25-Hydroxyvitamin D as a Biomarker of Vitamin D Status and Its Modeling to Inform Strategies for Prevention of Vitamin D Deficiency within the Population

Kevin D Cashman,[1,2] Ellen GHM van den Heuvel,[3] Ruud JW Schoemaker,[3] Damien P Prévéraud,[4] Helen M Macdonald,[5] and Jayashree Arcot[6]

[1]Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences and [2]Department of Medicine, University College Cork, Cork, Ireland; [3]FrieslandCampina, Amersfoort, Netherlands; [4]Center of Expertise and Research in Nutrition, Adisseo France SAS, Commentry, France; [5]School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom; and [6]Food Science and Technology Group, School of Chemical Engineering, University of New South Wales, Sydney, New South Wales, Australia

ABSTRACT

There is substantial evidence that the prevalence of vitamin D deficiency is unacceptably high in the population, and this requires action from a public health perspective. Circulating 25-hydroxyvitamin D [25(OH)D] is a robust and reliable marker of vitamin D status and has been used by numerous agencies in the establishment of vitamin D dietary requirements and for population surveillance of vitamin D deficiency or inadequacy. In a wider context, modeling of serum 25(OH)D data and its contributory sources, namely dietary vitamin D supply and UVB availability, can inform our understanding of population vitamin D status. The aim of this review is to provide the current status of knowledge in relation to modeling of such vitamin D–relevant data. We begin by highlighting the importance of the measurement of 25(OH)D and its standardization, both of which have led to new key data on the prevalence of vitamin D deficiency and inadequacy in North America and Europe. We then overview how state-of-the-art modeling can be used to inform our understanding of the potential effect of ergocalciferol and 25(OH)D on vitamin D intake estimates and how meteorological data on UVB availability, when coupled with other key data, can help predict population serum 25(OH)D concentration, even accounting for seasonal fluctuations, and lastly, how these in silico approaches can help inform policymakers on strategic options on addressing low vitamin D status through food-based approaches and supplementation. The potential of exemplar food-based solutions will be highlighted, as will the possibility of synergies between vitamin D and other dairy food–based micronutrients, in relation to vitamin D status and bone health. Lastly, we will briefly consider the interactions between season and vitamin D supplements on vitamin D status and health. *Adv Nutr* 2017;8:947–57.

Keywords: vitamin D deficiency, 25(OH)D assessment, mathematical modeling, vitamin D food fortification, UVB availability

Introduction

The major source of vitamin D in humans is the UVB component of sunlight; UVB radiation stimulates cutaneous synthesis of vitamin D_3 (cholecalciferol), which undergoes hydroxylation in the liver to 25-hydroxyvitamin D [25(OH)D] (1). Several environmental factors, such as latitude and prevailing weather conditions, determine whether UVB of sufficient strength is available to stimulate the conversion of 7-dehydrocholesterol in the skin to precholecalciferol (2). Personal attributes, such as skin pigmentation, age, clothing, working environment, physical activity, sunscreen use, and

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Address correspondence to KDC (e-mail: k.cashman@ucc.ie).

Abbreviations used: BMD, bone mineral density; EAR, Estimated Average Requirement; EC, European Commission; IGF-1, insulin-like growth factor-I; IOM, Institute of Medicine; NANS, National Adult Nutrition Survey; ODIN, Food-Based Solutions for Optimal Vitamin D Nutrition and Health Through the Life Cycle; RCT, randomized controlled trial; VDSP, Vitamin D Standardization Program; 25(OH)D, 25-hydroxyvitamin D; $25(OH)D_2$, 25-hydroxyergocalciferol; $25(OH)D_3$, 25-hydroxycholecalciferol.

sun exposure behavior, can also much reduce, if not prevent, cholecalciferol synthesis (2). Vitamin D also occurs in the diet, both naturally and as a fortificant, as cholecalciferol and vitamin D_2 (ergocalciferol) and in nutritional supplements.

The well-known late-winter nadir in circulating 25(OH)D concentrations means that substantial portions of the population resident at latitudes greater than ~40° rely on body stores and vitamin D in the diet to maintain healthy vitamin D status all year. Because body stores are dependent on sun exposure, the importance of the diet in maintaining vitamin D status above the level of deficiency is a corollary of UVB sunlight deficit (3). There is increasing evidence that the dietary supply is currently unable to offset the seasonal sunlight deficit, which increases with latitude and the duration of winter (4). There are very few rich natural sources of vitamin D; these are oily fish and cod liver oil (which are consumed sporadically), egg yolk, fortified foods, and UV-exposed mushrooms or UV-irradiated yeast, in which ergocalciferol is found. Food consumption surveys throughout Europe, Canada, the United States, and beyond have all consistently reported low vitamin D intake and widespread dietary inadequacy (4).

