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Vitamin D fortification of foods and prospective health outcomes

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ABSTRACT

Vitamin D is essential for bone health and has significant roles in non-skeletal health and organ function. Dermal synthesis through exposure to ultraviolet B light is the major natural source of vitamin D, while only a small portion of the necessary amount can be acquired by a diet without fortified foods. In recent years, vitamin D deficiency as a result of lifestyles with inadequate sun exposure, has received increased attention due to its association with the increased risk of serious chronic diseases. This review summarizes our current understanding of food fortification strategies with vitamin D and the resulting health impact. Conventional and biotechnological approaches can be used for the production of new and novel vitamin D rich or vitamin D fortified foods. The availability of a wider range of every-day consumed fortified foods as part of a "Daily D" public health policy can contribute to the improvement of vitamin D status and to prevention of vitamin D deficiency.

1. Introduction

Vitamin D is a fat soluble vitamin essential for bone health and has significant roles in non-skeletal health, organ function and prevention of diseases (Christakos et al., 2013). The major natural source of vitamin D is dermal synthesis through exposure to ultraviolet B light, while only a small portion of the necessary amount can be acquired by a diet without fortified foods (Holick, 2017). In recent years, vitamin D deficiency as a result of lifestyles with inadequate sun exposure, has received increased attention due to its association with the risk of serious chronic diseases. Since prolonged exposure to sunlight has been associated with risk for skin cancer, food fortification arises as an important option in obtaining vitamin D sufficiency. This review summarizes our current understanding of food fortification strategies with vitamin D and the resulting health impact. A search for original articles focusing on vitamin D fortification of foods and the subsequent health outcomes was performed in PubMed and Web of Science. The search terms used were "food fortification", "food enrichment", "fortified foods", "biofortification", "bread", "wheat flour", "juice", "milk", "yogurt", "eggs", "biofortified beef", "yeast", "biofortified bread", in combination with vitamin D. The most recent and relevant published results were selected and are presented in this review.

2. Chemistry and metabolism of vitamin D

Vitamin D is a generic term describing a class of biologically active secosteroids involved in calcium and bone metabolism and in other important biological functions. There are two forms of the vitamin, cholecalciferol or vitamin D_3 and ergocalciferol or vitamin D_2 (Fig. 1). Vitamin D is a 9,10-secosteroid, a steroid derivative in which the B ring is opened and the bond between C_9 and C_{10} of the steroid structure is cleaved. Cholecalciferol is the natural form of the vitamin and is produced in the skin of vertebrates with the action of UV radiation (290–315 nm) on the precursor molecule 7-dehydrocholesterol by a non-enzymatic mechanism (Christakos et al., 2016; Holick, 1987, 2017). Ergocalciferol is produced by irradiation of the plant sterol ergosterol. The importance of ergocalciferol lays in its wide use for food fortification and as a food supplement. Vitamin D cannot usually be adequately obtained from natural dietary sources due to the small number of such sources and the low content of the vitamin in most of them. Therefore, the main source of the vitamin in most countries is exposure to solar radiation (Holick, 2017). In this sense, vitamin D is not really a vitamin, since it can be produced by exposure to sunlight and it is not necessary to be acquired from nutritional sources. Vitamin D is a prohormone of the steroid hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of the vitamin (Christakos et al., 2016; DeLuca, 2004).

Both cholecalciferol and ergocalciferol are rapidly absorbed by the intestine. The mechanism of the absorption is not well understood and probably involves both passive diffusion and active mechanisms involving membrane carriers (Silva and Furlanetto, 2018). There are studies suggesting that the presence of fat in the food facilitates the

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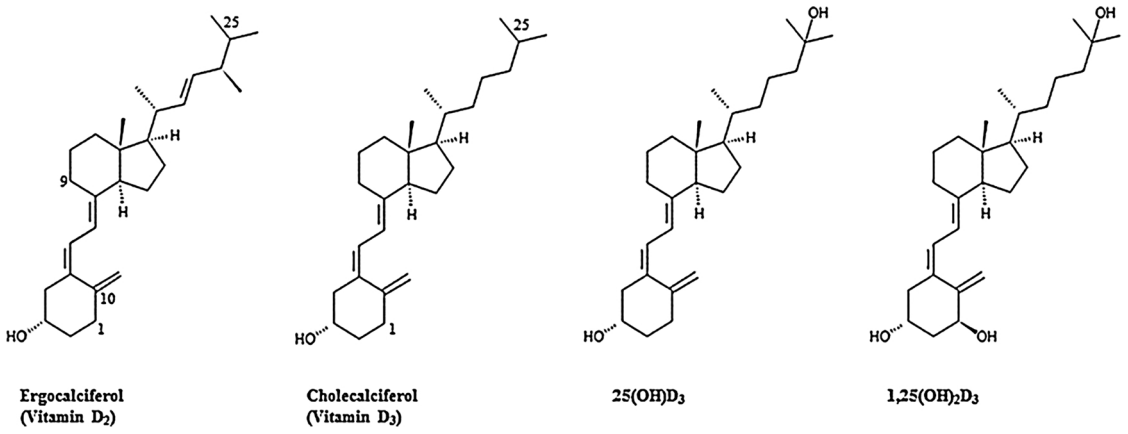

Fig. 1. Chemical formulae of vitamin D_2, vitamin D_3, $25(OH)D_3$ and $1,25(OH)_2D_3$.

absorption of vitamin D (Raimundo et al., 2015). Other studies have shown that vitamin D is bioavailable without the presence of significant amounts of dietary fat or even in the absence of it (Nikooyeh et al., 2016; Silva and Furlanetto, 2018; Tangpricha et al., 2003).

After cutaneous production of cholecalciferol or absorption by the intestine of cholecalciferol or ergocalciferol, the vitamin enters the circulation bound to the vitamin D binding protein (DBP). The DPB is a serum glycoprotein of the albuminoid family of binding proteins. It is an α2-globulin with 458 amino acids and molecular weight of 52–59 kDa (Delanghe et al., 2015; Speeckaert et al., 2014).

Upon production or absorption, cholecalciferol is transported to the liver where it is enzymatically hydroxylated by CYP2R1 to 25-hydroxyvitamin D_3, ($25(OH)D_3$) the major vitamin D compound in the circulation. The serum concentration of 25(OH)D is an indicator of the vitamin D status (Christakos et al., 2016). In a second enzymatic hydroxylation step, $25(OH)D_3$ is further hydroxylated in the kidney by CYP27B1 to $1,25(OH)_2D_3$, the hormonal form of vitamin D (Fig. 1). In the human body the D_2 and D_3 vitamins have a similar metabolism but the D_3 metabolites are thought to be more active and the D_3 vitamin is more efficient in restoring the serum levels of the major circulating metabolite, 25(OH)D (Silva and Furlanetto, 2018).

