

The Effect of Maternal Vitamin D Supplementation on Vitamin D Status of Exclusively Breastfeeding Mothers and Their Nursing Infants: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

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ABSTRACT

The optimal vitamin D supplementation plan during lactation is unclear. We investigated the effect of maternal vitamin D supplementation on mother-infant dyads' vitamin D status during lactation. All controlled trials that compared vitamin D supplements to placebo or low doses of vitamin D in breastfeeding mothers were included. Pooled effect size and the associated 95% CI for each outcome were estimated using random-effects models. A 1-stage random-effect dose-response model was used to estimate the dose-response relation across different vitamin D dosages and serum 25-hydroxy vitamin D [25(OH)D] concentrations. We identified 19 clinical trials with 27 separate comparison groups ($n = 3337$ breastfeeding mothers). Maternal vitamin D supplement dosages were associated with circulating 25(OH)D concentrations in breastfeeding women in a nonlinear fashion. Supplementation with 1000 IU of vitamin D/d increased serum 25(OH)D concentrations by 7.8 ng/mL, whereas there was a lower increase in concentrations at vitamin D doses of >2000 IU/d (3.07 and 2.05 ng/mL increases between 2000–3000 and 3000–4000 IU/d, respectively). A linear relation was observed between maternal vitamin D supplementation dosage and the infants' circulating 25(OH)D concentrations. Each additional 1000 IU of maternal vitamin D intake was accompanied by a 2.7 ng/mL increase in serum 25(OH)D concentration in their nursing infants. The subgroup analysis showed that maternal vitamin D supplementation was accompanied by a statistically significant increase in infants' 25(OH)D concentration in the trials with a duration of >20 wk, vitamin D supplementation >1000 IU/d, East Indian participants, maternal BMI <25 kg/m², and studies with an overall low risk of bias. Long-term maternal supplementation with vitamin D at a high dose (>6000 IU/d) effectively corrected vitamin D deficiency in both mothers and infants. Nevertheless, infants with 25(OH)D concentrations over 20 ng/mL may require a relatively low maternal dose to maintain vitamin D sufficiency. *Adv Nutr* 2022;13:568–585.

Statement of Significance: This study is the first dose-response analysis on the relation between circulating 25-hydroxy vitamin D [25(OH)D] and maternal vitamin D supplementation in mother-infant dyads. We also considered factors such as study design and population characteristics that may affect the outcomes of a given vitamin D trial that have been overlooked in previous reviews.

Keywords: vitamin D, breastfeeding women, breastfed infant, breast milk, 25-hydroxy vitamin D

Introduction

Vitamin D is a steroid hormone that regulates calcium and phosphorus homeostasis, which is essential in maintaining bone health (1). Besides the classical role of vitamin D

in bone homeostasis, vitamin D also has noncalcemic actions, such as modulating the immune system, inhibiting cancer progression, and regulating the cardiovascular and neurological systems (2, 3).

During the first year of life, infants are very dependent on the nutrient content of breast milk for their growth and development (4). Breast milk is usually considered a limited source of vitamin D for prolonged and exclusively breastfed infants who are not taking a vitamin D supplement (5). However, the vitamin D content of breast milk is closely related to maternal vitamin D intake, which is largely determined by maternal vitamin D supplementation (6). So, breastfed infants who do not receive supplemental vitamin D or adequate sunlight exposure or whose mothers have inadequate vitamin D status are at high risk of developing vitamin D deficiency (5).

Despite routine vitamin D supplementation programs for breastfeeding women and their nursing infants in many countries, including the USA (7), Canada (8), United Kingdom (8), and many European countries (9), vitamin D deficiency has remained a major public health challenge in many developed and developing countries (6, 10, 11). The RDA of 600 IU/d is designed to support serum 25-hydroxy vitamin D [25(OH)D] concentrations of 50 nmol/L in lactating women with vitamin D sufficiency (12). There is reasonable agreement between the maternal RDA and adequate intake (AI) for infants with serum 25(OH)D in the range of >20 ng/mL (12). However, there is a concern that current vitamin D recommendations may not be enough to meet vitamin D requirements in breastfeeding mothers and their infants suffering from vitamin D insufficiency and deficiency from the very beginning (13–16). Indeed, the RDAs for lactating women do not adequately cover all infants' needs (12).

