REVIEW ARTICLE

Effect of Vitamin D supplementation and fortification strategies on serum Vitamin D levels: A systematic review and meta-analysis

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Abstract

Background: Meta-analyses on effect of vitamin D intake of both vitamin D interventions (supplementation and fortification) on vitamin D status are lacking. *Aim and Objectives:* This study aimed to evaluate the effect of consuming Vitamin D fortified products and Vitamin D supplements on improving serum 25-hydroxyvitamin D in healthy people. *Material and Methods:* A systematic search was performed in three databases, including PubMed/Medline, Scopus, and Web of Science up to January 2024. The randomized controlled trials that compared the usage of Vitamin D supplements and Vitamin D-fortified products on levels of serum 25-hydroxyvitamin D were eligible for inclusion in this study. Studies that provided insufficient outcomes were excluded. *Results*: Meta-analysis was conducted using a random-effect model. Subgroup analysis was used to identify the sources of heterogeneity. The initial search strategy yielded 1,747 studies. After removing duplicates, a total number of six eligible articles were included in the meta-analysis. Both Vitamin D supplementation [WMD: 35.67; 95% CI: (25.52, 45.82); $P < 0.001$; ($I^2 = 96.4\%$, $P_{heterogeneity} < 0.001$) and Vitamin D fortification [WMD: 34.84; 95% CI: (25.91, 43.78); $P < 0.001$; ($I^2 = 98.4\%$, $P_{heterogeneity} < 0.001$)] significantly increased serum 25-hydroxyvitamin D levels. The results did not change based on the study location, duration, type of vehicle, dose of the fortification, gender, and number of participants' subgroups. *Conclusion:* Both Vitamin D fortification and Vitamin D supplementation can significantly increase 25-hydroxyvitamin D concentration. Policymakers might use these findings to tackle Vitamin D deficiency through supplementation and food fortification strategy.

Keywords: Vitamin D, Deficiency, Supplementation, Food fortification, Meta-analysis

Introduction

Vitamin D is an essential nutrient for calcium absorption and bone health [1]. According to the US Institute of Medicine (IOM) serum 25-hydroxyvitamin D[25(OH)D] levels < 30 nmol/l(12 ng/mL) is considered as Vitamin D deficiency. Recently published study reported global prevalence of Vitamin D deficiency as 15.7% [2-3]. In some countries, such as India, the prevalence of VDD was more than 90% in adolescence . Due to the limited availability of natural food sources rich in Vitamin D, such as fish, egg yolk, and some wild mushrooms, as well as low dermal synthesis of Vitamin D from exposure to sunlight year-round [1], therapeutic supplementation and food

fortification are the commonly used strategies for improving Vitamin D status [4-5]. Having a healthier lifestyle with more outdoor activities and optimal nutrition definitely improve Vitamin D status but will not eliminate Vitamin D deficiency. Sunlight exposure must be balanced with regard to potential negative consequences, such as risk of developing skin cancer [6]. Although the use of supplementation strategy was considered to improve Vitamin D status, particularly in high-risk subgroups, there are several related challenges such as ensuring population coverage, adherence, cost-effectiveness, regular usage, sustainability and risk for overdosing. Systematic Vitamin D food fortification is, however, an effective approach to improve Vitamin D status in the general population, and is recognized as a cost-effective nutrition intervention, especially for low- and, middle-income countries [6-7]. In this regard, the main challenges to the implementation of these strategies include uncertainty about the ideal dosage, as well as timing and duration of interventions for various population groups. Furthermore, due to the lack of a single guideline for each country, it is difficult to compare the effectiveness of interventions [8]. This study aimed to evaluate the effect of consuming vitamin D fortified products and vitamin D supplements on improving serum 25-hydroxyvitamin D in healthy people.

Method and Materials

Search strategy

The present study was designed and reported based on the Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA) guideline. The protocol of the study was approved by the deputy for research at Tabriz University of Medical Sciences, Tabriz, Iran (reference number: IR.TBZMED.REC.1400.653). To identify the relevant articles, a systematic search was carried out in such electronic databases as Scopus, PubMed/Medline, Web of Science, from inception up to January 24, 2024 using the following search strategy:
("Vitamin D" OR "Ergosterol" OR "Cholecalciferol") OR ("Vitamin d"[Title/Abstract]) OR ("cholecalciferol"[Title/Abstract]) OR ("hydroxycholecalciferol"[Title/Abstract]) OR (ergocalciferol[Title/Abstract]) OR ("25-hydroxyvitamin D2" [Title/Abstract]) OR ("Vitamin D3"[Title/Abstract]) OR ("hydroxycholecalciferol" [Title/Abstract]) OR ("calcifediol"[Title/ Abstract]) OR (calcidiol[Title/Abstract]) OR ("25-hydroxyvitamin d"[Title/Abstract]) OR (25(OH)D [Title/Abstract]) OR ("1,25-dihydroxyvitamin D"[Title/Abstract]) OR (calcitriol[Title/Abstract]) OR (25-hydroxycholecalciferol[Title/Abstract]) OR (25-OH Vitamin D3[Title/Abstract]) AND ("randomized controlled trial"[Publication Type]) OR ("controlled clinical trial"[Publication Type]) OR (random*[Title/Abstract]) OR (group*[Title/Abstract]) OR ("clinical trial"[Title/Abstract]) OR ("randomized controlled trial"[Title/Abstract]) OR ("controlled clinical trial"[Title/Abstract]) OR ("placebo"[Title/Abstract])) AND (oral[Title/Abstract]) OR (supplement*[Title/Abstract]) OR ("Mouth")AND (fortif*[Title/Abstract]) OR ("Food, Fortified") OR "Biofortification"). Wildcard term "*" was used to promote more sensitive search.

