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Published Version

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Guo, J., Lovegrove, J. A. ORCID: <https://orcid.org/0000-0001-7633-9455> and Givens, D. I. ORCID: <https://orcid.org/0000-0002-6754-6935> (2018) 25(OH)D3-enriched or fortified foods are more efficient at tackling inadequate vitamin D status than vitamin D3. *Proceedings of the Nutrition Society*, 77 (3). pp. 282-291. ISSN 0029-6651 doi: 10.1017/S0029665117004062 Available at <https://centaur.reading.ac.uk/74272/>

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To link to this article DOI: <http://dx.doi.org/10.1017/S0029665117004062>

Publisher: Cambridge University Press

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Conference on ‘Improving nutrition in metropolitan areas’ Postgraduate Symposium

25(OH)D₃-enriched or fortified foods are more efficient at tackling inadequate vitamin D status than vitamin D₃

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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 D₃) and calcifediol (25(OH)D₃) content of eggs, fish and milk increased in response to vitamin D₃ 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)D₃, not vitamin D₃ supplementation, resulted in a pronounced increase of 25(OH)D₃ in the eggs. Furthermore, evidence from randomised controlled trials indicated that a 25(OH)D₃ oral supplement could be absorbed faster and more efficiently raise serum 25(OH)D concentration compared with vitamin D₃ supplementation. Moreover, evidence showed the relative effectiveness of increasing vitamin D status using 25(OH)D₃ varied between 3.13 and 7.14 times that of vitamin D₃, probably due to the different characteristics of the investigated subjects or study design. Therefore, vitamin D-enrichment or fortified foods using 25(OH)D₃ would appear to have advantages over vitamin D₃. Further well-controlled studies are needed to assess the effects of 25(OH)D₃ enriched or fortified foods in the general population and clinical patients.

Enrichment: Fortification: 25(OH)D₃: Vitamin D₃: 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)D₃, 25-hydroxyvitamin D₃; RCT, randomised controlled trial.

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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 D₃ (25(OH)D₃) <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 D₃) and/or calcifediol (25(OH)D₃) supplements to laying hens, fish and cows. Second, the present review summarises evidence from the human randomised controlled trials (RCT), which include the effects of 25(OH)D₃ supplementation on increasing serum/plasma 25(OH)D₃ concentration.

Vitamin D absorption, synthesis and metabolism

Generally, the term vitamin D refers to both vitamin D₂ and vitamin D₃. Vitamin D₂ is produced by fungi, while vitamin D₃ is produced by human subjects and animals⁽¹⁴⁾. Human subjects usually synthesise vitamin D₃ in the skin⁽¹⁵⁾ where 7-dehydrocholesterol in the epidermis is converted to pre-vitamin D₃ when skin is exposed to sunlight. Then, pre-vitamin D₃ undergoes a temperature-dependent isomerisation to vitamin D₃ over a period of approximately 3 d⁽⁶⁾. Vitamin D (vitamin D₂ or vitamin D₃) 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)₂D by 25-hydroxyvitamin D-1 α -hydroxylase⁽⁶⁾, and the 1,25(OH)₂D 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 D₃ and 25(OH)D₃ concentrations of 9.7–18 and 4.3–13.2 $\mu\text{g/kg}$, respectively⁽¹⁹⁾. In addition, our recent UK retail study⁽²⁰⁾ showed vitamin D₃ and 25(OH)D₃

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) $\mu\text{g/kg}$) of vitamin D₃ than the egg yolks produced from outdoor systems (57.2 (SE 3.2) $\mu\text{g/kg}$), while 25(OH)D₃ 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 D₃ content of wild salmon to be three times higher than that of farmed salmon; however, the 25(OH)D₃ content of the salmon was not measured. In addition, other studies^(22,23) have shown the 25(OH)D₃ content of several species of marine and freshwater fish to be <0.02 $\mu\text{g/100 g}$. 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 $\mu\text{g/d}$ ⁽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 D₃ content (mean 14.3 $\mu\text{g/100 g DM}$) than eggs from the indoor group (mean 3.8 $\mu\text{g/100 g DM}$)⁽²⁵⁾. Furthermore, there are several studies which have shown that the vitamin D₃ content of eggs can be enhanced by feeding vitamin D₃ supplements to the hens (Table 1)^(26–32). The results of all studies revealed that egg yolk vitamin D₃ concentration was efficiently increased by vitamin D₃ dietary supplementation. The study of Yao *et al.* showed a linear dose–response relationship existed between vitamin D₃ dietary supplementation and vitamin D₃ concentrations of egg yolks⁽³⁰⁾. Moreover, as 25(OH)D₃ is a metabolite of vitamin D₃, the 25(OH)D₃ content in eggs can also be enhanced by supplementing the birds' diet with vitamin D₃. However, the response in 25(OH)D₃ content of egg yolk is much less than that of vitamin D₃. Browning and Cowieson⁽³¹⁾ showed that a 4-fold increase in vitamin D₃, and a 2-fold increase in 25(OH)D₃ in egg yolk resulted from a 4-fold increase in the vitamin D₃

