Proceedings of the Nutrition Society (2017), **76**, 392–399 doi:10.1017/S0029665117000349
© The Authors 2017 First published online 28 March 2017

Nutrition Society Summer Meeting 2016 held at University College Dublin on 11–14 July 2016

Conference on 'New technology in nutrition research and practice' Postgraduate Symposium

Vitamin D deficiency as a public health issue: using vitamin D_2 or vitamin D_3 in future fortification strategies

Louise R. Wilson*, Laura Tripkovic, Kathryn H. Hart and Susan A Lanham-New, on behalf of the D2-D3 Study Team

Department of Nutritional Sciences, University of Surrey, Guildford GU1 7XH, UK

The role of vitamin D in supporting the growth and maintenance of the skeleton is robust; with recent research also suggesting a beneficial link between vitamin D and other non-skeletal health outcomes, including immune function, cardiovascular health and cancer. Despite this, vitamin D deficiency remains a global public health issue, with a renewed focus in the UK following the publication of Public Health England's new Dietary Vitamin D Requirements. Natural sources of vitamin D (dietary and UVB exposure) are limited, and thus mechanisms are needed to allow individuals to achieve the new dietary recommendations. Mandatory or voluntary vitamin D food fortification may be one of the mechanisms to increase dietary vitamin D intakes and subsequently improve vitamin D status. However, for the food industry and public to make informed decisions, clarity is needed as to whether vitamins D_2 and D_3 are equally effective at raising total 25-hydroxyvitamin D (25(OH)D) concentrations as the evidence thus far is inconsistent. This review summarises the evidence to date behind the comparative efficacy of vitamins D_2 and D_3 at raising 25(OH)D concentrations, and the potential role of vitamin D food fortification as a public health policy to support attainment of dietary recommendations in the UK. The comparative efficacy of vitamins D_2 and D_3 has been investigated in several intervention trials, with most indicating that vitamin D_3 is more effective at raising 25(OH)D concentrations. However, flaws in study designs (predominantly under powering) mean there remains a need for a large, robust randomised-controlled trial to provide conclusive evidence, which the future publication of the D_2–D_3 Study should provide (BBSRC DRINC funded: BB/I006192/1). This review also highlights outstanding questions and gaps in the research that need to be addressed to ensure the most efficacious and safe vitamin D food fortification practices are put in place. This further research, alongside cost, availability and ethical considerations (vitamin D_3 is not suitable for vegans), will be instrumental in supporting government, decision-makers, industry and consumers in making informed choices about potential future vitamin D policy and practice.

Vitamin D_2: Vitamin D_3: 25-hydroxyvitamin D: Fortification

Overview of vitamin D

Role of vitamin D

The role of vitamin D in skeletal health is robust; the major biological function of the active form of vitamin D ($1,25(OH)_2D$) is to maintain serum calcium and phosphorus homeostasis, essential for bone mineralisation[1] and neuromuscular function[2]. The discovery that the vitamin D receptor is expressed in virtually all cells in the human body[3] has led to the recognition that vitamin D may have a role to play in many more processes than previously thought. This concurs with the growing

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; RCT, randomised-controlled trial; RNI, reference nutrient intake.

***Corresponding author:** Dr L. R. Wilson, email LWilson@yakult.co.uk

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

body of observational data showing associations between 25-hydroxyvitamin D (25(OH)D) concentrations and chronic diseases and conditions such as CVD, diabetes and colorectal cancer[4]. However, whether low 25(OH)D concentrations are a cause or effect of such conditions has not been established[4].

Sources of vitamin D

Vitamin D is the generic term used for both vitamin D_2 and vitamin D_3, also known as ergocalciferol and cholecalciferol, respectively. Vitamin D_2 is synthesised by the exposure of ergosterol in plants to UVB radiation, whereas vitamin D_3 is synthesised in the skin of human subjects and animals by the action of UVB radiation.

There are therefore two sources of vitamin D; diet and exposure to sunlight. In human subjects, the action of direct sunlight, containing UVB radiation of wavelengths 290–315 nm, on skin results in synthesis of vitamin D. The first step in this synthesis is the conversion of 7-dehydrocholesterol to pre-vitamin D_3, which is then converted to vitamin D_3 by a temperature-dependent isomerisation reaction.

Naturally occurring dietary sources of vitamin D include both vitamin D_2, found in plants and fungi, and vitamin D_3, found in meat, fish and eggs. However, few naturally occurring food sources of vitamin D are considered a rich source of vitamin D. The few foods considered a good source of vitamin D are mostly of animal origin and therefore contain vitamin D_3 such as oily fish, egg yolks and meat, liver and kidney.

Other sources include supplements or fortified foods, which will be discussed later in this review. Both vitamins D_2 and D_3 can be commercially synthesised for use as supplements and to fortify foods, by UVB irradiation of ergosterol from plants and fungi and 7-dehydrocholesterol from sheep's wool, respectively.

In most countries, exposure of skin to UVB radiation or supplement use are the main source of vitamin D and in the UK skin synthesis is the primary source. The latest UK National Diet and Nutrition Survey data collected in 2012/13 and 2013/14 showed mean dietary intakes of 3·1 and 2·5 μg/d in adult men and women, respectively[5], although lower dietary intakes of 1·25–1·6 μg/d have been reported for South Asian women in the UK[6].