This review is based on 4 vitamin D–related presentations from Session V of the 4th International Vitamin Conference held in Copenhagen, Denmark, 25-27 May 2016. We begin the review by highlighting the importance of measurement of circulating 25(OH)D and its standardization, both of which have led to new key data on the prevalence of vitamin D deficiency and inadequacy. We then provide an overview on how state-of-the-art modeling can be used to inform our understanding of the potential effect of the minor vitamer and metabolite of vitamin D in the food chain [i.e., ergocalciferol and 25(OH)D, respectively] on vitamin D intake estimates and of how changes in estimates in overall vitamin D intake, in the presence and absence of UVB availability, effects population serum 25(OH)D concentrations. This is important in terms of informing food-based approaches toward improving vitamin D status and preventing vitamin D deficiency. The potential of exemplar food-based solutions for addressing low vitamin D status, such as vitamin D–biofortified foods as well as traditional vitamin D fortification of more novel dairy-based foods, will be highlighted, and the possibility of synergies between vitamin D and other naturally present micronutrients present in dairy foods will be explored. Lastly, we briefly consider interactions between seasons and vitamin D supplements on vitamin D status and health.

Current Status of Knowledge

25(OH)D as a marker of vitamin D status and its assessment

There is consensus that serum or plasma 25(OH)D concentration should be used to assess vitamin D status because it reflects the contributions from both diet and synthesis in the skin (1, 5). A systematic review of existing and potentially novel functional markers of vitamin D status reported that serum 25(OH)D concentration increased in response to supplemental vitamin D intake in all the included randomized controlled trials (RCTs), irrespective of whether ergocalciferol or cholecalciferol was used, differing analytical techniques, study duration (6 wk to >2 y), or age group of the participants (6). Serum or plasma 25(OH)D concentration was used as an indicator of vitamin D status by the Institute of Medicine (IOM) DRI committee on calcium and vitamin D in North America (1) as well as the UK and European Union authorities (5, 7–9) to establish dietary reference values for vitamin D.

Several reports have shown that available 25(OH)D assays can yield markedly differing results (10–12), and this has confounded international efforts to develop evidenced-based guidelines (12). Importantly, the issue of international standardization of serum 25(OH)D measurement has been progressed by the Vitamin D Standardization Program (VDSP), a collaborative initiative between the Office of Dietary Supplements of the NIH, the CDC, the National Institute of Standards and Technology, and a number of the national health surveys around the world (12, 13). The VDSP has developed protocols for standardization of existing serum 25(OH)D data from national nutrition and health surveys and cohort studies (14–17), which allow for more valid between-country or -region comparisons of vitamin D status and prevalence of vitamin D deficiency (see below).

Serum 25(OH)D thresholds underpinning international vitamin D recommendations and some associated population surveillance estimates

The IOM DRI committee in the United States, with the use of bone health as the basis for developing DRI for vitamin D, suggested that people are at risk of deficiency at serum 25(OH)D concentrations <30 nmol/L; some, but not all, people are potentially at risk of inadequacy at serum 25(OH)D concentrations from 30 to ≤50 nmol/L; and that practically all people are vitamin D sufficient at concentrations >50 nmol/L (1). In contrast, the Endocrine Society Task Force on Vitamin D in the United States (18) suggests that individuals should be identified as vitamin-D-deficient at a cut-off level of 50 nmol/L serum 25(OH)D, and to maximize the effect of vitamin D on calcium, bone, and muscle metabolism, serum 25(OH)D concentration should exceed 75 nmol/L. A serum 25(OH)D threshold of 50 nmol/L in terms of adequacy of vitamin D has been adopted by several European agencies, including the European Food Safety Authority (9) [for recent reviews, see Cashman (19) and Hayes and Cashman (20)]. However, the Scientific Advisory Committee on Nutrition in the United Kingdom, after considering the evidence, suggested that the risk of poor musculoskeletal health was increased at serum 25(OH)D concentrations <25 nmol/L (5). There is universal agreement that we do not wish to have individuals in the populations with circulating 25(OH)D concentrations <25–30 nmol/L.

Estimates of the prevalence of vitamin D deficiency [based on data of VDSP standardized serum 25(OH)D <30 nmol/L] in representative population samples in the United States (n = 15,652) (14), Canada (n = 11,336) (15),

and Europe [n = 55,844; with the use of a collection of 14 nationally or regionally representative studies in the European Commission (EC)-funded ODIN (Food-Based Solutions for Optimal Vitamin D Nutrition and Health Through the Life Cycle) project; www.odin-vitd.eu] (16) have been reported recently as 5.9%, 7.4%, and 13%, respectively. As worrisome as these population estimates are, they do not capture the differences in prevalence arising from factors such as age, seasonality, geographical location, and ethnicity in these regions. For example, the prevalence of serum 25(OH)D concentrations <30 nmol/L increases from 8.2% in summer to 17.7% in winter in the European sample (16) and from 3.3% in summer to 9.3% in winter in the United States (14). Across ethnic groups, the prevalence of serum 25(OH)D concentrations <30 nmol/L in non-Hispanic white, Hispanic, and non-Hispanic black populations in the United States is 2.3%, 6.4%, and 24%, respectively (14). Young adults are at high risk: in the United States, the prevalence of serum 25(OH)D concentrations <30 nmol/L in those aged 1–11 y is only 0.7%, whereas it is 8.2% in those aged 20–39 y (14). The average yearly population prevalence of standardized serum 25(OH)D concentrations <50 nmol/L in Europe, the United States, and Canada is 40.4%, 24.0%, and 36.8%, respectively (14–16). Clearly, regardless of which threshold is used, strategies for vitamin D deficiency prevention are required (19). The typical average intake for populations within the EU and the United States are generally ~3–8 μg/d on average, depending on the country (21). There is a significant gap between the typical intake in both European and North American populations and the estimated average requirement (EAR) for vitamin D intake, which was set by the IOM at 10 μg/d for those aged ≥1 y (1).