Study selection

The inclusion criteria were: randomized controlled trials (RCTs) with a quasi, parallel, or cross-over design, studies focusing on simultaneous review of Vitamin D supplementations and Vitamin D fortified foods, studies with adequate information to compare the interventions at the baseline and

end of the trial, and studies with a control group. The exclusion criteria were: *in-vitro* and *in-vivo* studies, animal studies, duplicate, observational studies, reviews, letters, conference abstracts or case-reports, studies with Vitamin D supplementation along with another ingredient, and studies including pregnant and lactating mothers (Table 3).

Data extraction

After excluding all the irrelevant articles, the full text of the included articles was reviewed. Two investigators retrieved the required data using standard extraction table independently (BA and SK) and any discrepancy was solved by the third author (MA). The following information was extracted: 1) author's name, 2) publication date, 3) study location, 4) sample size, 5) study design, 6) mean age, 7) dosage, 8) duration of Vitamin D interventions, 9) study participants in Vitamin D supplementation, fortification, and control groups, and 10) change in mean ± standard deviation before and after interventions in both intervention and placebo groups.

Quality assessment

The included studies were evaluated for methodologic quality by two investigators (BA and RKH) independently. Cochrane Collaboration's tool for assessing risk of bias was employed to assess quality of selected studies ' [9]. Based on the recommendations of the Cochrane Handbook, the included studies were rated as high, low, and unclear risk of bias.

Statistical analysis

Statistical analysis was conducted employing random-effects model via restricted maximum likelihood method. Chi-square (Q) and I^2 statistics were used to estimate heterogeneity between studies [10], so that $I^2 > 50\%$ indicated a high heterogeneity. We calculated the mean changes in Standard Deviation (SD) before and after (baseline serum vitamin D levels, final serum vitamin D levels) the intervention in Vitamin D supplementation, fortification, and control groups to estimate the effect size with a 95% Confidence Interval (CI). In cases where means ± SD of studies were reported as figures, data were converted to numerical values via Graph Digitizer software. Since the number of included studies in the meta-analysis was less than 10, only subgroup analysis was applied to evaluate potential heterogeneity sources. Prespecified subgroup analysis was performed to report the effect sizes across Vitamin D dosage, study location, gender, treatment duration, and fortification vehicles. A sensitivity analysis was conducted by leaving out one study at a time to investigate the influence of any individual study on the outcome. To investigate the small study effect, Begg's adjusted rank correlation tests were used. Furthermore, publication bias was assessed by visual inspection of funnel plot. In case of the presence of small study effect or publication bias, trim-and-fill analysis was performed. After adjustment for multiple comparisons where applicable, $p < 0.05$ was considered as significant. All statistical analysis was performed using STATA 17.0.

Results

Study selection

Out of a total of 1,747 relevant articles identified in the initial search, 957 duplicates were removed.The 790 remaining records were screened, and 770 articles were excluded due to following reasons: unrelated title and abstract (n=689), animal studies (n=53), review studies (n=28). Consequently, 20 relevant publications remained for full-text evaluation. Fourteen studies

were excluded because of a lack of required data reporting and other necessary information as outlined in inclusion/exclusion criteria. Ultimately, six articles met the eligibility criteria and were included in the meta-analysis (Figure 1).

Study characteristics

The characteristics of included studies are presented in Table 1. All six studies reported the impact of Vitamin D supplementation and Vitamin D fortification in improving 25(OH)D concentration [11-16]. Sample sizes ranged from 7 to 32 participants (total sample size in intervention group: 146, control group: 132).

Due to the difference in the type of intervention, we included 2-effect size in the meta-analysis of the supplementation group for the studies by Itkonen *et al*., (2016) and Wagner *et al*., (2008) and in the fortification group for studies by Natri *et al*., (2006) and Wagner *et al*., (2008) [12, 14, 16]. The dosage of the Vitamin D interventions was 400-4000 International Units (IUs). Also, only one trial reported the use of ergocalciferol (Vitamin D2) [12], and five trials used cholecalciferol (Vitamin D3). The type of intervention across the included studies was a combination of supplementation and fortified foods with a control group. Initial and final serum 25(OH)D concentration was reported in all the six trials. The Vitamin D delivery vehicle was different, so that three trials used bread and three trials used canola oil, cheese, and yogurt. The duration of the intervention ranged from 3 to 8 weeks. While two trials included only women, four trials included both men and women (Table 2).