3. Biological functions of vitamin D

Vitamin D compounds have both genomic and non-genomic actions. $1,25(OH)_2D_3$, the hormonal form of vitamin D, exerts its action by binding to the vitamin D receptor (VDR), a member of the family of nuclear receptors (Pike et al., 2017).

By binding to the VDR, $1,25(OH)_2D_3$ activates a heterodimeric complex of the VDR and the Retinoic X Receptor (RXR) that binds to specific vitamin D response elements (VDREs) in the regulatory area of the gene controlled by $1,25(OH)_2D$ and either upregulates or downregulates the expression of target genes (Lin, 2016; Pike et al., 2017).

The major biological functions of $1,25(OH)_2D_3$ are regulation of bone metabolism and maintenance of skeletal health and are mainly achieved by enhancement of calcium and phosphorus absorption by the intestine and promotion of bone mineralization. It is not surprising that VDR is expressed in the intestine, bones, kidney and parathyroid glands. However VDR is expressed in a vast variety of cells and tissues in the human body, suggesting a role of $1,25(OH)_2D_3$ in extraskeletal health (Lin, 2016; Pike et al., 2017; Wang et al., 2012). Experiments in animal models as well as epidemiological and clinical data suggest roles for vitamin D in protection from infections, prevention of cancer, on the cardiovascular system and in certain autoimmune diseases (Christakos et al., 2013).

4. Vitamin D sources and vitamin D status

The serum concentration of 25(OH)D is a reliable biomarker of a person's vitamin D status (Christakos et al., 2016). Unlike other nutrients vitamin D (and thus 25(OH)D) can be obtained from a non-nutritional source, i.e. dermal synthesis. Additionally vitamin D has a range of biological functions beyond those in bone and these functions possibly require different levels of 25(OH)D. These facts create a controversy about the vitamin D intake that is necessary for the vitamin to exert all its biological functions and about the serum concentrations of 25(OH)D that define sufficiency and deficiency (Pludowski et al., 2018).

The European Food Safety Authority (EFSA) considers that a serum 25(OH)D concentration of 20 ng/mL (50 nmol/L) is a suitable target value for all population groups and based on that, the adequate intake (AI) is set to 600 IU (15 μg) for adults and 400 IU (10 μg) for infants (Bresson et al., 2016). The US Institute of Medicine (IOM) suggests a serum level of 25(OH)D of 16 ng/mL (40 nmol/L) as the targeted level for a median dietary requirement with the lower end of the requirement range being 12 ng/mL (30 nmol/L). Based on the above the Estimated Average Requirement (EAR) for vitamin D is 400 IU (10 μg)/day for all population groups while the Recommended Dietary Allowance (RDA) is 600 IU (15 μg)/day with the exception of persons aged over 71 years where the RDA is 800 IU (20 μg /day) (IOM, 2011). Guidelines focusing on the pleiotropic effects of vitamin D instead of mainly the bone effects suggest a serum 25(OH)D target level of 30 ng/mL (75 nmol/L), and daily vitamin D intake in the range of 400–2000 IU (10–50 μg) depending on age, ethnicity, body weight and disease status (Pludowski et al., 2018).

The vast majority of $25(OH)D_3$ in the circulation comes from exposure to sunlight (Battault et al., 2013; Christakos et al., 2016; Holick, 2017). The cutaneous production of vitamin D depends on demographic factors such as skin pigmentation and age, use of sunscreen and factors related to the solar radiation such as time of day, season, latitude, altitude as well as weather conditions and atmospheric pollution (Holick, 2017). Public awareness on the causative relation between skin cancer and UV radiation probably contributes to avoidance of exposure to sunlight. Vitamin D deficiency, as diagnosed by 25(OH)D levels below 20 ng/mL (50 nmol/L), is very common throughout the world even in sunny countries where the amount of regular sunshine was thought to be adequate to maintain the vitamin D status at the recommended levels (Holick, 2017; Lapatsanis et al., 2005; Manios et al., 2017a).

On the other hand, a healthy balanced diet is not enough to prevent vitamin D deficiency if it is not accompanied by exposure to sunlight, since most unfortified foods do not contain adequate amounts of vitamin D. Only some oily fishes such as salmon, herring, kipper, mackerel and sardines containing approximately between 200 and 1000 IU (5–25 μg) per 100 g, are sufficient sources of cholecalciferol while wild

mushrooms are considered to be natural ergocalciferol carriers (Milesevic et al., 2018; O'Mahony et al., 2011).

Several *in vivo*, epidemiological and clinical data suggest an association of vitamin D deficiency with increased risk for a large number of acute and chronic illnesses including rickets (Sahay and Sahay, 2012), childhood caries (Schroth et al., 2013), osteoporosis (Goltzman, 2018; Lips and van Schoor, 2011; Sunyecz, 2008), infections (Gunville et al., 2013), autoimmune diseases (Vanherwegen et al., 2017; Christakos et al., 2016), cardiovascular diseases (Majeed, 2017; Zittermann, 2018; Christakos et al., 2016), cancer (Atoum and Alzoughool, 2017; Feldman et al., 2014; Christakos et al., 2016), type 2 diabetes (Chiu et al., 2004; Forouhi et al., 2008) and neurological disorders (Di Somma et al., 2017).

Although there is no generally accepted consensus on the optimal serum levels of 25(OH)D, most researchers agree that vitamin D deficiency should be avoided. Specific approaches such as increased dietary and supplemental vitamin D intakes and encouragement of outdoor activities and hence more direct sun exposure (usually 5–10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3 times per week) could guarantee vitamin D sufficiency (Holick, 2004).

5. Vitamin D fortified foods

5.1. Food fortification

Fortification refers to the addition of nutrients or non-nutrient bioactive components to food and food constituents. Fortification can be used as a public health measure for the promotion of the intake of a nutrient in order to prevent or treat deficiencies but can also be used in order to appeal to consumers (Dwyer et al., 2015). Biofortification, or bio-addition refers to increasing the nutrient content of a food of plant or animal origin by selective breeding, feeding or treating or genetically engineering or by adding another food rich in a nutrient, instead of adding the vitamin during food processing (Calvo and Whiting, 2013; Cashman, 2015).

5.2. Fortification strategies

Cholecalciferol, ergocalciferol and their 25-hydroxylated metabolites have been used for food fortification. There are two recent reviews highlighting the results of several intervention trials regarding the comparative efficacy of vitamins D_2 and D_3 at raising 25(OH)D concentrations, with most indicating that vitamin D_3 is more effective at raising 25(OH)D concentrations (Silva and Furlanetto, 2018; Wilson et al., 2017).