Supplementation of breastfeeding mothers with vitamin D, infant supplementation, or concomitant supplementation of mothers and infants have been implemented as potential strategies to prevent hypovitaminosis D (6, 9). However, the concept of vitamin D transfer from mother to infant is not accounted for in the current DRI series, and separate recommendations are made for each group. There are several questions yet to be answered regarding whether maternal supplementation alone would improve the vitamin D status of both mothers and their nursing infants including the optimal dose needed to achieve adequate concentrations of circulating vitamin D in these target populations, which is the topic of our meta-analysis (17). The findings of primary studies on appropriate maternal or infant vitamin D supplementation regimens to ensure vitamin D sufficiency in these vulnerable populations present conflicting results (18–25). For example, in a study by Wagner et al., mothers

supplementing with 6400 IU/d cholecalciferol (vitamin D3) increased their vitamin D status to a concentration sufficient to meet vitamin D requirements in their exclusively breastfed infants (24). In other studies, vitamin D supplementation at doses of ≤ 4000 IU/d was not sufficient to maintain vitamin D adequacy in breastfeeding infants with low 25(OH)D concentration at baseline (20, 26).

To our knowledge, there are only a few studies that have pooled the data from vitamin D supplementation trials in mother-infant dyads to reach a comprehensive result. For example, a systematic review by O'Callaghan et al., investigated the effect of daily infant vitamin D supplementation compared with intermittent infant supplementation or maternal supplementation and suggested that maternal postpartum or intermittent infant vitamin D supplementation could serve as potential substitutes for daily infant vitamin D supplementation of 400 IU (17). To our knowledge, no review has considered the dose-response relation between infants' circulating 25(OH)D concentration and maternal vitamin D supplement dosages. Further, previous reviews have overlooked aspects such as the study design and the characteristics of the population that could influence vitamin D trial results. Hence, the comprehensive evaluation of current literature using statistical techniques to pool the data from several studies to re-examine the current strategies for preventing vitamin D deficiency in this vulnerable population is needed. Therefore, we aimed to illuminate these uncharted areas in the present systematic review and meta-analysis of the effect of vitamin D supplementation in breastfeeding mothers on the vitamin D status of breastfeeding mothers and their exclusively breastfed infants.

Objectives

Primary objectives.

We assessed the effect of maternal vitamin D supplementation on the circulating 25(OH)D changes in breastfeeding women or their exclusively breastfed infants (not receiving any other form of nutrition than human milk or another source of vitamin D) compared with controls (placebo or no treatment or vitamin D ≤ 600 IU/d).

Secondary objective.

Our secondary objectives were to assess the effect of maternal vitamin D supplementation on: 1) the circulating parathyroid hormone (PTH), calcium, and phosphorus changes in breastfeeding women and their exclusively breastfed infants (not receiving any other form of nutrition than human milk or another source of vitamin D) compared with controls (placebo or no treatment or vitamin D ≤ 600 IU/d), 2) the effect of maternal vitamin D supplementation on the anthracitic activity of breast milk compared with controls (placebo or no treatment or low dosage of vitamin D ≤ 600 IU/d), and 3) the heterogeneity across primary studies of the association between maternal vitamin D supplements and circulating 25(OH)D concentrations in mother-infant pairs and the potential causes.

This study was financially supported by the Alborz University of Medical Sciences.

Author disclosures: The authors report no conflicts of interest.

Supplemental Table 1 and Supplemental Figures 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/advances/>.

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Abbreviations used: AI, adequate intake; ARA, antirachitic activity; IOM, Institute of Medicine; PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis; PTH, parathyroid hormone; SMD, standardized mean difference; 25(OH)D, 25-hydroxy vitamin D.

Methods

The current systematic review protocol was adopted and conducted in concordance with PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) checklist guidelines for systematic reviews (27, 28). This protocol was registered on PROSPERO International Prospective Register of Systematic Reviews as CRD42020215784. Since patients are not involved in this protocol for a systematic review ethics approval is not required.

Selection criteria

We clarified the eligibility criteria of articles based on a pilot test of 4–6 articles for exclusion and inclusion criteria before the screening phase.

Type of study.

Studies were screened for selection using the PICO (Population, Intervention, Comparison, Outcomes) and the review objectives. All open-label or single-, double-, and triple-blind randomized and nonrandomized controlled trials comparing interventions that differed only in vitamin D content from controls (placebo and no treatment or vitamin D ≤ 600 IU/d) were included. We also included other experimental research designs, i.e. pilot studies. Studies including animal models, review articles, observational studies, case studies, and case reports were excluded from the current systematic review.

Type of participants.

The primary studies were those that included singleton mothers whose infants were exclusively breastfeeding and receiving no other form of nutrition than human milk or another source of vitamin D. Participants were also in good general health at recruitment. Mothers with metabolic disorders (e.g. pre-existing type I or II diabetes, hypertension, thyroid diseases, hypo- and hyperthyroidism) or taking anticonvulsants, antituberculosis drugs, or any vitamin D supplement containing >600 IU/d were also excluded.

Intervention and control.