Modeling the potential effect of ergocalciferol and 25(OH)D in the food chain on vitamin D intake estimates

Although much of the vitamin D in the diet (including in fortified foods and supplements) is in the form of cholecalciferol, ergocalciferol and 25(OH)D are also present and may be underestimated contributors to vitamin D nutritional status.

25(OH)D in food and effect on vitamin D nutriture. 25(OH)D is present in certain foods of animal origin, such as meat, offal, eggs, and, to a lesser extent, fish [for reviews, see Cashman (22) and Ovesen et al. (23)]. The total vitamin D activity of these animal foods in food compositional tables in the United Kingdom (and those in Denmark and Switzerland) accounts not only for the vitamin D content of the food, but also for the content of the 25(OH)D multiplied by a factor of 5 (24–26). However, the US food composition database does not account for the 25(OH)D content of food or apply an efficacy factor (27). Importantly, Taylor et al. (28) performed some modeling to include overall food-derived 25(OH)D content in intake estimates for US adults, which showed that there was a potentially meaningful increase (1.7–2.9 μg or 15–30% of the EAR) in vitamin D intake estimates. However, there is some debate around the use of the factor of 5, with alternate suggested factors ranging from 1 to 9 [for reviews, see Cashman (22), Ovesen et al. (23), and Heaney et al. (29)]. In a specifically designed RCT aimed at addressing this question, consumption of orally consumed synthetic 25-hydroxycholecalciferol [25(OH)D_3] was shown to be 5 times more effective than an equivalent amount of cholecalciferol at improving the serum 25(OH)D concentrations of older adults in winter (30).

Although the use of synthetic 25(OH)D as a food fortificant has not been approved yet, its use in animal feeds for certain species is permitted in Europe and the United States (31, 32), and this can increase the content of 25(OH)D_3 in the human food chain. For example, the addition of commercially available 25(OH)D_3 to the diet of hens has been shown to increase egg 25(OH)D_3 content (33–35). This approach of increasing the total vitamin D content of foods of animal origin by increasing the dietary vitamin D or 25(OH)D amount in animal feed has been referred to as biofortification (19). Importantly, a recent winter-based RCT of older adults (n = 55) showed that weekly consumption of 7 vitamin D–biofortified eggs, produced by hens provided with feed containing 25(OH)D_3 (or cholecalciferol) at the allowable maximum content, prevented the typical decline in serum 25(OH)D concentration during winter and any incidence of vitamin D deficiency (36). The control group in the study, who were requested to consume up to a maximum of 2 commercially available eggs/wk, had a significant decline in serum 25(OH)D over the 8 wk of winter, and 22% had vitamin D deficiency [serum 25(OH)D <25 nmol/L] at the endpoint (36).

Vitamin D_2 in food and effect vitamin D nutriture. It has been suggested that ergocalciferol is not very prevalent in the human food chain. However, data from a number of recent intervention studies as well from the National Adult Nutrition Survey (NANS) in Ireland, a nationally representative sample of Irish adults, suggest that the majority of subjects had measurable serum 25-hydroxyergocalciferol [25(OH)D_2] concentrations (37). Serum 25(OH)D_2, unlike 25(OH)D_3, is not directly influenced by skin exposure to UVB sunlight and thus has only dietary origins; however, quantifying dietary ergocalciferol is difficult because of the limitations of food composition data. Cashman et al. (37) used serum 25(OH)D_2 concentrations in the NANS participants to estimate the intake of ergocalciferol with the use of a mathematical modeling approach. This approach used recently published RCT data on the relation between ergocalciferol intake and the responses of serum 25(OH)D_2 concentrations in combination with data on serum 25(OH)D_2 concentration distribution in NANS. The projected median intake of ergocalciferol ranged from 1.7 to 2.3 μg/d, suggesting that it may have an effect on nutritional adequacy at a population level and thus warrants further investigation (37).

Modeling of serum 25(OH)D concentration to inform approaches toward improving vitamin D status

Modeling UVB and dietary vitamin D data to predict serum 25(OH)D concentrations in the population and changes arising from vitamin D fortification or supplementation. Even accounting for potentially underestimated contributions of dietary-derived 25(OH)D_3 and ergocalciferol to overall vitamin D intake in some populations, it is unrealistic to expect the habitual Western-style diet to supply vitamin D at 10 μg/d [i.e., the EAR (1)] across the population. For example, Roman Viñas et al. (38) showed that, of European national nutrition surveys reporting vitamin D intake data from 2000 on, 77–100% and 55–100% of adults (19–64 y of age) and the elderly people (>64 y of age), respectively, had intakes below the EAR. Consequently, there have been calls for the use of vitamin D supplements as a means of correcting low vitamin D intake and status in European populations, and, in fact, vitamin D supplement use has been recommended as national policy in certain countries, particularly for at-risk population groups (39). Although vitamin D supplementation has been shown to significantly improve vitamin D intake across a variety of age and sex groups, with dose-dependent increases in serum 25(OH)D concentrations (6), relying on supplements is not an appropriate public health strategy to increase intake across the population because supplements are only effective for those who consume them, and uptake within the population is generally too low to provide widespread population protection, as is outlined elsewhere (40). Based on the collective evidence from food-based RCTs, novel food fortification approaches may represent the best opportunity to increase the vitamin D supply to the population (41, 42).

