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# Vitamin D Food Fortification is an Effective Approach for Improving Vitamin D Status; A Systematic Review

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#### ARTICLE INFO

# SYSTEMATIC REVIEW

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#### **ABSTRACT**

**Background:** Low vitamin D (vit D) serum levels have been linked to various diseases such as multiple sclerosis, cardiovascular diseases, and diabetes. Maintenance of optimal vit D level should be supported by foods and supplements. Limited food sources of vit D made researchers focus on food fortification in recent years. Methods: A systematic review of randomized controlled trials reporting the impact of vit D food fortification on 25-hydroxy vitamin D [25(OH)D] level was conducted on PubMed, Scopus, ISI Web of Science and Google Scholar from inception up to August 2017. Results: Sixteen studies met the inclusion criteria and most of them (n =14) have represented the significant effect of food fortification on the improvement of vit D status of the participants. However, two studies failed to find any reasonable associations. Furthermore, it was observed that vit D food fortification has a greater impact on the people with lower 25(OH)D serum levels. Conclusions: Fortification of foods with vit D has a significant impact on serum 25(OH)D concentrations. Therefore, food enrichment is an economical and effective approach for different populations to prevent vit D deficiency.

**Keywords:** Vitamin D; 25-hydroxy vitamin D; 1, 25 dihydroxy vitamin D; Food fortification.

#### Introduction

Vitamin D (vit D) belongs to the class of fatsoluble steroids that is categorized as two forms; cholecalciferol (vit D<sub>3</sub>) and ergocalciferol (vit D<sub>2</sub>) which are derived from animal and plant sources, respectively (Holick, 2007, Jolfaie *et al.*, 2016). Although sunlight exposure is the main source of vit D (Baeke *et al.*, 2007, Singh *et al.*, 2011), access to other sources of vit D through diet is also possible (Holick, 2007, Spiro and Buttriss, 2014).

Natural Sunlight exposure (UV light of wavelength 290-315 nm) can penetrate the epidermis and photolysis 7-dehydrocholesterol to previtamin D<sub>3</sub>, which is metabolized in the liver after its entrance. Vit D<sub>3</sub> is then converted to 25-hydroxy vitamin D [25(OH)D] by 25-hydroxylases in the liver. Subsequently, 1, 25-dihydroxy vitamin D [1,25(OH)2D] is made in the kidneys by 1-hydroxylation of 25(OH)D (Bauer *et al.*, 2013, Spiro and Buttriss, 2014). Although 1,25(OH)2D is the

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active metabolite of vit D, the plasma concentration of 25(OH)D is the best clinical indicator of vit D status (Runia *et al.*, 2012).

Vit D deficiency is a worldwide concern and presents when serum 25(OH)D levels are below 50 nmol/L (20 ng/ml) (Christodoulou *et al.*). Dark pigmentation, lack of sufficient sun exposure, wearing clothes covering most of the body, sunscreen use, air pollution, and aging are known as common causes of vit D deficiency (Holick, 2002, Nicolaidou *et al.*, 2006, O'Riordan *et al.*, 2008, Sloka *et al.*, 2009, van der Meer *et al.*, 2006). Additionally, vegetarians and lactose intolerant people are highly at the risk of vit D deficiency (Alharbi and El-Sohemy, 2017, Outila, 2001).

Vit D deficiency adversely affects the health, leading to increased risk for several diseases including rickets in children or osteoporosis in adults, cardiovascular diseases, diabetes, cancer, and autoimmune diseases such as multiple sclerosis (Garland *et al.*, 2006, Garland *et al.*, 2009, Kriegel *et al.*, 2011, Norman *et al.*, 1974, Papandreou and Hamid, 2015, Pierrot-Deseilligny and Souberbielle, 2013, Wagner and Greer, 2008). Therefore, sufficient vit D status plays a major role in the prevention and treatment of various diseases (Holick, 2004, Holick *et al.*, 2011, Jolfaie *et al.*, 2016).

Several studies have suggested that vit D supplementation can increase circulating serum 25(OH)D levels (Björkman *et al.*, 2009, Havens *et al.*, 2012, Todd *et al.*, 2015). In addition to vit D supplementation, food fortification would be advisable to compensate vit D deficiency. Today, food fortification is considered to be a common strategy to improve and combat vit D deficiency. Vit D food fortification is an important and inexpensive strategy to certify the adequacy of vit D intake among populations (Biancuzzo *et al.*, 2010a, Calvo *et al.*, 2004, Upreti *et al.*, 2002).

Vit D3 is typically added to foods such as cheese and yogurt (Al-Khalidi *et al.*, 2015, Green *et al.*, 2010, Johnson *et al.*, 2005, Levinson *et al.*, 2016, Madsen *et al.*, 2013, Neyestani *et al.*, 2012, Rich-Edwards *et al.*, 2011, Wagner *et al.*, 2008), orange

juice (Biancuzzo et al., 2010b, Tangpricha et al., 2003, Tripkovic et al., 2017), bread (Itkonen et al., 2016, Natri et al., 2006, Nikooyeh et al., 2016) and mushrooms (Mehrotra et al., 2014, Urbain et al., 2011). The efficiency of vit D2 as a fortificant in raising and maintaining blood concentrations of 25(OH)D is not yet definitively proven (Itkonen et al., 2016, Mehrotra et al., 2014, Urbain et al., 2011). Due to the concern about the changes in cooking and preparation of foods, studies have used several fortification methods including the use of ultraviolet radiation and water-soluble vit D (Biancuzzo et al., 2010b, Mehrotra et al., 2014, Natri et al., 2006, Urbain et al., 2011).

