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An Updated Systematic Review and Meta-Analysis of the Efficacy of Vitamin D Food Fortification[1–3]

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Abstract

Food fortification is a potentially effective public health strategy to increase vitamin D intakes and circulating 25-hydroxyvitamin D [25(OH)D] concentrations. We updated a previous systematic review to evaluate current evidence from randomized controlled intervention studies in community-dwelling adults of the effect of fortified foods on 25(OH)D concentrations. Ovid MEDLINE, PubMed, CINAHL, Embase, and Cochrane Central Register of Controlled Trials were searched for randomized controlled intervention studies with vitamin D-fortified foods in free-living adults and data on circulating 25(OH)D. Two reviewers independently screened 441 papers for eligibility and extracted the relevant data. A meta-analysis of the absolute mean change in circulating 25(OH)D concentrations was conducted using a random effects model. Sixteen studies from 15 publications were included, of which 14 showed a significant effect of fortified foods on 25(OH)D concentrations. Heterogeneity was high ($P = <0.0001$, $I^2 = 89\%$) and was partly explained by dose, latitude (range, 3–60°), and baseline 25(OH)D (range, 24.0–83.6 nmol/L). When combined in a random effects analysis (n = 1513; 767 treated, 746 controls), a mean individual intake of ~11 μg/d (440 IU/d) from fortified foods (range, 3–25 μg/d) increased 25(OH)D by 19.4 nmol/L (95% CI: 13.9, 24.9), corresponding to a 1.2 nmol/L (95% CI: 0.72, 1.68) increase in 25(OH)D for each 1 μg ingested. Vitamin D food fortification increases circulating 25(OH)D concentrations in community-dwelling adults. Safe and effective food-based strategies could increase 25(OH)D across the population distribution and prevent vitamin D deficiency with potential benefit for public health. J. Nutr. 142: 1102–1108, 2012.

Introduction

Vitamin D deficiency is a public health issue that affects each stage of the lifecycle and crosses sex, economic, educational, and ethnic classifications, with huge potential human and economic cost implications. Although discussions on the reference ranges of circulating 25-hydroxyvitamin D [25(OH)D][6] that represent deficient, adequate, and optimal vitamin D status are ongoing, identification and prioritization of feasible and prudent public health measures for the prevention of serum 25(OH)D concentrations <30 nmol/L (the cutoff below which the risk of clinical vitamin D deficiency increases, manifesting as vitamin D-dependent rickets in children and osteomalacia in adults) are urgently required (1).

The recently revised Dietary Reference Intakes for vitamin D and calcium, published by the Institute of Medicine, proposed a serum 25(OH)D concentration of 50 nmol/L as the estimate of the serum 25(OH)D concentration that would meet the requirement of nearly all "normal healthy persons" (2). In the UK, the prevalence of serum 25(OH)D <50 nmol/L is 70–75% among 19- to 64-y-old adults during the January to March period (3). Data from NHANES III showed that 18–40% of adults in the southern United States have serum 25(OH)D concentrations <50 nmol/L between November and March (4).

Assuming minimal UV B sunlight exposure, the Institute of Medicine assigned serum concentrations of 25(OH)D at 40 and 50 nmol/L, which normally reflect exposure to vitamin D from a combination of sun-derived endogenous synthesis and diet, to specify the Estimated Average Requirement (EAR) and RDA for vitamin D. The EAR is 10 μg/d (40 IU/d) in all age and gender subgroups in the population >1 y old and the RDA is 15 and 20 μg/d for individuals aged 1–70 and ≥70 y, respectively (2).