To enable food fortification strategies to be evidence-based, mathematical models can be developed and used to identify the appropriate amounts of food fortification as well as potential vehicles to ensure adequacy of vitamin D intake in population groups. Such mathematical models can provide underpinning supportive data and complement intervention-based trials (43). The models relate vitamin D intake, arising from habitual food consumption as well as from various food fortification scenarios, to serum 25(OH)D concentrations, while accounting for the contribution of UVB-induced synthesis in the skin to the distribution of serum 25(OH)D concentrations within the population. The UVB data that underpin these mathematical models of population serum 25(OH)D concentrations can be from direct ground-based measurements or can themselves be modeled with the use of data from various satellites (44), and on an annual basis, these data show clear and consistent seasonal variation as well as striking latitudinal variation in vitamin D–effective UVB availability (**Figure 1**). Such modeled vitamin D–effective UVB availability over a typical 12 mo period has recently been mapped for several locations in Europe, ranging from 35°N to 69°N, and clearly highlights the considerable variability across the region as one moves from southerly to northerly latitudes (45).

With the use of stepwise approaches, models based on UVB availability data, hours of sunlight, and a key component, namely, the dose-response of serum 25(OH)D to UVB in adults, have been used to predict changes in population serum 25(OH)D concentrations throughout the year in the United Kingdom (46–48), Ireland (43), and Germany (49, 50), some of which have been validated against VDSP standardized serum 25(OH)D concentration data from nationally

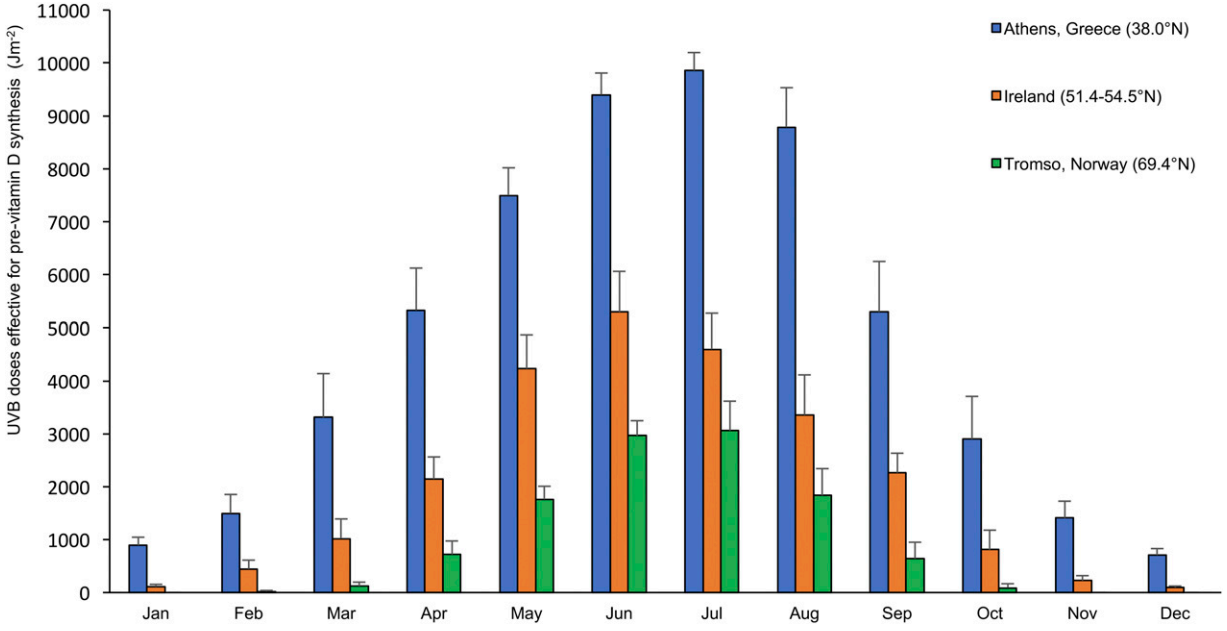

FIGURE 1 Monthly modeled UVB doses effective for precholecalciferol synthesis (Jm^{-2}) in Tromsø, Norway (latitude 69°N), Republic of Ireland (latitude 51–54°N), and Athens, Greece (latitude 38°N). Values are means (2003–2012 data) ± SDs, n = 28–31. Data are from O'Neill et al. (45).

representative nutrition surveys. By inclusion of one additional key component into a model, namely the dose-response of 25(OH)D to increased vitamin D intake, one can use the resulting integrated model to predict changes in the population concentrations of 25(OH)D that may arise from various dietary fortification approaches, while accounting for the seasonal variation in serum 25(OH)D concentrations due to UVB availability. This was illustrated recently when published estimates of the effect of 3 hypothetical vitamin D food fortification scenarios on vitamin D intake in a representative sample of Irish adults (51) were used in the Irish model as a test and showed how mathematical models can inform how vitamin D food fortification in various constructs may affect population serum 25(OH)D concentrations and the prevalence of vitamin D deficiency (43).

