Proceedings of the Nutrition Society (2018), **77**, 282–291 doi:10.1017/S0029665117004062

The Nutrition Society Summer Meeting was held at King's College, London on 10–12 July 2017

Conference on 'Improving nutrition in metropolitan areas' Postgraduate Symposium

25(OH)D_3-enriched or fortified foods are more efficient at tackling inadequate vitamin D status than vitamin D_3

Jing Guo[1]*, Julie A. Lovegrove[2] and D. Ian Givens[1]
[1]*Institute for Food, Nutrition and Health, University of Reading, Reading RG6 6AR, UK*
[2]*Hugh Sinclair Unit of Human Nutrition, Department of Food & Nutritional Sciences, University of Reading, Reading RG6 6AP, UK*

The ability to synthesise sufficient vitamin D through sunlight in human subjects can be limited. Thus, diet has become an important contributor to vitamin D intake and status; however, there are only a few foods (e.g. egg yolk, oily fish) naturally rich in vitamin D. Therefore, vitamin D-enriched foods via supplementing the animals' diet with vitamin D or vitamin D fortification of foods have been proposed as strategies to increase vitamin D intake. Evidence that cholecalciferol (vitamin D_3) and calcifediol (25(OH)D_3) content of eggs, fish and milk increased in response to vitamin D_3 supplementation of hens, fish or cows' diets was identified when vitamin D-enrichment studies were reviewed. However, evidence from supplementation studies with hens showed only dietary 25(OH)D_3, not vitamin D_3 supplementation, resulted in a pronounced increase of 25(OH)D_3 in the eggs. Furthermore, evidence from randomised controlled trials indicated that a 25(OH)D_3 oral supplement could be absorbed faster and more efficiently raise serum 25(OH)D concentration compared with vitamin D_3 supplementation. Moreover, evidence showed the relative effectiveness of increasing vitamin D status using 25(OH)D_3 varied between 3·13 and 7·14 times that of vitamin D_3, probably due to the different characteristics of the investigated subjects or study design. Therefore, vitamin D-enrichment or fortified foods using 25(OH)D_3 would appear to have advantages over vitamin D_3. Further well-controlled studies are needed to assess the effects of 25(OH)D_3 enriched or fortified foods in the general population and clinical patients.

Enrichment: Fortification: 25(OH)D_3: Vitamin D_3: Vitamin D deficiency

Vitamin D is usually synthesised in skin that is exposed to UV radiation, which has led to the term 'sunshine vitamin'[1]. Traditionally, the primary role of vitamin D is related to calcium absorption and bone health. Children and adults with vitamin D deficiency have an increased risk of developing rickets or osteomalacia[2]. Recently, a resurgence of childhood rickets has highlighted the need for adequate vitamin D status in many parts of the world[3–5]. Furthermore, mounting evidence from epidemiological studies indicates that vitamin D status is inversely associated with the risk of CVD, cancers and diabetes[1,6], although there is some uncertainty about what defines an adequate vitamin D status[7].

Vitamin D deficiency is prevalent and is considered a serious issue throughout the world[8–10], even in sunnier climates such as Australia and New Zealand[11].

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; RCT, randomised controlled trial.
***Corresponding author:** Dr J. Guo, email sarah.guo@reading.ac.uk

Recently, the Scientific Advisory Committee on Nutrition[7] reported that in the UK, 22–24 % of adults aged 19–64 years, and 17–24 % of those ≥65 years were vitamin D deficient (plasma 25-hydroxyvitamin D_3 (25(OH)D_3) <25 nmol/l). There are several factors that have contributed to the low vitamin D status commonly seen today, such as lifestyle changes (increased indoor lifestyle, sun screens use) and human characteristics (e.g. ageing, clothing, increased obesity, low-fat diet trend)[12]. Therefore, foods that contribute to vitamin D intake have become more important than before. However, there are only a few foods naturally rich in vitamin D, such as oily fish and egg yolks[13].

The aim of this review is first to critically evaluate the existing evidence on whether the vitamin D content of animal-derived foods can be increased by feeding cholecalciferol (vitamin D_3) and/or calcifediol (25(OH)D_3) supplements to laying hens, fish and cows. Second, the present review summaries evidence from the human randomised controlled trials (RCT), which include the effects of 25(OH)D_3 supplementation on increasing serum/plasma 25(OH)D_3 concentration.

Vitamin D absorption, synthesis and metabolism

Generally, the term vitamin D refers to both vitamin D_2 and vitamin D_3. Vitamin D_2 is produced by fungi, while vitamin D_3 is produced by human subjects and animals[14]. Human subjects usually synthesise vitamin D_3 in the skin[15] where 7-dehydrocholesterol in the epidermis is converted to pre-vitamin D_3 when skin is exposed to sunlight. Then, pre-vitamin D_3 undergoes a temperature-dependent isomerisation to vitamin D_3 over a period of approximately 3 d[6]. Vitamin D (vitamin D_2 or vitamin D_3) can also be obtained from the diet[15] and it is absorbed with long-chain TAG in the small intestine[16]. It is then incorporated into chylomicrons and transported in lymph to the blood and into the general circulation[17].

After entering the circulation, there are two hydroxylation reactions to convert vitamin D to the biologically active form[6]. The first hydroxylation reaction is in the liver where vitamin D is hydroxylated to 25(OH)D by the vitamin D-25-hydroxylase enzyme. The second hydroxylation reaction is in the kidney where 25(OH)D is converted to 1,25$(OH)_2$D by 25-hydroxyvitamin D-1α-hydroxylase[6], and the 1,25$(OH)_2$D metabolite is the biologically active form of vitamin D[18].

Foods of animal origin as dietary sources of vitamin D

Vitamin D content of vitamin D-enriched foods can differ considerably between food retailers. One US retail study analysed the vitamin D content of egg yolks collected from twelve individual retail supermarkets across the country and reported a broad range of vitamin D_3 and 25(OH)D_3 concentrations of 9·7–18 and 4·3–13·2 μg/kg, respectively[19]. In addition, our recent UK retail study[20] showed vitamin D_3 and 25(OH)D_3 concentrations of eggs were significantly different depending on the egg production systems. Egg yolks produced by birds kept in indoor systems had much lower concentrations (40·2 (SE 3·1) μg/kg) of vitamin D_3 than the egg yolks produced from outdoor systems (57·2 (SE 3·2) μg/kg), while 25(OH)D_3 concentrations of the eggs were higher in organic eggs only. Similarly, the vitamin D contents of fish have been shown to vary according to the production systems. The study of Lu *et al.*[21] indicated the vitamin D_3 content of wild salmon to be three times higher than that of farmed salmon; however, the 25(OH)D_3 content of the salmon was not measured. In addition, other studies[22,23] have shown the 25(OH)D_3 content of several species of marine and freshwater fish to be <0·02 μg/100 g. Therefore, foods generally regarded as rich sources of vitamin D may not be sustainable vitamin D contributors for the general population, due to variability in vitamin D content, which in turn may be influenced by production systems or different species (genotype). Furthermore, the National Diet and Nutrition Survey of the UK[24] reported that the average daily intake of vitamin D for adults was 3·1 μg for men and 2·6 μg for women, which is much lower than the UK vitamin D reference nutrition intake of 10 μg/d[7]. Therefore, vitamin D-enriched or fortified foods are needed to ensure an adequate vitamin D intake for the general population.