The intervention was vitamin D3 or ergocalciferol (D2) supplement (daily, weekly, and monthly), but not vitamin D metabolites, administered in any form (intravenous, intramuscular, or oral) or any dose. Control groups took a placebo or low vitamin D dosage (≤ 600 IU/d). Since maternal supplements at the current RDA are not sufficient for achieving 25(OH)D concentrations >20 ng/mL in individuals with vitamin D insufficiency (29), studies in which women in control groups received ≤ 600 IU/d vitamin D were also included. This issue was further addressed by subgroups and dose-response analysis.

Primary outcomes were either mother's or infant's serum 25(OH)D concentrations. Secondary outcomes included the anthracitic activity of breast milk, and serum PTH, calcium, and phosphorus concentrations in mothers and infants. Additionally, the effect of vitamin D supplementation on primary outcomes in different subgroups and identification

of the sources of heterogeneity were considered secondary outcomes.

Search strategy

We searched PubMed, Scopus, Web of Science, Embase, and 3 key journals, including *The American Journal of Clinical Nutrition*, *Nutrients*, and *The Journal of Nutrition*, from January 1983 until 1 July, 2020 with no restriction in the English language. Relevant search terms in accordance with an intervention, outcome, and participant components of the current systematic reviews were extracted from medical subject heading (Mesh) and Emtree terms. The key search terms included ("Vitamin D3" OR cholecalciferol OR "Vitamin D2") AND ("Hydroxyvitamin D" OR hydroxycholecalciferol OR "25 Hydroxyvitamin D2" OR "25-Hydroxyergocalciferol" OR "25 Hydroxyergocalciferol") AND (lactation OR "prolonged lactation" OR breastfeeding OR "breast milk" OR "postpartum women" OR "lactating mothers"). Also, bibliographies of all relevant prior systematic reviews and meta-analyses and primary studies identified by the search strategy were scanned for additional articles.

Study selection

Two authors (SA and PS) independently screened and selected studies for possible inclusion in the study using the PRISMA flow diagram (30). First, each review author independently reviewed the titles and abstracts of all primary articles to determine the eligibility of studies for inclusion. Then, the full text of potentially relevant articles was independently assessed by each author. Any disagreements between the reviewers were resolved via discussion to reach a consensus (31). With regards to duplicate publication of the same study, the stronger study (e.g. larger sample size, clarity of methods) was retained.

Quality assessment

Each selected article was independently assessed for quality by SA and EK using an adapted Cochrane Collaboration "Risk of bias" assessment tool (32). Each study was then classified into 1 of 3 categories of bias, i.e. high, low, and unclear risk of bias. Sensitivity analysis was conducted to assess the effect of trial quality on the effect size.

Data extraction

Data were extracted from the primary articles by 2 review authors independently (SA and EK), using the data extraction form. Information was collected on study identification (first author's name, year of publication, country in which the study was performed), study design (type of study, sample size, inclusion and exclusion criteria, duration of intervention, type and dosage of vitamin D supplements, season of intervention, baseline and changes in serum 25(OH)D concentration), population (age, race, BMI, baseline dietary intake, and laboratory measurements), intervention and comparator details (sample size for each treatment group, blinding, withdrawals, and dropouts). The data extraction was crosschecked independently.

Data analysis and synthesis

Descriptive analysis.

All studies included are presented in [Table 1](#), which displays details regarding study characteristics, i.e. trial duration; maternal age; intervention, cointervention, and outcomes; baseline and change in serum 25(OH)D concentration in the intervention group. Details regarding maternal BMI; infant age; inclusion and exclusion criteria; vitamin D measurement assay; and maternal and infants' 25(OH)D concentration at the final visit in intervention groups are also displayed in [Supplemental Table 1](#). The study quality based on the adapted Cochrane Collaboration "Risk of bias" assessment tool is also depicted.

Inferential statistics.

The overall mean difference was determined by subtracting the mean change in the control group from the mean change in the intervention group. We calculated the SD from SEs or a CI or IQR for both control and intervention groups. Data were presented as between-group differences and relative differences in primary and secondary outcomes were estimated. Since different measurement methods were used, Cohen's *d* effect size [standardized mean differences (SMDs)] with 95% CI was calculated by dividing the mean difference by the pooled SD (33). A 1-stage random-effect dose-response model was administered to assess the dose-response relation across different vitamin D dosages with serum 25(OH)D concentration.

Pooled effect size and the associated 95% CI (the overall effect sizes) for each outcome were estimated using a random-effect model. Cochran's *Q* statistic (*P* value of <0.05 to indicate statistical significance) and the *I*² statistic were used to assess the extent of heterogeneity throughout the studies. The *I*² statistic was judged as 0–40% unimportant heterogeneity, 40–70% as moderate, 70–90% as substantial, and >90% as considerable heterogeneity (34). Subgroup analyses were performed to identify the potential sources of heterogeneity. All data analyses were carried out using STATA (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP). *P* values ≤0.05 were considered statistically significant.

