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.

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

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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 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 summarises evidence from the human randomised controlled trials (RCT), which include the present review summaries evidence from the human randomised controlled trials (RCT), which include the present review summaries evidence from the human randomised controlled trials (RCT), which include the present review summaries evidence from the human randomised controlled trials (RCT). The results of all studies revealed that egg yolk vitamin 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) 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\(^17\). 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\(_3\)
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.

There are only a few studies 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. It is of note that most of vitamin D supplementation studies, summarised in Table 1, had higher vitamin D doses than the EU diet limit, 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 and Duffy et al. 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, 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). 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. 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. Similar high vitamin D3 supplementation doses were reported in another two studies, which also showed that elevated vitamin D3 content of the fish liver or whole fish had been achieved by supplementing vitamin D3 in the diet. However, 25(OH)D3 contents of the enriched fish were not measured in these studies, and the lack of evidence on the effects by feeding fish with 25(OH)D3 on the vitamin D content of the

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**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) |
|-------------------|-----------------------------|--------------------------|-----------------------------------------------|
| Mattila et al.    |                             |                          |                                               |
| 26.6              | 25(OH)D3                    | 6                        | 1.4                                           |
| 62.4              | 25(OH)D3                    | 6                        | 3.5                                           |
| 216.0             | 25(OH)D3                    | 6                        | 22.0                                          |
| 280.0             | 25(OH)D3                    | 4                        | 30.0                                          |
| 62.5              | 25(OH)D3                    | 4                        | 13.6                                          |
| 150.0             | 25(OH)D3                    | 4                        | 33.7                                          |
| 250.0             | 25(OH)D3                    | 9                        | 6.5                                           |
| 125.0             | 25(OH)D3                    | 9                        | 10.5                                          |
| 375.0             | 25(OH)D3                    | 9                        | 26.2                                          |
| Browning and Cowieson |                         | 3                        | 3.0                                           |
| 62.5              | 25(OH)D3                    | 3                        | 21.6                                          |
| 62.5              | 25(OH)D3                    | 3                        | 41.0                                          |
| 62.5              | 25(OH)D3                    | 3                        | 60.3                                          |
| 250.0             | 25(OH)D3                    | 3                        | 870.4                                         |
| Yao et al.        |                             |                          |                                               |
| 55.0              | 25(OH)D3                    | 9                        | 6.5                                           |
| 242.6             | 25(OH)D3                    | 9                        | 10.5                                          |
| 430.0             | 25(OH)D3                    | 9                        | 26.2                                          |
| 617.5             | 25(OH)D3                    | 9                        | 30.9                                          |
| 2555.0            | 25(OH)D3                    | 9                        | 81.6                                          |
| Mattila et al.    |                             |                          |                                               |
| –                 | 25(OH)D3                    | 6                        | ≤0.2                                          |
| 55.0              | 25(OH)D3                    | 6                        | 2.1                                           |
| Mattila et al.    |                             |                          |                                               |
| –                 | 25(OH)D3                    | 6                        | ≤0.2                                          |
| Duffy et al.      |                             |                          |                                               |
| 37.5              | 25(OH)D3                    | 4                        | 1.0*                                          |
| 75.0              | 25(OH)D3                    | 4                        | 2.0*                                          |
| 37.5              | 25(OH)D3                    | 4                        | 1.3*                                          |
| 75.0              | 25(OH)D3                    | 4                        | 0.7*                                          |

25(OH)D3, 25-hydroxyvitamin D3.

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

| References | Vitamin D3 supplement (µg/kg) | Feeding duration (weeks) | Vitamin D3 of fish (µg/100 g) |
|------------|-------------------------------|--------------------------|------------------------------|
| Horvä et al. (55) | 40 | 11 | 1 (fillet) |
| | 2210 | 11 | 21 (fillet) |
| | 28680 | 11 | 210 (fillet) |
| Vielma et al. (56) | 62.5 | 12 | 1 (liver) |
| | 6250 | 12 | 73 (liver) |
| | 62500 | 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. (50) | 200 | 9 | ≤25 (whole fish) |
| | 5000 | 9 | 80 (whole fish) |
| | 57000 | 9 | 650 (whole fish) |

* Estimated from graph.