Although the majority of the abovementioned models have all been exclusively based on white populations, recently, a model has been developed to account for the more complex and relevant proportion of European populations at increased risk of vitamin D deficiency due to skin color and ethnicity (48) (**Figure 2**). This is important because within Europe, North America, and other continents, dark-skinned ethnic groups are worryingly at a much increased risk of vitamin D deficiency than their white counterparts (**Table 1**). Although many of the environmental factors that contribute to this elevated risk, such as latitude (52), skin color (2), and cultural clothing practices (53) are not modifiable, in contrast, an important modifiable factor is the vitamin D supply in the diet and the obvious, albeit complex, solution is to develop strategies to address the low vitamin D intake in ethnic subgroups, thereby preventing deficiency safely throughout the population (48). Such a tailored, dark-skinned ethnic group mathematical model presents a viable approach to estimating changes in the population concentrations of 25(OH)D that may arise from various dietary fortification approaches and that are cognizant of appropriate food vehicles for these ethnic subgroups.

Novel food-based solutions for addressing low vitamin D status

As outlined above, there is a need for sustainable food-based strategies to bridge the gap between current and recommended intakes of vitamin D to minimize the prevalence of vitamin D deficiency (4, 19–22). While acknowledging the valuable contribution that vitamin D–fortified milk makes to vitamin D intake among consumers, particularly children, and the continued need for fortification of milk and other dairy products (54), strategic approaches to fortification, including biofortification of a wider range of foods, has the potential to increase vitamin D intake in the population and minimize the prevalence of low serum 25(OH)D concentration without increasing the risk of excessive dosing (4, 40).

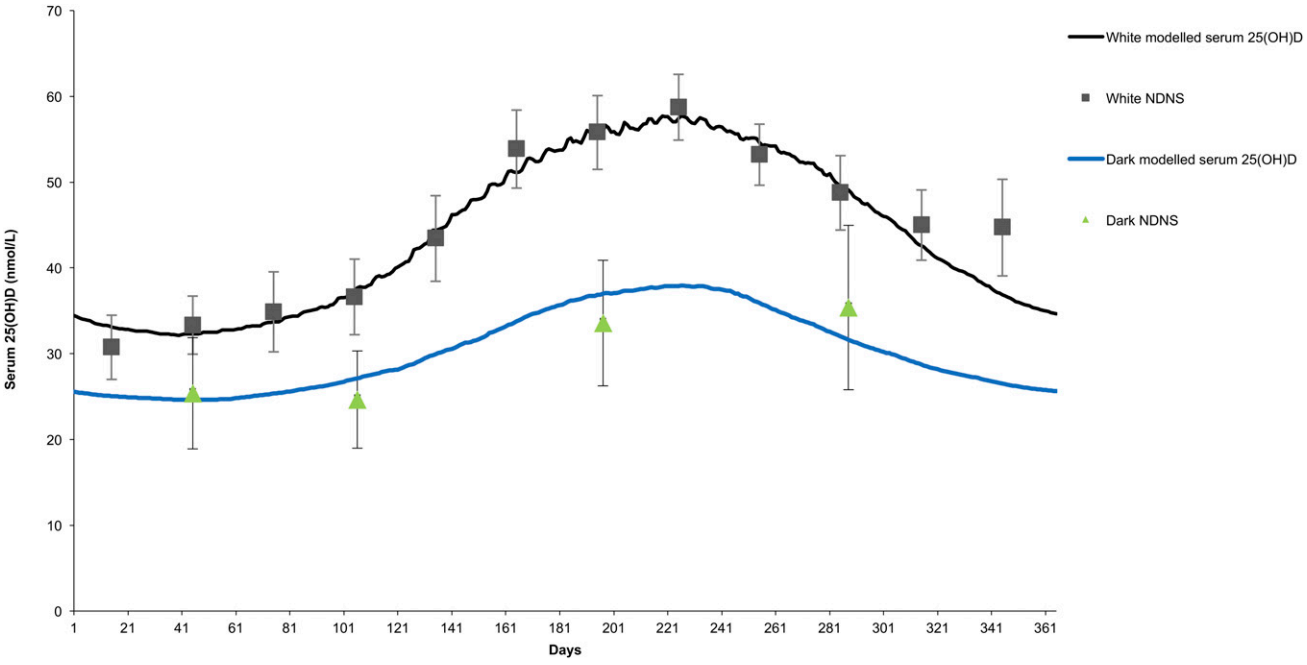

FIGURE 2 Modeled changes in serum 25(OH)D concentration over a typical year for white adults (white) and adults of Asian or black ethnic background (dark) in the United Kingdom with the use of daily mean UVB availability estimates [modified from O'Neill et al. (48)]. Squares (and associated error bars) are monthly geometric means ± 95% CIs of measured serum 25(OH)D concentrations for white adults (18–70 y of age) in the NDNS (n = 35–101/mo) in the United Kingdom. Triangles (and associated error bars) are geometric means ± 95% CIs of measured serum 25(OH)D concentrations for each season in the adults (18–70 y of age) of Asian or black ethnic background in the NDNS (n = 9–27/season). Seasons were used in place of monthly geometric means because of the much lower sample number for Asian or black ethnic adults and were defined as follows: winter (December, January, and February), spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November), with each triangle marker placed at the midpoint of each season. NDNS, National Diet and Nutrition Survey; 25(OH)D, 25-hydroxyvitamin D.