Enrichment of animal-derived foods as dietary sources of vitamin D

Vitamin D-enriched eggs

In general, there are two main methods to enrich the vitamin D content of eggs: increased sunlight exposure and vitamin D supplementation of the birds' diet. Because hens can synthesise vitamin D from natural sunlight exposure, free-range egg production system may be an inexpensive way to increase their vitamin D content. A study by Kuhn *et al.* assigned laying hens to a free-range treatment or an indoor treatment for over 4 weeks and found that eggs from the free-range group, which were exposed to sunlight, had significantly higher vitamin D_3 content (mean 14·3 μg/100 g DM) than eggs from the indoor group (mean 3·8 μg/100 g DM)[25]. Furthermore, there are several studies which have shown that the vitamin D_3 content of eggs can be enhanced by feeding vitamin D_3 supplements to the hens (Table 1)[26–32]. The results of all studies revealed that egg yolk vitamin D_3 concentration was efficiently increased by vitamin D_3 dietary supplementation. The study of Yao *et al.* showed a linear dose–response relationship existed between vitamin D_3 dietary supplementation and vitamin D_3 concentrations of egg yolks[30]. Moreover, as 25(OH)D_3 is a metabolite of vitamin D_3, the 25(OH)D_3 content in eggs can also be enhanced by supplementing the birds' diet with vitamin D_3. However, the response in 25(OH)D_3 content of egg yolk is much less than that of vitamin D_3. Browning and Cowieson[31] showed that a 4-fold increase in vitamin D_3, and a 2-fold increase in 25(OH)D_3 in egg yolk resulted from a 4-fold increase in the vitamin D_3

Table 1. Summary of enrichment studies investigating the impact of adding vitamin D to the diet of laying hens on the vitamin D content of egg yolks

References	Vitamin D supplement (μg/kg)		Feeding duration (weeks)	Vitamin D concentration of egg yolk (μg/100 g)	
	Vitamin D_3	25(OH)D_3		Vitamin D_3	25(OH)D_3
Mattila *et al.*[26]	26·6	–	6	1·4	0·5
	62·4	–	6	3·5	0·9
	216·0	–	6	22·0	1·5
Mattila *et al.*[27]	280·0	–	4	30·0	1·9
Mattila *et al.*[28]	62·5	–	4	3·8	–
	150·0	–	4	13·6	–
	375·0	–	4	33·7	–
Browning and Cowieson[31]	62·5	–	9	6·5	1·6
	125·0	–	9	10·5	2·1
	250·0	–	9	26·2	3·0
Yao *et al.*[30]	55·0	–	3	3·0	–
	242·5	–	3	21·6	–
	430·0	–	3	41·0	–
	617·5	–	3	60·3	–
	2555·0	–	3	870·4	–
Browning and Cowieson[31]	62·5	0	9	6·5	1·6
	62·5	34·5	9	6·0	3·3
	62·5	69·0	9	4·9	4·5
	125·0	0	9	10·5	2·1
	125·0	34·5	9	7·4	4·5
	125·0	69·0	9	8·1	5·8
	250·0	0	9	26·2	3·0
	250·0	34·5	9	23·6	3·7
	250·0	69·0	9	30·9	8·1
Mattila *et al.*[29]	–	55·0	6	≤0·2	2·1
	–	122·0	6	≤0·2	4·3
Duffy *et al.*[32]	37·5	–	4	1·0*	1·9*
	75·0	–	4	2·0*	1·9*
	37·5	37·5	4	1·3*	3·6*
		75·0	4	0·7*	4·4*

25(OH)D_3, 25-hydroxyvitamin D_3.
* Vitamin D content per egg.

in the diet (62·5–250 μg/kg). Similarly, evidence from another study showed that the vitamin D_3 in egg yolk was increased approximately 7-fold as a result of feeding a diet with a 3·5-fold higher vitamin D_3 content (from 62·4 to 216 μg/kg), while the corresponding increase in 25(OH)D_3 content was only about 1·5-fold[26].

There are only a few studies[29,31,32] examining the effect of feeding birds with diets supplemented with 25(OH)D_3. In the EU, 25(OH)D_3 has only recently been authorised for addition to poultry diets, and the maximum content of the vitamin D_3 and 25(OH)D_3 combination for laying hens is 80 μg/kg[33,34]. It is of note that most of vitamin D supplementation studies[27–31], summarised in Table 1, had higher vitamin D doses than the EU diet limit[33], thus, the potential for increasing vitamin D in eggs by adding vitamin D to the diet of laying hens is limited by EU regulations. Browning and Cowieson[31] and Duffy *et al.*[32] both showed an addition of 25(OH)D_3 to the vitamin D_3 supplement resulted in the elevation of the 25(OH)D_3 content of the egg yolk, but there was no significant increase in the vitamin D_3 content of the egg yolk. Other studies investigated dietary supplementation with 25(OH)D_3[29,32], and showed that only egg yolk 25(OH)D_3 was increased, but not vitamin D_3. Therefore, we speculate that 25(OH)D_3 in the diet can be absorbed directly by laying hens without transfer to vitamin D_3 in the circulation.

