation (87), although there are some isolated cases that seem to be transmitted as incomplete autosomal dominant. In some kindreds, there is a family history of consanguinity, multiple affected siblings and a specific geographical distribution. The clinical, radiological, histological and biochemical data, with the exception of serum levels of vitamin D and its metabolites, are identical to severe vitamin D deficiency; i.e., signs and symptoms of hypocalcaemia and defective mineralization of newly formed organic matrix of bone: in the growing skeleton – rickets and in remodelling bone – osteomalacia.

A notable difference in clinical presentation is that alopecia is present in more than 50% of patients with HCRR. The alopecia may be obvious at birth, but usually develops during the first months of life and may range from sparse hairs to total alopecia. In one or two patients additional ectodermal anomalies were observed. Serum biochemistry reveals hypocalcaemia, secondary hyperparathyroidism, hypophosphatemia and elevated levels of biochemical markers of bone formation. Serum levels of 25-hydroxyvitamin D are normal to markedly elevated, depending on prior treatment, in HVDDR type I and HCRR, and low to very low in HVDDR type II. Serum calcitriol levels are low to undetectable in both types of HVDDR and elevated to very high in patients with calcitriol resistance (HCRR), depending on prior treatment with vitamin D or its 1- α hydroxylated metabolites.

HVDDR and HCRR are now understood at the gene level (85, 86, 87, 88, 89). HVDDR type I is the end result of different defects in the enzyme 25-hydroxyvitamin D 1- α hydroxylase-causing deficiency of calcitriol and its physiological effects. The same mutation was found

in different members of the same kindred, but may be shared with additional unrelated kindreds. HVDDR type II is a very rare mutation in the liver enzyme vitamin D 25-hydroxylase, causing deficiency of 25-hydroxyvitamin D, the substrate for the kidney synthesis of calcitriol and, therefore, a deficiency of the active hormonal form of vitamin D, a similar situation to HVDDR type I (90). In HCRR, different mutations were found along the complex cascade of calcitriol physiological action, starting with the specific binding of calcitriol to the vitamin D receptor (VDR), dimerization with the retinoic X receptor, modulation of binding of the complex to the nucleus, to specific elements of the DNA (vitamin D-responsive element or VDRE), transcription, translation and post-translational events. Different defects were observed in almost each of these steps leading to severe aberrations in the physiological response of target tissues to 1,25-dihydroxyvitamin D. These studies became feasible with the demonstration that cells originating from tissues easily accessible contain VDRs that are similar, if not identical, to those of classical target tissues. The cells used were mainly dermal fibroblasts grown from skin biopsies and mitogen-stimulated peripheral T-lymphocytes. These cells are used to assess most of the steps in calcitriol action, as well as the molecular defects and a biological response measured mainly by dose-dependent induction of the enzyme 25-hydroxyvitamin D 24-hydroxylase in both target cells or an antiproliferative effect in mitogen-stimulated peripheral T-cells. Up to now, the response *in vivo* in patients treated with massive doses of 25-hydroxyvitamin D, or calcitriol and its active metabolites, or tested *in vitro*, was similar if not identical.

Treatment: HVDDR is a disease caused by a failure to produce an active hormone; thus, the most effective way is to ensure replacement therapy by administration of the active hormone – calcitriol and, in the case of HVDDR type I, also the 1- α hydroxylated vitamin D metabolites that are transformed in the liver into calcitriol (88). A complete cure of all clinical, radiological, histological and biochemical aberrations could be achieved and maintained by the administration of physiological replacement doses of calcitriol (0.25–1.0 μ g/day) or its active 1- α hydroxylated metabolites (0.5–2.0 μ g/day) plus calcium supplementation. As is obvious, the 1- α hydroxylated metabolites are contraindicated in HVDDR type II, but calcidiol (not available in all countries) will evoke and maintain a good physiological response. Thus, the clinical and biochemical data of patients with HVDDR type II will resemble vitamin D deficiency. The doses have to be adjusted depending on the severity of the defect

in bone matrix mineralization, age and weight of the patient. There is a need for close and frequent follow-up, especially in the first few months after treatment is initiated, for clinical and radiological signs as well as serum measurement of calcium, phosphorous, PTH, vitamin D metabolites and 24-h urinary calcium excretion. With full remission, the follow-up frequency can be reduced.