TABLE 1 Prevalence of vitamin D deficiency in European dark-skinned ethnic groups and their white counterparts[1]

Country, population subgroup	Study (reference)	Prevalence of serum 25(OH)D concentration <30 nmol/L, %
Finland		
Dark-skinned ethnic	Finnish Migrant Health and Wellbeing Study (16)	28.0 Somali (n = 364) 50.4 Kurdish (n = 500)
White	Health 2011 (17)	0.4 (n = 4102)
United Kingdom	National Diet and Nutrition Survey [4-y rolling program (16)]	
United Kingdom dark-skinned ethnic		35.7 Black (n = 28) 59.6 Asian (n = 52)
White		19.6 (n = 1359)
Norway (Oslo)	The Oslo Health Study (17)	
South Asian (Pakistani) immigrant		64.8 (n = 176)
White native, adult		1.3 (n = 866)
United States	Nutrition and Health Examination Survey 2007–2010 (≥1 y of age) (14)	
Non-Hispanic white		2.3 (n = 6711)
Hispanic		6.4 (n = 5138)
Non-Hispanic black		24.0 (n = 2997)

[1] Prevalence estimates are based on Vitamin D Standardization Program standardized serum 25(OH)D concentration data. n = total population sample. 25(OH)D, 25-hydroxyvitamin D.

In terms of traditional fortification of foods, bread and orange juice have also been shown to be effective in improving vitamin D status in a number of RCTs (55–58). Beyond these foods, eggs, beef, pig and sheep meat, poultry, milk, and cultured fish are potentially important targets for vitamin D–biofortified foods (20). In addition, the use of the vitamin D–fortified, reduced-fat cheese is worthy of consideration as an additional sustainable food-based strategy, beyond traditional dairy foods. For example, 2 systematic reviews examining the effectiveness of consuming vitamin D–fortified products in raising serum 25(OH)D concentrations in an RCT setting (41, 42) highlighted the fact that most studies have examined the effectiveness of vitamin D–fortified milk and yogurt, and there have been limited studies exploring the effectiveness of vitamin D–fortified cheese. Even with the studies that have been carried out with vitamin D–fortified cheese, the results have been quite mixed. This may relate to the quality of some of these studies, as suggested by Black et al. (41), as a limitation, but also to the fact that fortification of cheese with vitamin D has certain technological considerations, particularly for reduced-fat cheese varieties (59).

A recent RCT within the EC-funded ODIN project showed how the daily consumption of 60 g of cholecalciferol-fortified, reduced-fat Gouda cheese could counterbalance the expected decrease in serum 25(OH)D concentrations during 8 wk of winter in postmenopausal women (60), a group at risk of low vitamin D status and the associated risks of osteoporosis and related fractures. The study showed that although the average serum 25(OH)D concentration increased significantly (by 5.1 nmol/L) in the cholecalciferol-fortified cheese group (receiving an additional dose of 5.7 μg of cholecalciferol), it decreased significantly (by 4.6 nmol/L) in the control group, as would be expected in winter in individuals not taking additional vitamin D (habitual intake ~2 μg/d). None of the women in the cholecalciferol-fortified cheese group were vitamin D deficient [defined as serum 25(OH)D concentration <30 nmol/L] after the 8-wk study compared with 41% of women in the control group, a significant difference (P = 0.001) (60). Evidence of the effectiveness of food fortification approaches from RCTs that evaluate their effect on reducing the prevalence of vitamin D deficiency in the populations studied is a key priority (4), and the positive data from these recent RCTs provides a high level of evidence in relation to vitamin D–fortified, reduced-fat cheese (60) and vitamin D–biofortified eggs (36) among other potential food-based solutions to vitamin D deficiency. Data from these and other RCTs also underpin dietary vitamin D modeling analysis based on data from nationally representative dietary surveys, which in turn, when used in the mathematical models of population serum 25(OH)D concentration, can provide in silico projections of how these food interventions may affect the prevalence of vitamin D deficiency in the population. Such work is currently underway in the EC-funded ODIN project (61). Preliminary modeling of national food intake data from 4 European countries within the ODIN project has shown that consumption of vitamin D–biofortified foods together with traditional dairy fortification does not put the population at risk in terms of breaching the vitamin D tolerable upper intake level for adults [100 μg/d (1)], even for those taking vitamin D supplements containing 10 or 25 μg/d (M Kiely, ODIN, personal communication, 2017). This is of importance in light of the high percentage of some populations taking vitamin D–containing supplements (62). Increased food fortification together with high-dose vitamin D supplements (i.e., 50 μg/d) led to ~20% of individual breaching the Tolerable Upper Intake Level (M Kiely, ODIN, personal communication, 2017).

Dairy-based foods are good vehicles for vitamin D fortification not only because dairy foods are a major part of the diet for a high proportion of individuals in many, but clearly not all, population subgroups, but also as the bioavailability of vitamin D from dairy-based foods has been shown to be good in RCTs (41, 42, 60, 63). In addition, vitamin D may work

synergistically with dairy-based nutrients to improve bone health. For example, globally, an adequate supply of both calcium and vitamin D form part of the nutritional recommendations in relation to ensuring good bone health. The importance of both an appropriate dietary calcium intake and adequate serum 25(OH)D concentrations for skeletal health (64) has been confirmed by several meta-analyses (1, 65, 66).