Vitamin D-enriched fish

There are very few studies on enriching the vitamin D content of fish (Table 2)[35–38]. Mattila *et al.* fed rainbow trout with different doses of vitamin D_3 supplements up to 539 μg/kg, but no significant differences in the vitamin D_3 content of the fish fillet were observed[37]. In contrast, the study of Horvli *et al.* with Atlantic salmon showed a dose–response relationship between the vitamin D_3 in the diet up to 28·68 mg/kg and vitamin D_3 in the fish meat[35]. Similar high vitamin D_3 supplementation doses were reported in another two studies[36,38], which also showed that elevated vitamin D_3 content of the fish liver or whole fish had been achieved by supplemental vitamin D_3 in the diet. However, 25(OH)D_3 contents of the enriched fish were not measured in these studies[35–38], and the lack of evidence on the effects by feeding fish with 25(OH)D_3 on the vitamin D content of the

Table 2. Summary of enrichment studies investigating the impact of vitamin D supplemental fish feeding on vitamin D content of fish

References	Vitamin D_3 supplement (μg/kg)	Feeding duration (weeks)	Vitamin D_3 of fish (μg/100 g)
Horvli *et al.*[35]	40	11	1 (fillet)
	2210	11	21 (fillet)
	28 680	11	210 (fillet)
Vielma *et al.*[36]	62·5	12	1 (liver)
	6250	12	73 (liver)
	62 500	12	6900 (liver)
Mattila *et al.*[37]	89	16	6–15 (fish fillet)
	174	16	6–10 (fish fillet)
	539	16	7–16 (fish fillet)
Graff *et al.*[38]	200	9	≤25 (whole fish)*
	5000	9	80 (whole fish)*
	57 000	9	650 (whole fish)*

* Estimated from graph.

fish warrants further research. Again, supplement doses of the listed studies[35–38] in Table 2 were over the EU diet limit for farmed fish of 75 μg/kg[33], which will limit application in the market.

Vitamin D-enriched milk

A few studies have investigated the longer term effect of supplemental vitamin D_3 on the vitamin D content of the milk; the summary of these studies is presented in Table 3[39–42]. Hollis *et al.* showed a 10-fold enhancement of vitamin D_3 intake from 100 to 1000 μg/d resulted in a 7·5-fold increased vitamin D_3 concentration of the milk and a 2-fold increase in 25(OH)D_3[39]. Moreover, McDermott *et al.* compared three different doses of vitamin D_3 with a control diet, and showed an increased concentration of vitamin D_3 and 25(OH)D_3 in the milk[41]. However, the relationship between increasing dietary vitamin D_3 doses and milk vitamin D_3 or 25(OH)D_3 concentrations were not linear. Furthermore, the study of Weiss *et al.* investigated the effect of feeding 450 μg/d vitamin D_3 to pre-calving cows for 13 d which resulted in concentrations of vitamin D_3 and 25(OH)D_3 in the milk ranging from 0·33–0·45 to 0·36–1·02 μg/l, respectively[42]. In addition, the study included a diet treatment of 6 mg vitamin D_3 with a cation–anion difference of −138 mEq/kg daily for 13 d; the concentrations of 25(OH)D_3 in the milk were increased but the treatment effect disappeared after 28 d. Therefore, evidence from the limited number of studies[39–42] demonstrated that milk vitamin D concentrations can be increased by feeding dairy cows with vitamin D supplements. However, it is of note that the highest milk vitamin D_3 and 25(OH)D_3 concentrations were 0·47 and 3·69 μg/l, respectively (Table 3), which for one typical milk serving of 200 ml only contributes 0·09 and 0·74 μg vitamin D_3 and 25(OH)D_3, respectively, well below the current UK vitamin D reference nutrition intake of 10 μg/d[7]. Furthermore, the doses of vitamin D in those studies[41,42] were much higher than the maximum allowed vitamin D content in EU (0·01 mg/kg diet at 880 g DM/kg approximately equivalent to 2·27 mg/d)[34], which imposes an even greater restriction on the possibility of increasing vitamin D in milk by adding vitamin D supplements in the diet of dairy cows.

Evidence from human dietary intervention studies with vitamin D-enriched animal-derived foods

Despite numerous animal-based vitamin D-enrichment studies on vitamin D in eggs, fish and milk, there are few RCT on the effect of consuming vitamin D-enriched foods on the vitamin D status of the consumer. To our knowledge, only one recent study has investigated the weekly effect of consuming seven vitamin D_3 or seven 25(OH)D_3-enriched eggs on vitamin D status compared with commercial eggs of ≤2 egg/week[43]. After 8 weeks follow-up in winter, the results showed that while the serum 25(OH)D of the subjects who consumed commercial eggs decreased from a baseline of 41 (SD 14·1) nmol/l to 35 (SD 11·4) nmol/l, the serum 25(OH)D of subjects who consumed vitamin D_3-enriched eggs or 25(OH)D_3-enriched eggs was maintained. The serum 25(OH)D concentrations of subjects who consumed vitamin D_3- or 25(OH)D_3-enriched eggs were 50 (SD 21·4) nmol/l and 49 (SD 16·5) nmol/l, respectively. However, there was no significant difference between vitamin D_3- and 25(OH)D_3-enriched egg consumption on serum 25(OH)D concentrations.

Although there are a limited number of human dietary intervention studies on vitamin D-enriched foods, the study of Mattila *et al.*[29] demonstrated that the effect of foods enriched with either vitamin D_3 or 25(OH)D_3 on human vitamin D status depended on their relative effectiveness of raising serum or plasma 25(OH)D concentrations. A previous study[44] indicated that there was no consensus on the relative effectiveness of 25(OH)D_3 compared with vitamin D_3 for raising human serum or plasma 25(OH)D_3 concentrations. Furthermore, UK food composition tables[45] indicate that there is no certainty on the relative potency of 25(OH)D_3 compared with vitamin D_3, although it was assumed that 25(OH)D_3 had a potency of five times that of vitamin D_3 for calculating the total vitamin D of foods[45].

Human intervention studies on the relative effects of calcifediol and cholecalciferol supplementation on vitamin D status

Heterogeneity of intervention studies

Eleven RCT that investigated the effects of 25(OH)D_3 relative to vitamin D_3 were identified[46–56] (Table 4). Nine studies administered 25(OH)D_3 supplementation only, except two studies which provided a combination supplement of 25(OH)D_3 and calcium[46,49]. Five of the eleven studies[47,49–52] supplemented 25(OH)D_3 to generally healthy subjects, whereas the other six studies[46,48,53–56] supplemented 25(OH)D_3 to clinical patients. Most studies reported the serum or plasma

Table 3. Summary of enrichment studies investigating the impact of vitamin D supplementation to the diet of dairy cows on vitamin D content of milk

	Supplements to diet (μg/d)			Vitamin D concentration of milk (μg/l)		
References	Vitamin D_3	25(OH)D_3	Feeding duration	Vitamin D_3	25(OH)D_3	1,25(OH)$_2$ D_3
Hollis *et al.*[39]	100	–	NA	0·04	0·37	0·01
	1000	–	NA	0·32	0·68	0·004
Reeve *et al.*[40]	375	–	30 d	0·28	0·15	0·01
Mcdermott *et al.*[41]	0	–	14 weeks	0·08	0·25	0·10
	250	–	14 weeks	0·20	0·43	0·03
	1250	–	14 weeks	0·15	0·75	0·13
	6250	–	14 weeks	0·33	0·93	0·10
Weiss *et al.*[42]	450	–	13 d before calving	0·33–0·47	0·36–1·02	–
	–	DCAD + 6000	13 d before calving	–	0·61–3·69	–

25(OH)D_3, 25-hydroxyvitamin D_3; 1,25(OH)$_2$$D_3$, 1,25 dihydroxyvitamin D_3; DCAD, dietary cation–anion difference of −138 mEq/kg.