After the discovery of the anti-rachitic effects of vitamin D in the early 20th century, a variety of foods was fortified including milk and dairy products, margarine and even beer (Holick, 2004). Cow's milk became the main delivery vehicle for vitamin D since the 1940s in the United States and Canada and a carefully planned fortification policy has been introduced to eliminate rickets as a public health issue. Two different fortification practices, a voluntary approach and a stressing mandatory fortification are applied in USA and Canada respectively, but both are similar in providing fortified foods with proven efficacy. Currently it is estimated that ~60% of the intake of vitamin D from foods in the US and Canada could be attributed to fortified foods. The major contributors to vitamin D intake in the US are fluid milk and cereals. In Canada fortified milk and fortified margarine are the major contributors to vitamin D intake, as it was shown by the higher 25(OH)D levels, that were found in Canadians ingesting fortified milk as compared to those not ingesting (Calvo and Whiting, 2013).

On the other hand, across Europe, legislative and voluntary fortification policies and practices vary from country to country. In the 1940s, vitamin D fortification of margarine and fat spreads became a mandatory approach in the UK and Ireland, aiming mainly to lift the vitamin D content up to the level naturally found in butter and not to improve the vitamin D status of the population (Brown et al., 2013; Wilson et al., 2017). Allen et al. provided evidence regarding the fortification vehicle and the optimum concentration that could safely improve the vitamin D status in the United Kingdom. With the use of nationally representative data, the authors simulated a fortification level of 400 IU (10 μg) vitamin D/100 g wheat flour, and a significant reduction from 93% to 50% of the proportion of at-risk groups estimated to have vitamin D intakes below the Reference Nutrient Intakes (RNI) was achieved with no individual exceeding the tolerable upper intake level (UL). Additionally, the estimated theoretical 2.5th percentile of population winter serum 25(OH)D concentration was elevated from 8 to 10.8 ng/ml (20–27 nmol/L) post fortification, which was above the minimum reference threshold of 10 ng/ml (25 nmol/L), and vitamin D intakes were improved across all socioeconomic groups. Fortification of wheat flour at this concentration was determined to be more effective than that of milk or milk and flour combined at any of the concentrations assessed and was, therefore, selected as the optimal fortification concentration. The results of this study suggest that vitamin D fortification of wheat flour could be a potentially effective strategy to increase the vitamin D intakes and the status of United Kingdom population groups at risk of deficiency without raising the risk of exceeding current reference thresholds (Allen et al., 2015).

Brown et al., have modeled the effect of fortifying various carrier foods, including orange juice, bread and milk on vitamin D levels, since the adult population in Germany appears to have generally low vitamin D levels compared to the US. The aim of the study was to prevent deficiency in the entire population while ensuring that people remain under upper limits for consumption. The authors have also compared the conventional approach of constant food fortification with the seasonal fortification of these staple foods, since the UVB-induced dermal synthesis of vitamin D is decreased during the winter. By using this fortification model, it was estimated that an average individual needs approximately 948 IU (23.7 μg) of vitamin D daily to reach a 25(OH)D serum concentration of 30 ng/mL (75 nmol/L) in Germany. Bread seems suitable as carrier product for base supply, but the application of vitamin D fortification strategies for different foodstuffs to overcome the possibility of overdose risk is also suggested (Brown et al., 2013).

Grønborg et al. performed a graded modelling of dietary vitamin D intake by calculating the contribution of vitamin D from fortified foods in 855 Danish women aged 18–55 years and concluded that adequate and safe levels of intake can be achieved and risks for reaching the 4000 IU (100 μg) national and EFSA upper limit of intake were observed only with daily consumption of supplements of 3200 IU (80 μg) (Grøndborg et al., 2018).

In Southeast Asia given the lifestyle changes that are limiting the sun exposure and the marginal consumption of foods naturally containing vitamin D, Yang et al. estimated, based on dietary intakes available from several Southeast Asian countries, whether food fortification has the potential to increase daily vitamin D intakes among women of reproductive age and other targeted groups (Yang et al., 2013). In Vietnam it is estimated that 91.5% of women and 38% of children under 5 years of age consume vegetable oil daily (median consumption, 11.1 and 6 g/day, respectively) and therefore, fortification of the vegetable oil at a level of 300 to 400 IU (7.5–10 μg) /100 g could provide 7%–9% of the Institute of Medicine (IOM) Estimated Average Requirement (EAR) of vitamin D and 4%–5% of the IOM EAR for a woman (400 IU or 10 μg) and for a child under 5 years of age respectively. This study highlights that vitamin D-fortified vegetable oil could provide from 3% to approximately 21% of the EAR for vitamin D for an adult, having thus significant potential to improve the vitamin D status in Southeast Asian countries (Yang et al., 2013).

In order to be effective in preventing deficiency, food fortification needs to be tailored to the nutritional habits of each country and specific market. For this reason fortification strategies should take in account the above. For example in India, it was proposed that fortification of widely consumed foods such as wheat flour, maida, rice and rice

Table 1
Trials with vitamin D fortified foods.