It is currently unrealistic to expect the habitual Western-style diet to supply vitamin D at 10–20 μg/d across the population. Using data from the NHANES 2003–2006 (2 y and older), Fulgoni et al. (5) reported median intakes of ~1.75 μg/d of naturally occurring vitamin D and median and 90th percentile intakes of 6 and 16.25 μg/d, respectively, from all sources, including fortified foods and supplements. The prevalence of intakes below the EAR was 69.5%. According to Bailey et al. (6), <7% of the U.S. population over the age of 51 y met the previous Adequate Intake of 5 μg/d for vitamin D through diet alone. We reported median

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[3] Supplemental Table 1 and Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
[6] Abbreviations used: CPBA, competitive protein binding assay; EAR, Estimated Average Requirement; 25(OH)D, 25-hydroxyvitamin D.
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vitamin D intakes in a nationally representative sample of Irish adults at ~3 μg/d (5–95th percentiles were ~1–12.5 μg/d from all sources), with 74% consuming <5 μg/d (7). Recent data from the European Prospective Investigation into Cancer and Nutrition Study, which uses an integrated food composition database and dietary assessment methodology, showed mean intakes in men and women of ~5 and 3 μg, respectively, with considerable between-country variation (8).

Whiting et al. (9) showed that dietary supplements are a useful way of improving serum 25(OH)D concentrations in individuals, particularly in high-risk groups; however, relying on supplement consumption is not an appropriate public health strategy to increase intakes across the population distribution, because uptake does not typically exceed ~40% (10). To address the issue of vitamin D deficiency in the general population, many countries have opted for mandatory or voluntary food fortification (11,12). Indeed, fortified foods, including milk, yoghurt, butter, margarine, cheese, orange juice, bread, and breakfast cereals, constitute the major dietary source of vitamin D in the United States (13). In Finland, the impact of national fortification of fluid milks and margarines with vitamin D on intakes and circulating 25(OH)D concentrations has been evaluated in men (14), young children (15), and adolescent girls (16). Whereas substantial increases in intakes and circulating 25(OH)D were noted in the young men and children, benefits to adolescent girls were relatively minor, which the authors attributed to the low milk consumption in this population group (16).

The problem of fortifying a single staple, e.g. milk, or focusing on a commodity sector such as dairy, is that it does not increase the vitamin D supply in nonconsumers. For example, Babu and Calvo (17) suggested that fortification of wheat flour may be more efficacious in alleviating vitamin D deficiency in countries such as India and Jordan, where pasteurized milk is not widely consumed. Van Horn et al. (18) showed that African American girls relied more heavily on meat and beans as a source of vitamin D than white girls, emphasizing the need to account for diversity in food consumption patterns.

The potential of vitamin D-fortified foods to increase vitamin D status was recently highlighted by O'Mahony et al. (19) based on a descriptive review of evidence from randomized controlled trials. Previously, O'Donnell et al. (20) carried out a systematic review to assess the efficacy of food fortification on serum 25(OH)D concentrations and found evidence for benefit of fortification, although high heterogeneity was reported when the trials were combined in a meta-analysis. Subgroup analyses resulted in a reduction in heterogeneity when combining results from trials that used RIA to measure serum 25(OH)D and when combining results from trials using milk as the fortified food source. However, small numbers of relevant randomized controlled trials were available at the time, with 7 trials included in the meta-analysis and 4 trials included in the subgroup analyses mentioned.

In the interim, several additional randomized controlled trials have been published, some of which are large scale and of high quality. The aim of this systematic review and meta-analysis was to update the evaluation of the evidence for efficacy of vitamin D fortification, including recently published data, and to summarize key outcomes and data requirements in the context of new knowledge about recommendations for vitamin D intakes.

Methods

Data searches and study selection. Randomized controlled trials were included with the following criteria: a study population of free-living adults, comparators were foods fortified with cholecalciferol or ergocalciferol with vitamin D-unfortified food or regular diet, and circulating 25(OH)D concentrations as an outcome measure. Searches were conducted up to December 2011. A search strategy was developed in Ovid MEDLINE and was modified for PubMed, CINAHL, Embase, and Cochrane Central Register of Controlled Trials (**Supplemental Table 1**). Duplicates were removed in EndNote and study selection was independently conducted by 2 authors, first by a screen of the titles and abstracts, followed by a review of the full text of potentially relevant papers. In addition, a bibliographic check of all relevant papers was conducted. A record was kept of reasons for excluding studies. Authors were contacted where essential data were missing and studies with insufficient data to analyze the treatment effect compared with a control were eventually excluded.