25(OH)D concentration at both the beginning and end of the treatment, except one study[55], which only reported the 25(OH)D concentration at the end of the treatment. In terms of the vitamin D status measurement, most studies measured total 25(OH)D concentration, except two studies[49,52], which measured 25(OH)D_3. For the characteristics of the investigated subjects, five studies included both men and women[46,48,51,53,55], while the other studies only included men or women. In addition, most studies reported the age and BMI of the subjects, except two studies[46,48] that did not report the BMI range.

Acute pharmacokinetic action of cholecalciferol and calcifediol

An early study provided meals with single doses of 25(OH)D_3 of 1·5, 5 or 10 μg/kg body weight to generally healthy subjects and showed that the peak serum 25(OH)D_3 concentration was reached within 4–8 h after ingestion[57]. A later study by Jetter *et al.* compared the pharmacokinetic absorption of vitamin D_3 and 25(OH)D_3 by providing a single dose of 20 μg vitamin D_3 or 20 μg 25(OH)D_3 to postmenopausal women[52]. The time to reach maximum plasma 25(OH)D_3 concentration was 22 and 11 h for vitamin D_3 and 25(OH)D_3, respectively. In addition, the peak concentration of plasma 25(OH)D_3 (44 nmol/l) from 25(OH)D_3 supplementation was higher than vitamin D_3 supplementation (35 nmol/l), although they were not significantly different. This study further compared the effect of a higher single dose of 140 μg vitamin D_3 and 140 μg 25(OH)D_3 with the time to reach peak plasma 25(OH)D_3 being 21 and 4·8 h for vitamin D_3 and 25(OH)D_3 supplementation, respectively[52]. In addition, the maximum plasma concentration of 25(OH)D_3 for 25(OH)D_3 treatment (100 nmol/l) was significantly higher than for vitamin D_3 treatment (44 nmol/l). These results suggest that 25(OH)D_3 was absorbed more quickly than vitamin D_3 possibly because 25(OH)D_3 has higher solubility in aqueous media than vitamin D_3 due to its more polar chemical structure[58]. Furthermore, as this metabolite of vitamin D_3 is produced in the liver, the hepatic metabolism of vitamin D_3 to 25(OH)D_3 is circumvented and consequently the conversion from vitamin D_3 to 25(OH)D_3 would be negligible[59]. In patients with liver disease who had an impaired ability to synthesise 25(OH)D_3 from vitamin D_3[60], the study of Sitrin and Bengoa[61] verified that 25(OH)D_3 could be absorbed more efficiently than vitamin D_3 after oral supplementation. Therefore, supplementation with 25(OH)D_3 is not only more efficient at increasing vitamin D status in generally healthy people, but may also have a specific role in tackling lower vitamin D status in patients who are suffering from liver diseases.

Chronic effects and relative effectiveness of cholecalciferol and calcifediol treatments

Regarding the expected higher biological effect of 25(OH)D_3 in raising serum or plasma 25(OH)D level after long-term administration, several studies have confirmed that oral consumption of 25(OH)D_3 is highly effective in raising serum or plasma 25(OH)D level (Table 4)[46–56]. However, the majority of the evidence in support of a higher impact of 25(OH)D_3 supplementation compared with vitamin D_3 on serum or plasma 25(OH)D_3 level is from only four studies[51,52,54,56] where both 25(OH)D_3 and vitamin D_3 treatments were included in the same study (Table 5). The study of Barger-Lux *et al.*[47] provided three different doses of vitamin D_3 (25, 250, 1250 μg/d) or 25(OH)D_3 (10, 20, 50 μg/d) to the participants for 8 and 4 weeks, respectively. However, the effects of 25(OH)D_3 and vitamin D_3 treatments were not directly comparable as the interventions were not at the same dose or treatment time. Thus, the study of Barger-Lux *et al.*[47] was excluded from the relative effectiveness analysis. In order to compare the relative effectiveness of 25(OH)D_3 and vitamin D_3 supplementation on raising serum or plasma 25(OH)D concentrations, a dose–response factor was calculated for each μg of orally consumed 25(OH)D_3 or vitamin D_3 in four studies[51,52,54,56]. The dose–response factors of 25(OH)D_3 and vitamin D_3 were calculated by using endpoint 25(OH)D concentration minus baseline 25(OH)D concentration, divided by the dose of the supplementation (dose–response factor = Δ serum/plasma (mmol/l)/dose (μg)). Then, the relative

Table 4. Summary of study details and serum 25, hydroxyvitamin D (25(OH)D) concentration in long-term randomised controlled trials with calcifediol (25 hydroxyvitamin D_3 ($25(OH)D_3$)) supplementation in adults (order by year)