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 (45). 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) nmol/l to 35 (SD 11) 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) nmol/l and 49 (SD 16) 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 (46–50) (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 (47–50) 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
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)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 (nmol/l)/dose (µg)). Then, the relative

| 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 et al.\(^{59}\) | 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.
| References     | Subjects characteristics (trial time during the year, subjects (sex), age, BMI)                                                                 | 25(OH)D3 supplementation group | Control group (if available) | Baseline 25(OH)D3 treatment | Endpoint 25(OH)D3 treatment | Vitamin D3 treatment | Baseline 25(OH)D3 | Endpoint 25(OH)D3 |
|----------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------|-----------------|-----------------|
| Hahn et al. (46) | Whole year, patients (women and men) with glucocorticoid-induced osteopenia 46 years, BMI (NA)                                 | Duration 18 months 40 µg/d + 500 mg calcium/d | Baseline 25(OH)D(nmol/l) | Endpoint 25(OH)D(nmol/l) | Duration 8 weeks 25 µg/d | n | 9 | 39 | 205 | 13 | 67 | 96 |
| Barger-Lux et al. (47) | January–April, men 28 years, 26 kg/m² | 4 weeks 10 µg/d | 7 | 67 | 107 | 8 weeks 250 µg/d | 10 | 67 | 213 |
|                |                                                                                                                                | 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/m²                                                                       | 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/m²                                         | 16 weeks 500 µg/month | 18 | 45 | 105 |
| Cashman et al. (51) | January–April, women and men, 57 years, 29 kg/m²                                                                               | 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/m²                                                                   | 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/m²                                        | 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/m²                                                   | 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 |
| Ortego-Jurado et al. (55) | Whole year, patients (women and men) had autoimmune diseases, undergoing glucocorticoids therapy, 56 years, 28 kg/m² | 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/m²                                                             | 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/2 weeks | 10 | 38 | 214 |
|                |                                                                                                                                | 12 months 266 µg once/2 weeks | 10 | 38 | 233 |
|                |                                                                                                                                | 6 months 266 µg once/2 weeks | 10 | 40 | 165 |

* 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.
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 28-99 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

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**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 (nmol/l)/dose (µg).
† Relative effectiveness = Δ serum/plasma (nmol/l)/dose (µg).
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(53). 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(55).

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(60). 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 supplementation 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-fortified 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.

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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. Borradaile 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/sac-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, 538S–645S.
13. Schmid A & Walther B (2013) Natural vitamin D content in animal products. Adv Nutr 4, 463–462.
14. O’Mahony L, Stepnien 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 D3 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. Dielder P, Pedersen JI, Helgerud P et al. (1983) Absorption, distribution, and transport of vitamin D3 and 25-hydroxyvitamin D3 in rat. Am J Physiol 245, S538–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, Klim J, 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 D3 and 25-hydroxyvitamin D3 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, Rossov L et al. (2004) Effect of vitamin D2- and D3-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 D3-enriched diet on egg yolk vitamin D3 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. Dufly SK, Rajauria G, Clarke LC et al. (2017) The potential of cholecalciferol and 25-hydroxyvitamin D3 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 D3 (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 D3 in Atlantic salmon Salmo salar: effect of dietary period. 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, Hakkaraainen T et al. (1999) Possibilities to raise vitamin D content of rainbow trout
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(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. Mc Dermott CM, Beitz DC, Littledike ET et al. (1985) Effects of dietary vitamin-D₃ 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) Effects of feeding 25-hydroxyvitamin D₃ with a negative cation-union difference diet on calcium and vitamin D status of peri-parturient 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 D₃ active compounds – Which potency should be used for 25-hydroxyvitamin D₃? 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, Tietelbaum 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 D₃ (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 D₃ and vitamin D₂ 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 osteopenotvarstatin-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. Sitirin 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)D₃ versus vitamin D₂: 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 D₂ and vitamin D₃ 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, Boura S, Geusens PP et al. (2014) Serum 25(OH)D response to vitamin D₃ supplementation: a meta-regression analysis. Nutrition 30, 975–985.