Global vitamin D status and recommendations

Rates of vitamin D deficiency

Data from across the globe have shown that vitamin D deficiency is a worldwide issue. Although different cut-off points have been used to define deficiencies, these data are based on 25(OH)D concentrations below 25 nM/l. Studies have reported the prevalence of deficiency to range from 2 to 30 % across Europe[7], 38 to 80 % in women of child-bearing age in the Middle East[8,9], and 3·5 % in the USA, although when ethnicity was considered the prevalence of deficiency were highest among non-Hispanic black individuals at 15 %[10].

In the UK, the latest National Diet and Nutrition Survey[5] reported that 22 % of men and 15 % of women aged 19–64 years had total 25(OH)D concentrations below 25 nM/l all year-round. Furthermore, seasonal variation in 25(OH)D concentrations exists[11]; previous National Diet and Nutrition Survey data reported rates of deficiency increasing to 39 % in men and women between January and March[12]. Sub-groups of the population, particularly those considered at-risk of vitamin D deficiency, have shown even higher rates of deficiency; a longitudinal study (D-FINES) looking at dietary and sunlight contribution to seasonal vitamin D status in South Asian and Caucasian women of child-bearing age reported that 51·4 % of the South Asian women had 25(OH)D concentrations <25 nM/l in the summer months, and this rose to 64·5 % in the winter[13].

Vitamin D dietary recommendations

In July 2016, the Scientific Advisory Committee on Nutrition published the new UK recommendations for vitamin D[14]. Based on the health outcome of musculoskeletal health, the Scientific Advisory Committee on Nutrition concluded that the estimated average nutrient intake required to maintain 25(OH)D concentrations at or above 25 nM/l in winter for 97·5 % of the population aged between 11 years and older is 10 μg/d, and a reference nutrient intake (RNI) of 10 μg/d was set for everyone aged 1 year and above. Prior to this update, there was no RNI for vitamin D set for those aged 4–64 years[15], unless the individual was considered at-risk of deficiency. This was based on the assumption that sufficient vitamin D was made, through skin synthesis, and stored during the summer months to sustain 25(OH)D concentrations during the winter months, which is now known not to be the case[11,13].

This new RNI brings the UK in-line with other European and international recommendations being previously the only country in Europe without a dietary recommendation for all aged 4–6 years[7].

Vitamins D_2 and D_3

Comparative metabolism

The chemical structures of vitamins D_2 and D_3 are similar but not identical; vitamin D_3 has an additional double bond and methyl group, and it is postulated that this different structure results in vitamin D_3 being the preferred substrate at several stages of the pathway of metabolism of vitamin D.

Both vitamins D_2 and D_3, irrespective of source, undergo the same metabolism process from the venous circulatory system, a two-step hydroxylation process. Firstly, vitamins D_2 and D_3 are transported by the vitamin D-binding protein to the liver where they are converted to 25(OH)D_2 and 25(OH)D_3, respectively, by the action of 25-hydroxylase. These are then transported, again by the vitamin D-binding protein, to the kidneys where they are converted to the biologically active form

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

of vitamin D ($1,25(OH)_2D$), by the action of 1-α-hydroxlyases.

There is data to suggest that the differences in the side chains of the two forms of vitamin D directly affect the rate of vitamin D hydroxylation at the liver, with vitamin D_3 thought to be the preferred substrate for hepatic 25-hydroxylase[16,17]. Vitamin D_3 and its metabolites also have a higher binding affinity to the vitamin D-binding protein than vitamin D_2[18]. In addition to these metabolic differences between the two forms of vitamin D, the degradation of vitamin D_3 requires an addition step/process to that of vitamin D_2, which suggest that the degradation rate of vitamin D_2 may be higher than that of vitamin D_3[19].

Therefore, there are several biologically plausible mechanisms by which vitamin D_3 may have a greater capacity than vitamin D_2 to raise and maintain 25(OH)D concentrations, as reviewed by Houghton and Vieth[18], although randomised-controlled trial (RCT) data have not conclusively supported this theory, as discussed below.

Comparative efficacy at raising 25-hydroxyvitamin D concentrations

Historically, vitamins D_2 and D_3 were considered as equally effective at raising 25(OH)D concentrations[20]. However, since the 1980s there have been a number of intervention trials, including RCT, published that have investigated specifically this, as shown in Table 1, and these have shown conflicting results. Although the majority of intervention trials comparing the two forms of vitamin D have provided data suggesting that vitamin D_3 is superior to vitamin D_2 in raising 25(OH)D concentrations[21–36], there have also been four trials that have produced data supportive of vitamins D_2 and D_3 being equally effective[37–40]. There are no studies which have shown that vitamin D_2 is more effective than vitamin D_3.