		$25(OH)D_3$ supplementation group					Control group (if available)				
References	Subjects characteristics (trail time during the year, subjects (sex), age, BMI)	Duration	$25(OH)D_3$ treatment	*n*	Baseline 25(OH)D (nmol/l)	Endpoint 25(OH)D (nmol/l)	Duration	Vitamin D_3 treatment	*n*	Baseline 25(OH)D (nmol/l)	Endpoint 25(OH)D (nmol/l)
Hahn *et al.*[46]	Whole year, patients (women and men) with glucocorticoid-induced osteopenia 46 years, BMI (NA*)	18 months	40 µg/d + 500 mg calcium/d	9	39	205					
Barger-Lux *et al.*[47]	January–April, men 28 years, 26 kg/m^2	4 weeks	10 µg/d	7	67	107	8 weeks	25 µg/d	13	67	96
		4 weeks	20 µg/d	6	67	143	8 weeks	250 µg/d	10	67	213
		4 weeks	50 µg/d	4	67	273	8 weeks	1250 µg/d	14	67	710
Jean *et al.*[48]	March–September, haemodialysis patients (women and men) 67 years, BMI (NA)	6 months	16 µg /d	149	30	126					
Cavalli *et al.*§[49]	April–July, postmenopausal women 65–75 years, 25 kg/m^2	12 weeks	125 µg/week + 500 mg calcium/d	25	50	76					
		12 weeks	250 µg/month + 500 mg calcium/d	28	51	70					
		12 weeks	500 µg/month + 500 mg calcium/d	27	52	77					
Russo *et al.*[50]	January–April, women (7 premenopausal and 11 postmenopausal), 24–72 years, 24 kg/m^2	16 weeks	500 µg/month	18	45	105†					
Cashman *et al.*[51]	January–April, women and men, 57 years, 29 kg/m^2	10 weeks	20 µg/d	12	38	135	10 weeks	20 µg/d	13	50	69
Jetter *et al.*‡§[52]	January–July, postmenopausal women 50–70 years, 18–29 kg/m^2	16 weeks	20 µg/d	5	31	173	16 weeks	20 µg/d	5	35	77
Catalano *et al.*[54]	September–March, osteopenic and dyslipidaemic postmenopausal women 59 years, 27 kg/m^2	24 weeks	140 µg once weekly	29	56	126	24 weeks	140 µg once weekly	28	51	61
Banon *et al.*[53]	Whole year, patients (women and men) had HIV-infected, 44 years, 15–44 kg/m^2	Summer	400 µg once/month	123	37	86	Summer	NA	242	53	99
		Fall	400 µg once/month	123	37	69	Fall	NA	242	53	84
		Winter	400 µg once/month	123	37	45	Winter	NA	242	53	55
		Spring	400 µg once/month	123	37	57	Spring	NA	242	53	78
Ortego-Jurado *et al.*[55]	Whole year, patients (women and men) had autoimmune diseases, undergoing glucocorticoids therapy, 56 years, 28 kg/m^2	Spring–summer	8·85 µg/d	49	NA	84	Spring–summer	20 µg/d	86	NA	71
		Fall–winter	8·85 µg/d	49	NA	89	Fall–winter	20 µg/d	86	NA	61
Navarro-Valverde *et al.*[56]	Whole year, postmenopausal osteoporotic women, 67 years, 26 kg/m^2	6 months	20 µg/d	10	37	161	6 months	20 µg/d	10	41	80
		12 months	20 µg/d	10	37	188	12 months	20 µg/d	10	41	86
		6 months	266 µg once/week	10	38	214					
		12 months	266 µg once/week	10	38	233					
		6 months	266 µg once/2 weeks	10	40	165					
		12 months	266 µg once/2 weeks	10	40	211					

* NA, not available.
† Estimated from graph.
‡ Same study of (Jetter *et al.*[52]) and (Bischoff-Ferrari *et al.*[62]).
§ Study has measured vitamin D status as $25(OH)D_3$.

Table 5. Summary of randomised controlled trials with both calcifediol (25 hydroxyvitamin D_3 (25(OH)D_3)) and vitamin D_3 in adults to calculate the relative effectiveness of 25(OH)D_3 and vitamin D_3 supplementation in raising serum 25, hydroxyvitamin D (25(OH)D) level

References	Treatment (dose, duration)	Serum 25(OH)D raising (nmol/l) per 1 μg*	Relative effectiveness†
Cashman *et al.*[51]	20 μg 25(OH)D_3/d × 10 weeks	4·82[a]	4·99
	20 μg vitamin D_3/d × 10 weeks	0·97[b]	
Jetter *et al.*[52]	20 μg 25(OH)D_3/d × 15 weeks	7·12[a]	3·40
	20 μg vitamin D_3/d × 15 weeks	2·51[b]	
Catalano *et al.*[54]	140 μg 25(OH)D_3/week × 24 weeks	0·50[a]	7·14
	140 μg vitamin D_3/week × 24 weeks	0·07[b]	
Navarro-Valverde *et al.*[56]	20 μg 25(OH)D_3/d × 6 months	6·19[a]	3·13
	20 μg vitamin D_3/d × 6 months	1·98[b]	
	20 μg 25(OH)D_3/d × 12 months	7·54[a]	3·29
	20 μg vitamin D_3/d × 12 months	2·29[b]	

* Dose–response factor = Δ serum/plasma (mmol/l)/dose (μg).
† Relative effectiveness = a/b within same study.

effectiveness of 25(OH)D_3 to vitamin D_3 was calculated by dividing the dose–response factor of 25(OH)D_3 by that of vitamin D_3.

The highest relative effectiveness was found in the study by Catalano *et al.*[54]. Weekly treatment of 140 μg 25(OH)D_3 or 140 μg vitamin D_3 supplements was provided to osteopenic and dyslipidaemic postmenopausal women for 24 weeks. Supplementation with 25(OH)D_3 raised serum 25(OH)D from a baseline of 56–126 nmol/l, while vitamin D_3 treatment increased serum 25(OH)D to a lower extent, from baseline 51 to 61 nmol/l. Thus, the relative effectiveness factor derived from this study was 7·14, i.e. dietary 25(OH)D_3 was 7·14 times more effective at increasing serum 25(OH)D than dietary vitamin D_3.

Vitamin D dietary recommendations are generally between 10 and 20 μg/d[10], yet, there are few studies which have compared the effectiveness of dietary 25(OH)D_3 and vitamin D_3 using doses of 20 μg in their treatments. Cashman *et al.*[51] provided daily supplements of 20 μg vitamin D_3 or 20 μg 25(OH)D_3 to adult men and women with a mean age of 57 years and with baseline serum 25(OH)D of 28·9 nmol/l during winter. After 10 weeks of supplementation, the subjects' serum 25(OH)D increased to 135 and 69 nmol/l for the 25(OH)D_3 and vitamin D_3 treatments, respectively. A relative effectiveness factor of 4·99 was calculated representing the relative effectiveness of each μg of dietary 25(OH)D_3 relative to dietary vitamin D_3 for raising serum 25(OH)D concentration. However, lower relative effectiveness factors were achieved in other studies using the same dose of 20 μg vitamin D_3 and 25(OH)D_3. Jetter *et al.* supplemented healthy postmenopausal women with 20 μg 25(OH)D_3 or 20 μg vitamin D_3 for 16 weeks during the winter[52]. They found that for the 25(OH)D_3 treatment, plasma 25(OH)D_3 increased to 173 nmol/l from a baseline of 31 nmol/l, whereas for the vitamin D_3 treatment, plasma 25(OH)D_3 increased to 77 nmol/l from a baseline level of 35 nmol/l. The relative effectiveness factor of each μg of 25(OH)D_3 was 3·40 compared with vitamin D_3 in raising plasma 25(OH)D_3 level. A similar low relative effectiveness factor was found in another study where post-menopausal osteoporotic women were given either 20 μg vitamin D_3 or 20 μg 25(OH)D_3 over 6 or 12 months[56]. The serum concentration of 25(OH)D for the 25(OH)D_3 treatment reached 161 and 188 nmol/l from a baseline of 37 nmol/l after 6 or 12 months administration, respectively, while the comparable values for the vitamin D_3 treatment were an increase to 80 and 86 nmol/l from a baseline of 41 nmol/l. So the relative effectiveness factor of 25(OH)D_3 relative to vitamin D_3 treatment at 6 and 12 months were 3·13 or 3·29, respectively.