Massive doses of vitamin D or 25-hydroxyvitamin D (1000–3000 µg/day or 200–700 µg/day, respectively), 100–300 times the recommended daily dose plus calcium supplementation are required to achieve and maintain chemical and biochemical remission in patients with hereditary vitamin D dependent rickets (HVDDR). Based on the above, this approach is not recommended.

In patients with end-organ resistance to calcitriol (HCRR), less than 50% of affected individuals presented a complete clinical, radiological and biochemical response to treatment with massive doses of vitamin D, 25-hydroxyvitamin D, calcitriol or its 1- α hydroxylated active metabolites (89). These doses cause an increase in the circulating calcitriol levels, up to more than 100 times the upper normal limit.

This remission may be maintained for years, depending on continuous treatment. There are a very few reports of continuous remission, even after stopping the megadoses of calcitriol or vitamin D metabolites. However, as noted earlier, the majority of patients are resistant even to very high doses of whatever vitamin D-related product is used. Calcium and bone homeostasis may be restored in such patients by using very high oral doses of calcium (up to 5–10g/day) or intravenous calcium infusions. For example, in one baby, complete remission of the disease and normalization of biochemical aberrations has been achieved by the administration of massive doses of calcium salts, mainly by the intravenous route, 1g of elementary calcium given i.v. each night.

To judge if a patient is responding, 3–5 months of treatment is necessary, with the administration of vitamin D and calcium as discussed above. This duration of treatment also helps in differentiating HCRR from other situations like ‘hungry bone syndrome’ that may appear on therapy initiation in patients with very severe calcium or vitamin D deficiency. When a patient undergoes a therapeutic trial, there is a need for frequent and close follow-up, to check clinical and radiological signs as well as serum levels of calcium, phosphorous, PTH and vitamin D metabolites to monitor adverse events, responsiveness and compliance. There is no need to carry out a therapeutic trial in patients with HCRR if the *in vitro* response of target cells, as discussed above,

will continue to be identical to the *in vivo* response to treatment in these patients.

Recent major and ongoing vitamin D RCTs

Recent major vitamin D RCTs published from 2013 to 2017

A literature search revealed 475 publications from January 1, 2013 to June 12, 2017 (Scopus). In addition, about 3000 vitamin D randomized controlled trials (RCTs) were registered with the ClinicalTrials.gov registry by 2017. The primary end-points of these published RCTs included the following health outcomes: Frailty and falls, CVD, maternal and infant health, musculoskeletal diseases, type 2 diabetes mellitus, cancer, critical illness, osteoarthritis of the knee, respiratory diseases, infection and immunity.

A summary of findings from major trials (selected on the basis of >100 participants and numbers of citations) follows:

Frailty/falls: A 12-month, double-blind trial of 200 ambulatory men and women (mean age 78 years) given monthly 24 000IU cholecalciferol vs 60 000IU cholecalciferol or 24 000IU cholecalciferol and 300 µg calcifediol (27). The primary end point (change in Short Physical Performance Battery scores; including gait speed and repeated chair stands time) was no different across groups. Repeated chair stands showed less improvement in the 60 000IU and 24 000IU+calcifediol groups. The secondary endpoint of falls showed an increased percentage of fallers in the 60 000IU and 24 000IU+calcifediol groups (67 and 66%, respectively, vs 24 000IU group (48%; $P=0.048$)). Despite the lack of a control group, these data suggest monthly high doses of vitamin D should be avoided in the elderly and may also have implications for safety reporting in ongoing large-scale RCTs where this dose is also being trialled (e.g. D-HEALTH).

A trial sequential meta-analysis of 20 trials ($n=29\,535$) (91) showed the effect estimate for vitamin D with or without calcium on falls does not alter the relative risk by $\geq 15\%$. In a sensitivity analysis using a relative risk reduction of 10%, the effect estimate also lay within the futility boundary. Subsequent analyses using a risk reduction threshold of 15%, showed no effect in trials of vitamin D supplementation (16 trials, $n=22\,291$) and in trials of vitamin D with calcium (6 trials, $n=9919$). Unfortunately, this meta-analysis also included two trials using high annual bolus doses of vitamin D, which were associated with an increased falls risk and are no longer recommended. In an older Cochrane review of 159 trials

with 79 193 participants, vitamin D did not reduce rate of falls (RR 1.00, 95% CI 0.90–1.11; 7 trials; 9324 participants) or risk of falling (RR 0.96, 95% CI 0.89–1.03; 13 trials; 26 747 participants), but may do so in individuals with lower baseline vitamin D levels (92).