The main purpose of the present systematic review was to evaluate the findings from available studies regarding the effect of food fortification with vit D on serum 25(OH)D levels.

#### **Materials and Methods**

Search strategy: A systematic search was performed among the published studies in the following electronic databases: **PubMed** (www.pubmed.com), Scopus (www.scopus.com), ISI Web of Science (www.isiknowledge.com) and Google Scholar (www.scholar.google.com) from inception up to August 2017. The search was done without restriction on language or publication year, using the combined MeSH and non-MesSH terms as follows: (vitamin D, vitamin D2, vitamin D3, vitamin-D2, vitamin-D3, 25-hydroxy vitamin D, 25hydroxyvitamin D2, 25-hydroxyvitamin D3, 1,25dihydroxy vitamin D, 1,25-dihydroxy vitamin D2, 1,25-dihydroxy vitamin D3, 25(OH)D, 25(OH)D2, 25(OH)D3, 1,25(OH)2D, 1,25(OH) 2D2, 1,25(OH) 2D3, hydroxycalcidol, 7-dehydrocholesterol, ergosterol, calcitriol, 25-hydroxy cholecalciferol, calcidiol, cholecalciferol, ergocalciferol) AND ("fortified food", "fortified foods", "Enriched Food", "Enriched Foods", "enriched food", "enriching food", "enhanced foods", "complementary food").

Broad screening of titles and abstracts were carried out by Talenezhad N and Mohammadi M to exclude irrelevant studies. Discrepancies between reviewers were resolved by the third author (Ramezani-Jolfaie N). In order to look for potential eligible articles and

to increase the sensitivity of the search; the references of the retrieved studies were also checked.

Eligibility criteria: The literature that met the following criteria were considered for inclusion in the present systematic review: 1) original articles; 2) randomized controlled clinical trials (RCTs); 3) studies evaluating the effect of fortified foods with vit D on serum 25(OH)D. The studies that did not measure vit D levels, were non-human (in vitro and animal), used fortified food with vit D and another nutrient were excluded from the research. Moreover, the studies that besides vit D food enrichment, had used vit D supplementation were also excluded from the present research since the effect of enrichment could not be controlled separately.

Data extraction: Two reviewers (Talenezhad N, Ramezani-Jolfaie N) independently extracted the data in each study and participant characteristics including the author's last name, publication date, the country in which study was conducted, study design, study duration, number of participants, gender (female/male/both), age (mean or range), type of fortified food, dose, type of vit D, baseline, endpoint and changes in 25(OH)D levels. Data were then tabulated for further evaluations.

Quality assessment: The Cochrane Collaboration's tool for assessing the risk of bias was employed to describe the methodological quality of eligible trials. This tool consists of six domains including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and selective reporting. The included studies were classified into three categories: "yes" (low risk of bias), "no" (high risk of bias) or "unclear" (uncertain risk of bias) (Higgins and Green, 2011). The quality of each study was evaluated as good (low risk for more than two domains), fair (low risk for two domains), and weak (low risk for less than two domains).

# Results

Study selection and characteristics: A total of 791 articles were retrieved based on electronic data search. After further reading of title and abstract,

deduplication and exclusion of irrelevant articles, 44 studies were totally remained for the full-text screening, and 30 articles that met exclusion criteria were dropped out. Eventually, after including two papers through hand search, 16 studies were selected for the present systematic review. The description of the study selection is illustrated in **Figure 1**.

**Table 1** presents the characteristics of the sixteen chosen articles which were published between 2003 and 2017 (Al-Khalidi et al., 2015, Biancuzzo et al., 2010b, Green et al., 2010, Itkonen et al., 2016, Johnson et al., 2005, Levinson et al., 2016, Madsen et al., 2013, Mehrotra et al., 2014, Natri et al., 2006, Neyestani et al., 2012, Nikooyeh et al., 2016, Rich-Edwards et al., 2011, Tangpricha et al., 2003, Tripkovic et al., 2017, Urbain et al., 2011, Wagner et al., 2008). From these articles, eight studies had examined the effect of dairy products fortifications on 25(OH)D (Al-Khalidi et al., 2015, Green et al., 2010, Johnson et al., 2005, Levinson et al., 2016, Madsen et al., 2013, Neyestani et al., 2012, Rich-Edwards et al., 2011, Wagner et al., 2008), three on bread (Itkonen et al., 2016, Natri et al., 2006, Nikooyeh et al., 2016), two on mushrooms (Mehrotra et al., 2014, Urbain et al., 2011) and three on orange juice (Biancuzzo et al., 2010b, Tangpricha et al., 2003, Tripkovic et al., 2017). Four studies were carried out in the United States (Biancuzzo et al., 2010b, Johnson et al., 2005, Mehrotra et al., 2014, Tangpricha et al., 2003), two studies in Canada (Al-Khalidi et al., 2015, Wagner et al., 2008), two in Iran (Neyestani et al., 2012, Nikooyeh et al., 2016), two in Finland (Itkonen et al., 2016, Natri et al., 2006) and one study was conducted in Germany (Urbain et al., 2011), one in Israel (Levinson et al., 2016), one in United Kingdom (Tripkovic et al., 2017), one in Denmark (Madsen et al., 2013), one in Mongolia (Rich-Edwards et al., 2011), and one in New Zealand (Green et al., 2010). All trials were designed as parallel-group studies, and duration of their intervention ranged from two weeks to sixteen weeks.