Food Source	Reference	Fortification model (vitamin D dose)	Duration and Population	Study groups (product portion)	Serum 25(OH)D
Fortified Yogurt	Mostafai et al. (2018)	Each 100 g pack of yogurt contained 1000 IU (25 μg) vitamin D	12 weeks Patients with confirmed prediabetes $n = 90$	1) Group A: Fortified yogurt with vitamin D 2) Group B: Plain yogurt 3) Group C: Oral vitamin D supplement	1) Pre-intervention: 18.15 ± 13.47 ng/mL (45.3 nmol/L) Post-intervention: 30.79 ± 12 ng/mL (76.9 nmol/L) 2) Pre-intervention: 17.57 ± 16.43 ng/mL (43.9 nmol/L) Post-intervention: 15.55 ± 10 ng/mL (38.8 nmol/L) 3) Pre-intervention: 18.73 ± 12.82 ng/mL (46.8 nmol/L) Post-intervention: 33.03 ± 18.08 ng/mL (82.4 nmol/L)
	Bonjour et al. (2015)	Each 125 g of yogurt provides 400 IU (10 μg) of vitamin D_3	84 days Aged white women at risk of fragility fractures over 60 years (mean age 73.4) $n = 48$	1) Fortified Yogurt (FY) Group: two yogurts of 125 g each, providing 400 IU (10 μg) of vitamin D_3 and 800 mg of elemental Ca 2) Control Yogurt (CY) Group: 0 μg of supplemental vitamin D_3 and 280 mg of elemental Ca	D0 to D84, serum 25(OH)D increased: FY: from 13.7 to 22.5 ng/mL (from 34.3 ± 2.4 to 56.3 ± 2.4 nmol/L) CY: from 14 to 16.5 ng/mL (from 35.0 ± 2.5 to 41.3 ± 3.0 nmol/L)
	Bonjour et al. (2018)	Each 125 g servings of Vitamin D_3-fortified yogurt contained 200 and 400 IU (5 and 10 μg) daily doses respectively	24 weeks (The 16 intervention weeks lasted from early January to mid-August, the 8 follow-up weeks, without product, from late August to mid-October) Menopausal women (mean age: 61.5) $n = 133$	1) Gr.Suppl.0: time controls maintaining dietary habits 2) Gr.Suppl.5: consuming one 125-g servings of $VitD_3$-fortified yogurts with 5 μg daily doses. 3) Gr.Suppl.10: consuming two 125-g servings of $VitD_3$-fortified yogurts with 10 μg daily doses.	Serum 25(OH)D ≥ 20 ng/mL (50 nmol/L) was 37.8, 54.5, and 63.6% in Gr.Suppl.0, Gr.Suppl.5, and Gr.Suppl.10, respectively
Fortified yogurt drink	Hajimohammadi et al. (2017)	Vitamin D_3-fortified doogh (yogurt drink) containing 500 IU (12.5 μg)/250 m L	12 weeks Diabetic patients $n = 100$	1) Group of fortified yogurt drink (FD) : 500 IU vitamin D and 170 mg calcium in each 250 cc bottle 2) Plain yogurt drink (PD): 170 mg calcium and no vitamin D/250 cc	1) FD Group: Before: 15.2 ng/mL (38.0 ± 22.8 nmol/L) After: 13.38 ng/mL (33.4 ± 22.8 nmol/L) 2) PD Group: Before: 15.4 ng/mL (38.5 ± 20.2 nmol/L) After: 28.8 ng/mL (72.0 ± 23.5 nmol/L)
Fortified Cheese	Manios et al. (2017b)	60 grams of enriched cheese provided a daily dose of 228 IU (5.7 μg) of vitamin D_3	8 consecutive winter weeks from January to March Postmenopausal women (55–75 years old) $n = 79$	1) Control group (CG: $n = 39$) consumed, as part of their usual diet, 60 g of non-enriched Gouda-type cheese 2) Intervention group (IG: $n = 40$) consumed, 60 g of vitamin D_3 enriched Gouda-type cheese	There was a differential response of mean (95 % CI) serum 25(OH)D levels in the IG and CG, with the former increasing and the latter decreasing significantly [i.e., by 2 ng/mL or 5.1 (3.4, 6.9) nmol/L vs. −1.84 ng/mL or -4.6 (-6.4, -.8) nmol/L, $p < 0.001$, respectively]
Fortified milk	Piirainen et al. (2007)	Since February 2003, fluid milks (0.5 mg/100 g) and margarines (10 mg/100 g)	During wintertime, before in 2001–2002 and after the initiation of fortification in 2003–2004 Finnish 4-year-old children $n = 118$	Two cohorts of children were studied during wintertime, one before ($n = 82$) in 2001–2002 and the other after ($n = 36$) the initiation of fortification in 2003–2004	The serum 25(OH)D concentration was higher after fortification (26 ng/mL or 64.9 (95% CI 59.7–70.1) nmol/l) compared to prior (21.9 ng/mL or 54.7 (95% CI 51.0–58.4) nmol/l; p = 0.002)
	Khadgawat et al. (2013)	Milk fortified either with 600 IU (15μg) or 1000 IU (25 μg) of vitamin D	12 weeks healthy school children, aged 10-14 years $n = 713$ (boys-300; girls-413)	1) Group A ($n = 237$) received 200 ml of unfortified milk per day 2) Group B ($n = 243$) received 200 ml of milk fortified with 600 IU (15 μg) of vitamin D per day 3) Group C ($n = 233$) received 200 ml of milk fortified with 1000 IU (25 μg) of vitamin D per day	Subjects having serum 25(OH)D levels > 20 ng/ml (50 nmol/L) Group A: 5.9 % Group B: 69.95 % Group C: 81.11 % vs 6.32 %, 4.9 % and 12 %, respectively, at baseline
	Jaaskelainen et al. (2017)	Vitamin D fortification of fluid milk products and fat spreads started in 2003 in Finland to improve vitamin D status	Between 2000 and 2011 Finnish adult population aged ≥30 years $n =$ sample comprising 6134 and 4051	–	91% of supplement nonusers who consumed fluid milk products, fat spreads, and fish based on Finnish nutrition recommendations reached serum 25(OH)D concentrations > 20 ng/mL (50 nmol/L) in 2011

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Table 1 (*continued*)