Systolic hypertension: A double-blind, placebo-controlled trial of 159 men and women (mean age 77 years) with vitamin D deficiency and baseline systolic blood pressure (SBP) >140 mmHg at baseline given 100 000 IU cholecalciferol at baseline, and at 3, 6 and 9 months (93). The change in SBP was not different (–0.7 mmHg (95% CI: –5.2, 3.8), $P=0.76$). There was no difference in pulse wave velocity, blood lipids, inflammatory markers or insulin sensitivity. In conclusion, this small negative RCT suggests vitamin D repletion does not reduce SBP.

CVD: A large, double-blind, placebo-controlled trial (ViDAL) enrolled 5108 participants (58% male; mean age 65.9 years) treated with a 200 000 IU loading dose of cholecalciferol followed by monthly 100 000 IU doses for a median of 3.3 years (94). Only ~25% of participants were vitamin D deficient at baseline. Mean baseline 25-hydroxyvitamin D was 63 nmol/L, with only 2% <25 nmol/L. There was no difference in incidence of CVD between groups 303 (11.8%) vs 293 (11.5%); HR=1.02 (95% CI: 0.87, 1.20). The primary study outcome was negative, but this outcome focused on a large number of different CVD outcomes, and it is unlikely vitamin D deficiency could have a benefit for all of them.

Maternal/infant health: A large double-blind, placebo-controlled trial of 1135 women (mean age 30 years) recruited at 14-week gestation were treated with 1000 IU cholecalciferol per day vs placebo (95). The plasma 25-hydroxyvitamin D increased significantly, by 22.9 nmol/L, in the supplementation group, but did not change in the placebo group. The proportion of women with plasma 25-hydroxyvitamin D >50 nmol/L increased from 36.5 to 83.4% in the supplementation group. There were no differences in BMC, BMD, lean mass or fat mass in infants at birth. However, vitamin D prevented winter and spring declines in plasma 25-hydroxyvitamin D. The season of birth influenced the effects of vitamin D on BMC at birth, resulting in increases in BMC from 57 to 64 g in babies born in winter. In conclusion, this study showed a stratified approach to vitamin D supplementation in pregnancy is useful. However, a larger dose of 2000 IU/day may result in a larger proportion of women achieving vitamin D sufficiency. Prevention of the gestational reduction in 25-hydroxyvitamin D in women who deliver in winter could improve BMC of the baby; however, further studies are required to confirm the beneficial

skeletal effects of antenatal vitamin D supplementation on offspring.

Musculoskeletal: There were secondary outcomes recorded in the ViDAL Study of 5108 participants (58% male; mean age 65.9 years) treated with a 200 000 IU loading dose of cholecalciferol followed by monthly 100 000 IU doses for a median 3.3 years (96). Only ~25% of participants were vitamin D deficient at baseline. Only 2% <25 nmol/L and only 17 hip fractures occurred; no calcium supplements were given. Reported falls were no different: 1312 (52%) vitamin D vs 1326 (53%) placebo; HR=0.99 (0.92, 1.07) $P=0.82$. Non-vertebral fractures were also similar; 156 (6%) vitamin D vs 136 (5%) placebo; HR: 1.19 (95% CI: 0.94–1.50) $P=0.15$. However, the interpretation of these outcomes has some limitations. These were secondary trial outcomes; the trial was underpowered to detect differences in falls and fractures, and bolus D dosing was used, which based on recent RCT data in the elderly would not now be recommended in that group.

