



## Conference on ‘New technology in nutrition research and practice’ Postgraduate Symposium

# Vitamin D deficiency as a public health issue: using vitamin D<sub>2</sub> or vitamin D<sub>3</sub> in future fortification strategies

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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<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> has been investigated in several intervention trials, with most indicating that vitamin D<sub>3</sub> 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<sub>2</sub>-D<sub>3</sub> 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<sub>3</sub> 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<sub>2</sub>: Vitamin D<sub>3</sub>: 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)<sub>2</sub>D) is to maintain serum calcium and

phosphorus homeostasis, essential for bone mineralisation<sup>(1)</sup> and neuromuscular function<sup>(2)</sup>. The discovery that the vitamin D receptor is expressed in virtually all cells in the human body<sup>(3)</sup> 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.

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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<sup>(4)</sup>. However, whether low 25(OH)D concentrations are a cause or effect of such conditions has not been established<sup>(4)</sup>.

#### *Sources of vitamin D*

Vitamin D is the generic term used for both vitamin D<sub>2</sub> and vitamin D<sub>3</sub>, also known as ergocalciferol and cholecalciferol, respectively. Vitamin D<sub>2</sub> is synthesised by the exposure of ergosterol in plants to UVB radiation, whereas vitamin D<sub>3</sub> 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<sub>3</sub>, which is then converted to vitamin D<sub>3</sub> by a temperature-dependent isomerisation reaction.

Naturally occurring dietary sources of vitamin D include both vitamin D<sub>2</sub>, found in plants and fungi, and vitamin D<sub>3</sub>, 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<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> 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<sup>(5)</sup>, although lower dietary intakes of 1.25–1.6 µg/d have been reported for South Asian women in the UK<sup>(6)</sup>.

### **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<sup>(7)</sup>, 38 to 80 % in women of child-bearing age in the Middle East<sup>(8,9)</sup>, and 3.5 % in the USA, although when ethnicity was considered the prevalence of deficiency were highest among non-Hispanic black individuals at 15 %<sup>(10)</sup>.

In the UK, the latest National Diet and Nutrition Survey<sup>(5)</sup> 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<sup>(11)</sup>; previous National Diet and Nutrition Survey data reported rates of deficiency increasing to 39 % in men and women between January and March<sup>(12)</sup>. 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<sup>(13)</sup>.

#### *Vitamin D dietary recommendations*

In July 2016, the Scientific Advisory Committee on Nutrition published the new UK recommendations for vitamin D<sup>(14)</sup>. 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<sup>(15)</sup>, 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<sup>(11,13)</sup>.

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<sup>(7)</sup>.

### **Vitamins D<sub>2</sub> and D<sub>3</sub>**

#### *Comparative metabolism*

The chemical structures of vitamins D<sub>2</sub> and D<sub>3</sub> are similar but not identical; vitamin D<sub>3</sub> has an additional double bond and methyl group, and it is postulated that this different structure results in vitamin D<sub>3</sub> being the preferred substrate at several stages of the pathway of metabolism of vitamin D.

Both vitamins D<sub>2</sub> and D<sub>3</sub>, irrespective of source, undergo the same metabolism process from the venous circulatory system, a two-step hydroxylation process. Firstly, vitamins D<sub>2</sub> and D<sub>3</sub> are transported by the vitamin D-binding protein to the liver where they are converted to 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, 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

of vitamin D (1,25(OH)<sub>2</sub>D), by the action of 1- $\alpha$ -hydroxylases.

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<sub>3</sub> thought to be the preferred substrate for hepatic 25-hydroxylase<sup>(16,17)</sup>. Vitamin D<sub>3</sub> and its metabolites also have a higher binding affinity to the vitamin D-binding protein than vitamin D<sub>2</sub><sup>(18)</sup>. In addition to these metabolic differences between the two forms of vitamin D, the degradation of vitamin D<sub>3</sub> requires an addition step/process to that of vitamin D<sub>2</sub>, which suggests that the degradation rate of vitamin D<sub>2</sub> may be higher than that of vitamin D<sub>3</sub><sup>(19)</sup>.

Therefore, there are several biologically plausible mechanisms by which vitamin D<sub>3</sub> may have a greater capacity than vitamin D<sub>2</sub> to raise and maintain 25(OH)D concentrations, as reviewed by Houghton and Vieth<sup>(18)</sup>, 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<sub>2</sub> and D<sub>3</sub> were considered as equally effective at raising 25(OH)D concentrations<sup>(20)</sup>. 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<sub>3</sub> is superior to vitamin D<sub>2</sub> in raising 25(OH)D concentrations<sup>(21–36)</sup>, there have also been four trials that have produced data supportive of vitamins D<sub>2</sub> and D<sub>3</sub> being equally effective<sup>(37–40)</sup>. There are no studies which have shown that vitamin D<sub>2</sub> is more effective than vitamin D<sub>3</sub>.