In summary, of the studies reviewed, the relative effectiveness of 25(OH)D_3 to vitamin D_3 for raising vitamin D status (Table 5), ranged from 3·13 to 7·14. Previous studies have demonstrated that the season may have influences on vitamin D status[13,14]. There were two studies conducted during the winter which may have minimised any confounding influence of cutaneous vitamin D synthesis from UV radiation [47,51]. Other studies have longer intervention periods of 6 months or more, which could not have avoided some cutaneous synthesis. Furthermore, baseline status may be another factor that influences the relative effectiveness factor. The study of Catalano *et al.* had the highest factor of 7·14 in the present review, and the baseline concentration of 25(OH)D of the study participants was higher (>50 nmol/l) than the others[54]. Therefore, the different relative effectiveness seen in different studies may be due to the different characteristics or genotypes of the subjects, or different study designs.

Overall, evidence suggests that dietary 25(OH)D_3 can more effectively increase serum 25(OH)D concentrations than vitamin D_3 and may also be absorbed faster reaching a serum or plasma 25(OH)D plateau earlier than vitamin D_3 supplementation. Furthermore, supplementation with 25(OH)D_3 may also have more benefits to human health compared with vitamin D_3 in a general healthy population. Bischoff-Ferrari *et al.* reported that 20 μg 25(OH)D_3 supplementation over 4 months led to a 5·7 mmHg decrease in systolic blood pressure and improvements in several markers of innate immunity in healthy postmenopausal women[62].

For patients with different diseases and receiving long-term medication, studies[63–65] showed that several drugs (e.g. antiepileptic agents, glucocorticoids, antiretroviral

or anti-oestrogen drugs) interfered with vitamin D metabolism, which resulted in patients being more likely to have low vitamin D status. Thus, it is not only important to increase vitamin D status in the generally healthy population but also in patients with specific illnesses and receiving certain medication. Therefore, the studies using 25(OH)D_3 treatments in patients were also summarised in Table 4[46,48,53–56], and those studies consistently reported that chronic 25(OH)D_3 supplementation effectively increased serum 25(OH)D concentrations. For example, Ortego-Jurado *et al.* showed a lower daily dose of 8·85 μg 25(OH)D_3 to be more effective than a 20 μg dose of vitamin D_3 for increasing vitamin D status in patients with autoimmune disease who were treated with a low dose of glucocorticoids throughout the year[55]. Similarly, the study of Banon *et al.* showed that a monthly dose of 400 μg 25(OH)D_3 was safe and effective at improving vitamin D status of HIV-infected patients throughout the year[53].

Furthermore, supplementation with 25(OH)D_3 may have additional benefits on patients' health. Previously, 25(OH)D_3 was recommended for patients with kidney disease since 25(OH)D_3 has a direct action on bone metabolism[66]. Hahn *et al.* provided a daily 40 μg 25(OH)D_3 and 500 mg calcium supplement to patients who had glucocorticoid-induced osteopenia for 18 months[46]. The treatment markedly increased vitamin D status from 39 to 205 nmol/l. In addition, this study showed that the 25(OH)D_3 treatment improved mineral and bone metabolism. Jean *et al.* also offered haemodialysis patients who suffered from vitamin D deficiency with a daily dose of 16 μg 25(OH)D_3 for 6 months; vitamin D status reached 126 nmol/l from 30 nmol/l, at the same time 25(OH)D_3 supplementation corrected the excess bone turnover[48]. Similarly, a study by Catalano *et al.*[54] provided 140 μg 25(OH)D_3 supplements for 24 weeks to osteopenic and dyslipidaemic postmenopausal women, and results showed that 25(OH)D_3 improved plasma lipid levels (increased HDL-cholesterol (P = 0·02) and decreased LDL-cholesterol (P = 0·02)) in osteopenic and dyslipidaemic postmenopausal women when added to an ongoing atorvastatin treatment.

As an alternative to vitamin D-enriched foods, vitamin D fortification of foods may also be an option for tackling vitamin D deficiency throughout the world. In general, fortification of foods refers to mandatory and voluntary fortification. The contribution of vitamin D-fortified foods to vitamin D intake by the public varies considerably between countries as there are different food standard policies[10], and in practice, vitamin D_2 or vitamin D_3 are used for fortification. Evidence from one previous meta-analysis of RCT showed that vitamin D_3 supplementation is more effective at raising vitamin D status than vitamin D_2[67]. However, a further comprehensive systematic review and meta-analysis of thirty-three RCT[68] showed that the effect of vitamin D_3 supplement on serum 25(OH)D_3 response was limited by the supplemental dose, duration, age of subjects and baseline level. In addition, the meta-analysis showed a greater serum or plasma 25(OH)D increase when the intervention study used a dose of 20 μg/d vitamin D_3 or even higher, with subjects aged >80 years and an administration period of at least 6–12 months or subjects had lower baseline 25(OH)D status (<50 nmol/l) than subjects aged <80 years, administration period <6 months or subjects had higher baseline 25(OH)D status (≥50 nmol/l)[68]. Therefore, better strategies are needed to raise vitamin D status of the public throughout life, and 25(OH)D_3-fortified foods warrant further research.

Conclusions

Vitamin D insufficiency has become a world problem, especially where sunlight exposure is limited by geographic reasons (latitude), personal characteristics (skin pigmentation, ageing) or behaviour (sunscreen use, cultural reasons). However, there are a few natural foods rich in vitamin D. Thus, vitamin D-enriched foods produced through a food chain approach such as feeding animals vitamin D supplements or vitamin D-fortified foods are needed to guarantee an adequate dietary intake of vitamin D by the general population.

The present review summarised the available and limited number of RCT investigating the effect of 25(OH)D_3 supplementation on serum or plasma 25(OH)D concentration. We concluded that it is difficult to get consensus on the effectiveness of 25(OH)D_3 supplementation relative to vitamin D_3 for raising vitamin D status, due to various influencing factors such as different person characteristics (age, BMI), baseline vitamin D status and time of the year. However, it is unquestionable that 25(OH)D_3 supplementation is more efficient at raising serum 25(OH)D concentrations and also appears to be absorbed faster by than the same dose of vitamin D_3. Second, by reviewing available evidence on vitamin D-enriched eggs, fish or milk, it is practical and possible to increase the vitamin D content of eggs, fish or milk by addition of vitamin D supplements to the diet of poultry, fish or dairy cows. However, the limitations of adding vitamin D to animal feed should be considered in future enrichment studies. Furthermore, there are a few RCT investigating the impact of these vitamin D-enriched foods on improving vitamin D status. Therefore, 25(OH)D_3-enriched or fortified foods should be further explored in the future, and additional RCT should be conducted to investigate the effect of 25(OH)D_3-enriched or fortified foods on vitamin D status of the general population and patients with long-term health conditions.