Risk of bias assessment: The quality assessment

of the included articles for all domains of Cochrane risk of bias tool is summarized in Table 2. Most of the studies (n=12) had good quality (low risk for more than 2 items) (Biancuzzo et al., 2010b, Green et al., 2010, Johnson et al., 2005, Madsen et al., 2013, Mehrotra et al., 2014, Neyestani et al., 2012, Nikooyeh et al., 2016, Rich-Edwards et al., 2011, Tangpricha et al., 2003, Tripkovic et al., 2017, Urbain et al., 2011, Wagner et al., 2008), two of them were fair (low risk for 2 items) (Itkonen et al., 2016, Levinson et al., 2016) and others (n=2) were classified as poor quality (low risk for less than 2 items) (Al-Khalidi et al., 2015, Natri et al., 2006). The articles having weak quality score did not report blinding of outcome assessment, allocation concealment, and random sequence generation.

The effect of bread fortification with vit D on 25(OH) D levels: Three studies investigated the effect of fortified bread with vit D on serum 25(OH)D concentrations (Itkonen et al., 2016, Natri et al., 2006, Nikooyeh et al., 2016). Natri et al. (Natri et al., 2006) observed that bioavailability of vit D from both wheat and rye bread is equal, although rye bread contains more fiber. Their three-week investigation emphasized that the consumption of either fortified wheat or rye bread with vit D (12µg/100g) can significantly improve serum 25(OH)D levels in 41 healthy women. In line with their study, Nikooyeh et al. (Nikooyeh et al., 2016) revealed that over longer intervention among 90 healthy men and women who consumed 50 g bread fortified with 25µg vit D for eight weeks, there was a significant raising in the circulation of 25(OH)D concentrations.

Another study assessed the bioavailability of vit  $D_2$  from UV-irradiated yeast present in bread during eight-week intervention among 33 young adult females (Itkonen *et al.*, 2016). Participants were divided into four groups with different interventions: placebo pill and regular bread;  $D_2$  supplement and regular bread;  $D_3$  supplement and regular bread and placebo pill and  $D_2$ -biofortified bread. The results represented that vit  $D_2$  fortified bread (25µg  $D_2$ /d) modestly increased serum

25(OH)D<sub>2</sub>, but not as much as vit D supplement (6.4 vs. 31.3 nmol/l, respectively).

The effect of mushroom fortification with vit D on 25(OH)D levels: Two studies assessed the effect of consuming fortified mushrooms with vit D on 25(OH)D changes in the body (Mehrotra et al., 2014, Urbain et al., 2011). Twenty-six young adults with low serum 25(OH)D ( $\leq$  50 nmol/l) and normal serum calcium concentration (2.2-2.7 nmol/l) were enrolled in a randomized, placebocontrolled study to determine the bioavailability of vit D<sub>2</sub> from vit D<sub>2</sub>-enhanced mushrooms by ultraviolet irradiation (Urbain et al., 2011). An experimental soup was made by fresh mushrooms irradiated with a UV-B dose of 1.5 J/cm<sup>2</sup>, increasing vit  $D_2$  content from <1 to  $491 \mu g/100 g$ . It was observed that serum 25(OH)D concentrations rose significantly after ingesting 28000 IU of D<sub>2</sub>/week for four weeks via experimental soup (3.9 nmol/l) vs. supplement (4.7 nmol/l).

Another research was conducted among prediabetic individuals, included 43 nonsmoking participants, with at least two features of metabolic syndrome (Mehrotra *et al.*, 2014). Two groups of participants who consumed entrees made by 100 g of UVB-treated mushrooms containing 600 IU D<sub>2</sub> or 4000 IU D<sub>2</sub> for sixteen weeks had modest or no increase in 25(OH)D<sub>2</sub> or total 25(OH)D.

The effect of orange juice fortification with vit D on 25(OH)D levels: Tangpricha et al. (Tangpricha et al., 2003) carried out a study to determine the bioavailability of vit  $D_3$  in orange juice and vit  $D_2$  in whole milk, skim milk, and corn oil on toast. Eighteen healthy adults were asked to drink 240 ml of whole milk or skim milk that contained 25000 IU ergocalciferol or 25000 IU vit  $D_2$  that had been dissolved in 0.1 ml corn oil and applied to toast. After ingestion of fortified whole milk, skim milk and corn oil on toast, there were not any significant changes in blood vit  $D_2$  concentrations. However, remarkable differences appeared in blood vit  $D_3$  among the participants who consumed fortified orange juice.