Food Source	Reference	Fortification model (vitamin D dose)	Duration and Population	Study groups (product portion)	Serum 25(OH)D
			adults from the Health 2000 and Health 2011 surveys		
	Reyes-Garcia et al. (2018)	500 mL semi-skimmed milk/day enriched with 150–600 IU (3.75–15 μg) vitamin D_3	24 months Postmenopausal healthy women $n = 500$	1) Low-dose group (L) ($n = 152$): semi-skimmed milk enriched with calcium (120 mg/100 mL) and vitamin D (30 IU/100 mL) 2) Vitamin D group (A) ($n = 157$): semi-skimmed milk enriched with calcium (180 mg/100 mL) and vitamin D (120 IU/100 mL) 3) Vitamin D + FOS group (B) ($n = 152$): semi skimmed milk enriched with calcium (180 mg/100 mL), vitamin D (120 IU/100 mL), and FOS (5 g/L)	Serum 25(OH)D concentrations did not change in the Low-dose group, but increased in group A and group B, $p < 0.001$
Fortified orange juice	Tangpricha et al. (2003)	Fortification with 1000 IU (25 μg) vitamin D_3	12 weeks 22–60 years, healthy subjects $n = 30$	1) Control group (n = 12) consumed 240 mL orange juice fortified with 350 mg Ca and 2) Intervention group (n = 14) consumed 240 mL orange juice fortified with 350 mg Ca and 1000 IU vitamin D_3 for 12 weeks	Control group had a 45% seasonal increase in 25(OH) D concentrations (20 to 29.2 ng/mL or 50.0 ± 10.0 to 73.0 ± 8.0 nmol/L; $p < 0.01$). Intervention group had serum 25(OH)D_3 concentrations increased by 150% (14.8 to 37.6 ng/mL or 37.0 ± 8.0 to 94.0 ± 20 nmol/L; $p < 0.01$)
	Biancuzzo et al. (2010)	1000 IU (25 μg) vitamin D_3 or vitamin D_2 in orange juice or capsule	11 weeks (started at 14 February 2007) Healthy subjects aged 18–79 y (15-20/group) $n = 105$	1) Placebo capsule + orange juice without vitamin D (placebo orange juice) 2) Placebo capsule + orange juice containing 1000 IU vitamin D_3/236.6 mL 3) Placebo capsule + orange juice containing 1000 IU vitamin D_2/236.6 mL 4) 1000 IU vitamin D_3 capsule + placebo orange juice 5) 1000 IU vitamin D_2 capsule + placebo orange juice	No significant difference in serum 25(OH)D_3 was observed either between subjects who consumed vitamin D_3-fortified orange juice and vitamin D_3 capsules ($p > 0.1$) or those who consumed vitamin D_2-fortified orange juice and vitamin D_2 capsules ($p > 0.1$) respectively
Fortified bread	Madsen et al. (2013)	The fortified milk had a vitamin D_3 concentration 15 IU (0.38 μg)/100 mL The fortified bread had 240IU (6 μg) vitamin D_3/100 g	6 months starting in September $n = 782$ children and adults (4–60 y old) recruited as 201 families	1) Fortification Group: Vitamin D-fortified milk and bread 2) Control Group: Vitamin D non-fortified milk and bread	< 1% of subjects in the fortification group and 25% of subjects in the control group had 25(OH)D concentrations < 12 ng/mL (30 nmol/L) and 16% and 65% of subjects, respectively, had 25(OH)D concentrations < 20 ng/mL (50 nmol/L)
	Nikooyeh et al. (2016)	1000 IU (25 μg) per 50 g of bread	8 weeks Healthy subjects aged 20–60 years $n = 90$	1) Fortified bread (FP): 50 g bread fortified with 25 μg vitamin D_3 + placebo daily ($n = 30$) 2) Supplement (SP): 50 g plain bread + 25 μg vitamin D supplement daily ($n = 30$) 3) Control (CP): 50 g plain bread + placebo daily ($n = 30$)	The within-group changes of serum 25(OH)D concentrations were: FP Group: 15.6 ng/mL (39.0 ± 22.6 nmol/) ($p < 0.001$) SP Group: 11.5 ng/mL (28.9 ± 31.2 nmol/L) ($p < 0.001$) CP Group: -3.68 ng/mL (-9.2 ± 12.3 nmol/L)
	Costan et al. (2008)	One bun fortified with 5000 IU (125 μg) vitamin D_3	12 months Nursing home residents with 25(OH)D concentrations < 50 nmol/L $n = 45$ (28 women and 17 men, aged 58-89 years)	Residents who consumed daily one bun that had been fortified with 5000 IU (125 μg) vitamin D_3	Serum 25(OH)D reached optimal status (> 30 ng/mL or 75 nmol/L)
	Moulas et al. (2015a)	25,000 IU (625 μg) vitamin D_3	12, 24 and 48 hours Healthy adult volunteers $n = 26$	1) Volunteers consuming 25,000 IU of pure vitamin D_3 2) Volunteers consuming fortified bread containing the same amount of vitamin D_3	The concentration of cholecalciferol in the blood of volunteers after consumption of fortified bread showed a maximum at 12 hours and, although lower, was not statistically different than the concentration after consumption of vitamin D_3

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Table 1 (*continued*)

Food Source	Reference	Fortification model (vitamin D dose)	Duration and Population	Study groups (product portion)	Serum 25(OH)D
Biofortified bread	Itkonen et al. (2016)	1000 IU (25 μg) D_2 in the daily portion of 87 g bread	8-week (during winter February–April 2014) Healthy 20–37–year-old women in Helsinki (60 °N) $n = 33$	1) Placebo pill and regular bread = 0 μg D_2 or D_3/day 2) D_2 supplement and regular bread = 25 μg D_2/day 3) D_3 supplement and regular bread = 25 μg D_3/day 4) placebo pill and D_2- biofortified bread = 25 μg D_2/day	D_2 is less potent in increasing total serum 25(OH)D concentrations than D_3, also indicating a decrease in the percentage contribution of serum 25(OH)D_3 to the total vitamin D pool
Biofortified salmon	Graff et al. (2016)	150 grams of salmon twice a week containing either 1080 IU (27μg) cholecalciferol (low D_3) or 4200-4560 IU (105–114 μg) (high D_3) plus vitamin K_1 plus calcium.	12 weeks Postmenopausal women Caucasian ethnicity, age range 50-65 years $n = 122$	Women were individually randomized into three salmon groups (150 grams/two times/week) also receiving calcium supplements : 1) High D_3 + high K_1 (HD/HK) 2) Low D_3 + high K_1 (LD/HK) or 3) High D_3 (0.35-0.38 mg/kg/fillet) + low K_1 (HD/LK) and one tablet group: 1) 800 IU (20 μg) vitamin D and 1000 mg calcium/day	Increased levels of serum 25(OH)D in all groups from pre- to post-intervention, except in the LD/ HK group. Results indicate that intake of standard Atlantic salmon alone is not enough to improve vitamin D status
Vitamin D enhanced eggs	Hayes et al. (2016)	Addition of either vitamin D_3 (3000 IU 75μg/kg diet) or 25(OH)D_3 (75 μg/kg diet) to the hens' feed at amounts that are allowable by European Council directive	8 weeks (winter RCT) Adults aged 45–70 y $n = 55$	1) Control group: 2 eggs/wk 2) Vitamin D_3 -eggs group: 7 vitamin D_3-enhanced eggs/wk 3) 25-D_3- eggs group: 7 25(OH)D_3-enhanced eggs/wk	Serum 25(OH)D in the control group significantly decreased by 10.57 ng/mL or 26.4 ± 6.7 nmol/L ($p = 0.001$), while there was no change in the 2 groups who consumed vitamin D-enhanced eggs ($p > 0.1$ for both)

flour could result in improvement of bone density and general health with minimal risk at a modest cost (Ritu and Gupta, 2014).

A consensus group, representing 11 international scientific organizations, proposes the fortification of staple foods with vitamin D and calcium, as appropriate based on dietary patterns (Munns et al., 2016).

5.3. Food fortification and vitamin D status

Food fortification strategies can only be considered successful if the targets of improving vitamin D intake and increasing 25(OH)D levels are achieved. Studies involving the use of vitamin D fortified foods are presented below and summarized in Table 1.