In 2012, Tripkovic *et al.* conducted a systematic review and meta-analysis of RCT data comparing the efficacy of vitamins D_2 and D_3 in raising 25(OH)D concentrations[41]. Although ten studies were identified within the systematic review, only seven studies had sufficient and available data to be included within the meta-analysis. The primary analysis, of all seven studies regardless of dosing frequency, showed that vitamin D_3 led to a greater absolute change in 25(OH)D concentrations than vitamin D_2, with a weighted mean difference of 15·23 (95 % CI 6·12, 24·34; $Z = 3{\cdot}28$; $I^2 = 81$ %; $P = 0{\cdot}001$). To determine any confounding effect of dosing frequency, separate analyses were performed on: (a) studies giving bolus doses and (b) studies with daily supplementation. For bolus studies alone vitamin D_3 remained significantly more effective than vitamin D_2, with a weighted mean difference of 34·10 (95 % CI 16·39, 51·83; $Z = 3{\cdot}77$; $I^2 = 77$ %; $P = 0{\cdot}0002$). However, when the analysis was completed on the daily dosing RCT data alone, the differentiation between the two forms of vitamin D was moderated with a non-significant weighted mean difference of 4·83 (95 % CI −0·98, 10·64; $Z = 1{\cdot}63$; $I^2 = 41$ %; $P = 0{\cdot}10$).

Key limitations of the meta-analysis were identified and discussed by the authors. Firstly, there were few studies for inclusion within the analysis, and of the studies that were available these were small and unpowered with respect to study population size (*n* 19–89). There was also substantial between-study heterogeneity, with diverse intervention strategies, including varied doses of vitamin D, frequencies of supplementation and methods of administration, and all the studies used supplementation doses in excess of current recommendations[14]. Taking these factors into consideration, the authors concluded that far larger, more robust trials are required to not only measure 25(OH)D concentrations in response to vitamins D_2 and D_3, but also to explore potential mechanisms behind any differences seen.

Since the 2012 meta-analysis, there have been at least another ten further intervention trials comparing the efficacy of vitamins D_2 and D_3 in raising 25(OH)D concentrations[23,26,27,29–31,33,34,38,40]. However, findings remain equivocal with eight of the studies showing vitamin D_3 to be more effective at raising or maintaining 25(OH)D concentrations compared to vitamin D_2[23,26,27,29–31,33,34], and two of these trials reporting no significant difference between the two forms[38,40]. However, a direct comparison between the total change in 25(OH)D concentrations in response to vitamins D_2 and D_3 was not reported in the analysis by Fisk *et al.* which returned neutral findings[38].

These more recent studies have addressed some, but not all, of the limitations identified from the 2012 systematic review and meta-analysis. Specifically, over half of the studies since 2012 examined supplementation doses closer to the range of current global recommendations. However, underpowered sample sizes remain an issue; the largest published RCT comparing the efficacy of vitamins D_2 and D_3 on raising 25(OH)D concentrations had a total of 107 subjects across three intervention groups[27] as shown in Table 1. There remains a need for a large, robust RCT to provide more conclusive evidence in which confidence in results can be sought and the publication of the BBSRC Diet and Health Research Industry Club (DRINC) funded D2–D3 Study (BBSRC DRINC: BB/I006192/1, ISRCTN23421591); an RCT in *n* 335 healthy white Caucasian and South Asian women, should provide just that[42].

Potential mechanisms from randomised-controlled trial data

Within some of these intervention trials the mechanisms by which vitamins D_2 and D_3 might lead to different effects on total 25(OH)D concentrations have been explored. Where methods such as LC-MS/MS for measuring 25(OH)D concentration are implemented, 25(OH)D_2 and 25(OH)D_3 concentrations are measured, which are added together to determine total 25(OH)D concentrations. In studies where these two metabolites have been measured, the vitamin D_2 interventions have led to an increase in 25(OH)D_2 concentrations and the vitamin D_3 interventions have led to an increase in 25(OH)D_3 concentrations[21,24,27,29,37,38], as would be expected.

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

Table 1. Study characteristics and outcomes of intervention trials comparing the effects of vitamins D_2 and D_3 on 25-hydroxyvitamin D (25(OH)D) concentrations