Quality of studies

The results of quality assessment of the included articles were reported according to the Cochrane Collaboration's risk of bias tool (Table 3). Random allocation and proper blinding were observed in three and six studies, respectively. There was no selective reporting bias in the included articles.

Intervention efficacy and subgroup analyses

Impact of Vitamin D supplementation in improving serum 25-hydroxyvitamin D levels

The results of meta-analysis showed that Vitamin D supplementation had an increasing effect on 25(OH)D levels. The serum 25(OH)D weighted mean difference from the random-effects analysis was 35.67 nmol/L [WMD: 35.67 nmol/L, 95% CI: 25.52, 45.82; $p < 0.01$] (Figure 2A). High between-study heterogeneity was observed due to the large variation in the study designs (I^2= 96.4%, $p <$ 0.001). Study location, dosage, and duration were recognized as the sources of heterogeneity following subgroup analysis (Table 4). Subgroup analysis showed significant effects of Vitamin D supplementation on improving 25(OH)D levels in dosages of 10, 25, and 100 nmol/l in all the studied countries, as well as in females and both gender subgroups. However, using sensitivity analysis through removing each of the studies conducted by Itkonen *et al*., (2016), Natri *et al*., (2006), Nikooyeh *et al*., (2016), Nikooyeh *et al*., (2023), Ghasemifard *et al*., (2022) and Wagner *et al*., (2008) the overall results of the meta-analysis did not change ($p < 0.05$) [11-16]. No significant small study effect was observed between studies using Begg's test (p = 0.386). In addition, visual inspection of funnel plot showed that there was a between-study publication bias (Figure 2B). Due to the presence of asymmetry in funnel plot, trim-and-fill analysis was performed, and the use of imputed effect size (in two studies) decreased the asymmetry observed in the funnel plot (WMD: 29.51; 95% CI: 14.56, 44.46; $p < 0.05$).

Impacts of Vitamin D food fortification in improving serum 25-hydroxyvitamin D levels

The results of meta-analysis revealed that Vitamin D food fortification had a significant effect on improving 25(OH)D levels [WMD= 34.84 nmol/l; 95% CI: 25.91, 43.78; $p < 0.001$] (Figure 2C). Between-study heterogeneity was significant (I^2= 98.4%, $p < 0.001$) where study location, dosage, duration, and vehicle type were recognized as the source of heterogeneity following subgroup analysis (Table 4). Subgroup analysis revealed that bread, cheese, canola oil, and yogurt, as Vitamin D fortification vehicles in dosages of 10, 25, and 100 nmol/l, had a greater effect in increasing 25(OH)D levels in all the countries studied, as well as in both gender subgroups. There was no significant small study effect on the results using Begg's model (p = 0.1). However, visual inspection of funnel plot revealed that there was a between-study publication bias (Figure 2D). Interestingly, after performing trim-and-fill analysis, the overall results were statistically significant (WMD= 35.68 µmol/l; 95% CI: 18.67, 52.69; p <0.05).