A recent systematic review and meta-analysis of 33 randomized trials (51 145 community-dwelling participants aged >50 years) showed no significant association of either calcium or vitamin D with risk of hip fracture compared with placebo or no treatment (calcium: RR, 1.53 (95% CI: 0.97–2.42); vitamin D: RR, 1.21 (95% CI: 0.99–1.47)) (97). Nor was there any significant association of combined calcium and vitamin D with hip fracture compared with placebo or no treatment (RR, 1.09 (95% CI: 0.85–1.39)). No significant associations were found between calcium, vitamin D, or combined calcium and vitamin D supplements and incidence of non-vertebral, vertebral or total fractures. These negative findings were not altered by adjusting for the calcium or vitamin D dose, sex, fracture history, dietary calcium intake and baseline serum 25-hydroxyvitamin D. However, this meta-analysis included two trials, which may have adversely affected the outcomes – the RECORD study, a trial of secondary, not primary, fracture prevention and the largest trial (WHI), which used a suboptimal dose of vitamin D (400 IU per day), based on the current IOM recommendation.

Body composition: A double-blind, placebo-controlled trial of 218 postmenopausal women (mean age 60 years) recruited with serum 25-hydroxyvitamin D between 10 and 32 ng/mL were treated with 2000 IU cholecalciferol per day vs placebo for 12 months (98). All participants underwent a diet and exercise weight loss programme. There were no differences in weight loss (8.2% vs 8.4%, $P=0.66$); change in lean mass (–0.8 kg vs +1.1 kg, $P=0.53$); appendicular lean mass both groups (both –0.1 kg, $P=0.11$) and no difference in upper body strength (–0.9

lbs vs -3.6 lbs; $P=NS$). However, leg strength significantly decreased in vitamin D-supplemented subjects (-2.6 lbs vs $+1.8$ lbs; $P=0.03$), and there was greater spinal bone loss in the vitamin D group (-0.02 g/cm² vs -0.01 g/cm²; $P=0.01$). Although a greater magnitude of change in serum 25-hydroxyvitamin D was significantly associated with greater weight loss in the vitamin D group ($P=0.03$), a greater increase in 25-hydroxyvitamin D was not significantly associated with any other outcome. Vitamin D supplementation resulted in greater rates of spinal bone loss and decreases in leg strength, which was unanticipated; however, the biological significance of these small changes is unclear.

Diabetes mellitus: A double-blind, placebo-controlled trial was conducted in 275 adults with type 2 diabetes mellitus, serum HbA1c $\leq 8.0\%$ and stable for 3 months (99). Exclusion criteria included an eGFR ≤ 30 mL/min/1.73 m². They were treated with 50 000 IU cholecalciferol monthly for 6 months vs placebo. There was no change in the primary end point, HbA1c ($\beta=0.4$ ($-0.6, 1.5$) $P=0.42$). No significant differences were seen in other indicators of glycaemic control and anthropometric variables. There was no effect of vitamin D on diabetes control in this short duration trial. Confirmation is required by larger trials of longer duration (D2d).

Cancer: In a large double-blind, placebo-controlled trial of 2303 postmenopausal women (mean age 65 years), subjects were treated with 2000 IU cholecalciferol per day and 1500 mg per day of calcium or placebo for 4 years (100). A new diagnosis of cancer was confirmed in 45 (3.9%) in the vitamin D group and 64 (5.6%) in placebo (difference, 1.69% ($-0.06\%, 3.46\%$); $P=0.06$). Kaplan-Meier incidence over 4 years was 0.042 (0.032–0.056) in vitamin D vs 0.060 (0.048–0.076); $P=0.06$. Although the study was negative, it certainly suggests a trend for vitamin D to reduce cancer, which needs to be confirmed in ongoing, larger RCTs (D-HEALTH, VITAL).

Acute respiratory infections: Although an earlier, smaller meta-analysis of 15 trials including 7053 individuals did not show an effect of vitamin D in reducing acute respiratory tract infections (101), a recent larger meta-analysis of 25 eligible RCT did show a decrease (total of 11 321 participants, with individual patient data obtained for 10 933 (96.6%)) (102). Vitamin D reduced the risk of acute respiratory tract infections (RTIs) among all participants (OR=0.88, 95% CI: 0.81–0.96; $P<0.001$). Protective effects were seen in those receiving daily or weekly vitamin D without additional bolus doses (OR=0.81, 95% CI: 0.72–0.91), but not in those receiving bolus doses. Protective effects were greatest in those

with baseline serum 25-hydroxyvitamin D <25 nmol/L (OR=0.30, 95% CI: 0.17–0.53) than in those with baseline 25-hydroxyvitamin D ≥ 25 nmol/L (OR=0.75, 95% CI: 0.60–0.95; P interaction=0.006). These data show vitamin D supplementation protected against acute RTI, and patients with severe vitamin D deficiency and those not receiving bolus doses experienced the most benefit from supplementation.