Data synthesis and quality assessment. Vitamin D intake was quantified in μg and 25(OH)D concentrations were reported as mean ± SD or mean (95% CI) in nmol/L. Habitual dietary intakes of vitamin D were not added to the doses used in the fortified foods as these data were usually not reported in studies. Some data transformations were necessary to enter data into Cochrane Review Manager (http://www.cc-ims.net/revman) for analysis. Where necessary (21–23), SD was calculated from SEM using the formula: SD = SEM × $\sqrt{n}$. Absolute change (mean ± SD) was calculated from baseline and endpoint data where necessary (24–31). The formula used to calculate the SEM of the change using baseline and endpoint data was: SEM = $\sqrt{[(\text{SD of baseline mean}/\sqrt{n}\text{ baseline})^2 + (\text{SD of endpoint mean}/\sqrt{n}\text{ endpoint})^2 - (\text{SD of baseline mean}/\sqrt{n}\text{ baseline}) \times (\text{SD of endpoint mean}/\sqrt{n}\text{ endpoint})]$. In one case (32), absolute change (mean ± SD) was calculated from the percentage of change using the formula: absolute change = (percentage change/100) × baseline mean. This formula was also applied to the 95% CI; CI were then converted to SD using the formula SD = [(top CI – bottom CI) × $\sqrt{n}$]/3.92. The Jadad scale was used to assess the quality of the included studies (33). This instrument assesses the quality of randomized controlled trials in relation to randomization, blinding, and the reporting of withdrawals and dropouts. Scores range from 1 to 5, with scores ≥3 indicating higher quality.

Data analysis. Treatment effects were summarized as the mean difference with 95% CI by using the absolute change values for control and treatment groups. A meta-analysis was carried out with Cochrane software, Review Manager version 4.2 (Cochrane Collaboration) with random-effects analysis to determine the overall weighted mean difference. In addition to forest plots, the presence of statistical heterogeneity was examined using the chi-square statistic with $P < 0.10$ indicating significant heterogeneity. The I^2 statistic was also assessed: an I^2 of 0% indicated no heterogeneity, whereas 25, 50, and 75% were considered low, moderate, and high heterogeneity, respectively (34). Three subgroup analyses stratifying studies on the basis of mean baseline 25(OH)D concentrations ≥ or <50 nmol/L, on latitude (greater than or equal to or <40°), and dose ≥ or <10 μg/d were planned a priori to explore potential differences in treatment effect between the studies. The relation between vitamin D intake from the fortified foods and achieved serum 25(OH)D concentration was determined using a regression model that controlled for study effect.

Results

Results of the literature search. The searches conducted in Ovid MEDLINE, PubMed, CINAHL, Embase, and the Cochrane Central Register of Controlled Trials in November 2011 resulted in 441 papers after duplicate removal. An initial screen of the titles and abstracts reduced the number of potentially relevant papers to 39. After carefully examining these articles, 29 were excluded for various reasons; **Supplemental Figure 1** shows the study selection procedure and reasons for exclusion. A further 5 papers were identified, 3 of which were cited by O'Donnell et al. (20). In total, 15 papers met the inclusion criteria (21–32,35–37). Kruger et al. (27) reported data in 2 distinct population groups in separate

locations (**Table 1**). In studies with 2 treatment arms, either with various calcium concentrations (30), cholecalciferol or ergocalciferol (35), or different breads (22), data were used from the vitamin D intervention arm with its true control only. Foods, doses, and circulating concentrations of 25(OH)D from the 15 publications are presented in **Table 2**.

Study characteristics. There was a high level of variability between the studies, as most were designed to address hypotheses relating to calcium and/or vitamin D fortification and health outcomes as opposed to the impact of fortification on serum 25(OH)D concentrations specifically. Studies that were vitamin D focused accounted for season; 7 were conducted at latitudes ≥40° north (21–23,26,29,35,36). Four studies were conducted for 1 y or more (21,24,25,32).