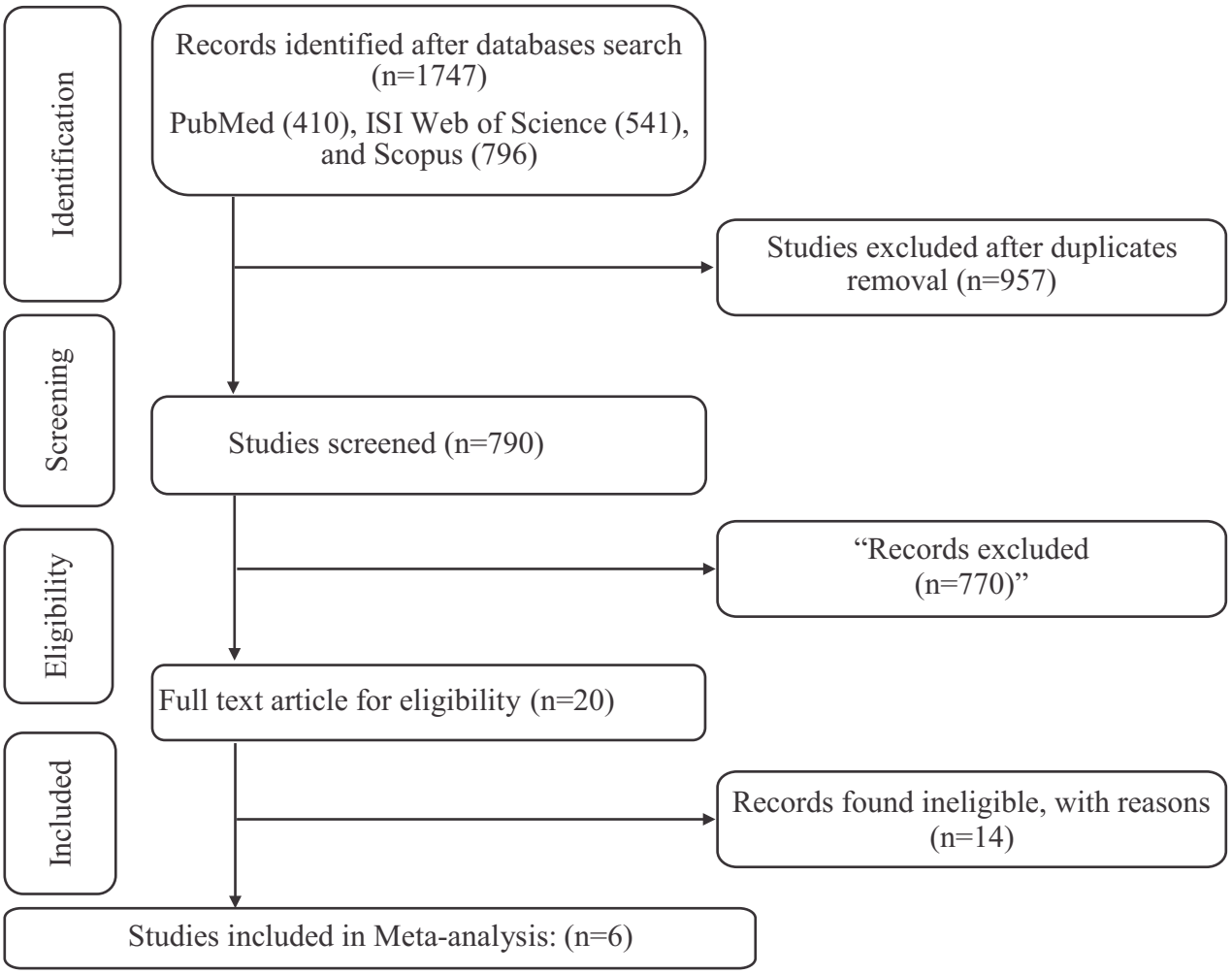

Figure 1: Flow chart of study selection for inclusion trials in the meta-analysis

Table 1: Characteristics of the included studies in the meta-analysis

Author	Location	Gender	Inter-vention number	Control number	Dosage (micro-gram)	Duration/ week	Vehicle	Intervention type	Mean age in intervention group	Mean age in placebo group
Itkonen *et al*. [22] (2016)	Finland	F	9	7	25	8		Vitamin D2 supplementation	25.6 ± 4.2	24.9 ± 4.8
			8	7				Vitamin D3 supplementation	30.8 ± 3.7	24.9 ± 4.8
			9	7			Bread	Fortification	25.6 ± 4.2	27.6 ± 6
Natri *et al*. [24] (2005)	Finland	F	11	9	10	3		Vitamin D3 supplementation	31.1 ± 1.8	29.0 ± 1.7
			11	9			Wheat	Fortification	28.8 ± 1.8	29.0 ± 1.7
			10	9			Rye	Fortification	27.3 ± 6 0.6	29.0 ± 1.7
Nikooyeh *et al*. [25] (2016)	Iran	F/M	30	30	25	8		Vitamin D3 supplementation	37.3 ± 10.9	39.4 ± 11.6
			30	30				Fortification	37.2 ± 10.5	39.4 ± 11.6
Wagner *et al*. [26] (2008)	Canada	F/M	20	10	100	8		Vitamin D3 supplementation	23.6 ± 6 3.5	24.6 ± 4.5
			20	20			Cheddar cheese	Fortification	23.6 ± 6 3.5	24.6 ± 4.5
			10	20			Low-fat cheddar cheese	Fortification	30.6 ± 11.7	24.6 ± 4.5
Ghase-mifard *et al*. [23] (2022)	Iran	F/M	31	32	25	12		Vitamin D3 supplementation	25.7 ± 3.88	25.60 ± 3.99
			30	32			Canola oil	Fortification	24.8 ± 3.75	25.60 ± 3.99
Nikooyeh *et al*. [21] (2023)	Iran	F/M	27	27	25	8		Vitamin D3 supplementation	41.0 ± 7.6	40.9 ± 10.1
			27	27			Yogurt	Fortification	45.0 ± 5.6	44.1 ± 6.6

Table 2: Description of interventions and total S-25(OH)D[1] concentrations in six randomized placebo-controlled trials in healthy adults