In addition, another study was conducted on fortified orange juice with vit  $D_2$  and vit  $D_3$ 

(Biancuzzo et al., 2010b). Eighty-six healthy participants were randomly assigned into one of the five groups: 1) placebo capsule + orange juice without vit D, 2) placebo capsule + orange juice containing 1000 IU vit D<sub>3</sub>, 3) placebo capsule + orange juice containing 1000 IU vit D2, 4)1000 IU vit D<sub>3</sub> capsule + placebo orange juice, and 5) 1000 IU vit D<sub>2</sub> capsule + placebo orange juice. After eleven weeks of intervention, analysis of the area demonstrated under the curved that bioavailability of vit D<sub>2</sub> and vit D<sub>3</sub> in orange juice and capsules were similar and raised 25(OH)D effectively. Also, there were no considerable changes in serum 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> in the placebo groups, which means that sunlight exposure and diet could not significantly affect the vit D status.

Another research was carried out in 2017 by Tripkovic et al. (Tripkovic et al., 2017) indicating that whether vit  $D_2$  or vit  $D_3$  added to juice or biscuit is effective in increasing serum total 25(OH)D. The results showed that 335 women who received 15  $\mu$ g vit D2 or vit D3 added to juice and biscuit for twelve weeks had improvement in their vit D status.

The effect of dairy products fortification with vit D on 25(OH)D levels: Neyestani et al. (Neyestani et al., 2012) carried out a study to determine the effect of vit D fortification either with or without calcium on certain inflammatory markers among 90 Iranian individuals with type 2 diabetes. All subjects were randomly divided into three groups of 1) receiving two 250-ml bottles of Doogh (Persian vogurt drink) per day (PD, containing 150 mg calcium and no detectable vit D<sub>3</sub>/250ml); 2) vit D-fortified Doogh (DD, containing 500 IU vit D<sub>3</sub> and 150 mg calcium/250ml); and 3) calcium + vit D<sub>3</sub>-fortified Doogh (CDD, containing 500 IU vit D<sub>3</sub> and 250 mg calcium/250ml). There was a detectable improvement in vit D status of DD and groups following twelve weeks CDD intervention. Moreover, inflammatory markers and retinol binding protein-4 concentrations significantly decreased in those groups.

Green et al. also demonstrated that 73 women

who consumed fortified milk ( $5\mu g$  vit  $D_3$ ) for twelve weeks had 10 nmol/L higher serum 25(OH)D concentrations compared those consumed the placebo (Green *et al.*, 2010).

In the two researches conducted by Johnson, the bioavailability of vit D from fortified process cheese and its effects on 25(OH)D status in the elderly subjects was assessed (Johnson et al., 2005). One hundred older men and women randomly received 85 g of 600 IU vit D fortified cheese, non-fortified cheese or no cheese during two months of intervention. Unexpectedly, a greater decrease in serum 25(OH)D was observed among the vit D fortified cheese group. The researchers speculate that this decrement may be related to higher baseline serum 25(OH)D concentrations. An additional randomized cross over the trial was also conducted to determine the bioavailability of vit D2 and its absorption from process cheese and fortified water dilution (Johnson et al., 2005). A total sample of eight people, divided into two groups of young and old, randomly received either vit D-fortified cheese or water. Consistent with the results obtained from serial blood sampling collected during 24-hours after the intervention, there was a similar peak in the serum vit D of younger (23 to 50 yr) and older (72 to 84 yr) adults, and vit D2 absorbed more significantly from cheese than from water.

Another study was done by Wanger et al. (Wagner et al., 2008) also investigated the bioavailability of vit D from fortified cheese. Eighty adults were randomly assigned to one of six weekly interventions of: 1) fortified cheddar cheese (DC) (34g, n=20); 2) fortified low-fat cheese (DLF) (41g, n=10); liquid vit D supplement (1ml), taken with food (DS+) (n=20) or without food (DS-) (n=10); placebo cheddar cheese (n=10); or placebo supplement (n=10). Over eight weeks of intervention, in the placebo groups, baseline 25(OH) D levels of 55.0±25.3 nmol/l declined to 50.7±24.2 nmol/l. In the vit D-treated groups, the mean increases in 25(OH)D values were as follows: 65.3±24.1 (DC), 69.4±21.7  $59.3\pm23.3$  (DS+) and  $59.3\pm19.6$  nmol/l (DS-) which presents that fortified cheese boost vit D status as adequately as a supplement, making it a proper choice for vit D fortification.

The other research was performed to assess the bioavailability and safety of vit  $D_3$  from fortified mozzarella cheese baked on pizza (Al-Khalidi *et al.*, 2015). The research demonstrated that ingesting 200 IU or 28000 IU vit  $D_3$ -fortified mozzarella cheese for eight weeks could increase 25(OH)D levels by  $5.1\pm11$  nmol/l in the low-dose group (n=47; P-value=0.003) and by  $73\pm22$ nmol/l in the high-dose group (n=49; P-value<0.0001).