Study	Intervention Groups	Participants	Duration	Results
Armas *et al.*[21]	(1) No supplement, (2) one tablet of 1,250 µg vit D_2, (3) ten tablets of 125 µg vit D_3	*n* 30, 20–61 y All M	28 d	28-d AUC was significantly greater for vit D_3 group than vit D_2 group ($P < 0{\cdot}002$)
Biancuzzo *et al.*[37]	(1) Placebo capsule + placebo OJ, (2) Placebo capsule + 25 µg/d vit D_3 OJ, (3) Placebo capsule + 25 µg/d vit D_2 OJ, (4) 25 µg/d vit D_3 capsule + placebo OJ, (5) 25 µg/d vit D_2 capsule + placebo OJ	*n* 86, 18–79 y 59 F & 27 M	11 wk	No significant difference was shown in AUC for 25(OH)D when vit D_2 and vit D_3 were compared, irrespective of vehicle (capsule or juice)
Binkley *et al.*[22]	(1) 40 µg/d vit D_2, (2) 40 µg/d vit D_3, (3) 1250 µg/mo vit D_2, (4) 1250 µg/mo vit D_3	*n* 65, >65 y 43 F & 21 M	12 mo	Vit D_3 was significantly more effective than vit D_2 at raising 25(OH)D concentrations for the daily dosage ($P = 0{\cdot}05$) and for daily and monthly dosage groups combined ($P = 0{\cdot}01$). NS for monthly dosage group
Cipriani *et al.*[23]	(1) single oral dose of 1500 µg vit D_2, (2) single IM dose of 1500 µg vit D_2, (3) single oral dose of 1500 µg vit D_3, (4) single IM dose of 1500 µg vit D_3	*n* 24, 50–78 y 18 F & 6 M	120 d	Mean AUC 25(OH)D values considering all times points were significantly higher after vit D_3 than vit D_2, for both oral and intramuscular groups ($P < 0{\cdot}0001$)
Fisk *et al.*[38]	(1) 5 µg/d vit D_2 malted milk drink, (2) 5 µg/d vit D_3 malted milk drink, (3) 10 µg/d vit D_2 malted milk drink (4) 10 µg/d vit D_3 malted milk drink, (5) placebo malted milk drink	*n* 40, 18–65 y 23 F & 17 M	4 wk	The increment and iAUC for total 25(OH)D in the vit D_2 groups did not differ from those in the D_3 groups
Glendenning *et al.*[24]	(1) vit D_2 25 µg/d, (2) vit D_3 25 µg/d	*n* 70, 82–84 y Sex unknown	3 mo	Vit D_3 supplementation was associated with a 31 % greater increase in 25(OH)D than vit D_2 supplementation ($P = 0{\cdot}01$)
Heaney *et al.*[25]	(1) One capsule of 1250 µg vit D_2, (2) five capsules of 1250 µg vit D_3	*n* 33, 49·5 ± 9·8 y 30 F & 3 M	12 wk	12-wk induced AUC was significantly greater for the vit D_3 group than for the vit D_2 group ($P < 0{\cdot}001$). Vit D3 was calculated as 87 % more potent at raising 25(OH)D
Holick *et al.*[39]	(1) Placebo, (2) 25 µg/d vit D_2 capsule, (3) 25 µg/d vit D_3 capsule, (4) 12·5 µg/d D_2 + 12·5 µg/d D_3 capsule	*n* 68, 18–84 y 47 F & 21 M	11 wk	At the end of the intervention, there was no significant difference in 25 (OH)D concentrations between vit D_2 and D_3 groups
Itkonen *et al.*[26]	(1) 25 µg/d vit D_2 bio-fortified bread + placebo pill, (2) regular bread + 25 µg/d vit D_2 pill, (3) regular bread + 25 µg/d vit D_3 pill, (4) regular bread + placebo pill	*n* 33, 20–37 y All F	8 wk	Vit D_3 was more effective at raising 25(OH)D concentrations than placebo or vit D_2 bio-fortified bread. Mean change in total 25(OH)D in the D_2 pill and D_3 pill group were + 9·6 nM/l and +17·0 nM/l, respectively
Lehmann *et al.*[27]	(1) 50 µg/d vit D_2 pill, (2) 50 µg/d vit D_3 pill, (3) placebo pill	*n* 107, 19–67 y 68 F & 39 M	8 wk	At 8 wk, 25(OH)D was significantly higher in the vit D_3 group than the vit D_2 and placebo groups ($P < 0{\cdot}01$). Absolute change in total 25(OH)D was significantly lower in the vit D_2 group than the vit D_3 group
Leventis & Keily[28]	(1) single IM injection of 7500 µg vit D_2, (2) single 100-ml oral dose of 7500 µg vit D_3	*n* 69, 23–82 y 58F & 11M	24 wk	Greater increases in serum 25(OH)D were achieved with vit D_3 intervention
Logan *et al.*[29]	(1) 25 µg/d vit D_2 pill, (2) 25 µg/d vit D_3 pill, (3) placebo pill	*n* 61, 18–50 y M & F	25 wk	Total 25(OH)D concentrations were 21 nM/l lower in those receiving vit D_2 compared with those receiving vit D_3 ($P < 0{\cdot}001$)
Mehrotra *et al.*[30]	(1) 15 µg/d vit D_2 in UVB-treated mushrooms + placebo pill, (2) 100 µg/d vit D_2 in UVB-treated mushrooms + placebo pill, (3) untreated mushrooms + 15 µg/d vit D_3 pill, (4) untreated mushrooms + 100 µg/d vit D_3 pill	*n* 43, 30–90 y 29 F & 14 M	16 wk	An increase in total 25(OH)D concentration was shown in both the vit D_3 intervention groups, but not in either vit D_2 group. No direct statistical comparison results were available
Nimitphong *et al.*[40]	(1) 10 µg/d vit D_2, (2) 10 µg/d vit D_3	*n*39, 15–70 y 32 F & 7 M	3 mo	There was no significant difference in 25(OH)D concentrations or change in 25(OH)D from baseline between the vit D_2 and D_3 groups ($P = 0{\cdot}08$)
Oliveri *et al.*[31]	(1) 2500 µg vit D_2 on d0 + 120 µg/d vit D_2 oral drops from d7 to 20, (2) 2500 µg vit D_3 on d0 + 120 µg/d vit D_3 oral drops from d7 to 20, (3) Placebo oral drops daily from d7 to 20	*n* 33, 24–46 y 25 F & 8 M	77 d	At d7 and d21, 25(OH)D concentrations were not significantly different between the vit D_2 and D_3 groups. At d77, after 56 d without supplementation, 25(OH)D concentration was significantly higher in the vit D_3 group than both the vit D_2 and placebo groups ($P < 0{\cdot}05$)
Romagnoli *et al.*[32]	(1) Single oral dose of 7500 µg vit D_3, (2) Single IM dose of 7500 µg vit D_3 (3) Single oral dose of 7500 µg vit D_2 (4) Single IM dose of 7500 µg vit D_2	*n* 32, 66–97 y All F	60 d	Vit D3 significantly more potent at raising serum 25(OH)D concentrations than was vit D_2 for both oral and IM administration