Reference	Intervention type	[2]Mean-25(OH)D baseline	[3]SD-25(OH)D baseline	Mean-25(OH)D baseline	SD-25(OH)D baseline	Mean-25(OH)D Final	SD-25OH)D Final	Mean-25OH)D Final	SD-25OH)D Final
		Intervention		Placebo		Intervention		Placebo	
Itkonen *et al*., (2016)	**Fortification**	63.5	11.3	66.2	18.6	51.76	2.47	23.2	3.48
		66.3	14.8	66.2	18.6	68.8	3.18	23.2	3.48
Natri *et al.,* (2006)		29.6	2.6	27.1	3.7	50.07	1.63	27.18	2.9
Nikooyeh *et al.,* (2016)		35	38.7	34.7	30.5	63.9	31	24.5	21.8
Wagner *et al*., (2008)		55.7	21.2	57.6	28.7	111.36	8.49	50.37	5.84
		53.4	40.1	57.6	28.7	111.36	8.49	50.37	5.84
Ghasemifard *et al.*, (2022)		30.24	14.29	31.62	14.32	35.27	12.6	31.01	11.41
Nikooyeh *et al.,* (2023)		31.8	18.9	33.2	27.8	62.3	20.8	25.7	21.8
Itkonen *et al*., (2016)	**Supplementation**	64.6	15.1	66.2	18.6	51.76	2.47	23.2	3.48
Natri *et al.,* (2006)		29	3	27.1	3.7	44.73	3.66	27.18	2.9
		28.9	3.5	27.1	3.7	46.33	2.85	27.18	2.9
Nikooyeh *et al.,* (2016)		33.9	21.9	34.7	30.5	72.9	23.1	25.4	21.8
Wagner *et al*., (2008)		50.7	18.9	52.4	22.7	116.13	6.36	50.37	5.84
		57.5	18.4	52.4	22.7	126.21	9.01	50.37	5.84
Ghasemifard *et al.*, (2022)		30.8	10.66	31.62	14.32	33.65	10.28	31.1	11.41
Nikooyeh *et al.,* (2023)		39.7	13.6	36.9	23.5	70.6	19.2	32.9	25.2

Table 3: Results of quality assessment of six included studies showed using Cochrane Collaboration's risk of bias tool

	Random sequence generation	Allocation concealment	Blinding of participants and Researchers	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Itkonen *et al*., (2016)	+	?	+	?	+	+	?
Natri *et al.,* (2006)	+	?	+	+	?	+	?
Wagner *et al*., (2008)	+	+	+	+	+	+	+
Ghasemifard *et al.*, (2022)	+	+	+	+	+	+	?
Nikooyeh *et al.,* (2016)	+	+	+	?	?	+	+
Nikooyeh *et al.,* (2023)	+	?	+	?	?	+	?

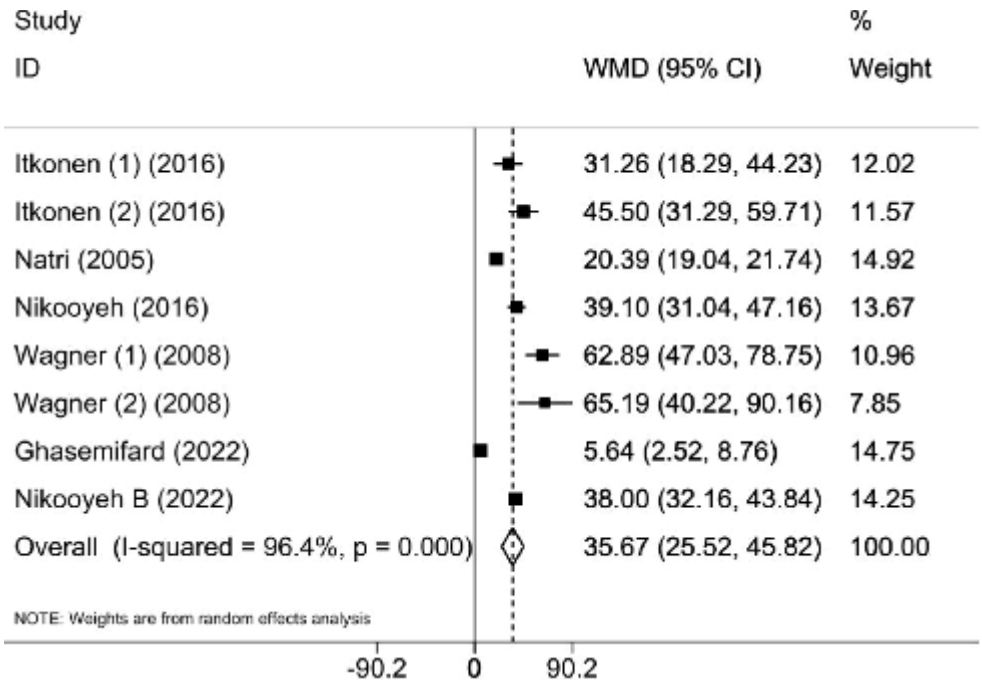

Figure 2A: Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) of the effect of vitamin D supplementation strategy on S- 25(OH)D