Fortification of milk with vitamin D constitutes a safe and effective strategy to deal with vitamin D deficiency. Piirainen et al. studied two cohorts of 4-year-old children in Finland before and after the national initiation of fortification of milk and margarine and found that after fortification, only 30.6% of the children achieved the recommended intake, while mean serum 25-hydroxyvitamin D concentration increased from 22 ng/mL (54.7 nmol/L) to 26 ng/mL (64.9 nmol/L) (Piirainen et al., 2007). In another prospective double-blind randomized control trial, 713 healthy school children aged 10–14 years were randomized to receive either unfortified milk (group A) or milk fortified with 600 IU (15 μg) (group B) and 1000 IU (25 μg) (group C) of vitamin D per day for 12 weeks. The percentage of subjects having serum 25(OH)D levels > 20 ng/ml (50 nmol/L) following supplementation was found 5.9% in group A, 69.95% in group B, and 81.11% in group C in comparison to 6.32%, 4.9% and 12%, respectively, at baseline (Khadgawat et al., 2013).

Jaaskelainen et al. investigated the changes in vitamin D status between 2000 and 2011 in the Finnish adult population after the 2003 initiation of fortification of fluid milk and fat spreads. The results have shown that in 2011 standardized serum 25(OH)D concentrations of > 16 ng/mL (40 nmol/L) and > 20 ng/mL (50 nmol/L) were achieved by 97% and 91% of the study population respectively, whereas the equivalent proportions in 2000 were 68% and 44%, respectively. The mean increase in serum 25(OH)D in daily fluid milk consumers (n = 1017) was 8 ng/ml (20 nmol/L) which was 2.4 ng/ml (6 nmol/L) higher than in non-consumers (n = 229) 5.6 ng/mL (14 nmol/L) $p < 0.001$, suggesting thus the success of the fortification policy (Jaaskelainen et al., 2017).

Since some population groups do not consume fortified milk due to lactose intolerance, studies have also shown that foods of plant origin such as orange juice and bread could be suitable vehicles for vitamin D fortification. Vitamin D is generally stable in juice (Biancuzzo et al., 2010; Moulas et al., 2015b; Tangpricha et al., 2003). In a bioavailability study, when subjects consumed daily orange juice fortified with 1000 IU (25 μg) vitamin D_3 for 12 weeks, serum 25(OH)D_3 concentrations increased by 150% and serum parathyroid hormone concentrations decreased by 25% compared with baseline (Biancuzzo et al., 2010; Moulas et al., 2015b; Tangpricha et al., 2003).

In a randomized, placebo-controlled, double-blind study conducted in healthy adults Biancuzzo et al. compared the bioavailability of vitamin D_2 and vitamin D_3 from orange juice with that of vitamin D_2 and vitamin D_3 supplements. The volunteers received 1000 IU (25 μg) vitamin D_3 or vitamin D_2, or placebo in orange juice or capsule for 11 weeks at the end of winter. The results indicate that vitamin D in orange juice is as bioavailable as is vitamin D in capsules while vitamin D_2 and vitamin D_3 in orange juice were found to raise serum 25(OH)D concentrations with effectiveness similar to that observed for vitamin D in capsules (Biancuzzo et al., 2010).

Additionally to orange juice, bread represents a good candidate for vitamin D fortification due to its common consumption. Vitamin D_3 is bioavailable from bread. In a bioavailability study, 26 apparently healthy adult volunteers consumed either 25,000 IU (625 μg) of pure vitamin D_3 or fortified bread containing the same amount of vitamin D_3 and the results have shown comparable serum levels of cholealciferol in the two groups at the tested time points of 12, 24 and 48 h, with the maximum concentration achieved at 12 h after the consumption (Moulas et al., 2015a).

In a randomized controlled trial of Madsen et al., recruiting 201 families (782 children and adults aged 4–60 years old) a higher serum 25(OH)D concentration was observed in the group consuming fortified bread and milk (27 ng/mL or 67.6 nmol/L) compared to the control group (16.7 ng/mL or 41.7 nmol/L) at the end of the winter season. This strategy resulted in a daily intake of 300 IU (7.5 μg) vitamin D in 78% of children and 56% of the adults in the fortification group while 84% of the subjects in this group had a serum 25(OH)D concentration > 20 ng/mL (50 nmol/L) (Madsen et al., 2013). In a randomized, double-blind, placebo-controlled trial conducted over 8 weeks, 90 healthy subjects were randomized to receive either fortified bread (FP group), supplement (SP) or unfortified bread (CP). After 8 weeks a remarkable improvement of vitamin D status and a decline in serum iPTH concentrations as compared to baseline levels was observed in the FP and SP groups. The authors conclude that fortification of flour with vitamin D could be a viable option for safely improving vitamin D intakes and the status of the Iranian population (Nikooyeh et al., 2016). In a study by Costan et al., 45 nursing home residents aged 58–89 years with 25(OH)D concentrations < 20 ng/mL (50 nmol/L) consumed daily one bun that had been fortified with 5000 IU (125 μg) vitamin D_3. After one year supplementation serum 25(OH)D achieved optimal status (> 30 ng/mL or 75 nmol/L) and bone health improved significantly (Costan et al., 2014).

Table 1 summarizes clinical and nutritional trials involving vitamin D fortified foods.

5.4. Biofortification

Although traditional fortification practices in which vitamin D is exogenously added to foodstuffs will continue to be an important strategy for increasing vitamin D intake, the introduction of novel vitamin D fortification approaches, including the use of biofortification or bio-addition, attracts attention. To date the most thoroughly examined vitamin D-biofortified food is without doubt, eggs. Mattila et al., evaluated the effect of cholecalciferol-enriched hen feed on egg quality. The top cholecalciferol content in egg yolk (ca 1200 IU (30 μg)/100 g) was reached 8–13 days from starting the high-cholecalciferol diet, while after 112 days feeding the cholecalciferol content gradually decreased to ca 880 IU (22 μg)/100 g. Vitamin D did not affect the health of the hens and the sensory properties, the fatty acid composition and the eggshell strength of the eggs (Mattila et al., 2003). In an another study, Mattila et al., investigated the effect of substitution of vitamin D with 25(OH)D_3 on the vitamin D content of commercial eggs and chicken meat. The vitamin D_3 contents of two commercial egg yolk pools were 196 IU/100 g (4.9 ± 0.14 μg/100 g) and 160 IU/100 g (4.0 ± 0.10 μg/100 g), and the 25(OH)D_3 contents were 1.3 ± 0.19 μg/100 g and 1.0 ± 0.07 μg/100 g. The chicken meat pools contained 8–12 IU of vitamin D_3/100 g (0.2–0.3 μg/100 g), whereas the content of 25(OH)D_3 was ≤0.2 μg/100 g. These studies showed that 25(OH)D_3 was effectively transferred from the hens' diet to yolk (Mattila et al., 2011). In the study by Browning et al., a total of 162 hens were fed with three different levels of vitamin D_3 in combination with three levels of 25(OH)D_3. This resulted in a significant increase of the content of vitamin D_3 and 25(OH)D_3 in the egg yolk, while no significant differences in egg quality parameters were observed. Depending on the dietary concentrations used, this approach could produce eggs containing between 100 and 500 IU (2.5 and 12.5 μg) vitamin D, providing scope to meet the recommended daily requirement of vitamin D for children or adults (Browning and Cowieson, 2014). Duffy and colleagues have additionally demonstrated increased antioxidant activity of the 25(OH)D_3 enriched eggs that were laid by hens fed with feed containing the upper allowable limit of 75 μg/kg feed. The highest (5.06 μg/egg) total vitamin D content of egg yolk was also achieved with this feed, without