Table 1. (*Cont.*)

Study	Intervention Groups	Participants	Duration	Results
Shieh *et al.*[33]	(1) 1250 µg vit D_2 twice weekly (2) 1250 µg vit D_3 twice weekly	*n* 38, ≥*18 y*	5 wk	Vit D_3 led to a greater increase in total 25(OH)D concentration than vit D_2 ($P = 0{\cdot}001$)
Stepien *et al.*[34]	(1) 15 µg/d vit D_2 mushroom powder, (2) placebo mushroom powder, (3) 15 µg/d vit D_3 capsule, (4) placebo capsule	*n* 90, 40–65 y 27 F & 15 M	4 wk	A significant increase in 25(OH)D concentration was only shown in the vit D_3 capsule group ($P < 0{\cdot}05$). Post-intervention 25(OH)D concentration was significantly higher in the vit D_3 capsule group than the other groups ($P < 0{\cdot}05$)
Tjellesen *et al.*[35]	(1) 100 µg/d vit D_3, (2) 100 µg/d vit D_2	*n* 19, 22–49 y All F	8 wk	Vit D_3 was linked to a greater increase in serum 25(OH)D compared with that in the vit D_2 intervention group. No direct statistical comparison results were available
Trang *et al.*[36]	(1) Untreated, (2) 4000 IU vit D_2, (3) 4000 IU vit D_3	*n* 89, 38–69 y 48 F & 23 M (18 unknown)	14 d	Greater increase in serum 25(OH)D concentrations with vit D_3 group than with vit D_2 group ($P = 0{\cdot}03$)

Key: vit, vitamin; M, males; F, females; d, days; wk, weeks; mo, months; OJ, orange juice; 25(OH)D, 25-hydroxyvitamin D; IM, intramuscular.

However, a decreasing effect of vitamin D_2 interventions on 25(OH)D_3 concentrations has been noted in several intervention trials[21,22,27,35], although not all[24,37,38]. The same has not been shown for vitamin D_3; vitamin D_3 interventions have not shown a decreasing effect on 25(OH)D_2 concentrations; however, baseline 25(OH)D_2 concentrations tend to be far lower at baseline (typically <5 nM/l) and so the opportunity for a decreasing effect is not available. Although even where mean baseline 25(OH)D_2 concentrations were slightly higher (13·3 nM/l) no change was shown in the vitamin D_3 intervention group[24].

This decline in 25(OH)D_3 concentrations reported in those taking vitamin D_2 could explain why vitamin D_3 is more effective at raising total 25(OH)D concentrations, and although the exact mechanisms are unknown this could reflect either competitive binding for 25-hydroxylase or the vitamin D-binding protein, or changes in degradation rate as discussed previously. Further research is needed to elucidate the exact mechanisms and to understand the impact of these changes on overall health, and not just total 25(OH)D concentrations.

Fortification

Across Europe, legislative and voluntary fortification policies and practices vary from country to country. In 1940, vitamin D fortification of margarine and fat spreads became mandatory in the UK and Ireland, although only to bring the vitamin D content up to the level naturally found in butter and not with the aim of improving population intakes. Currently most margarines and fat spreads are still fortified voluntarily despite the mandatory requirement being revoked in 2014[43]; however, it is important to note that although these would be considered fortified food, the amount of vitamin D added is 7·5–10 µg per 100 g, and thus the contribution to population intakes of vitamin D is minimal.

Vitamin D, in the form of either vitamin D_2 or D_3, is legally permitted to be added to foods on a voluntary basis (Annex 1 of Regulation (EC) No 1925/2006, amended by the Commission Regulation (EC) No 1170/2009), and foods which are most commonly fortified include breakfast cereals and more recently, non-dairy milks. However, as the amount of vitamin D added in to fortified products is low, fortified foods still contribute very little (0·8 µg/d) to the dietary intake of the UK adult population[12].