In 2012, Tripkovic *et al.* conducted a systematic review and meta-analysis of RCT data comparing the efficacy of vitamins D<sub>2</sub> and D<sub>3</sub> in raising 25(OH)D concentrations<sup>(41)</sup>. 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<sub>3</sub> led to a greater absolute change in 25(OH)D concentrations than vitamin D<sub>2</sub>, with a weighted mean difference of 15.23 (95% CI 6.12, 24.34;  $Z = 3.28$ ;  $I^2 = 81\%$ ;  $P = 0.001$ ). 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<sub>3</sub> remained significantly more effective than vitamin D<sub>2</sub>, with a weighted mean difference of 34.10 (95% CI 16.39, 51.83;  $Z = 3.77$ ;  $I^2 = 77\%$ ;  $P = 0.0002$ ). 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;  $Z = 1.63$ ;  $I^2 = 41\%$ ;  $P = 0.10$ ).

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<sup>(14)</sup>. 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<sub>2</sub> and D<sub>3</sub>, 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<sub>2</sub> and D<sub>3</sub> in raising 25(OH)D concentrations<sup>(23,26,27,29–31,33,34,38,40)</sup>. However, findings remain equivocal with eight of the studies showing vitamin D<sub>3</sub> to be more effective at raising or maintaining 25(OH)D concentrations compared to vitamin D<sub>2</sub><sup>(23,26,27,29–31,33,34)</sup>, and two of these trials reporting no significant difference between the two forms<sup>(38,40)</sup>. However, a direct comparison between the total change in 25(OH)D concentrations in response to vitamins D<sub>2</sub> and D<sub>3</sub> was not reported in the analysis by Fisk *et al.* which returned neutral findings<sup>(38)</sup>.

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<sub>2</sub> and D<sub>3</sub> on raising 25(OH)D concentrations had a total of 107 subjects across three intervention groups<sup>(27)</sup> 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<sup>(42)</sup>.

#### *Potential mechanisms from randomised-controlled trial data*

Within some of these intervention trials the mechanisms by which vitamins D<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> and 25(OH)D<sub>3</sub> 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<sub>2</sub> interventions have led to an increase in 25(OH)D<sub>2</sub> concentrations and the vitamin D<sub>3</sub> interventions have led to an increase in 25(OH)D<sub>3</sub> concentrations<sup>(21,24,27,29,37,38)</sup>, as would be expected.

**Table 1.** Study characteristics and outcomes of intervention trials comparing the effects of vitamins D<sub>2</sub> and D<sub>3</sub> on 25-hydroxyvitamin D (25(OH)D) concentrations