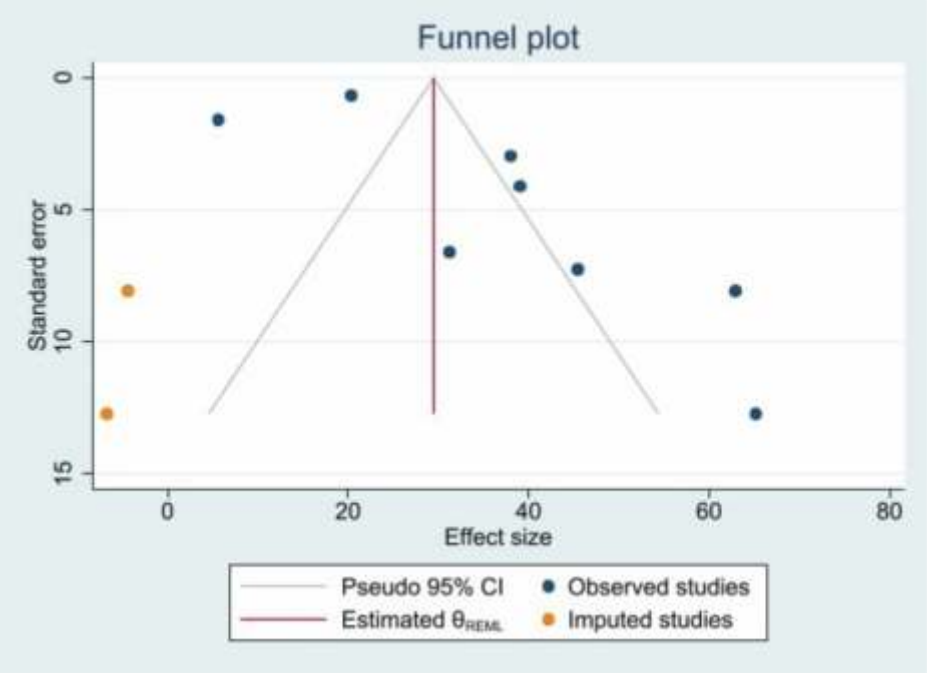

Figure 2B: Funnel plot of effect of Vitamin D supplementation strategy on serum 25(OH)D

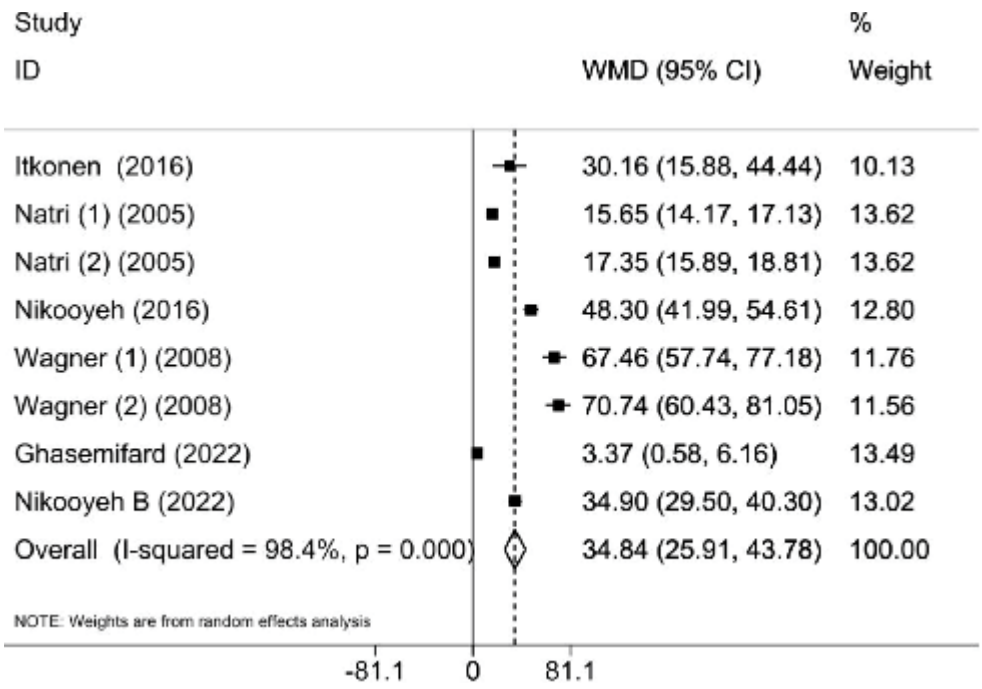

Figure 2C: Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) of the effect of vitamin D fortification strategy on S- 25(OH)D