Financial Support

This review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. J. G. was supported by the Barham Benevolent Foundation studentship.

Conflicts of Interest

None.

Authorship

J. G. conceived and wrote the manuscript. All authors critically reviewed and approved the final version of the manuscript.

References

1. Borradale D & Kimlin M (2009) Vitamin D in health and disease: an insight into traditional functions and new roles for the 'sunshine vitamin'. *Nutr Res Rev* **22**, 118–136.
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. Robinson PD, Hogler W, Craig ME *et al.* (2006) The re-emerging burden of rickets: a decade of experience from Sydney. *Arch Dis Child* **91**, 564–568.
4. Ward LM, Gaboury I, Ladhani M *et al.* (2007) Vitamin D-deficiency rickets among children in Canada. *CMAJ* **177**, 161–166.
5. Prentice A (2013) Nutritional rickets around the world. *J Steroid Biochem Mol Biol* **136**, 201–206.
6. Holick MF & Chen TC (2008) Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* **87**, 1080S–1086S.
7. Scientific Advisory Committee on Nutrition (2016) https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report (accessed August 17).
8. Forrest KY & Stuhldreher WL (2011) Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* **31**, 48–54.
9. Hilger J, Friedel A, Herr R *et al.* (2014) A systematic review of vitamin D status in populations worldwide. *Br J Nutr* **111**, 23–45.
10. Cashman KD & Kiely M (2016) Tackling inadequate vitamin D intakes within the population: fortification of dairy products with vitamin D may not be enough. *Endocrine* **51**, 38–46.
11. Nowson CA, McGrath JJ, Ebeling PR *et al.* (2012) Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust* **196**, 686–687.
12. Holick MF (1995) Environmental-factors that influence the cutaneous production of vitamin-D. *Am J Clin Nutr* **61**, 638s–645s.
13. Schmid A & Walther B (2013) Natural vitamin D content in animal products. *Adv Nutr* **4**, 453–462.
14. O'Mahony L, Stepien M, Gibney MJ *et al.* (2011) The potential role of vitamin D enhanced foods in improving vitamin D status. *Nutrients* **3**, 1023–1041.
15. Holick MF, MacLaughlin JA, Clark MB *et al.* (1980) Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. *Science* **210**, 203–205.
16. Haddad JG, Matsuoka LY, Hollis BW *et al.* (1993) Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* **91**, 2552–2555.
17. Dueland S, Pedersen JI, Helgerud P *et al.* (1983) Absorption, distribution, and transport of vitamin D_3 and 25-hydroxyvitamin D_3 in rat. *Am J Physiol* **245**, 5 Pt1, E463–E467.
18. Jones G, Strugnell SA & DeLuca HF (1998) Current understanding of the molecular actions of vitamin D. *Physiol News* **78**, 1193–1231.
19. Exler J, Phillips KM, Patterson KY *et al.* (2013) Cholesterol and vitamin D content of eggs in the US retail market. *J Food Comp Anal* **29**, 110–116.
20. Guo J, Kliem KE, Lovegrove JA *et al.* (2017) Effect of production system, supermarket and purchase date on the vitamin D content of eggs at retail. *Food Chem* **221**, 1021–1025.
21. Lu Z, Chen TC, Zhang A *et al.* (2007) An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? *J Steroid Biochem Mol Biol* **103**, 642–644.
22. Mattila P, Piironen V, Uusi-Rauva E *et al.* (1995) Cholecalciferol and 25-hydroxycholecalciferol contents in fish and fish products. *J Food Comp Anal* **8**, 232–243.
23. Bilodeau L, Dufresne G, Deeks J *et al.* (2011) Determination of vitamin D_3 and 25-hydroxyvitamin D_3 in foodstuffs by HPLC UV-DAD and LC-MS/MS. *J Food Comp Anal* **24**, 441–448.
24. Bates B, Lennox A, Bates C *et al.* (2014) *National Diet and Nutrition Survey (2008/2009-2011/2012)*. London: Public Health England Publications.
25. Kuhn J, Schutkowski A, Kluge H *et al.* (2014) Free-range farming: a natural alternative to produce vitamin D-enriched eggs. *Nutrition* **30**, 481–484.
26. Mattila P, Lehikoinen K, Kiiskinen T *et al.* (1999) Cholecalciferol and 25-hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. *J Agric Food Chem* **47**, 4089–4092.
27. Mattila P, Lehikoinen K, Kiiskinen T *et al.* (2003) Effect of cholecalciferol-enriched hen feed on egg quality. *J Agric Food Chem* **51**, 283–287.
28. Mattila P, Valaja J, Rossow L *et al.* (2004) Effect of vitamin D_2- and D_3-enriched diets on egg vitamin D content, production, and bird condition during an entire production period. *Poult Sci* **83**, 433–440.
29. Mattila P, Vakonen E & Valaja J (2011) Effect of different vitamin D supplementations in poultry feed on vitamin D content of eggs and chicken meat. *J Agric Food Chem* **59**, 8298–8303.
30. Yao LX, Wang T, Persia M *et al.* (2013) Effects of vitamin D_3-enriched diet on egg yolk vitamin D_3 content and yolk quality. *J Food Sci* **78**, C178–C183.
31. Browning LC & Cowieson AJ (2014) Vitamin D fortification of eggs for human health. *J Sci Food Agric* **94**, 1389–1396.
32. Duffy SK, Rajauria G, Clarke LC *et al.* (2017) The potential of cholecalciferol and 25-hydroxyvitamin D_3 enriched diets in laying hens, to improve egg vitamin D content and antioxidant availability. *Innov Food Sci Emerg Technol* https://doi.org/10.1016/j.ifset.2017.07.007.
33. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) (2012) Scientific Opinion on the safety and efficacy of vitamin D_3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories, based on a dossier submitted by DSM. *EFSA J* **10**, 2968, 26 pp.
34. The Commission of the European Communities (2017) Commission Implementing Regulation (EC) No 2017/1492. The authorisation of cholecalciferol as a feed additive for all animal species. *Off J Eur Union L216/19*.
35. Horvli O, LIE O & Aksnes L (1998) Tissue distribution of vitamin D_3 in Atlantic salmon Salmo salar: effect of dietary level. *Aquacul Nutr* **4**, 127–131.
36. Vielma J, Lall SP, Koskela J *et al.* (1998) Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **163**, 309–323.
37. Mattila P, Piironen V, Hakkarainen T *et al.* (1999) Possibilities to raise vitamin D content of rainbow trout