Levinson et al. (Levinson et al., 2016) also assessed the bioavailability of vit  $D_3$  from fat-free yogurt, in re-assembled casein micelles (rCMs) compared to polysorbate-80 (PS80/Tween80) which is commonly used a synthetic emulsifier. Serum 25(OH)D status of participants consuming fat-free yogurt with 50000 IU of either VD<sub>3</sub>-rCM, VD<sub>3</sub>-PS8 showed increases of  $\sim 8$  ng/ml after two

weeks, and no significant differences were found between mean changes of 25(OH)D among the individuals who consumed rCM yogurt versus PS80.

On the other hand, Madsen et al. (Madsen et al., 2013) investigated the association of fortified milk and bread with vit D status of 782 children and adults. Participants were recruited from 201 families and were randomly assigned to vit Dfortified or non-fortified milk and bread for six months. When comparing from baseline to completion of the intervention, a greater serum 25(OH)D level was seen in the fortification group (67.6 nmol/L) than in the control group (41.7 nmol/L). Similar finding was also reported by Rich-Edwards et al. (Rich-Edwards et al., 2011). improvements There 25(OH)D were in concentrations among 579 children in Mongolia who received 300 IU vit D via fortified milk.

Table 1. Characteristics of the studies that were included in the systematic review

Baseline 25(OH)D Endpoint 25(OH)D (mean±SD or 95% CIs) 95% CIs)	1) Pl 13.5. 13.5. 2) viv 59.7. 3) viv 61.9 4) viv 74 (6	nmol/L  id 1) Fortified $(V, \eta_{\pm} V', \iota)$ ment 2) Supplement $(V, \eta_{\pm} V', \iota)$ $(V, \eta_{\pm} V', \iota)$ 3) Control $(V, \eta_{\pm} V', \iota)$	.23 .8 .74	
25(OH)D Baseline analysis 45%	mol/L  1) Placebo 44.8 (37.5, 52.1) 2) vit D2 Juice 44.9 (37.8,52.0) 3) vit D2 biscuit 46.1 (38.9,53.4) 4) vit D3 Juice 42.3 (35.4, 49.2) 5) vit D3 biscuit 41.9 (34.9, 48.9)	nmol/L  1) Fortified  rr, 4±r1, 4  2) Supplement ro, .±r^, v  3) Control rt, v±r, v	Chemilumines ng/L cence Changes: immunoassay 1) 7.51±1.23 (CLIA) 2) 8.01±0.8 3) -0.65±0.74	IA nmol/L
Fortification 25(C dose, type of anal vitamin D	15 mg vit D3 HPLC-15 mg vit D3 MS/MS	25 µg vit D3 HPLC	2	1) 25 µg vit D2 OCTEIA In bread immunoenzy
Food Fo fortified v	Juice 15 n biscuit 15 n	Bread 25 μ	Fat-free 50000 yogurt with vit D3 vit D3	Bread ') 25 µg vit D2 (87 g) in bread
Study r duration (week)	12	8 (c) t (c) (c)	6	∞
Age (y) (range or mean±SD)	20-64	20-60 Fortified $(r', r_{\pm}! ., \circ)$ Supplement $(r', r_{\pm}! ., \circ)$ Control $(r', r_{\pm}! ., q)$	18-61	20-37
Number of participants (sex ratio)	335 1) Placebo (n=65) 2) vit D2 Juice (n=67) 3) vit D2 biscuit (n=66) 4) vit D3 Juice (n=70) 5) vit D3 biscuit (n=70)	90 (49 F/41 M) s 1) Fortified (n=30) 2) Supplement (n=30) 3) Control (n=30)		
y Study design	Parallel i five groups	Parallel three groups	Parallel three groups	Parallel four groups
on Country	United tive Kingdom	Iran ry-	Israel	Finland ry-
The first author Season (year)	Tripkovic Two (2017) consecutive (Tripkovic et winters al., 2017)	Nikooyeh Winter (2016) (February- (Nikooyeh <i>et</i> March) <i>al.</i> , 2016)	Levinson NR (2016) (Levinson et al., 2016)	(2016) Winter (February-