Study	Intervention Groups	Participants	Duration	Results
Armas <i>et al.</i> <sup>(21)</sup>	(1) No supplement, (2) one tablet of 1,250 µg vit D <sub>2</sub> , (3) ten tablets of 125 µg vit D <sub>3</sub>	n 30, 20–61 y All M	28 d	28-d AUC was significantly greater for vit D <sub>3</sub> group than vit D <sub>2</sub> group ( <i>P</i> < 0.002)
Biancuzzo <i>et al.</i> <sup>(37)</sup>	(1) Placebo capsule + placebo OJ, (2) Placebo capsule + 25 µg/d vit D <sub>3</sub> OJ, (3) Placebo capsule + 25 µg/d vit D <sub>2</sub> OJ, (4) 25 µg/d vit D <sub>3</sub> capsule + placebo OJ, (5) 25 µg/d vit D <sub>2</sub> 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 <sub>2</sub> and vit D <sub>3</sub> were compared, irrespective of vehicle (capsule or juice)
Binkley <i>et al.</i> <sup>(22)</sup>	(1) 40 µg/d vit D <sub>2</sub> , (2) 40 µg/d vit D <sub>3</sub> , (3) 1250 µg/mo vit D <sub>2</sub> , (4) 1250 µg/mo vit D <sub>3</sub>	n 65, >65 y 43 F & 21 M	12 mo	Vit D <sub>3</sub> was significantly more effective than vit D <sub>2</sub> at raising 25(OH)D concentrations for the daily dosage ( <i>P</i> = 0.05) and for daily and monthly dosage groups combined ( <i>P</i> = 0.01). NS for monthly dosage group
Cipriani <i>et al.</i> <sup>(23)</sup>	(1) single oral dose of 1500 µg vit D <sub>2</sub> , (2) single IM dose of 1500 µg vit D <sub>2</sub> , (3) single oral dose of 1500 µg vit D <sub>3</sub> , (4) single IM dose of 1500 µg vit D <sub>3</sub>	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 <sub>3</sub> than vit D <sub>2</sub> , for both oral and intramuscular groups ( <i>P</i> < 0.0001)
Fisk <i>et al.</i> <sup>(38)</sup>	(1) 5 µg/d vit D <sub>2</sub> malted milk drink, (2) 5 µg/d vit D <sub>3</sub> malted milk drink, (3) 10 µg/d vit D <sub>2</sub> malted milk drink (4) 10 µg/d vit D <sub>3</sub> 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 <sub>2</sub> groups did not differ from those in the D <sub>3</sub> groups
Glendenning <i>et al.</i> <sup>(24)</sup>	(1) vit D <sub>2</sub> 25 µg/d, (2) vit D <sub>3</sub> 25 µg/d	n 70, 82–84 y Sex unknown	3 mo	Vit D <sub>3</sub> supplementation was associated with a 31 % greater increase in 25(OH)D than vit D <sub>2</sub> supplementation ( <i>P</i> = 0.01)
Heaney <i>et al.</i> <sup>(25)</sup>	(1) One capsule of 1250 µg vit D <sub>2</sub> , (2) five capsules of 1250 µg vit D <sub>3</sub>	n 33, 49.5 ± 9.8 y 30 F & 3 M	12 wk	12-wk induced AUC was significantly greater for the vit D <sub>3</sub> group than for the vit D <sub>2</sub> group ( <i>P</i> < 0.001). Vit D <sub>3</sub> was calculated as 87 % more potent at raising 25(OH)D
Holick <i>et al.</i> <sup>(39)</sup>	(1) Placebo, (2) 25 µg/d vit D <sub>2</sub> capsule, (3) 25 µg/d vit D <sub>3</sub> capsule, (4) 12.5 µg/d D <sub>2</sub> + 12.5 µg/d D <sub>3</sub> 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 <sub>2</sub> and D <sub>3</sub> groups
Itkonen <i>et al.</i> <sup>(26)</sup>	(1) 25 µg/d vit D <sub>2</sub> bio-fortified bread + placebo pill, (2) regular bread + 25 µg/d vit D <sub>2</sub> pill, (3) regular bread + 25 µg/d vit D <sub>3</sub> pill, (4) regular bread + placebo pill	n 33, 20–37 y All F	8 wk	Vit D <sub>3</sub> was more effective at raising 25(OH)D concentrations than placebo or vit D <sub>2</sub> bio-fortified bread. Mean change in total 25(OH)D in the D <sub>2</sub> pill and D <sub>3</sub> pill group were +9.6 nmol/l and +17.0 nmol/l, respectively
Lehmann <i>et al.</i> <sup>(27)</sup>	(1) 50 µg/d vit D <sub>2</sub> pill, (2) 50 µg/d vit D <sub>3</sub> 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 <sub>3</sub> group than the vit D <sub>2</sub> and placebo groups ( <i>P</i> < 0.01). Absolute change in total 25(OH)D was significantly lower in the vit D <sub>2</sub> group than the vit D <sub>3</sub> group
Leventis & Keily <sup>(28)</sup>	(1) single IM injection of 7500 µg vit D <sub>2</sub> , (2) single 100-ml oral dose of 7500 µg vit D <sub>3</sub>	n 69, 23–82 y 58F & 11M	24 wk	Greater increases in serum 25(OH)D were achieved with vit D <sub>3</sub> intervention
Logan <i>et al.</i> <sup>(29)</sup>	(1) 25 µg/d vit D <sub>2</sub> pill, (2) 25 µg/d vit D <sub>3</sub> pill, (3) placebo pill	n 61, 18–50 y M & F	25 wk	Total 25(OH)D concentrations were 21 nmol/l lower in those receiving vit D <sub>2</sub> compared with those receiving vit D <sub>3</sub> ( <i>P</i> < 0.001)
Mehrotra <i>et al.</i> <sup>(30)</sup>	(1) 15 µg/d vit D <sub>2</sub> in UVB-treated mushrooms + placebo pill, (2) 100 µg/d vit D <sub>2</sub> in UVB-treated mushrooms + placebo pill, (3) untreated mushrooms + 15 µg/d vit D <sub>3</sub> pill, (4) untreated mushrooms + 100 µg/d vit D <sub>3</sub> 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 <sub>3</sub> intervention groups, but not in either vit D <sub>2</sub> group. No direct statistical comparison results were available
Nimitphong <i>et al.</i> <sup>(40)</sup>	(1) 10 µg/d vit D <sub>2</sub> , (2) 10 µg/d vit D <sub>3</sub>	n39, 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 <sub>2</sub> and D <sub>3</sub> groups ( <i>P</i> = 0.08)
Oliveri <i>et al.</i> <sup>(31)</sup>	(1) 2500 µg vit D <sub>2</sub> on d0 + 120 µg/d vit D <sub>2</sub> oral drops from d7 to 20, (2) 2500 µg vit D <sub>3</sub> on d0 + 120 µg/d vit D <sub>3</sub> 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 <sub>2</sub> and D <sub>3</sub> groups. At d77, after 56 d without supplementation, 25(OH)D concentration was significantly higher in the vit D <sub>3</sub> group than both the vit D <sub>2</sub> and placebo groups ( <i>P</i> < 0.05)
Romagnoli <i>et al.</i> <sup>(32)</sup>	(1) Single oral dose of 7500 µg vit D <sub>3</sub> , (2) Single IM dose of 7500 µg vit D <sub>3</sub> (3) Single oral dose of 7500 µg vit D <sub>2</sub> (4) Single IM dose of 7500 µg vit D <sub>2</sub>	n 32, 66–97 y All F	60 d	Vit D <sub>3</sub> significantly more potent at raising serum 25(OH)D concentrations than was vit D <sub>2</sub> for both oral and IM administration