(*Oncorhynchus mykiss*) by elevated feed cholecalciferol contents. *J Sci Food Agric* **79**, 195–198.
38. Graff IE, Hoie S, Totland GK *et al.* (2002) Three different levels of dietary vitamin D-3 fed to first-feeding fry of Atlantic salmon (*Salmo salar* L.): effect on growth, mortality, calcium content and bone formation. *Aquac Nutr* **8**, 103–111.
39. Hollis BW, Roos BA, Draper HH *et al.* (1981) Vitamin D and its metabolites in human and bovine milk. *J Nutr* **111**, 1240–1248.
40. Reeve LE, Jorgensen NA & Deluca HF (1982) Vitamin-D compounds in cows milk. *J Nutr* **112**, 667–672.
41. Mcdermott CM, Beitz DC, Littledike ET *et al.* (1985) Effects of dietary vitamin-D_3 on concentrations of vitamin-D and its metabolites in blood-plasma and milk of dairy-cows. *J Dairy Sci* **68**, 1959–1967.
42. Weiss WP, Azem E, Steinberg W *et al.* (2015) Effect of feeding 25-hydroxyvitamin D_3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. *J Dairy Sci* **98**, 5588–5600.
43. 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.
44. Jakobsen J (2007) Bioavailability and bioactivity of vitamin D_3 active compounds – Which potency should be used for 25-hydroxyvitamin D_3? *Int Congr Ser* **1297**, 133–142.
45. McCance RA & Widdowson EM (2015) *Composition of Foods*, 7th ed. Cambridge: The Royal Society of Chemistry.
46. Hahn TJ, Halstead LR, Teitelbaum SL *et al.* (1979) Altered mineral metabolism in glucocorticoid-induced osteopenia. Effect of 25-hydroxyvitamin D administration. *J Clin Invest* **64**, 655–665.
47. Barger-Lux MJ, Heaney RP, Dowell S *et al.* (1998) Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporos Int* **8**, 222–230.
48. Jean G, Terrat JC, Vanel T *et al.* (2008) Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: effects on mineral metabolism and bone markers. *Nephrol Dial Transplant* **23**, 3670–3676.
49. Cavalli L, Cavalli T, Marcucci G *et al.* (2009) Biological effects of various regimes of 25-hydroxyvitamin D_3 (calcidiol) administration on bone mineral metabolism in postmenopausal women. *Clin Cases Miner Bone Metab* **6**, 169–173.
50. Russo S, Carlucci L, Cipriani C *et al.* (2011) Metabolic changes following 500 mug monthly administration of calcidiol: a study in normal females. *Calcif Tissue Int* **89**, 252–257.
51. Cashman KD, Seamans KM, Lucey AJ *et al.* (2012) Relative effectiveness of oral 25-hydroxyvitamin D_3 and vitamin D_3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *Am J Clin Nutr* **95**, 1350–1356.
52. Jetter A, Egli A, Dawson-Hughes B *et al.* (2014) Pharmacokinetics of oral vitamin D(3) and calcifediol. *Bone* **59**, 14–19.
53. Banon S, Rosillo M, Gomez A *et al.* (2015) Effect of a monthly dose of calcidiol in improving vitamin D deficiency and secondary hyperparathyroidism in HIV-infected patients. *Endocrine* **49**, 528–537.
54. Catalano A, Morabito N, Basile G *et al.* (2015) Calcifediol improves lipid profile in osteopenicatorvastatin-treated postmenopausal women. *Eur J Clin Invest* **45**, 144–149.
55. Ortego-Jurado M, Callejas-Rubio JL, Rios-Fernandez RR *et al.* (2015) Oral calcidiol is more effective than cholecalciferol supplementation to reach adequate 25(OH)D levels in patients with autoimmune diseases chronically treated with low doses of glucocorticoids: a 'Real-Life' study. *J Osteoporos* http://dx.doi.org/10.1155/2015/729451.
56. Navarro-Valverde C, Sosa-Henriquez M, Alhambra-Exposito MR *et al.* (2016) Vitamin D3 and calcidiol are not equipotent. *J Steroid Biochem Mol Biol* **164**, 205–208.
57. Haddad JG & Rojanasathit S (1976) Acute administration of 25-hydroxycholecalciferol in man. *J Clin Endocrinol Metab* **42**, 284–290.
58. Cianferotti L, Cricelli C, Kanis JA *et al.* (2015) The clinical use of vitamin D metabolites and their potential developments: a position statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) and the International Osteoporosis Foundation (IOF). *Endocrine* **50**, 12–26.
59. Heaney RP, Armas LA, Shary JR *et al.* (2008) 25-hydroxylation of vitamin D3: relation to circulating vitamin D3 under various input conditions. *Am J Clin Nutr* **87**, 1738–1742.
60. Nair S (2010) Vitamin D deficiency and liver disease. *Gastroenterol Hepatol (NY)* **6**, 491–493.
61. Sitrin MD & Bengoa JM (1987) Intestinal absorption of cholecalciferol and 25-hydroxycholecalciferol in chronic cholestatic liver disease. *Am J Clin Nutr* **46**, 1011–1015.
62. Bischoff-Ferrari HA, Dawson-Hughes B, Stocklin E *et al.* (2012) Oral supplementation with 25(OH)D_3 versus vitamin D_3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. *J Bone Miner Res* **27**, 160–169.
63. Mehrotra R, Kermah D, Budoff M *et al.* (2008) Hypovitaminosis D in chronic kidney disease. *Clin J Am Soc Nephrol* **3**, 1144–1151.
64. Grober U & Kisters K (2012) Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinology* **4**, 158–166.
65. Griz L, Bandeira F, Diniz ET *et al.* (2013) Prevalence of vitamin D deficiency is higher in patients with Paget's disease of bone compared with age-matched controls. *Arq Bras Endocrinol Metabol* **57**, 509–512.
66. Torregrosa JV, Bover J, Cannata AJ *et al.* (2011) Spanish Society of Nephrology recommendations for controlling mineral and bone disorder in chronic kidney disease patients (S.E.N.-M.B.D.). *Nefrologia* **31**, Suppl. 1, 3–32.
67. 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.
68. Shab-Bidar S, Bours S, Geusens PP *et al.* (2014) Serum 25 (OH)D response to vitamin D_3 supplementation: a meta-regression analysis. *Nutrition* **30**, 975–985.