Knee osteoarthritis: In a double-blind, placebo-controlled trial, 413 adults, aged 50–79 years, were treated with 50 000 IU cholecalciferol per month or placebo for 2 years (103). Patients had knee pain for the previous 6 months ranging from 20 to 80 (out of 100 on VAS); ACR class of I, II or III and a serum 25-hydroxyvitamin D of 12.5–60 nmol/L at baseline. There was no difference in change in WOMAC pain score between groups (-49.9 for the vitamin D group vs -35.1 for placebo; between-group difference, -14.8 (-32.5 to 2.9); $P=0.10$). The change in MRI tibial cartilage volume was not different between groups (-242.6 mm³ vitamin D vs -301.4 mm³ with placebo (between-group difference, 58.8 mm³ (-13.9 to 131.4); $P=0.11$). Vitamin D supplementation therefore had no effect on either pain score or cartilage volume in patients with knee osteoarthritis.

A double-blind, placebo-controlled trial was performed in 146 adults treated with a 2000 IU cholecalciferol per day, to reach a target level of 36–100 ng/mL vs placebo for 2 years (104). There were no differences in WOMAC pain score -0.87 ($-2.12, 0.38$) $P=0.17$ or function -3.11 ($-6.52, 0.30$) $P=0.07$. There were also no differences in chair stand, 20 m walk times or in MRI-derived parameters of knee cartilage, including tibial cartilage, femoral cartilage or combined cartilage volumes. This study showed no effect of vitamin D supplementation on pain score or cartilage volumes in patients with knee osteoarthritis.

Two recent systematic reviews (105, 106) and meta-analyses of the effects of vitamin D supplementation on knee osteoarthritis, in four RCTs involving 1136 patients, showed vitamin D supplementation of more than 2000 IU vitamin D₃ per day resulted in small decreases in the WOMAC pain score and function in patients with knee OA. However, there was no beneficial effect on the prevention of tibial cartilage loss. Therefore, there is currently a lack of evidence to support the use of vitamin D supplementation in preventing the progression of knee OA.

Conclusions

There is discordance between the findings of observational studies associating vitamin D deficiency with positive

Table 1 Ongoing, large, long-term, randomized, placebo-controlled trials of vitamin D supplementation.