It has been suggested that additional strategic approaches to fortification, including bio-fortification, of a wider range of foods, have the potential to increase vitamin D intakes in the population[44]. Bio-fortification is the process by which nutritional quality is enhanced through agronomic or modern biotechnology techniques, as opposed to being added manually at a later stage of product processing. A thorough review of food-based solutions for vitamin D deficiency has recently been published by Hayes and Cashman earlier this year[45] and includes a review of the need for traditional fortification

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

but also provides an overview of recent advances in the field of bio-fortification, which may have greater consumer appeal. To date, there are several methods of bio-fortification that have begun to be explored, including vitamin D_3 enhancement in eggs and meat through addition of vitamin D to animal feeds[45], and the use of UV radiation to enhance the vitamin D_2 content of foods such as mushrooms, which has recently been examined in a systematic review and meta-analysis[46]. Although the majority of these developments have proved successful at improving vitamin D status in RCT, one recent RCT, feeding bread baked with UV-treated yeast, resulted in no significant change in 25(OH)D concentrations, despite the vitamin D_2 content of the baked bread being confirmed by HPLC[26,47]. This raises concerns about the bio-accessibility of these bio-fortified foods and highlights the need for human RCT data to provide proof of efficacy and safety prior to products reaching the market.

There are two key projects that have, and continue to, significantly contribute to the evidence base for the potential role of vitamin D fortified foods: (1) the Optimal Fortification with vitamin D (OPTIFORD; www.optiford.org) European project investigated the feasibility of fortification as a strategy for improving vitamin D status; among their findings they concluded that bread was a safe and feasible vehicle for fortification[48]; (2) the food-based solutions for optimal vitamin D nutrition and health throughout the life cycle project (ODIN; www.odin-vitd.eu), an EU funded project consisting of a multi-disciplinary team across 18 countries, aiming to develop food-based strategies with agri-food producers and the food industry that provide proof of efficacy and safety. To date the research team have published several key studies including a RCT with vitamin D-enhanced eggs[49] and a meta-analysis of studies examining the effects of UV-exposed mushrooms[46] on vitamin D status. The final report from the ODIN project is due in 2017.

Supplementation

Supplementation is another potential strategy to support the UK population in achieving the dietary recommendation and subsequently improving vitamin D status. Although there are no data showing the number of people in the UK currently taking vitamin D supplements specifically, National Diet and Nutrition Survey data have shown that 23 % of adults aged 19–64 years and 39 % of adults over 65 years take at least one dietary supplement[12]. Currently the UK advise daily supplements for those considered at-risk of vitamin D deficiency such as young children, pregnant and breastfeeding women, housebound elderly and those with darker skin tones. However, the awareness and adherence to this current guidance is poor[50] and so the impact of this advice, and any potential future advice, will only be realised if individuals are willing to take supplements and remember to do so. Universal provision of supplements has been identified as a successful strategy in some European countries where they are recommended for infants (Norway, Germany, Austria and Switzerland) and children up to the age of 5 years (Sweden). The potential universal use of vitamin D supplementation in the UK is therefore worthy of further research, as discussed in greater detail in two recent reviews[7,50].

Conclusion

The introduction of 10 μg/d as the new RNI for the UK population aged 1 year and above introduces a new era for vitamin D recommendations[51]. The Scientific Advisory Committee on Nutrition Vitamin D Working Group recognised that achieving the new RNI of 10 μg/d from natural dietary sources alone would be a challenge and so they have recommended that the UK government, namely Public Health England and the Department of Health, give consideration to strategies to support the UK population with achieving this RNI[14]. One of these potential strategies is fortification of foods, via mandatory or voluntary means. There has been significant progress in the research to support the use of vitamin D in food fortification, including the growth of potential bio-fortified foods, but there are outstanding questions and gaps in the research that need to be addressed to ensure the most efficacious and safe fortification practices are put in place. In addition, this review also highlights the need for further clarity as to the relative efficacy of vitamins D_2 and D_3 in raising total 25(OH)D concentrations, which the publication of the D2–D3 Study results will support. This further research will be key, alongside considerations, including cost, availability and ethics (vitamin D_3 is not suitable for vegans), in allowing government, decision-makers, industry and consumers to make informed choices about potential future vitamin D policy and practice.

Acknowledgements

L. W. would like to acknowledge and thank the D2–D3 Study Team: Professor Susan Lanham-New (Principal Investigator), Dr Kathryn Hart, Dr Laura Tripkovic and Dr Ruan Elliott (University of Surrey), Professor Colin Smith and Dr Giselda Bucca (University of Brighton), Dr Simon Penson and Dr Gemma Chope (Campden BRI), Dr Jacqueline Berry (University of Manchester) and Professor Elina Hyppönen (University of South Australia). L. W. also acknowledges the support of the National Institute of Health Research Clinical Research Network (NIHR CRN) and would like to thank the following parties for their great help and kind assistance in the identification, recruitment and retention of participants onto the D2-D3 Study: Mrs Shahnaz Bano (Surrey County Council, UK), Mrs Fatima Bukhari and Rukhsana Hanjra (Islamic Resource Centre, Kingston, UK). The D2–D3 Study team are extremely grateful to Professor Peter Schroder and Mr James Phillips (BBSRC DRINC Programme), and Professors John Mathers (University of Newcastle) and Professor Hilary Powers (University of Sheffield) for

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

their critical comments in the design of the D2–D3 Study and its implementation.

Financial Support

L. W. would like to acknowledge and thank the BBSRC Diet and Health Research Industry Club (DRINC) for funding the D2–D3 Study (BB/I006192/1) and her studentship. The BBSRC DRINC had no role in the writing of this article.

Conflicts of Interest

None.