All studies were conducted in adult populations, as specified by the inclusion criteria. Six studies were conducted in women only (22,24,26–28,31) and 2 in men only (25,32). In 9 studies, mean baseline 25(OH)D concentrations were <50 nmol/L (21–23,27,30,31,35–37). Dietary intake of vitamin D was reported at baseline in 5 studies (22,28,30,31,36) and sunlight exposure was reported in 4 studies (21,29,30,36).

The daily dose of vitamin D in the fortified foods ranged from 3 to 25 μg (per 100 g or serving, or dose achieved from consumption of fortified food): 8 studies used ≤10 μg (21,22,24,26–29,31). Twelve studies used dairy products as a food source (21,24–32,36,37) and orange juice was used in 2 studies (23,35) and bread was used once (22). In 5 studies, the control group continued with their usual diet with no placebo product (24,25,28,31,32).

A range of assay types was used to measure 25(OH)D, including RIA (22,24–26,29,31,32), HPLC (23,30,35,37), competitive protein binding assay (CPBA) (21,36), Roche Elecsys 2010 COBAS system (27), and chemiluminescence immunoassay (28).

Study quality. The method of randomization was reported in 5 studies (23–25,31,35). Seven studies were reported as blinded (21–23,26,29,30,35), but methods for blinding were unclear. An independent dose check for vitamin D concentrations in the fortified foods was reported in 8 studies (21–23,25,30,32,35,37). All studies reported data on dropouts; 3 had a dropout rate of >15% (21,29,36). Five studies scored <3 on the Jadad scale (27,28,32,36,37), and although the remainder achieved a score of ≥3, it should be noted that this scale does not assess compliance, which is an important factor in food-based interventions. The compliance rate was reported in 10 studies (24–28,30–32,35,37). Because compliance fell from ~75% at 1 mo to ~45% by 24 mo in Woo et al. (31), we used data from the 3-mo time point for the purposes of the meta-analysis.

A number of additional quality issues were identified in specific studies. In the study by Natri et al. (22), baseline vitamin D intake was significantly higher in the intervention group compared with the control group (10.8 μg/d compared with 1.8 μg/d). In the studies by Tangpricha et al. (23) and McKenna et al. (29), higher serum 25(OH)D concentrations at baseline were reported in the control group compared with the intervention group; however, a statistical comparison of serum 25(OH)D concentrations was not reported. McKenna et al. (29) did not quantify consumption of the fortified milk; the authors reported that participants were encouraged to drink at least 2 L/wk and reported a mean daily intake of vitamin D from fortified milk of ~3 μg.

Efficacy of interventions. The outcome variable for this review was circulating 25(OH)D concentration. A total of 16 independent studies were included from 15 publications. When combined in a random effects analysis (n = 1513, 767 treated and 746 controls) (**Fig. 1**), the treatment effect was 19.4 nmol/L (13.9, 24.9), corresponding to a 1.2 nmol/L (0.72, 1.68) increase in 25(OH)D for each 1 μg/d ingested [mean serum 25(OH)D (nmol/L) = 1.198 (vitamin D intake) + 2.711; adjusted R^2 = 0.67; $P < 0.001$] (**Fig. 2**). There was a high level of heterogeneity across the 16 studies ($P = < 0.0001$; I^2 = 89%), so the resulting treatment effect should be considered with caution. However, point estimates for all but 2 randomized controlled trials (26,28) showed a significant effect of supplementation on circulating 25(OH)D. There was a significant difference in the decrease in serum 25(OH)D between treatment and control groups in Green et al. (26), who administered 5 μg/d in New Zealand. However, there was no significant difference in the decrease in serum

TABLE 1 Outline summary of 16 selected randomized intervention studies from 15 publications using vitamin D-fortified foods[1]