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 <i>et al.</i> ⁽²⁶⁾	26.6	–	6	1.4	0.5
	62.4	–	6	3.5	0.9
	216.0	–	6	22.0	1.5
Mattila <i>et al.</i> ⁽²⁷⁾	280.0	–	4	30.0	1.9
Mattila <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽²⁹⁾	–	55.0	6	≤0.2	2.1
	–	122.0	6	≤0.2	4.3
Duffy <i>et al.</i> ⁽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)D₃, 25-hydroxyvitamin D₃.

* Vitamin D content per egg.

in the diet (62.5–250 µg/kg). Similarly, evidence from another study showed that the vitamin D₃ in egg yolk was increased approximately 7-fold as a result of feeding a diet with a 3.5-fold higher vitamin D₃ content (from 62.4 to 216 µg/kg), while the corresponding increase in 25(OH)D₃ 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)D₃. In the EU, 25(OH)D₃ has only recently been authorised for addition to poultry diets, and the maximum content of the vitamin D₃ and 25(OH)D₃ 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)D₃ to the vitamin D₃ supplement resulted in the elevation of the 25(OH)D₃ content of the egg yolk, but there was no significant increase in the vitamin D₃ content of the egg yolk. Other studies investigated dietary supplementation with 25(OH)D₃^(29,32), and showed that

only egg yolk 25(OH)D₃ was increased, but not vitamin D₃. Therefore, we speculate that 25(OH)D₃ in the diet can be absorbed directly by laying hens without transfer to vitamin D₃ 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 D₃ supplements up to 539 µg/kg, but no significant differences in the vitamin D₃ 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 D₃ in the diet up to 28.68 mg/kg and vitamin D₃ in the fish meat⁽³⁵⁾. Similar high vitamin D₃ supplementation doses were reported in another two studies^(36,38), which also showed that elevated vitamin D₃ content of the fish liver or whole fish had been achieved by supplemental vitamin D₃ in the diet. However, 25(OH)D₃ 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)D₃ 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 <i>et al.</i> ⁽³⁵⁾	40	11	1 (fillet)
	2210	11	21 (fillet)
	28 680	11	210 (fillet)
Vielma <i>et al.</i> ⁽³⁶⁾	62.5	12	1 (liver)
	6250	12	73 (liver)
	62 500	12	6900 (liver)
Mattila <i>et al.</i> ⁽³⁷⁾	89	16	6–15 (fish fillet)
	174	16	6–10 (fish fillet)
	539	16	7–16 (fish fillet)
Graff <i>et al.</i> ⁽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 D₃ 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 D₃ intake from 100 to 1000 µg/d resulted in a 7.5-fold increased vitamin D₃ concentration of the milk and a 2-fold increase in 25(OH)D₃⁽³⁹⁾. Moreover, McDermott *et al.* compared three different doses of vitamin D₃ with a control diet, and showed an increased concentration of vitamin D₃ and 25(OH)D₃ in the milk⁽⁴¹⁾. However, the relationship between increasing dietary vitamin D₃ doses and milk vitamin D₃ or 25(OH)D₃ concentrations were not linear. Furthermore, the study of Weiss *et al.* investigated the effect of feeding 450 µg/d vitamin D₃ to pre-calving cows for 13 d which resulted in concentrations of vitamin D₃ and 25(OH)D₃ 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 D₃ with a cation–anion difference of –138 mEq/kg daily for 13 d; the concentrations of 25(OH)D₃ 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 D₃ and 25(OH)D₃ 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 D₃ and 25(OH)D₃, 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 D₃ or seven 25(OH)D₃-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 D₃-enriched eggs or 25(OH)D₃-enriched eggs was maintained. The serum 25(OH)D concentrations of subjects who consumed vitamin D₃- or 25(OH)D₃-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 D₃- and 25(OH)D₃-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 D₃ or 25(OH)D₃ 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)D₃ compared with vitamin D₃ for raising human serum or plasma 25(OH)D₃ concentrations. Furthermore, UK food composition tables⁽⁴⁵⁾ indicate that there is no certainty on the relative potency of 25(OH)D₃ compared with vitamin D₃, although it was assumed that 25(OH)D₃ had a potency of five times that of vitamin D₃ 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)D₃ relative to vitamin D₃ were identified^(46–56) (Table 4). Nine studies administered 25(OH)D₃ supplementation only, except two studies which provided a combination supplement of 25(OH)D₃ and calcium^(46,49). Five of the eleven studies^(47,49–52) supplemented 25(OH)D₃ to generally healthy subjects, whereas the other six studies^(46,48,53–56) supplemented 25(OH)D₃ 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