Authorship

L. W. wrote the manuscript. L. T., K. H. and S. L. N. assisted with manuscript editing.

References

1. DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* **80**, 1689S–1696S.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA *et al.* (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**, 1911–1930.
3. Norman AW (2008) From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* **88**, 491S–499S.
4. Autier P, Boniol M, Pizot C *et al.* (2014) Vitamin D status and ill health: a systematic review. *Lancet Diab Endocrinol* **2**, 76–89.
5. Bates B, Cox L, Nicholson S *et al.* (2016) National Diet and Nutrition Survey Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013–2013/2014) A survey carried out on behalf of Public Health England and the Food Standards Agency.
6. Macdonald HM, Mavroeidi A, Fraser WD *et al.* (2011) Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? *Osteo Int* **22**, 2461–2472.
7. Spiro A & Buttriss JL (2014) Vitamin D: an overview of vitamin D status and intake in Europe. *Nutr Bull* **39**, 322–350.
8. Bassir M, Laborie S, Lapillonne A *et al.* (2001) Vitamin D de ciency in Iranian mothers and their neonates: a pilot study. *Acta Paediatr* **90**, 577–579.
9. Molla AM, Al Badawi M, Hammoud MS *et al.* (2005) Vitamin D status of mothers and their neonates in Kuwait. *Pediatr Int* **47**, 649–652.
10. Jain RB (2016) Recent vitamin D data from NHANES: variability, trends, deficiency and sufficiency rates, and assay compatibility issues. *J Adv Nutr Hum Metab* **2**, e1208.
11. Hyppönen E & Power C (2007) Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* **85**, 860–868.
12. Bates B, Lennox A, Prentice A *et al.* (2014) National Diet and Nutrition Survey Rolling Programme (NDNS RP). Results from years 1–4 (combined) for Scotland (2008/9–2011/12). Public Health England and Food Standards Agency in Scotland.
13. Darling AL, Hart KH, Macdonald HM *et al.* (2013) Vitamin D deficiency in UK South Asian Women of childbearing age: a comparative longitudinal investigation with UK Caucasian women. *Osteo Int* **24**, 477–488.
14. Scientific Advisory Committee on Nutrition (2016) *Vitamin D and Health Report*. London: The Stationary Office.
15. Scientific Advisory Committee on Nutrition (2007) *Update on Vitamin D. Position Statement*. London: The Stationary Office.
16. Holmberg I, Berlin T, Ewerth S *et al.* (1986) 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D_2 and D_3. *Scand J Clin Lab Invest* **46**, 785–790.
17. Cheng JB, Levine MA, Bell NH *et al.* (2004) Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci USA* **101**, 7711–7715.
18. Houghton LA & Vieth R (2006) The case against ergocalciferol (vitamin D_2) as a vitamin supplement. *Am J Clin Nutr* **84**, 694–697.
19. Horst RL, Reinhardt TA, Ramberg CF *et al.* (1986) 24-Hydroxylation of 1, 25-dihydroxyergocalciferol. An unambiguous deactivation process. *J Biol Chem* **261**, 9250–9256.
20. Park EA (1940) The therapy of rickets. *J Am Med Assoc* **115**, 370–379.
21. Armas LA, Hollis BW & Heaney RP (2004) Vitamin D2 is much less effective than vitamin D_3 in humans. *J Clin Endocrinol Metab* **89**, 5387–5391.
22. Binkley N, Gemar D, Engelke J *et al.* (2011) Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or 50,000 IU monthly in older adults. *J Clin Endocrinol Metab* **96**, 981–988.
23. Cipriani C, Romagnoli E, Pepe J *et al.* (2013) Long-term bioavailability after a single oral or intramuscular administration of 600,000 IU of ergocalciferol or cholecalciferol: implications for treatment and prophylaxis. *J Clin Endocrinol Metab* **98**, 2709–2715.
24. Glendenning P, Chew GT, Seymour HM *et al.* (2009) Serum 25-hydroxyvitamin D levels in vitamin D-insufficient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. *Bone* **45**, 870–875.
25. Heaney RP, Recker RR, Grote J *et al.* (2011) Vitamin D3 is more potent than vitamin D2 in humans. *J Clin Endocrinol Metab* **96**, E447–E452.
26. Itkonen ST, Skaffari E, Saaristo P *et al.* (2016) Effects of vitamin D 2-fortified bread v. supplementation with vitamin D_2 or D_3 on serum 25-hydroxyvitamin D metabolites: an 8-week randomised-controlled trial in young adult Finnish women. *Br J Nutr* **115**, 1232–1239.
27. Lehmann U, Hirche F, Stangl GI *et al.* (2013) Bioavailability of vitamin D_2 and D_3 in healthy volunteers, a randomized placebo-controlled trial. *J Clin Endocrinol Metab* **98**, 4339–4345.
28. Leventis P & Kiely PDW (2009) The tolerability and biochemical effects of high-dose bolus vitamin D_2 and D_3 supplementation in patients with vitamin D insufficiency. *Scand J Rheumatol* **38**, 149–153.
29. Logan VF, Gray AR, Peddie MC *et al.* (2013) Long-term vitamin D_3 supplementation is more effective than vitamin