Urbain	Neyestani (2012) (Neyestani <i>et al.</i> , 2012)	Madsen (2013) (Madsen <i>et al.</i> , 2013)	Mehrotra (2014) (Mehrotra et al., 2014)	Al-Khalidi (2015) (Al-Khalidi <i>et al.</i> , 2015)	
Winter (late Germany	NR	Winter	November 2011-April 2012	NR	
Germany	Iran	Denmark	United States	Canada	
Parallel	Parallel three groups	Parallel two groups	Parallel four groups	Parallel two groups	
27	90 1) Plain doogh (n=30) 2) Vit D fortified doogh (n=30) 3) Vit D + calcium fortified doogh (n=30)	726 1) Fortification (n=355) 2) Control (n=371)	1) Mushrooms 500 IU vit D2 + placebo (n=^\) 2) Mushrooms 2600 IU vit D2 + placebo (n=^\r) 3) 1200 IU vit D3/d capsules (n=^\) 4) 7300 IU vit D3/d capsules (n=^\)	96 1) Low-dose (n=47) 2) High-dose (n=49)	
1) 28.6±4.3	30-60 1) 50.8±6.7 2) 51.5±5.4 3) 49.9±6.2	Children and adults (4-60)	30-90 (€9±1Y)	NR	
S	17	26	16	∞	
Mushrooms	Persian yogurt drink or doogh (wo 250 ml bottle/d)	Milk and bread	Mushroom (100 g)	Pizza (mozzarella cheese)	
hrooms 28000 IU (700		Children aged 4- HPLC 17: 1) Fortification 10.2 µg 2) Control 2.2 µg Adults aged 18- 60: 1) Fortification 8.1 µg 2) Control 2.2 µg	1) 500 IU D2 2) 2600 IU D2 3) 1200 IU D3 4) capsules 7300 IU	1) 200 IU vit D3 2) 28000 IU vit D3	
Radioimmuno nmol/L	HPLC	HPLC	RIA and LC/MS/MS	NR	
nmol/L	nmol/L 1) 41.6±44.5 2) 44.4±28.7 3) 44.5±43.7	nmol/L Children aged 4-17: 1) Fortification YY, \( \lambda \) (64.0, 88.9) 2) Control YY, \( \lambda \) (61.8, 83.9) Adults aged 18-60: 1) Fortification YY, \( \text{Y} \) (61.9, 88.3) 2) Control Y**, \( \text{Y} \) (60.0, 86.7)	ng/mL 1) NR 2) 17.0±4.9 3) 16.1±3.5 4) 18.8±4.2	NR	4) D3 supplement $66.6\pm14.8$
nmol/L	nmol/L 1) 37.2± 44 2) 77.7±28.6 3) 74.6±39.5	nmol/L Children aged 4-17: 1) Fortification 1∨, 1 (56.2, 79.4) 2) Control ٤∀, √ (30.9, 58.9) Adults aged 18-60: 1) Fortification 1¬, ↑ (56.2, 79.4) 2) Control ٤٠, √ (28.8, 60.3)	ng/mL 1) Not change 2) 18.5±4.1 3) 28.5±1.1 4) 32.5±3.8	N <sub>R</sub>	+9.6

# Vitamin D food fortification

1) 56.7±7.2 2) 58.0±11.2 3) 28.7±8.7	ng/mL 1) 8±4 2) 20±6 3) 29±10 4) 21±6 5) 12±4	nmol/L 1) 65 (57, 73) 2) 53 (46, 62)	ng/mL 1) 18.1 ± 6.4 2) 230.7 ± 8.5 3) 26.4 ± 7.4 4) 28 ± 11.0 5) 27.4 ± 10.5	nmol/L 1) 115.62 ± 2.10 2) 126 ± 8.34 3) 115.21 ± 4.50
1) 34.0±11.0 2) 28.7±10.0 3) 38.7±14.2	ng/mL 1) 8±4 2) 8±4 3) 10±5 4) 7±3 5) 8±4	nmol/L 1 1) 76 (66, 87) 2) 74 (65, 85)	ng/mL 1) 19.8 ± 9.6 2) 17.9 ± 11.1 3) 15.8 ± 10.0 4) 19.6 ± 11.1 5) 16.6 ± 9.9	nmol/L 1) 50.7 ±18.9 2) 57.5 ±18.4 3) 55.7 ±21.2
assay (DiaSorin Inc)	Liquid chromatograp hy-tandem mass spectrometry	DiaSorin radioimmunoa ssay (Stillwater, MN)	Liquid chromatograp hy tandem mass spectroscopy	Radioimasay (DiaSorin)
mg) vit D2 fortified food 28000 IU vit D2 via a supplement	1) 300 IU weekly per milk 2) 300 IU supplement	75 g of fortified milk powder provided 200 IU vit D3	1000 IU vit Dr 1000 IU vit D2	28,000 IU vit D3 weekly (4000 IU/d)
(dnos)	Milk	Milk	Orange juice	Cheese
	from January through March	12	Ξ	∞
2) 31.1±6.7 3) 32.4±6.0	Children (9-11)	18-45	18-84 1) 40.8±10.8 2) 41.4±12.6 3) 40.1±15.6 4) 40.1±18.0 5) 38.9±12.3	1) 28.7±11.4 2) 30.6±11.7 3) 31.8±12.5 4) 23.6 6 3.5
1) 28000 IU (700 mg) vit D2 in soup (n=8) 2) 28000 IU vit D2 via a supplement (n=9) 3) placebo (n=9)	579 1) Unfortified Mongolian milk (n=101) 2) Fortified Mongolian milk (n=140) 3) Fortified UHT milk (n=37) 4) Daily vit D supplement (n=109) 5) Seasonal vit D supplement (n=92)	73 F 1) Fortified milk (n=37) 2) Placebo milk (n=36)	105 1) Placebo (n=15) 2) vit D3 orange juice (n=18) 3) vit D2 orange juice (n=17) 4) vit D3 in capsules (n=20) 5) vit D2 in capsules (n=16)	80 (40 F/ 40 M) 1) Fortified cheddar cheese (34 g; n=20) 2) Fortified low-fat
three groups	Parallel five groups	Parallel two groups	Parallel five groups	Parallel six groups
	Mongolia	New Zealand	United States	Canada
January- early March 2010)	Winter	January to April	February	Began in the fourth week of January and ended in
(2011) (Urbain et al., 2011)	Rich- Edwards Janet (2011) (Rich- Edwards et al., 2011)	Green (2010) (Green <i>et al.</i> , 2010)	Biancuzzo (2010) (Biancuzzo et al., 2010b)	Wagner (2008) (Wagner et al., 2008)