Riboflavin and its flavoenzymes may play a role in the biosynthesis of vitamin D [for a review, see Pinto and Cooper (67)]. Deficits in riboflavin intake and metabolism as well as defects in flavoenzyme activity result in marked structural alterations within the skeletal and central nervous systems similar to those of disorders (inborn errors) in the biosynthetic pathways that lead to cholesterol, steroid hormones, vitamin D, and their metabolites (67). A high protein intake via the production of the osteotropic hormone, insulin-like growth factor-I (IGF-I), which is important for bone formation, has been shown to positively interact with vitamin D metabolism. High circulating IGF-I concentration may be a contributory factor for the enhanced renal production of 1,25-dihydroxyvitamin D (68). The synergy between protein and vitamin D was confirmed in a retrospective analysis with the use of data from a 3-y RCT with calcium and vitamin D supplementation (69). The 342 healthy people (aged ≥65 y) who completed the trial were stratified based on tertiles of protein intakes (as assessed by FFQ), and this analysis revealed an additional effect of high protein intake on top of the vitamin D and calcium intervention for bone mineral density (BMD) at the femoral neck and for total body BMD (69).

Finally, there may be synergy between vitamins D and K. In a case-control study, Torbergsen et al. (70) examined the possible synergistic effect of vitamins D and K. They found that circulating phylloquinone and 25(OH)D, independently and synergistically, were associated with a lower risk of hip fracture in elderly subjects (70). In addition, 2 RCTs showed that vitamin K in combination with vitamin D was better than either vitamin alone in terms of effects on BMD of the lumbar spine (71) and of the ultradistal radius (72). Vitamin D may also work synergistically with other micronutrients, such as vitamin A, but that is beyond the scope of the present review and has been discussed elsewhere (73).

Thus, at a mechanistic level, interactions between vitamin D and calcium, as well as other micronutrients, may have implications for the biosynthesis and regulation of circulating 25(OH)D, but also directly for bone health outcomes (1, 64, 70–75). Milk and dairy are known for their bone-augmenting qualities, but also contain the abovementioned nutrients capable of interacting with vitamin D. Therefore, we reviewed the literature in a systematic way with a view toward substantiating the synergy of vitamin D with dairy nutrients in relation to bone in a RCT setting and, in so doing, providing additional evidence that dairy foods are a good choice for vitamin D fortification. The systematic literature review on vitamin D and dairy nutrients in relation to bone (the details of the search terms used are available from 2 of the authors, EGHMvdH and RJWS, on request) identified 5, 2, 0, 1, and 2 RCTs in which vitamin D with at least calcium, vitamin K, protein, zinc, or phosphorus, respectively, were used as the intervention. Only 2 RCTs changed either the vitamin D or calcium intake without changing the intake of other nutrients. One study of Chinese adolescents compared an intervention with calcium-fortified dairy supplying 245 mg/d of calcium with or without additional vitamin D (3.3 μg/d). The vitamin D enrichment resulted in lower concentrations of bone-specific alkaline phosphatase (a marker of bone formation) (76) and more favorable changes in BMD and bone mineral content (77, 78). The effects were mainly on the lower limbs (79). Another RCT, which kept vitamin D constant at 5 μg/d and changed the calcium intake (1110 compared with 655 mg/d), reported an enhanced bone mineral gain at the hip sites in girls, but had no observable effect in boys (80). Three studies in elderly women, aged 61–99 y, increased the daily calcium concentrations [i.e., an extra 66 mg (81) to 240 mg/d (82, 83)] as well as vitamin D via fortified soft cheese or yogurt. The addition of 10 μg cholecalciferol to yogurt resulted in decreases in 2 markers of bone resorption in older women in a community-dwelling home (83) or elderly institutionalized women (82). A prospective, crossover RCT of institutionalized women showed that a soft, plain cheese fortified with 2.5 μg cholecalciferol, compared with a nonfortified equivalent cheese, led to an increase in serum IGF-I and significant decreases in markers of bone resorption (81). These studies confirm a synergistic role of vitamin D and calcium in terms of reducing the rate of bone resorption and turnover, at least in women with low intake of calcium or vitamin D status. An increased rate of bone turnover in adults may be a risk factor for fracture (84) because it exacerbates bone loss (85).

Two studies included vitamin K in addition to vitamin D in a dairy matrix (86). A short-term study (16 wk) of young women aged 20–35 y showed no additional benefit of phylloquinone added to milk, which was fortified with calcium and vitamin D, on the rate of bone turnover (86), whereas in a 12-mo RCT of postmenopausal women, daily consumption of dairy foods containing 800 mg calcium and 10 μg vitamin D plus 100 μg menaquinone or phylloquinone was favorable for lumbar spine BMD and a marker of bone resorption as compared with dairy fortified with calcium and vitamin D only (87).

There were 3 studies on dairy foods containing vitamin D with different amounts of phosphorus or zinc (88–90), but the findings were such that it was not possible to draw firm conclusions on the possible interaction of vitamin D with zinc or phosphorus in terms of bone health outcomes.

Interactions between seasons and supplements on vitamin D status and health

As mentioned above, vitamin D can be provided by UVB sunlight and dietary sources, including fortified foods and vitamin D supplements. The main differences between the 2 sources are summarized in **Table 2**, and the relative contribution of each source on an individual's vitamin D status depends very much on their lifestyle (91).