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press

D_2 in maintaining serum 25-hydroxyvitamin D status over the winter months. *British Journal of Nutrition* **109**(06), 1082–1088.
30. Mehrotra A, Calvo MS, Beelman RB *et al.* (2014) Bioavailability of vitamin D_2 from enriched mushrooms in prediabetic adults: a randomized controlled trial. *Eur J Clin Nutr* **68**, 1154–1160.
31. Oliveri B, Mastaglia SR, Brito GM *et al.* (2015) Vitamin D3 seems more appropriate than D_2 to sustain adequate levels of 25(OH)D: a pharmacokinetic approach. *Eur J Clin Nutr* **69**, 697–702.
32. Romagnoli E, Mascia ML, Cipriani C *et al.* (2008) Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) in the elderly. *J Clin Endocrinol Metab* **93**, 3015–3020.
33. Shieh A, Chun RF, Ma C *et al.* (2016) Effects of high-dose vitamin D_2 versus D_3 on total and free 25-hydroxyvitamin D and markers of calcium balance. *J Clin Endocrinol Metab* **101**, 3070–3078.
34. Stepien M, O'Mahony L, O'Sullivan A *et al.* (2013) Effect of supplementation with vitamin D_2-enhanced mushrooms on vitamin D status in healthy adults. *J Nutr Sci* **2**, 29.
35. Tjellesen L, Hummer L, Christiansen C *et al.* (1986) Serum concentration of vitamin D metabolites during treatment with vitamin D_2 and D_3 in normal premenopausal women. *Bone Miner* **1**, 407–413.
36. Trang HM, Cole DE, Rubin LA *et al.* (1998) Evidence that vitamin D_3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D_2. *Am J Clin Nutr* **68**, 854–858.
37. Biancuzzo RM, Young A, Bibuld D *et al.* (2010) Fortification of orange juice with vitamin D_2 or vitamin D_3 is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr* **91**, 1621–1626.
38. Fisk CM, Theobald HE & Sanders TA (2012) Fortified malted milk drinks containing low-dose ergocalciferol and cholecalciferol do not differ in their capacity to raise serum 25-hydroxyvitamin D concentrations in healthy men and women not exposed to UV-B. *J Nutr* **142**, 1286–1290.
39. Holick MF, Biancuzzo RM, Chen TC *et al.* (2008) Vitamin D_2 is as effective as vitamin D_3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab* **93**, 677–681.
40. Nimitphong H, Saetung S, Chanprasertyotin S *et al.* (2013) Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D_3 or D_2 supplementation. *Nutr J* **12**, 39.
41. Tripkovic L, Lambert H, Hart K *et al.* (2012) Comparison of vitamin D_2 and vitamin D_3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr* **95**, 1357–1364.
42. Tripkovic L, Wilson LR, Hart K *et al.* (2015) The D2-D3 Study: a randomised, double-blind, placebo-controlled food-fortification trial in women, comparing the efficacy of 15ug/d vitamin D_2 vs vitamin D_3 in raising serum 25OHD levels. *Proc Nutr Soc* **74**, OCE1, E16.
43. Department for Environment, Food and Rural Affairs (2014) Consultation on revoking Regulation 4 of the Spreadable Fats (Marketing Standards) and Milk and Milk Products (Protection of Designations) (England) Regulations 2008 – margarine fortification. Summary of response. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/287673/margari%20ne-fortification-sum-resp-201403.pdf
44. Cashman KD & Kiely M (2013) EURRECA-estimating vitamin D requirements for deriving dietary reference values. *Crit Rev Food Sci Nutr* **53**, 1097–1109.
45. Hayes A & Cashman KD (2017) Food-based solutions for vitamin D deficiency: putting policy into practice and the key role for research. *Proc Nutr Soc* **76**, 54–63.
46. Cashman KD, Kiely M, Seamans KM *et al.* (2016) Effect of ultraviolet light-exposed mushrooms on vitamin D status: liquid chromatography-tandem mass spectrometry reanalysis of biobanked sera from a randomized controlled trial and a systematic review plus meta-analysis. *J Nutr* **146**, 565–575.
47. Lipkie TE, Ferruzzi MG & Weaver CM (2016) Low bioaccessibility of vitamin D_2 from yeast-fortified bread compared to crystalline D_2 bread and D_3 from fluid milks. *Food Funct* **7**, 4589–4596.
48. Natri AM, Salo P, Vikstedt T *et al.* (2006) Bread fortified with cholecalciferol increase the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplements. *J Nutr* **136**, 123–127.
49. Hayes A, Duffy S, O'Grady M *et al.* (2016) Vitamin D-enhanced eggs are protective of wintertime serum 25-hydroxyvitamin D in a randomized controlled trial of adults. *Am J Clin Nutr* **104**, 629–637.
50. Buttriss JL (2015) Vitamin D: sunshine vs. diet vs. pills. *Nutr Bull* **40**, 279–285.
51. Lanham-New SA & Wilson LR (2016) Vitamin D – has the new dawn for dietary recommendations arrived? *J Hum Nutr Diet* **29**, 3–6.

Proceedings of the Nutrition Society

https://doi.org/10.1017/S0029665117000349 Published online by Cambridge University Press