Trial (country), sponsor	Trial registration number	Type of trial*	Actual sample size (target)	Inclusion criteria	Active intervention [§]	Max allowable vitamin D from supplements	Treatment duration (years)	Total Follow-up (years)	Primary outcome	Major secondary outcomes	Outcome assessment	Estimated completion date (start date)
Vitamin D and Omega-3 (VITAL) (US) (111) NIH	NCT01169259	Community-based	25 874 (-20 000)	Age ≥55 year women, ≥50 year men; free of cancer and CVD	D ₃ 2000 IU/day, marine omega-3 fatty acid 1 g/day; 2×2 factorial	800 IU/day	5	10	Cancer, MACE	Specific cancers (colorectal, breast, prostate); total cancer mortality; CVD outcomes	Self-reported via annual survey. Primary end-points are confirmed by review of records	2018 (2010)
D-HEALTH (Australia) (112) NHMRC	ACTRN12613 000743763	Community-based	21 315 (25 000)	Age 60–84 years	D ₃ 60 000 IU/month	500 IU/day (2000 IU/day if prescribed)	5	10	All-cause mortality	Total cancer, colorectal cancer	Linkage via national registries	2025 (2014)
Vitamin D and type 2 diabetes (D2d) (US) (110) NIH	NCT01942694	Efficacy	2423 (2382)	Age >25 year, 2-out-of-3 American Diabetes Association criteria for pre-diabetes*	D ₃ 4000 IU/day	1000 IU/day	~3 (event driven trial)	~3	Diabetes	Cancer, MACE, specific cancer, CVD outcomes, quality of life	Central laboratory measurements (diabetes), adjudication (cancer/MACE)	2019 (2013)
Diabetes Prevention with Active Vitamin D (DPVD) (Japan) (115) University of Occupational and Environmental Health	UMIN000010758	Efficacy	Enrolling (750)	Age 30–90 year IGT (2-h glucose 140–199 mg/dL) plus fasting glucose <126 mg/dL and HbA1c <6.5%	Eidocalcitol (1,25(OH) ₂ D ₃) 0.75 µg/day	Not provided	2.8		Diabetes	Normoglycaemia, adverse events, HOMA-IR	Local laboratory measurements ^{sw}	Not available (2013)
Vitamin D ₃ – Omega3 – Home Exercise – Healthy Ageing and Longevity Trial (DO-HEALTH) (Europe) (116)	NCT01745263	Efficacy	2157 (2157)	Age >70 year	D ₃ 2000 IU/day; marine omega-3 fatty acid 1 g/day, exercise programme; 2×2×2 factorial	800 IU/day	3	3	Non-vertebral fracture, functional decline, blood pressure, cognitive decline, infection	See below		2017 (2012)
FIND (Finland) (117) Academy of Finland	NCT01463813	Community-based	2495 (18 000)	Age ≥65 year women, ≥60 year men	D ₃ 3200 IU/day, D ₃ 1600 IU/day	800 IU/day	5	5	Cancer, CVD		Self-reported via annual survey; linkage via national registries	2018 (2012)
The International Polycap Study 3 TIPS-3 (11 countries worldwide) (118)	NCT01646437	Efficacy	Enrolling (5000)	Age ≥60 year women, ≥55 years men; high CVD risk	D 60 000 IU/month, polycap (thiazide, atenolol, ramipril, simvastatin) aspirin, 2×2×2 factorial	800 IU/day	5	10	MACE+ (polycap vs placebo), MACE and cancer	MACE (polycap vs placebo), MACE and cancer	Not provided	2020 (2012)
The Vitamin D and Longevity Trial (VIDAL) Feasibility Study, (UK) National Institute of Health Research (119)	ISRCTN46328341	Feasibility	1615 (NA)	65–84 year	D 100 000 IU/month	NA	2		Feasibility, all-cause mortality	Cancer, infection	Linkage via national registries; GP records	2017 (2013)

VITAL study secondary outcomes: CVD outcomes, diabetes, hypertension, autoimmune disorders, asthma, fractures, cognitive function (*N* = 3000), bone density, diabetic neuropathy, mood disorder, infections, echocardiogram, macular degeneration, anaemia, atrial fibrillation, mammographic density, D-Health study secondary outcomes: CVD events (high blood pressure, heart failure, arrhythmia, angina, heart attack, stroke, transient ischaemic attack), depression, anxiety, upper respiratory illness, chronic obstructive pulmonary disease, hyperthyroidism, hypothyroidism, diabetes, pain, falls, fractures, arthritis, self-reported health status, number of hospitalizations, glaucoma, cataract, insomnia, sleep apnoea. DO-HEALTH secondary outcomes: hip fracture, vertebral fracture, total fracture, bone mineral density, rate of falls, reaction time and grip strength, muscle mass, gait speed, hypertension, depression, upper respiratory infections, severe infections, knee buckling, NSAID use, gastro-intestinal symptoms, oral health, tooth loss, fasting glucose, insulin level, body composition, kidney function, quality of life, disability, nursing home admission, repeat fractures, functional recovery after fracture, MACE, dementia, osteoarthritis.

*Community-based trials are being conducted by mail (e.g., participants are not seen in-person, study pills are mailed to participants; outcomes are assessed by self-report); †all trials include a placebo and are double-blind; ‡American Diabetes Association criteria for pre-diabetes include fasting glucose 100–125 mg/dL; 2-h glucose 140–199 mg/dL; HbA1c 5.7–6.4%.