Reference	Location	Population described	Age (y)	Duration	Season	25(OH)D analysis
(37)	Iran (Tehran, 35° 40′ N)	Diabetic adults	29–67	12 wk	October to March	HPLC
(30)	Iran (Tehran, 35° 40′ N)	Diabetic adults	30–60	12 wk	October to March	HPLC
(35)	USA (Boston, MA, 42° 21′ N)	Healthy adults	18–84	11 wk	End of winter	HPLC
(26)	New Zealand (Dunedin, 45° 52′ S)	Women	18–47	12 wk	January to April	RIA
(27)	Indonesia (Jakarta, 6° 10′ S) Philippines (Manila,14° 36′ N)	Postmenopausal women	>55	16 wk	Not reported	Roche Elecsys 2010
(32)	Australia (Geelong, 38° 9′ S)	Healthy Caucasian men	50–79	1 y	Not reported	RIA
(28)	Greece (Athens, 37° 58′ N)	Postmenopausal women	55–65	5 mo	October to February	Chemiluminescence immunoassay
(31)	China (Hong Kong, 22° 20′ N; Beijing, 39° 55′ N)	Young Chinese women	20–35	3 mo	Recruited February to June	RIA
(22)	Finland (Helsinki, 60° 10′ N)	Healthy women	25–45	3 wk	February to March	RIA
(25)	Australia (Melbourne, 37° 49′ S)	Ambulatory Caucasian men	>50	2 y	Not reported	RIA
(24)	Malaysia (Kuala Lumpur, 3° 7′ N)	Postmenopausal women	55–65	2 y	Not reported	RIA
(23)	USA (Boston, MA, 42° 21′ N)	Healthy adults	22–60	12 wk	Began in March	HPLC
(36)	Netherlands (Wageningen, 51° 58′ N)	Older adults	≥70	17 wk	January to June	CPBA
(21)	Ireland (Dublin, 53° 22′ N)	Older adults	66–91	1 y	April to April	CPBA
(29)	Ireland (Dublin, 53° 22′ N)	Healthy adults	17–54	5 mo	October to March	RIA

[1] CPBA, competitive protein binding assay; N, north; 25(OH)D, 25-hydroxyvitamin D; S, south.

TABLE 2 Foods, doses, and circulating concentrations of 25(OH)D in 16 randomized controlled intervention studies from 15 publications with vitamin D-fortified foods in community-dwelling adults[1]