Tangpricha (2003) (Tangpricha et al., 2003)	Johnson (2005) (Johnson <i>et</i> <i>al.</i> , 2005)	Natri (2006) (Natri <i>et al.</i> , 2006)	
Began in the United second week States of March	winter	February— March	the first week of April
United States	United States	Finland	
Parallel three groups	Parallel three groups	Parallel four groups	
1) vit D fortified juice (n=14) 2) Unfortified juice (n=12)	110 1) vit D-fortified process cheese (n=\(\tau^{\gamma}\)) 2) Non-fortified process cheese (n=\(\tau^{\gamma}\)) 3) No process cheese (n=\(\tau^{\gamma}\))	=	cheese (41 g; n=10) 5) 26.2 $\pm$ 6.5 3) vit D supplement 6) 24.6 $\pm$ 4.5 with food (n=20) 4) vit D supplement without food (n=10) 5) Placebo cheddar cheese (n=10) 6) Placebo
22-60 (29.0±9.0)	IV 60	25-45 1) 27.3±0.6 2) 28.8±1.8 3) 29.0±1.7 4) 31.1±1.8	5) 26.2 ±6.5 6) 24.6 ±4.5
12	∞	-1	
Orange juice and skim milk	Process cheese	Bread	
Orange juice 1000 IU vit D3 and skim milk	600 IU vit D	10 mg vit D in all groups except the control group	
The intraassay CV of 8% and an interassay CV of 12%	HPLC	Immunoradio metric method (DiaSorin)	
nmol/L 1) 7.0±8.0 2) 50.0±10	ng/ml 1) 23±1.4 2) 20±1.2 3) 18±1.3	nmol/L 1) 29.0±3.0 2) 28.9±3.5 3) 27.1±3.7 4) 29.6±2.6	4) 53.4 ±40.1 5) 52.4 ±22.7 6) 57.6 ±28.7
nmol/L 1) 94.0±20 2) 73.0±8.0	ng/ml 1) 21±1.4 2) 22±1.1 3) 19±1.2	nmol/L 1) 45.44±3.16 2) 49.31±1.50 3) 27.59±3.0 4) 43.96±3.33	4) 111.36 ± 8.55 5) NR 6) NR

<sup>&</sup>lt;sup>1</sup>Analytical methods for analyzing circulating 25(OH)D levels, F: female, M: male, NR: not reported, HPLC: high-performance liquid chromatography

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Table 2. Risk of bias assessment for included RCTs

	L (2017) (Tripkovic	οί αι. (2016) (Vikooyeh et αl.,	(2016) (Levinson et al.,	11konen et (2016)	18. (2015) (2015) (2015) (2015) (2015)	(2014) (Mehrotra et al.,	(2013) (2013) (1.2013) (1.2013)	(2012) (Neyestani et al.,	1anet (1102) (Kich-	nisav) (1102) to nisdv() (1100 ln	(2010) (Biancuzzo et al.,	Wagner (2008) (Wagner et	(8002) (8004) (8005)	(2002) (2005) (2005)	s (2003) oiriggnsT)	<sup>h</sup> 345 <sup>4</sup> (2010) (Green et (Oroc 1 <sub>0</sub>
Random sequence generation (selection bias)	+	+	¢.	ç.	ذ	+	+	٠	+	+	+	+	¢.	ذ	+	¿
Allocation concealment (selection bias)	ċ	ċ	ċ	<i>ċ</i> ·	ċ	ċ	ċ	ċ	ċ	ċ	ċ	ċ	ć:	ċ	ċ	ċ
Blinding of participants and personnel ( <b>Performance bias</b> )	+	+	c·	c·	ć·	+	ć·	ċ	¢.	c.	c·	+	c.	ċ	+	+
Blinding of outcome assessment (Detection bias)	ċ	ć·	c·	¢·	ć.	ċ	¢.	ċ	¢.	¢.	¢.	ċ	¢·	ċ	¢.	ċ
Incomplete outcome data (Attrition bias)	+	ċ	ć	+	+	+	+	ć	+	+	+	+	+	+	+	+
Selective reporting (Reporting bias)	+	+	+	+	ċ	+	+	+	+	+	+	+	+	+	+	+
	4	$\kappa$	_	7		4	$\infty$		3	$\mathcal{E}$	3	4	7	7	4	$\omega$
Overall quality	Good	Good	Weak	Fair	Weak	Good	Good	Weak	Good	Good	Good	Good	Fair	Fair	Good	Good