ADA, American Diabetes Association; BMI, body mass index; CVD, cardiovascular disease; HbA1c, haemoglobin A1c; IGT, impaired glucose tolerance; IU, international units; NIH, United States National Institutes of Health.

disease outcomes and the findings of RCTs in vitamin D deficiency, which are largely negative to date. This may be due to reverse causality with the illness itself contributing to low vitamin D levels. The results of many RCTs have also been inconsistent. However, overall evidence from RCTs shows vitamin D reduces fractures (when administered with calcium), in the institutionalized elderly, but not in community-dwelling adults >50 years (107). Our consensus is that, although controversial (108, 109), vitamin D also reduces acute RTIs (if not given as bolus monthly or annual doses), and may reduce falls in those with the lowest serum 25-hydroxyvitamin D levels. Currently, there is no consistent or conclusive evidence for a positive vitamin D impact on other diseases or on overall mortality.

Future opportunities

Vitamin D is a nutrient and any beneficial effects of vitamin D would be anticipated to be greatest in those with the most severe deficiency (25-hydroxyvitamin D <30 nmol/L or <12 ng/mL). The large ongoing RCTs with 21 000–26 000 participants, described in the next section, did not recruit based on baseline serum 25-hydroxyvitamin D levels, but will contain a large subset of participants with vitamin D deficiency and are adequately powered to meet their primary end-points.

Ongoing RCTs with vitamin D supplementation

There are several larger, ongoing, placebo-controlled trials that test the safety and efficacy of vitamin D supplementation on a variety of outcomes (Table 1). There are two general types of trials with different strengths and weaknesses. Efficacy trials (e.g., D2d) (110) place emphasis on optimizing internal validity by evaluating participants in-person and employing rigorous ways to assess outcomes that include laboratory assessment by a central laboratory.

In community-based trials (e.g. VITAL, D-HEALTH) (111, 112), the study is conducted 'by mail' and participants are not evaluated in-person. Study pills are mailed to participants living in the community and outcomes are self-reported based on surveys or through search of Electronic Health Records. Community-based studies enrol a very large number of participants at a fraction of the cost of large-scale efficacy trials. However, such studies often have several weaknesses including less than optimal adherence with study treatment, considerable personal use of supplements (113, 114) and reliance on self-reported or administrative data to assess outcomes, which may limit accuracy. All trials limit out-of-study personal use of

vitamin D; however, given the popularity of vitamin D supplements in the general population, 'contamination' of the intervention by personal use of supplements is a serious concern as the cohort may be sufficiently replete at baseline and throughout the trial.

Many trials (e.g., VITAL, D-HEALTH) (111, 112, 115, 116, 117, 118, 119) will conduct analyses for a very large number of secondary outcomes, and the possibility of false-negative results due to multiplicity of testing is also a real concern. Therefore, there is a need for reproducibility of results before any positive finding is widely accepted.

Answers relating to areas of agreement:

- Is vitamin D₃ superior to D₂? Pharmacokinetics, clinical data.

Vitamin D₃ treatment results in larger increases in serum 25OHD than does vitamin D₂. In addition, the oral route of administration is more effective at rapidly increasing serum 25OHD than the intramuscular route, with a 2-month lag time for the latter.

- What is the role for 25-hydroxyvitamin D (calcidiol) in replacement and its availability?

In patients with advanced liver disease in whom the hepatic hydroxylation of vitamin D is impaired, calcidiol would be a logical form because it bypasses that hydroxylation step in the liver, as it is in the setting of glucocorticoid-induced inhibition of hepatic 25-hydroxylase. Calcidiol is more effective in increasing serum 25OHD than vitamin D.

- Is there a place for active vitamin D metabolites in treating vitamin deficiency?

The use of calcitriol or alfacalcidol is recommended only in patients with renal insufficiency and secondary hyperparathyroidism in whom renal 1 α -hydroxylation of vitamin D is impaired. However, even in this clinical setting, there is some evidence that native vitamin D has the capability to normalize serum parathyroid hormone values, at least when the hyperparathyroidism is not severe. Hypercalciuria and hypercalcaemia may complicate therapy with active vitamin D metabolites.

- Is daily dosing of vitamin D preferred to larger weekly, monthly or annual doses?

After 3 months, daily or equivalent monthly doses of vitamin D₃ achieved the same serum 25OHD concentrations. However, avoiding large annual or monthly doses of vitamin D is recommended in the

elderly, in whom an increased risk of falling has been reported with bolus dosing.