	Intervention group							Control group					
Reference	Food	Added vitamin D	Added Ca	Baseline 25(OH)D)	Endpoint 25(OH)D	Absolute mean change in 25(OH)D	*n*	Food	Added Ca	Baseline 25(OH)D	Endpoint 25(OH)D	Absolute mean change in 25(OH)D	*n*
		μg/d	*mg*		*nmol/L*				*mg*		*nmol/L*		
(37)	Yogurt drink	25	170	38.5 ± 20.0	72.0 ± 23.5	32.6 ± 18.3	50	Placebo yoghurt drink	170	38.0 ± 22.8	33.4 ± 22.8	−2.7 ± 16.6	50
(30)	Yogurt drink	12.5	150	44.4 ± 28.7	77.7 ± 28.6	33.3 ± 28.7[3]	30	Placebo yoghurt drink	150	41.6 ± 44.5	37.2 ± 44.0	−4.4 ± 44.3[3]	30
(35)	Orange juice	25	350	44.8 ± 27.8	76.8 ± 21.3	32.0 ± 25.3	18	Placebo orange juice	350	49.5 ± 24.0	45.3 ± 16.0	−4.3 ± 14.5	15
(26)	Milk powder	5	0	76.0 ± 32.6	65.0 ± 23.1	−11.0 ± 27.4[3]	32	Placebo milk powder	0	74.0 ± 30.6	53.0 ± 23.8	−21.0 ± 27.3[3]	34
(27) (Indonesia)	Milk powder	9.6	1200	45.1 ± 11.0	57.8 ± 11.4	12.7 ± 11.0[3]	27	Placebo milk powder	54	43.3 ± 11.0	37.2 ± 11.6	−6.2 ± 11.2[3]	29
(27) (Philippines)	Milk powder	9.6	1200	62.0 ± 15.7	86.1 ± 17.0	24.1 ± 16.4[3]	30	Placebo milk powder	54	59.2 ± 15.7	71.0 ± 17.0	11.8 ± 16.4[3]	30
(32)	Milk	20	1000	83.6 ± 32.7	NR	12.8 ± 26.9[4]	44	Usual diet	NA	85.7 ± 40.3	NR	−6.2 ± 19.0[4]	42
(28)	Dairy products	7.5	1200	69.8 ± 22.3	63.5 ± 19.8	−6.25 ± 21.1[3]	39	Usual diet	NA	63.5 ± 22.0	55.3 ± 19.5	−8.3 ± 20.9[3]	36
(31)	Milk powder	5	1000	32.0 ± 11.0	44.0 ± 11.0	12.0 ± 10.7[3]	193	Usual diet	NA	31.0 ± 11.0	36.0 ± 12.0	5.0 ± 11.4[3]	203
(22)	Wheat bread	10	0	29.0 ± 9.9	NR	16.3 ± 21.9	11	Placebo wheat bread	0	27.1 ± 11.1	NR	−0.3 ± 12.0	9
(25)	Milk	20	1000	77.2 ± 22.6	81.5 ± 16.7	4.3 ± 19.5[3]	75	Usual diet	NA	76.1 ± 23.5	64.2 ± 18.2	−13.7 ± 20.5[3]	72
(24)	Skim milk powder	10	1200	69.1 ± 16.1	86.4 ± 22.0	17.3 ± 19.7[3]	91	Usual diet	NA	68.4 ± 15.7	71.2 ± 21.7	2.8 ± 19.4[3]	82
(23)	Orange juice	25	350	37.0 ± 29.9	94.0 ± 74.8	57.0 ± 26.2	14	Placebo orange juice	350	50.0 ± 34.6	73.0 ± 27.7	22.5 ± 17.3	12
(36)	Dairy products	12.5[2]	225	37.0 ± 20.0	NR	35.0 ± 18.0	37	Placebo dairy products	0	36.0 ± 20.0	NR	5.0 ± 9.0	34
(21)	Milk	5	0	24.0 (no SD)	NR	22.3 ± 10.9	24	Placebo milk	0	25.0 (no SD)	NR	6.8 ± 10.9	18
(29)	Milk	3	0	77.0 ± 35.0	62.0 ± 26.0	−15 ± 31.5[3]	52	Placebo milk	0	85.0 ± 39.0	54.0 ± 25.0	−31.0 ± 34.2[3]	50

[1] Data are presented as mean ± SD. NA, not applicable; NR, not reported; 25(OH)D, 25-hydroxyvitamin D.
[2] Dose based on data from de Jong et al. (42).
[3] Calculated from baseline and endpoint data.
[4] Calculated from percentage change.

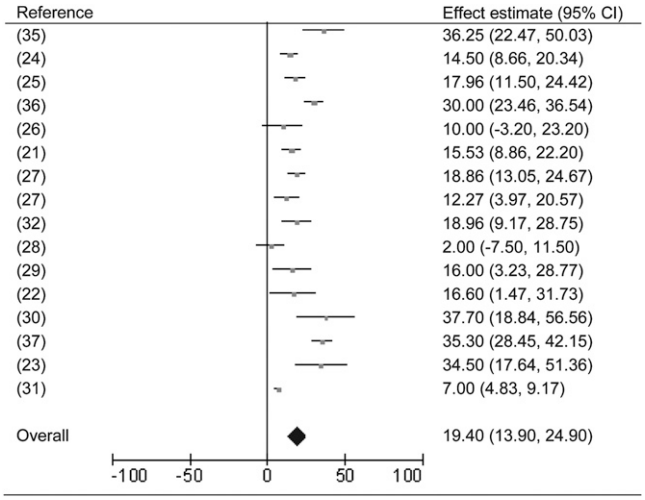

FIGURE 1 Change in circulating 25(OH)D (nmol/L) associated with food fortification with vitamin D. Weighted mean difference in absolute change estimated; mean dose of ~11 μg/d; I^2 (variation in effect size attributable to heterogeneity) = 89%; chi-square statistic P value = 0.00001; n = 16. Values are means (95% CI). 25(OH)D, 25-hydroxyvitamin D.