References	Supplements to diet (µg/d)			Vitamin D concentration of milk (µg/l)		
	Vitamin D ₃	25(OH)D ₃	Feeding duration	Vitamin D ₃	25(OH)D ₃	1,25(OH) ₂ D ₃
Hollis <i>et al.</i> ⁽³⁹⁾	100	–	NA	0.04	0.37	0.01
	1000	–	NA	0.32	0.68	0.004
Reeve <i>et al.</i> ⁽⁴⁰⁾	375	–	30 d	0.28	0.15	0.01
Mcdermott <i>et al.</i> ⁽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 <i>et al.</i> ⁽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)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25 dihydroxyvitamin D₃; 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)D₃. 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)D₃ of 1.5, 5 or 10 µg/kg body weight to generally healthy subjects and showed that the peak serum 25(OH)D₃ concentration was reached within 4–8 h after ingestion⁽⁵⁷⁾. A later study by Jetter *et al.* compared the pharmacokinetic absorption of vitamin D₃ and 25(OH)D₃ by providing a single dose of 20 µg vitamin D₃ or 20 µg 25(OH)D₃ to postmenopausal women⁽⁵²⁾. The time to reach maximum plasma 25(OH)D₃ concentration was 22 and 11 h for vitamin D₃ and 25(OH)D₃, respectively. In addition, the peak concentration of plasma 25(OH)D₃ (44 nmol/l) from 25(OH)D₃ supplementation was higher than vitamin D₃ 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 D₃ and 140 µg 25(OH)D₃ with the time to reach peak plasma 25(OH)D₃ being 21 and 4–8 h for vitamin D₃ and 25(OH)D₃ supplementation, respectively⁽⁵²⁾. In addition, the maximum plasma concentration of 25(OH)D₃ for 25(OH)D₃ treatment (100 nmol/l) was significantly higher than for vitamin D₃ treatment (44 nmol/l). These results suggest that 25(OH)D₃ was absorbed more quickly than vitamin D₃ possibly because 25(OH)D₃ has higher solubility in aqueous media than vitamin D₃ due to its more polar chemical structure⁽⁵⁸⁾. Furthermore, as this metabolite of vitamin D₃ is produced in the liver, the hepatic metabolism of vitamin D₃ to 25(OH)D₃ is circumvented and consequently the

conversion from vitamin D₃ to 25(OH)D₃ would be negligible⁽⁵⁹⁾. In patients with liver disease who had an impaired ability to synthesise 25(OH)D₃ from vitamin D₃⁽⁶⁰⁾, the study of Sitrin and Bengoa⁽⁶¹⁾ verified that 25(OH)D₃ could be absorbed more efficiently than vitamin D₃ after oral supplementation. Therefore, supplementation with 25(OH)D₃ 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)D₃ in raising serum or plasma 25(OH)D level after long-term administration, several studies have confirmed that oral consumption of 25(OH)D₃ 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)D₃ supplementation compared with vitamin D₃ on serum or plasma 25(OH)D₃ level is from only four studies^(51,52,54,56) where both 25(OH)D₃ and vitamin D₃ treatments were included in the same study (Table 5). The study of Barger-Lux *et al.*⁽⁴⁷⁾ provided three different doses of vitamin D₃ (25, 250, 1250 µg/d) or 25(OH)D₃ (10, 20, 50 µg/d) to the participants for 8 and 4 weeks, respectively. However, the effects of 25(OH)D₃ and vitamin D₃ 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)D₃ and vitamin D₃ supplementation on raising serum or plasma 25(OH)D concentrations, a dose–response factor was calculated for each µg of orally consumed 25(OH)D₃ or vitamin D₃ in four studies^(51,52,54,56). The dose–response factors of 25(OH)D₃ and vitamin D₃ 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 D₃ (25(OH)D₃)) supplementation in adults (order by year)

References	Subjects characteristics (trial time during the year, subjects (sex), age, BMI)	25(OH)D ₃ supplementation group					Control group (if available)				
		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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽⁴⁸⁾	March–September, haemodialysis patients (women and men) 67 years, BMI (NA)	6 months	16 µg/d	149	30	126					
Cavalli <i>et al.</i> ^{§(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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ^{‡§(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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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)D₃.

Table 5. Summary of randomised controlled trials with both calcifediol (25 hydroxyvitamin D₃ (25(OH)D₃)) and vitamin D₃ in adults to calculate the relative effectiveness of 25(OH)D₃ and vitamin D₃ 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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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	
	20 µg vitamin D ₃ /d × 12 months	2.29 ^b	

* Dose-response factor = Δ serum/plasma (nmol/l)/dose (µg).