- Does the baseline level of serum 25-hydroxyvitamin D influence the choice and frequency of dose?

Supplementation with vitamin D increases serum 25-hydroxyvitamin D levels, but the increment depends upon the baseline serum 25-hydroxyvitamin D concentration. Lower baseline serum 25-hydroxyvitamin D levels are associated with larger increases in serum 25-hydroxyvitamin D for a given dose of vitamin D. Loading doses may be considered in severe vitamin D deficiency. A total loading dose of 30-40 000 IU of vitamin D₃ should be sufficient to replace near total absence of vitamin D to normal 25-hydroxyvitamin D concentrations (50–75 nmol/L).

- Does BMI or race influence the dose or mode of administration?

Body weight affects the serum 25-hydroxyvitamin D response to both loading and maintenance doses of vitamin D, with larger doses being required. Compared to Caucasians, blacks have lower total 25-hydroxyvitamin D levels attributed primarily to lower cutaneous biosynthesis. Individuals from Saudi Arabia and China are also more likely to have vitamin D deficiency and may require higher doses of vitamin D initially, but there are no ethnic differences in the dose response to vitamin D.

- What is the role of fortification in increasing vitamin D?

While food fortification practices are modifying the epidemiological surveys of vitamin D status throughout the world, it is unrealistic to expect that such measures alone will satisfy recommended daily vitamin D requirements across all populations. Therefore, the use of vitamin D supplements is often required to ensure normal vitamin D status.

- How much is too much vitamin D?

Vitamin D is very safe. However, the most frequent cause of vitamin D overdosing is exogenous, with extremely high doses (e.g. 50 000 IU given per day instead of per week) and consequent production of very high levels of serum 25-hydroxyvitamin D (>250 nmol/L). Symptoms of hypercalcaemia may be present and often take a long time to remit in view of the prolonged half-life of vitamin D.

- What adverse outcomes are important?

A recently recognized adverse consequence of excessive exogenous vitamin D dosing is an increased risk of fracture and falls observed in the elderly when large, 'bolus' doses of vitamin D are employed. This form of excessive vitamin D is not accompanied by changes in serum or urine calcium concentrations. The relationship between vitamin D and mortality is biphasic, with an increased mortality observed under both vitamin D insufficiency and excess.

Research agenda

Variables that influence dosing and levels:

1. Is there a race × vitamin D supplementation interaction on extra-skeletal outcomes (such as CVD, diabetes, cancer)?
2. How does body composition influence 25-hydroxyvitamin D level after supplementation with vitamin D and during weight loss?
3. Do different types of weight loss (medical, surgical) affect 25-hydroxyvitamin D level differently?

Extra-skeletal effects:

1. What is the effect of long-term daily vitamin D supplementation on non-skeletal outcomes, such as T2DM, cancer and CVD?
2. What is the optimal dose and serum level that balances extra-skeletal benefits (e.g. T2DM) vs risks (e.g., CVD)?

Primary hyperparathyroidism:

1. How will the epidemiology of clinical presentations of PHPT change as many countries begin to screen the population with serum calcium measurements?
2. Are low levels of vitamin D in the asymptomatic variant of PHPT associated with microstructural skeletal abnormalities, along with higher PTH levels?
3. As generally higher levels of vitamin D in PHPT have become apparent, will the clinical phenotype of asymptomatic PHPT change?

Hypoparathyroidism:

1. What is the natural history of hypoparathyroidism with or without rhPTH(1–84)?
2. What delivery systems or administration protocols will reduce urinary calcium in hypoparathyroidism?
3. How will reductions in vitamin D needs with rhPTH(1–84) lead to improved skeletal health and

a reduction in concerns about target and off target organ calcifications?

4. Will rhPTH(1–84) and subsequent reduction in need for vitamin D also improve the outlook for renal function in hypoparathyroidism?

Randomized controlled clinical trials:

1. Will the large ongoing vitamin D RCTs be able to determine if effects of supplementation are greatest in those individuals with the lowest serum 25-hydroxyvitamin D levels (<30nmol/L)?
2. Will the large number of secondary outcomes in these large RCTs increase the risk for false negative results?

Declaration of interest

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