Table 1. (Cont.)

Study	Intervention Groups	Participants	Duration	Results
Shieh <i>et al.</i> <sup>(33)</sup>	(1) 1250 µg vit D <sub>2</sub> twice weekly (2) 1250 µg vit D <sub>3</sub> twice weekly	n 38, ≥18 y	5 wk	Vit D <sub>3</sub> led to a greater increase in total 25(OH)D concentration than vit D <sub>2</sub> (P = 0.001)
Stepien <i>et al.</i> <sup>(34)</sup>	(1) 15 µg/d vit D <sub>2</sub> mushroom powder, (2) placebo mushroom powder, (3) 15 µg/d vit D <sub>3</sub> 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 <sub>3</sub> capsule group (P < 0.05). Post-intervention 25(OH)D concentration was significantly higher in the vit D <sub>3</sub> capsule group than the other groups (P < 0.05)
Tjellesen <i>et al.</i> <sup>(35)</sup>	(1) 100 µg/d vit D <sub>3</sub> , (2) 100 µg/d vit D <sub>2</sub>	n 19, 22–49 y All F	8 wk	Vit D <sub>3</sub> was linked to a greater increase in serum 25(OH)D compared with that in the vit D <sub>2</sub> intervention group. No direct statistical comparison results were available
Trang <i>et al.</i> <sup>(36)</sup>	(1) Untreated, (2) 4000 IU vit D <sub>2</sub> , (3) 4000 IU vit D <sub>3</sub>	n 89, 38–69 y 48 F & 23 M (18 unknown)	14 d	Greater increase in serum 25(OH)D concentrations with vit D <sub>3</sub> group than with vit D <sub>2</sub> group (P = 0.03)

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<sub>2</sub> interventions on 25(OH)D<sub>3</sub> concentrations has been noted in several intervention trials<sup>(21,22,27,35)</sup>, although not all<sup>(24,37,38)</sup>. The same has not been shown for vitamin D<sub>3</sub>; vitamin D<sub>3</sub> interventions have not shown a decreasing effect on 25(OH)D<sub>2</sub> concentrations; however, baseline 25(OH)D<sub>2</sub> 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<sub>2</sub> concentrations were slightly higher (13.3 nm/l) no change was shown in the vitamin D<sub>3</sub> intervention group<sup>(24)</sup>.

This decline in 25(OH)D<sub>3</sub> concentrations reported in those taking vitamin D<sub>2</sub> could explain why vitamin D<sub>3</sub> 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<sup>(43)</sup>; 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<sub>2</sub> or D<sub>3</sub>, 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<sup>(12)</sup>.

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<sup>(44)</sup>. 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<sup>(45)</sup> and includes a review of the need for traditional fortification



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<sub>3</sub> enhancement in eggs and meat through addition of vitamin D to animal feeds<sup>(45)</sup>, and the use of UV radiation to enhance the vitamin D<sub>2</sub> content of foods such as mushrooms, which has recently been examined in a systematic review and meta-analysis<sup>(46)</sup>. 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<sub>2</sub> content of the baked bread being confirmed by HPLC<sup>(26,47)</sup>. 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<sup>(48)</sup>; (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<sup>(49)</sup> and a meta-analysis of studies examining the effects of UV-exposed mushrooms<sup>(46)</sup> 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<sup>(12)</sup>. 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<sup>(50)</sup> 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<sup>(7,50)</sup>.

### 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<sup>(51)</sup>. 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<sup>(14)</sup>. 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<sub>2</sub> and D<sub>3</sub> 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<sub>3</sub> 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.

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### Conflicts of Interest

None.

### Authorship

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

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