TABLE 2 Key differences in sunlight and dietary supply as contributors to vitamin D nutritional status

Sunlight	Diet
UVB is only available seasonally at higher latitude	Available year-round
UVB is only available at higher latitude from 1–6 mo of the year at latitudes ranging from 37–60°N, respectively: no vitamin D can be made in the skin[1]	Few foods naturally contain vitamin D
To make vitamin D, a person needs to be outside and expose the skin to sunlight	Very little vitamin D is obtained from most normal diets
A person cannot overdose on sunlight-derived vitamin D (but cancer risk is increased)	Vitamin D supplements make a significant contribution to vitamin D status
	A person can overdose if they take large amounts orally (high-dose vitamin D supplements for long periods)

[1] Data are from O'Neill et al. (45).

As summer UVB-rich sunlight is the major contributor to vitamin D status for most of the population who do not cover up when outside, the associations between vitamin D status and a reduced risk of chronic disease (e.g., cancer, cardiovascular, and autoimmune diseases) are confounded by being outside and being exposed to sunlight. Not only are those who are unwell more likely to spend time indoors, but there may be health benefits of light that are independent of vitamin D. To tease out the effect of vitamin D from sunlight, one needs a study specifically designed to account for the effect of season. The Vitamin D and Cardiovascular risk study was a 1-y RCT in Scotland (latitude 57°N) (92). All 305 female participants (aged 60–70 y) started at the same time between January and March, the point in the year when serum 25(OH)D is at its lowest (mean ± SD: 34 ± 15 nmol/L) and were assessed every 2 mo. The study found that serum 25(OH)D in the placebo group went up in the summer to a mean peak of 55 ± 18 nmol/L and returned to 32 ± 15 nmol/L by the following winter, whereas serum 25(OH)D reached plateaus for the groups receiving daily vitamin D (68 ± 16 nmol/L for 400 IU/d; 77 ± 18 nmol/L for 1000 IU/d) (data are means ± SDs). The small incremental differences between the 3 groups in summer compared with the large gap between placebo and both treatment groups in winter shows that there is interaction between light-derived vitamin D and oral vitamin D, i.e., the 2 sources are not additive.

The study found no change in markers of cardiovascular risk (total, HDL, and LDL cholesterol, TGs, apoA-1, and vitamin B-100), insulin resistance (homeostatic model assessment), or inflammation (high-sensitivity C-reactive protein, IL-6, and soluble intracellular adhesion molecule-1) either between the treatment groups or during the year, with one exception (92). Blood pressure went down in summer (mean ± SD systolic blood pressure decreased by 6.6 ± 10.8 mm Hg) and went back up in winter for all 3 groups, indicating that there are vascular effects of seasons that are independent of circulating 25(OH)D. BMD was a secondary outcome (93). Only the group taking the 1000-IU/d dose of vitamin D showed no hip bone loss compared with the mean 0.6% loss seen in both the 400-IU/d and placebo groups. In this case, the bone benefits do not appear to be directly linked to change in 25(OH)D concentration.

There is evidence to suggest that skin autoimmune conditions may be affected by UVB exposure through additional mechanisms, independent from the effects of light-derived vitamin D. A small study of outpatients undergoing UVB therapy, during which the UVB dose differed throughout treatment according to resistance to erythema, showed that the increase in the number of regulatory T cells was related to the change in circulating 25(OH)D, whereas the decrease in cytokine IL-10 was associated with the dose of UVB the patient received (94).

Conclusions

Although circulating 25(OH)D is a robust and reliable marker of vitamin D status, standardization of serum 25(OH)D data are extremely important in terms of within- and between-country comparisons of the prevalence of vitamin D deficiency. By using standardized serum 25(OH)D data and depending on the 25(OH)D threshold selected (30 or 50 nmol/L), vitamin D deficiency in Europe and North America can be classified as a mild (5–19.9%) or severe (>40%) public health problem based on WHO criteria (95). Regardless of which threshold is used, strategies for vitamin D deficiency prevention are required. Fortification, including biofortification, of a wider range of foods is likely to have the potential to increase vitamin D intakes across the population distribution and, in so doing, minimize the prevalence of vitamin D deficiency. Recent RCTs provide high-level evidence in relation to vitamin D–fortified, reduced-fat cheese and vitamin D–biofortifed eggs (36, 60). Evidence of the effectiveness of other food fortification approaches from RCTs that evaluate their effect on reducing the prevalence of vitamin D deficiency in the populations studied is undoubtedly a key priority. The interactions between vitamin D and other micronutrients in dairy-based foods in relation to beneficial effects on bone underscore their importance as vitamin D–fortified foods. Dietary modeling analysis based on data from nationally representative dietary surveys can provide in silico projections of how these food-based vitamin D interventions may affect the degree of vitamin D intake inadequacy in the population. Furthermore, although we acknowledge their simplicity and limitations, computational models can inform vitamin D food fortification strategies by assessing their potential effect on population serum 25(OH)D concentrations and the prevalence of vitamin D deficiency in the absence and presence of sufficient UVB availability. Those computational models, which can account for ethnic dark-skinned subpopulation groups,

are particularly important because these subpopulation groups are at a much higher risk of vitamin D deficiency than their white counterparts. Such modeling of nationally representative vitamin D intake estimates, UVB availability, and ultimately, population serum 25(OH)D concentration can contribute to our understanding of population vitamin D status and means of improving such status. Finally, there is emerging evidence that there may be interactions between sunlight and oral supply of vitamin D, such that seasonal health benefits could be independent of vitamin D status, an area deserving of future research.

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