having any impact on egg quality parameters. The consumption of a single egg enriched with 25(OH)D_3 has the potential to cover 25% to 33% of the recommended daily intake of vitamin D and in parallel improves the intake of natural antioxidants (Duffy et al., 2017). However limited data evaluating the effect of the consumption of such eggs on vitamin D status of healthy individuals are available. In a recent study, Hayes et al., performed an 8 week randomized control trial in adults aged 45–70 y (n = 55) consuming either less than 2 eggs per week, 7 vitamin D_3-enhanced eggs per week, or seven 25(OH)D_3- enhanced eggs per week. The trial was conducted during the winter months. The groups consuming 7 vitamin D_3- or 25(OH)D_3- enhanced eggs per week maintained serum 25(OH)D concentrations higher than the 10 ng/mL (25 nmol/L) European Union threshold for deficiency and were protected against the decline in serum 25(OH)D concentrations that was observed in the group that consumed less than 2 eggs per week (Hayes et al., 2016).

Irradiation of ergosterol with UV in mushrooms and baker's yeast to stimulate their endogenous vitamin D_2 content, constitutes another useful biofortification practice to control vitamin D deficiency particularly in those who do not consume meat or animal derived foods for cultural reasons, but also in the wider population as a cost-effective approach when applied to staple foods such as bread.

In a study by Mehrotra et al, 43 prediabetic vitamin D deficient adults were randomized to intake for 16 weeks UVB-treated mushrooms containing initially 600 IU D_2 or 4000 IU D_2 or untreated mushrooms and 600 IU D_3 or 4000 IU D_3. D_2-UVB-mushroom consumption resulted in modest or no increases in 25(OH)D_2 or total 25(OH)D in comparison to positive control subjects. Cooking was reported to significantly decrease the vitamin D_2 content of UV irradiated mushrooms (Mehrotra et al., 2014).

Recently Itkonen et al., in an 8-week randomized-controlled trial conducted in young adult females during winter in Finland, evaluated whether UVB-activated D_2 of yeast added to bread could improve serum 25(OH)D status as a cost effective and more ecological strategy for improving vitamin D intake and status in the general population. The daily ingestion of bread containing 1000 IU (25 μg) of D_2 derived from UVB-irradiated baker's yeast only very modestly increased mean serum 25(OH)D_2 concentration (by 2.5 ng/mL or 6.4 nmol/L), whereas mean total 25(OH)D concentration did not increase nor were there any changes in mean 25(OH)D_3 or serum parathyroid hormone concentrations. However, supplementation with 1000 IU (25 μg) of D_2 as a capsule led to a more prevalent increase of the mean 25(OH)D_2 and total 25(OH)D concentrations (by 12.5 and 3.8 ng/ml or 31.3 and 9.6 nmol/L, respectively) over the same time frame, thus suggesting a poor bioavailability of D_2 from the UVB-irradiated yeast in bread. The baking process of the bread or a potentially indigestible form of D_2 in the yeast preparation could be possible reasons for these findings (Itkonen et al., 2016). In a further study, Lipkie et al., have shown that vitamin D bioaccessibility was significantly higher from bovine milks and infant formula (71–85%) than from yeast-fortified sandwich breads (6–7%). Bioaccessibility was also found approximately 4 times lower from yeast-fortified bread as compared to crystalline vitamin D_2 fortified bread, while intact yeast cells were observed in the digesta of yeast fortified bread. The authors suggest that the poor bioavailability of yeast D_2 in comparison to other vitamin D_2 sources could be possibly attributed to entrapment within a less digestible yeast matrix and not only to metabolic differences between vitamins D_2 and D_3 (Lipkie et al., 2016). In a previous study of our lab, we developed Greek type fortified bread with the addition of vitamin D either as vitamin D_3 in encapsulated form, or as a novel food additive, Istafern Vita D Plus Concentrate, which is yeast (Saccharomyces cerevisiae) that contains vitamin D_2 produced by irradiation of the yeast ergosterol with UV light. Evaluation of the sensory characteristics of the fortified bread has shown no differences with the non-fortified one, while the vitamin D content (200 IU or 5 μg per 70 g portion) as assessed by the HPLC/MS analysis was found quite stable with a small loss during shelf life (Moulas et al., 2015a).

In a similar trend, the potential of biofortification of animal-derived foods, such as pork, beef and farmed fish with vitamin D compounds is currently under investigation. In a recent study, Duffy et al., investigated the UVB- mushroom-derived vitamin D_2 as a potential efficacious, cost-effective and renewable source for application in the production of vitamin D-biofortified beef as compared to synthetic vitamin D_2 and vitamin D_3 alternatives. To this end, thirty heifers were allocated to one of three dietary treatments: (1) basal diet + 4000 IU (100 μg) of vitamin D_3 (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2 (Vit D_2); and (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms (Mushroom D_2) for a 30 day pre-slaughter period. Results have demonstrated that supplementation of heifer diets with vitamin D_3 led to significantly higher *Longissimus thoracis* total vitamin D content (by 38–56%; $p < 0.05$) of the resulting beef steak than that from either vitamin D_2 or mushroom D_2 treatment sources. These findings were probably associated with the significantly lower (by 20–36%) serum total 25(OH)D found in these two treatment groups compared to that of the vitamin D_3-supplemented group. Irrespective of vitamin D source, carcass characteristics, sensory and meat quality parameter remained unaffected by the dietary treatments. The authors concluded that biofortification of heifer diets with 4000 IU (100 μg) of vitamin D_3 will contribute approximately 20% of the current EAR of 10 μg/d from a typical serving size of vitamin D_3-biofortified meat (Duffy et al., 2018).

Burild et al., found that the vitamin D metabolite content of pork meat depends on the ingested form of the vitamin. The authors found that increasing the vitamin D_3 or 25(OH)D_3 content of pig feed 49 days before slaughter increased the tissue content of vitamin D_3 and 25(OH)D_3 respectively and the increase was more prominent for the form of vitamin that was included to the feed (Burild et al., 2016).