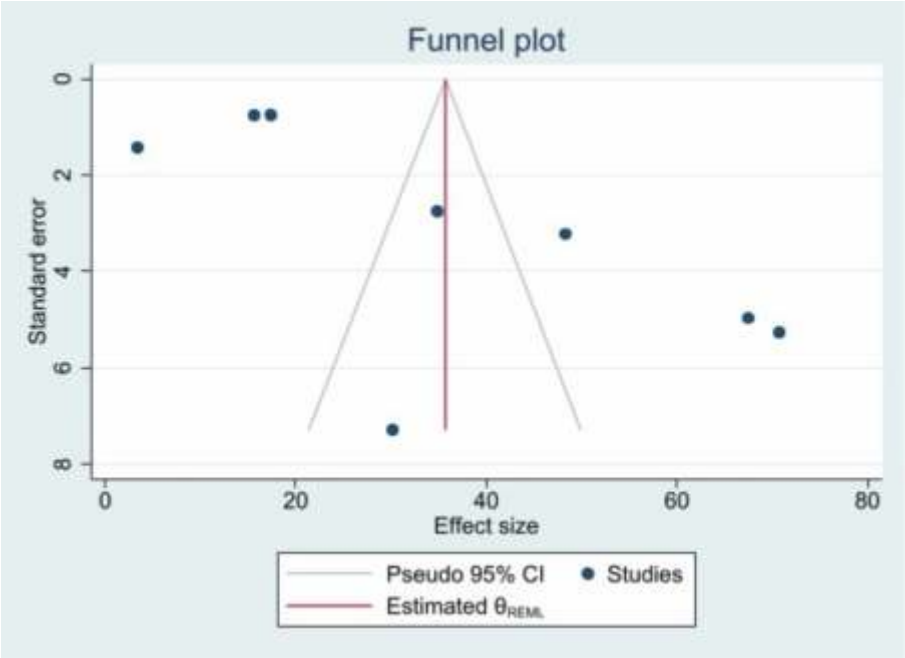

Figure 2D: Funnel plot of effect of Vitamin D fortification strategy on serum 25(OH)D

Table 4: Subgroup analyses of vitamin D supplementation and fortification on 25(OH)D

	Number of effect sizes	[1]WMD (95%CI)	*p*	Heterogeneity	
				P heterogeneity	I^2
Vitamin D supplementation					
Overall effect	8	35.67(25.52, 45.82)	<0.001	<0.001	96.4%
Study location					
Finland	3	31.15(15.96, 46.33)	<0.001	0.001	86.2%
Iran	3	27.42(2.37, 52.46)	0.032	<0.001	98.4%
Canada	2	63.55(50.16, 76.94)	<0.001	0.87	0.00%
Dose (µg/day)					
10	1	20.39(19.04, 21.73)	<0.001	<0.001	0.0%
25	5	31.56(12.49, 50.62)	<0.001	<0.001	97.3%
100	2	63.55(50.16, 76.94)	<0.001	0.87	0.0%
Sex					
Both	5	-11.26(-21.9, -0.63)	<0.001	0.001	97.7%
Female	3	-13.80(-24.99, -2.62)	<0.001	<0.001	86.2%
Vitamin D food fortification					
Overall effect	8	34.84(25.91, 43.78)	<0.001	<0.001	98.4.2%
Study location					
Finland	3	16.81(14.54, 19.13)	<0.001	0.048	67.0%
Iran	3	28.75(-0.12, 57.63)	0.05	<0.001	99.1%
Canada	2	69.00(61.93, 76.07)	<0.001	0.65	0.0%
Dose (µg/day)					
10	2	16.50(14.84, 18.17)	<0.001	0.1	61.1%
25	4	29.08(4.62, 53.53)	0.02	<0.001	98.7%
100	2	69.00(61.93, 67.07)	<0.001	0.65	0.00%
Vehicle					
Bread	4	26.51(18.87, 34.15)	<0.001	<0.001	97%
Cheese	2	69.00(61.93, 76.07)	<0.001	0.65	0.0%
Canola oil	1	3.37(0.58, 6.15)	0.018	<0.001	0.0%
Yogurt	1	34.90(29.50, 40.29)	<0.001	<0.001	0.0%
Sex					
Both	5	44.71(17.80, 71.62)	<0.001	<0.001	99.1%
Female	3	16.84 (14.54, 19.13)	0.280	0.048	67%

[1]WMD; Weighed Mean Difference, I^2; chi square.

Discussion

This is the first meta-analysis comparing the effects of Vitamin D supplementation and fortification on improving the serum 25(OH)D concentration. Based on the results reported in the included studies, Vitamin D supplementation revealed a significant positive relationship in increasing the serum 25(OH)D concentration. Moreover, subgroup analysis indicated a significant increasing effect of the Vitamin D supplementation in dosages of 10, 25, and 100 nmol/l. In addition, Vitamin D fortification had a significant positive relationship with serum 25(OH)D concentration. Subgroup analysis by type of vehicle and sex showed a significant positive correlation in bread, cheese, canola oil, and yogurt regardless of country and sex in the dosages of 10, 25, and 100 nmol/l.