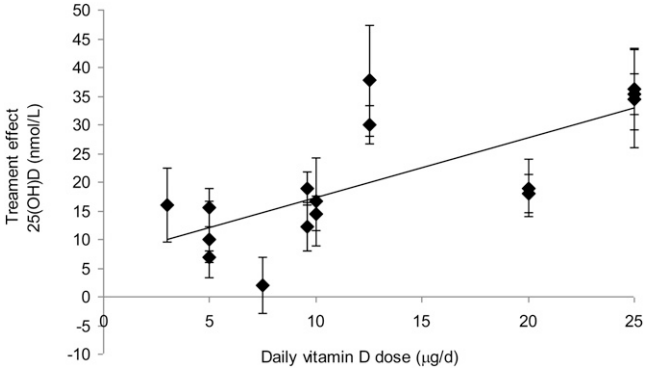

FIGURE 2 Dose-response of 25(OH)D to additional vitamin D at doses between 3 and 25 μg/d delivered in fortified foods in 16 randomized controlled studies. 25(OH)D, 25-hydroxyvitamin D.

25(OH)D between treatment and control groups in the Athens-based study by Manios et al. (28), whose treatment group received 7.5 μg/d.

When combined with latitude, the treatment effect was slightly higher in studies conducted ≥40° compared with those at lower latitude [22.4 (14.8, 30.0) and 17.3 (10.4, 24.3), respectively]. However, heterogeneity remained high at 91% for both. The treatment effect was substantially higher in studies where mean baseline 25(OH)D concentrations were <50 nmol/L compared with those ≥50 nmol/L [24.9 (15.6, 34.1) and 13.6 (9.5, 17.7), respectively]. Heterogeneity was 94% among studies with a mean baseline 25(OH)D <50 nmol/L but was much lower at 35% among studies with a mean baseline 25(OH)D ≥50 nmol/L. When grouped by dose, heterogeneity was 78% in those studies using ≥10 μg/d and 73% in those studies using <10 μg/d. The overall treatment effect was 25.9 (19.3, 32.4), which was substantially higher than for those studies using <10 μg/d [11.6 (6.7, 16.6)]. Studies that used RIA to measure serum 25(OH)D (n = 7) were grouped to investigate whether assay type was a source of additional heterogeneity, but within this group of studies heterogeneity was 83%. The small number of studies overall precluded further analysis of subgroups.

Discussion

On the basis of 16 separate randomized controlled studies from around the world, the current analysis shows that foods fortified with vitamin D increase circulating 25(OH)D concentrations in a dose-dependent manner. When combined in a random effects analysis, the mean increase of 25(OH)D concentrations in the treated compared with control group was 19.4 nmol/L. However, the combined studies demonstrated high statistical heterogeneity and the overall result should be used with caution. This was expected due to the variability in environmental, clinical, and methodological characteristics of the studies, which were conducted for different purposes and at varying latitudes, seasons, durations, doses, and food sources, with or without added calcium, comparing with usual diet or placebo food, and study populations with differing baseline 25(OH)D concentrations. In an attempt to investigate the sources of heterogeneity, a number of subgroup analyses were performed, but in most cases heterogeneity remained high. The treatment effect was substantially higher in studies using ≥10 μg/d, in those conducted at latitudes ≥40°, and where baseline 25(OH)D concentrations were <50 nmol/L.

It was not possible to evaluate the impact of food fortification interventions on reducing the prevalence of inadequate vitamin D intakes and vitamin D deficiency in the populations studied, because most studies were not set up a priori to test this hypothesis. Eight studies were designed to assess the impact of a fortified food on a specific health outcome, of which 6 studies focused on skeletal outcomes (24,25,27,28,31,32). Of these, 4 reported a significant improvement in bone mineral density and/or biochemical indices of bone metabolism in the treatment group (24,25,27,28). Two studies were conducted in participants with type 2 diabetes, both of which found a significant improvement in glycemic status in the treatment group (30,37).