5.5. Health effects of food fortification with vitamin D

Several randomized-controlled trials (RCTs) have examined the effects of vitamin D fortified foods on the protection from various health problems.

Osteoporosis is a very common disease that affects especially elder women and has been related to increased bone fracture risk. Adequate vitamin D status has been shown to implicate in bone fragility risk reduction (Lips and van Schoor, 2011; Sunyecz, 2008). The following recent RCTs deal with the effects of vitamin D on bone mass and bone metabolism indices when supplemented to susceptible population groups via fortified dairy products. A randomized double-blind controlled trial was conducted to evaluate whether fortification of yogurts with vitamin D and calcium exerts an additional lowering effect on serum parathyroid hormone (PTH) and bone resorption markers (BRM) as compared to iso-caloric and iso-protein dairy products in aged white women at risk of fragility fractures. Daily consumption of two fortified yogurts for three months providing 520 mg of Ca and 400 IU (10 μg) of vitamin D_3 daily prevented the development of secondary hyperparathyroidism and accelerated bone resorption as compared to non-fortified equivalent foods (Bonjour et al., 2015). In a further 24-week randomized controlled trial, using again fortified yogurt, Bonjour et al., demonstrated that consumption of vitamin D fortified yogurt caused a positively dose-dependent and inversely baseline-dependent increase in serum 25(OH)D concentrations. The effect of consumption of fortified yogurt was also season-dependent thus making the consumption of vitamin D_3-fortified foods during winter, even at 200 IU (5 μg) /day a prevalent necessity (Bonjour et al., 2018). Additionally, in a randomized controlled study conducted by Manios et al., 79 postmenopausal women (55–75 years old) were assigned to participate during the typical winter months in Greece (from January to March) for eight consecutive weeks, receiving either 60 g of non-enriched or vitamin D_3 enriched Gouda-type cheese. This is the only study of vitamin D-enriched cheese to report the impact on prevalence of vitamin D deficiency as the primary outcome measure, while health-related quality of

life (HRQL) indices were examined as secondary outcomes. The authors concluded that the daily consumption of vitamin D-enriched, reduced-fat Gouda-type cheese, providing a daily dose of 228 IU (5.7 μg) of vitamin D_3, in addition to the usual dietary intake of ~80 IU (2 μg) /day vitamin D, significantly increased mean serum 25(OH)D concentrations preventing wintertime vitamin D deficiency (Manios et al., 2017b).

Most of the studies described above had relatively short follow-up periods. In a recent two-year randomized controlled study, including 500 healthy postmenopausal women, Reyes-Garcia et al., evaluated the effect of milk enriched in calcium and vitamin D on 25(OH)D plasma concentrations, bone parameters (bone mass density (BMD) and bone turnover markers), and cardiovascular risk factors (glucose metabolism and lipid profile) in postmenopausal healthy women. A daily intake of milk enriched with vitamin D_3 (600 IU (15 μg) /day) induced a significant improvement in vitamin D status and a small increase in femoral neck BMD after 24 months (Reyes-Garcia et al., 2018).

Graff et al., successfully tailored Atlantic salmon fillet to contain different levels of vitamin D and vitamin K. The authors also investigated whether intake of vitamin D_3 and vitamin K_1 enriched salmon or vitamin D_3 and vitamin K tablets decreased bone biomarkers (urinary *N*-telopeptides, deoxypyridinoline, serum bone-specific alkaline phosphatase, and osteocalcin) compared to a low vitamin D_3 intake. To this end, 122 healthy postmenopausal women were randomized into four groups: three salmon groups (150 g/two times/week) and one tablet group (800 IU vitamin D and 1000 mg calcium/day). The salmon groups also received calcium supplements. The salmon had three different vitamin D_3/vitamin K_1 combinations: high D_3 + high K_1, low D_3 + high K_1, or high D_3 + low K_1.

Intake of salmon containing high levels of vitamin D_3 (0.35–0.38 μg/kg fillet) and supplements containing equivalent amounts of vitamin D had a similar positive effect on bone biomarkers in postmenopausal women. The authors conclude that an increased level of vitamin D_3 in feed for salmonids will contribute to an improved vitamin D_3 status and may improve human bone health (Graff et al., 2016).

Although several studies have suggested a role of vitamin D in the pathogenesis and treatment of type 2 diabetes (Boucher et al., 1995; Chiu et al., 2004; Forouhi et al., 2008), limited data regarding the effects of vitamin D-fortified foods in preventing diabetes are available. In a study of Mostafai et al. conducted in Iran, where diabetes, pre-diabetes and vitamin D deficiency constitute prevalent issues, the authors compared the effects of yogurt fortified with vitamin D (Group A), yogurt alone (Group B), and oral vitamin D supplementation (Group C) on the glycemic and anthropometric indices in pre-diabetic individuals. At the start of this study, 69% of Group A and 70.4% of Group C participants had severe vitamin D deficiency. By the end of the intervention, these figures were decreased at similar levels down to 10.3% and 7.4%, respectively, representing thus the significant effect of the daily intake of 1000 IU (25 μg) of vitamin D through fortified yogurt on serum levels of 25(OH)D_3. Given that there was no significant difference in the duration of exposure to sunlight among all three groups, improvements in serum levels of 25(OH)D_3 between baseline and after three months' intake of vitamin D could be attributed to the intervention (Mostafai et al., 2018). In another single blind randomized clinical trial conducted in type 2 diabetes patients, Hajimohammadi et al. found significant improvement of circulating 25(OH)D after consumption of a local type of yogurt (doogh) fortified with vitamin D. They also observed improvement of insulin sensitivity indices like fasting glucose and Quantitative Insulin Check Index (QUICKI) in the fortified doogh group compared to the plain doogh group. That in return might regulate beneficially appetite hormones like leptin and ghrelin as suggested by the observed lower ratio of leptin to ghrelin in the group that consumed fortified yogurt (Hajimohammadi et al., 2017).

6. Conclusion

Vitamin D deficiency is a major public health issue that can be addressed with food fortification. Vitamin D fortification of food is technically feasible with conventional and biofortification methods and can be used to address deficiency in large population segments without modifications in life styles and consumption patterns. We have proposed the "Daily D" concept by developing and studying for bioavailability a range of every-day consumed vitamin D fortified foods. A relevant fortification strategy should include a variety of staple foods and should take into account the dietary patterns of all the population groups in each country. There is a need for more research on fortified foods and biotechnology can offer solutions in the production of new and novel vitamin D rich or vitamin D fortified foods.

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