Previous review studies conducted by Balachandar *et al.*, explored the relative efficacy of vitamin D2 and vitamin D3 on improving the serum concentration of vitamin D metabolites and indicators on 1277 healthy human. The results of study showed vitamin D3 intervention was more efficacious in raising serum vitamin D levels and regulating PTH levelscompared to vitamin D2, irrespective of the dosage and vehicle of supplementation [17]. Gallagher *et al*., (2013) supplemented a smaller dose of Vitamin D (400-800 IU/d) in comparison with medium and higher dosages and found improving plasma 25(OH)D concentration in obese women [18]. Bischoff-Ferrari *et al*., (2012) showed that oral supplementation of (20 µg) Vitamin D promoted the level of 25(OH)D and demonstrated beneficial effects on innate immune function, systolic blood pressure, and lower extremity function [19]. Based on the study by Close *et al*., (2013) supplementation of 20000 and 40000 IU Vitamin D over a six-week period led to high plasma 25(OH)D concentration above 50 nmol/l [20]. A study performed by Garg et al., showed that Supplementing 60,000 IU of vitamin D3 per week for 4–8 weeks, followed by 600 IU daily through fortified milk, is an effective approach for improving vitamin D status in Indian adolescents [4]. Another study that investigate impact of consumption of vitamin D fortified food on S.25(OH)D levels in Indian school children showed vitamin D fortification of milk was an effective strategy in improving vitamin D status in children aged 10–14 years [5]. Study performed by Giri *et al.*, showed intake of vitamin D supplement (60,000 IU) with a weekly dose for two months may be improve serum vitamin D among laboratory personnel in Nepal [21]. The study by Itkonen *et al*., (2016) showed that Vitamin D3 supplementation was more effective than D2 in increasing serum 25(OH)D concentration [12].

Vitamin D status is usually assessed by serum 25(OH)D levels. 25(OH)D circulates in the body by different mechanisms. Up to 85 percent of 25(OH)D is bound to Vitamin D binding protein, 10-15 percent of Vitamin D is carried by albumin, and very small fraction of 25(OH)D is unbounded [22]. In a review study conducted by Al-Azzawi et al., it was emphasized that vitamin D3 is essential in many physiological possess and general health. The function of vitamin D3 included regulating of calcium levels, supporting bone strength, and relation with immune system and chronic disease [23]. In cytosolic genomic pathway, Vitamin D Receptors (VDR) are the targets of calcitriol. At first, it increases the phosphorylation of VDR and then VDR form heterodimerization with Retinoid-X Receptor (RXR). Finally, it translocates in the nuclear complex. This complex (VDR-Calcitriol-RXR) binds to Vitamin D Response Element Proteins (VDRE-P) to target genes for activating

coactivator or corepressor to express mRNA and regulate target genes and a variety of genes functions. The functions regulated by Vitamin D are phosphorous and calcium metabolisms. Moreover, adaptor proteins, which play a role in autophagy in the same mechanisms, can activate VDR-RXR complex to target genes in hepatic stellate cells. In addition, Vitamin D in non-gene related mechanisms binds to VDR of membrane and induces changes in signaling pathway of cells, including Mitogen Activated Protein Kinase (MAPK) and calcium. It interacts with protein-protein function of certain phenotypes in the intracellular signaling [23-25]. Some previous studies found that Vitamin D deficiency led to cardiovascular diseases. Observational studies showed endothelial dysfunction in case of insufficiency of Vitamin D concentration [26]. Also, low serum Vitamin D was considered in patients with type 2 diabetes which is associated with diabetic nephropathy [27]. Vitamin D deficiency elevated morbidity and mortality from critical illness [28]. In addition, Vitamin D plays important roles in regulating innate and adaptive immune system. Furthermore, VDR in gut epithelium showed protective effects on the mucosal barrier keeping integrity and regulating innate immune [29]. The exogenous source of Vitamin D is obtained through diet, fortification of foods, and supplementation. The richest sources of Vitamin D are eggs, fatty fish, liver, and sun exposed mushrooms. However, other way for including Vitamin D in human food chain is fortification. It usually occur by using ergocalciferol (vegetables sources) and cholecalciferol such as butter, margarine, milk, and breakfast cereals. Fortification makes these foods richest sources of Vitamin D [30].

The main strengths of this study include: this is the first meta-analysis comparing the effects of fortification and supplementation of Vitamin D where we included the subgroup analysis performed by countries and vehicles of Vitamin D, and no small study effect was demonstrated on the Begg's analysis. The main limitations of this study included: we witnessed some publication bias on the funnel plots, there was high heterogeneity between studies, and the studies had been performed on different dosages and vehicles having potential effects on the results.

Conclusion

In summary, both Vitamin D fortification and supplementation have significant effect on serum 25(OH)D levels. Vitamin D fortification vehicles including bread, cheese, canola oil, and yogurt, showed important effects on 25(OH)D levels in both genders. In addition, Vitamin D supplementation demonstrated a significant increasing effect on 25(OH)D concentration regardless of location, gender, and dosage. It appears that supplementation with 10, 25, and 100 nmol /d Vitamin D is helpful to reach the minimum serum 25(OH)D levels but we could not definitely recommend the best dose of Vitamin D supplementation by this review.

Limitations

The sample size of the current meta-analysis was small considering the sample size of the included studies. We suggest to conduct well-designed studies with a large sample size in the future."

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