The strength of the current evaluation is that a systematic, thorough search of the literature was undertaken to identify all studies meeting the inclusion criteria for the review. Five of the leading medical and health databases were searched to ensure that all published articles would be found; however, only English language articles were included, leading to the potential exclusion of some relevant studies. All included data were derived from published literature, which potentially introduces publication bias. It should be noted that because most studies were not focused on 25(OH)D concentrations as an outcome measure, it was challenging in some cases to extract complete data for the purpose of this review. The quality of the studies using fortified foods was variable and some standard quality items for validity of randomized controlled trials, including blinding, allocation concealment, and compliance, were inadequately addressed. For example, no studies included in this review reported the methods used for allocation concealment, and evidence suggests that inadequate allocation concealment leads to an overestimation of the treatment effect (38). In addition, blinding methods were unclear in all studies. Compliance was not reported in 5 studies and was extremely low in one (45% at the final time point) (31).

Some key factors specific to vitamin D were unreported in most studies: only 5 studies reported usual dietary intake of vitamin D and 4 reported sunlight exposure, which is particularly important in studies conducted at high latitudes. Two studies conducted at ≥40° did not mention sunlight exposure (26,35) and another 2 studies at this latitude did not report excluding participants who had recent or planned exposure to higher-than-usual levels of sunshine (21,36). Seven studies did not report an independent dose check for vitamin D concentrations in

the fortified foods, raising some quality concerns, because actual vitamin D amounts are often outside the stated fortification range (39). A number of studies lacked essential quality assurance data, such as comparisons between groups in baseline 25(OH)D concentrations and poor clarification of dose intake.

The biomarker used to assess vitamin D status was circulating 25(OH)D concentration (plasma or serum). Circulating 25(OH)D is generally accepted as the most reliable marker of vitamin D exposure, which was confirmed in a recent systematic review of biomarkers of vitamin D status (40). However, circulating 25(OH)D is highly protein bound, making measurement challenging. No standardized assay method is available and results vary depending on the assay and laboratory used. A cross-calibration of the 25(OH)D assays of 5 laboratories showed that the mean 25(OH)D concentration was 80% higher using CPBA compared with HPLC, and RIA gave intermediate values (41). The studies included in this review employed a number of different assay types, including RIA, HPLC, and CPBA, with the majority of studies using RIA ($n = 7$) and HPLC ($n = 4$). For the purpose of this review, the measurements were assumed to be similar enough to warrant combining, despite potential variation due to the different assays and laboratories used in each study. The use of different assay types may have influenced the validity of the study outcomes but did not affect statistical heterogeneity in the current analysis.

Given the gap between the new recommended intakes of vitamin D and typical mean intakes of ~4–7.5 μg/d, depending on the country, sustainable food-based strategies to increase intakes in the population and minimize the prevalence of serum 25(OH)D concentrations <30 nmol/L are urgently required. Dietary advice and supplementation are unlikely to increase intakes across the distribution, because rich food sources of vitamin D are few and infrequently consumed and the proportion of supplement users is relatively low. There is a need for stronger data on the effect of vitamin D-fortified food on circulating 25(OH)D concentrations, deficiency prevention, and potential health benefits. High-quality study design and conduct and transparent reporting are paramount. Basic quality items relating to randomized controlled trials should be clearly reported, including blinding, allocation concealment, and compliance. Factors that may affect the impact of vitamin D-fortified food on 25(OH)D concentrations, such as sun exposure, dietary intake of vitamin D, and baseline 25(OH)D concentrations, should be taken into account and clearly reported. Consideration should be given to the potential confounding generated by the food used, which may include other nutrients, such as calcium and phosphorous, which are relevant to vitamin D metabolism. Ideally, a placebo product should be used for the control group and the vitamin D content of the treatment food should be independently checked to confirm the dose. Careful consideration must be given to the range of products used for fortification and the concentration of vitamin D used in each to optimize the effectiveness and minimize risk of excessive intakes. This can be achieved only by modeling usual food consumption intakes in representative populations and evaluating potential fortification initiatives by carrying out high-quality, food-based, randomized controlled studies in the community that measure the impact on circulating concentrations of 25(OH)D in the population to achieve efficacy without compromising safety.

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