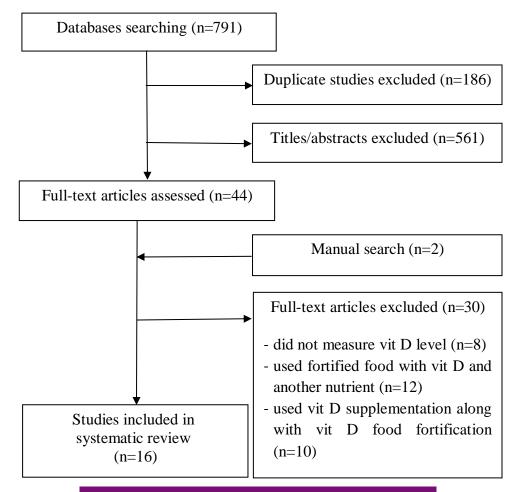


Figure 1: Flow chart of the study selection process

# Discussion

The current systematic review was undertaken to evaluate the effect of food fortification with vit D on serum 25(OH)D levels. According to the findings of reviewed studies, vit D food fortification can be an effective and cost-effective approach provide sufficient 25(OH)D concentrations and reduce the number of people suffering vit D deficiency. It was also observed that vit D<sub>3</sub> fortified foods had better efficacy than vit D<sub>2</sub> fortified foods. Overally, fourteen studies supported the benefits of food enrichment (Al-Khalidi et al., 2015, Biancuzzo et al., 2010b, Green et al., 2010, Johnson et al., 2005, Levinson et al., 2016, Madsen et al., 2013, Natri et al., 2006, Neyestani et al., 2012, Nikooyeh et al., 2016, Rich-Edwards et al., 2011, Tangpricha et al., 2003, Tripkovic et al., 2017, Urbain et al., 2011, Wagner

et al., 2008) and only two studies failed to find reasonable changes as a result of food fortification on serum 25(OH)D levels (Itkonen et al., 2016, Mehrotra et al., 2014). Food fortification with vit D significantly increases serum 25(OH)D levels, helping perpetuation of vit D status among the populations.

Different foods have been suggested to be fortified with vit D, such as milk, mushrooms, bread, and orange juice. Dairy products fortification is very common in the United States, but many people avoid consuming milk due to lactose intolerance and vegetation cause (Gibson, 1994, Pawłowska *et al.*, 2016). On the other hand, there was a concern that low PH of orange juice (~4) would limit stability of added vit D. However HPLC analysis indicated that vit D<sub>3</sub> remained unchangeable after storage for 30 days at 4°C

(Tangpricha *et al.*, 2003). Therefore, some researchers have suggested that since bread is the primary food and also available in many countries, it is a good choice for fortification (Mocanu and Vieth, 2013, Natri *et al.*, 2006, Nikooyeh *et al.*, 2016).

Vit D is bioavailable from cheddar, low-fat cheese, whole milk and skim milk and also fat content of milk and its products cannot change the bioavailability (Tangpricha et al., 2003, Wagner et al., 2008). In addition, some studies showed that vit D fortified bread, orange juice and mushrooms improved 25(OH)D status in different population groups (Biancuzzo et al., 2010b, Natri et al., 2006, Nikooyeh et al., 2016, Tangpricha et al., 2003, Urbain et al., 2011). Whereas UVB-activated D2 yeast added to bread did not increase total 25(OH)D concentrations which may be results of low bioavailability of vit D from UVB irradiated yeast in bread (Itkonen et al., 2016). On the other hand, in another RCT, vit D2-UVB-mushrooms did not increase total 25(OH)D which may be attributed to cooking loss and probable low absorption of vit D (Mehrotra et al., 2014).

In addition, the efficacy of vit D fortification varied among the included studies, which might be related to differences in doses, type of vit D, type of food, fortification method, age and baseline vit D status of participants. According to the results of eligible studies, the higher 25(OH)D concentrations were achieved among participants with lower baseline 25(OH)D levels and no differences were reported between men and women (Natri *et al.*, 2006, Tangpricha *et al.*, 2003).

Despite beneficial effects of vit D fortification in the prevention of many diseases, it may cause some serious side effects on human health; consequently, it has limitation of consumption for some individuals. Just as some researchers reported the withdrawal of participants from their studies because of gastrointestinal discomfort, especially those who consumed fortified dairy products (Daly *et al.*, 2006, Johnson *et al.*, 2005, Lau *et al.*, 2001).

The limitations of this systematic review are the lack of information on dietary intake of vit D of the

participants, the baseline vit D status of participants that varied in the included studies which may impact on the 25(OH)D responses to fortified foods, thethe power of detecting small changes in 25(OH)D is different between laboratories, and the results varied by countries depending on their lifestyle, dietary habits and genetic background which are all being involved in responses to vit D fortification.

The current study comprehensively investigated the effect of food fortification with vit D on 25(OH)D levels based on all available RCTs to date, which have high levels of credibility. Cochrane's risk of bias tool was also used to investigate the methodological quality of the trials which is considered as the strength of the present review.

### Conclusion

The present systematic review of RCTs demonstrated that fortification of foods with vit D has significantly improved 25(OH)D serum status and has similar efficacy with vit D supplements. Moreover, it was observed that food fortification has a greater impact on the people with lower 25(OH)D serum levels. Therefore, food enrichment is an economical and effective approach for different populations to prevent vit D deficiency. Further studies are still needed to determine the best food and optimal dosage of vit D for fortification.

# **Conflict of Interest**

The authors declare that there is no conflict of interest.

# **Author Contributions**

Mohammadi M designed the search strategy; Mohammadi M and Talenezhad N performed electronic search and study selection; Talenezhad N and Ramezani-Jolfaie N extracted the data; Sakhaei R and Ramezani-Jolfaie N wrote the manuscript, and all authors read and approved the final manuscript.

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