† Relative effectiveness = a/b within same study.

effectiveness of 25(OH)D₃ to vitamin D₃ was calculated by dividing the dose-response factor of 25(OH)D₃ by that of vitamin D₃.

The highest relative effectiveness was found in the study by Catalano *et al.*⁽⁵⁴⁾. Weekly treatment of 140 µg 25(OH)D₃ or 140 µg vitamin D₃ supplements was provided to osteopenic and dyslipidaemic postmenopausal women for 24 weeks. Supplementation with 25(OH)D₃ raised serum 25(OH)D from a baseline of 56–126 nmol/l, while vitamin D₃ 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)D₃ was 7.14 times more effective at increasing serum 25(OH)D than dietary vitamin D₃.

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)D₃ and vitamin D₃ using doses of 20 µg in their treatments. Cashman *et al.*⁽⁵¹⁾ provided daily supplements of 20 µg vitamin D₃ or 20 µg 25(OH)D₃ 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)D₃ and vitamin D₃ treatments, respectively. A relative effectiveness factor of 4.99 was calculated representing the relative effectiveness of each µg of dietary 25(OH)D₃ relative to dietary vitamin D₃ 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 D₃ and 25(OH)D₃. Jetter *et al.* supplemented healthy postmenopausal women with 20 µg 25(OH)D₃ or 20 µg vitamin D₃ for 16 weeks during the winter⁽⁵²⁾. They found that for the 25(OH)D₃ treatment, plasma 25(OH)D₃ increased to 173 nmol/l from a baseline of 31 nmol/l, whereas for the vitamin D₃ treatment, plasma 25(OH)D₃ increased to 77 nmol/l from a baseline level of 35 nmol/l. The relative effectiveness factor of each µg of 25(OH)D₃ was 3.40 compared with vitamin D₃ in raising plasma 25(OH)D₃ level. A similar low relative effectiveness factor was found in another study where post-menopausal osteoporotic women were given either 20 µg vitamin D₃ or 20 µg

25(OH)D₃ over 6 or 12 months⁽⁵⁶⁾. The serum concentration of 25(OH)D for the 25(OH)D₃ 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 D₃ 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)D₃ relative to vitamin D₃ 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)D₃ to vitamin D₃ 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)D₃ can more effectively increase serum 25(OH)D concentrations than vitamin D₃ and may also be absorbed faster reaching a serum or plasma 25(OH)D plateau earlier than vitamin D₃ supplementation. Furthermore, supplementation with 25(OH)D₃ may also have more benefits to human health compared with vitamin D₃ in a general healthy population. Bischoff-Ferrari *et al.* reported that 20 µg 25(OH)D₃ 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)D₃ treatments in patients were also summarised in Table 4^(46,48,53–56), and those studies consistently reported that chronic 25(OH)D₃ 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)D₃ to be more effective than a 20 µg dose of vitamin D₃ 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)D₃ was safe and effective at improving vitamin D status of HIV-infected patients throughout the year⁽⁵³⁾.

Furthermore, supplementation with 25(OH)D₃ may have additional benefits on patients' health. Previously, 25(OH)D₃ was recommended for patients with kidney disease since 25(OH)D₃ has a direct action on bone metabolism⁽⁶⁶⁾. Hahn *et al.* provided a daily 40 µg 25(OH)D₃ 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)D₃ 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)D₃ for 6 months; vitamin D status reached 126 nmol/l from 30 nmol/l, at the same time 25(OH)D₃ supplementation corrected the excess bone turnover⁽⁴⁸⁾. Similarly, a study by Catalano *et al.*⁽⁵⁴⁾ provided 140 µg 25(OH)D₃ supplements for 24 weeks to osteopenic and dyslipidaemic postmenopausal women, and results showed that 25(OH)D₃ 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 D₂ or vitamin D₃ are used for fortification. Evidence from one previous meta-analysis of RCT showed that vitamin D₃ supplementation is more effective at raising vitamin D status than vitamin D₂⁽⁶⁷⁾. However, a further comprehensive systematic review and meta-analysis of thirty-three RCT⁽⁶⁸⁾ showed that the effect of vitamin D₃ supplement on serum 25(OH)D₃ 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 D₃ 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)D₃-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)D₃ supplementation on serum or plasma 25(OH)D concentration. We concluded that it is difficult to get consensus on the effectiveness of 25(OH)D₃ supplementation relative to vitamin D₃ 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)D₃ 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 D₃. 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)D₃-enriched or fortified foods should be further explored in the future, and additional RCT should be conducted to investigate the effect of 25(OH)D₃-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.

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