Sensitivity analysis

Subgroup analyses were performed to assess the impact of methodological quality of primary studies on results.

Assessment of reporting bias

Publication bias was determined with funnel plots and Egger's and Begg's test and plots. The Trim and Fill method was utilized to correct for publication bias as it was not negligible.

Results

The search of PubMed, Scopus, Web of Science, and Embase provided a total of 5973 records. After removing duplicates, 5219 citations remained. Of these, 5152 studies that did not meet our inclusion criteria were discarded after screening the title and abstract. The full texts of the remaining 67 articles were assessed in more detail, and 47 studies were excluded because they did not meet the inclusion criteria (e.g. inappropriate study design or supplementation regimen) described. Twenty-three additional articles were also identified by checking the references of relevant articles and studies that cited these articles. No unpublished relevant studies were obtained. Overall, 19 studies met the inclusion criteria and were included in the current systematic review. In addition, we identified 19 clinical trials with 27 separate comparison groups (*n* = 3337 breastfeeding mothers) that reported mothers' or infants' serum 25(OH)D concentrations as an outcome for the quantitative analysis ([Figure 1](#)).

Characteristics of included studies

The descriptive data for the 19 qualified trials are presented in [Table 1](#) and Supplemental Table 1. Five studies were carried out ≤2010 (18, 19, 24, 35, 36) and 14 were performed in or after 2011 (20, 21, 23, 25, 37–46). The average age of study participants was 29.8 ± 2.7 y. Nine studies were randomized placebo-controlled trials (20, 25, 35, 37, 38, 40, 43, 45, 46), 4 had an open control group (18, 19, 36, 44), and 6 compared outcomes between different doses of vitamin D (21, 23, 24, 39, 41, 42). Nine studies mainly included a white population (18–20, 24, 25, 38, 39, 41, 42); others were conducted in Thailand (40), Japan (36), India (37, 43, 45), South Africa (35), Qatar (21), Turkey (44), and Bangladesh (46). The duration of the interventions ranged from 4 wk to 47 wk, and the dosage of vitamin D supplementation varied between 400 and 6400 IU/d. Vitamin D was administered at multiple doses in 6 studies (19, 23, 25, 35, 39, 41). Most studies administered a daily dose of vitamin D (18–21, 23, 24, 35, 36, 38–42, 44, 45), whereas 1 (46) and 3 trials (25, 37, 43) used weekly and monthly vitamin D supplementation, respectively. Three studies also provided 400 IU/d of vitamin D to mothers in the control group (23, 24, 39). Vitamin D supplementation was given to infants in control groups in 6 trials (300–400 IU/d) (18, 19, 21, 23, 24, 35) and infants in both intervention and control groups received vitamin D supplements in 2 studies (20, 44). The mothers of both intervention and control groups received calcium supplements (200–500 mg/d) as cointerventions in 3 studies (20, 37, 46). There were 14 studies reporting changes in serum 25(OH)D in infants (18–21, 23–25, 35, 37, 39, 42, 43, 45, 46). In trials reporting baseline 25(OH)D concentrations, the average concentrations in mothers and infants were 19.59 ± 9.26 and 14.54 ± 8.42 ng/mL, respectively. Mothers' 25(OH)D concentrations at baseline were <10 ng/mL in 2 studies (11.1%) (37, 43); 10–20 ng/mL in 11 studies (61.1%) (18–21, 35, 36, 38, 40, 44–46); and >20 ng/mL in 5 studies (27.7%) (23–25, 41, 42). In studies

TABLE 1 Summary of studies included in the systematic review and meta-analysis of the effect of maternal vitamin D supplementation on the circulating 25(OH)D changes in breastfeeding women and their nursing infants¹

Authors, year	n	Maternal age, year (mean ± SD)	Country	Intervention period	Intervention		Cointerventions		Outcomes		Circulating 25(OH)D, ng/mL (mean ± SD)			
					Mothers	Infants	Mothers	Infants	Mothers	Infants	Baseline		Changes over time in intervention group	
											Mothers	Infants	Mothers	Infants
Rothberg et al., 1982 (35)	28	11.9 ± 6.0	South Africa	From delivery to 6 wk p.p.	Vitamin D3 500 or 1000 IU/d vs. placebo	Infants in control group were given 400 IU/d of vitamin D	No	Serum 25(OH)D, Ca, & P	Serum 25(OH)D, Ca, & P	500 IU/d: 11.9 ± 6.0 1000 IU/d: 11.9 ± 6.0	500 IU/d: 8.9 ± 7.1 1000 IU/d: 10.00 IU/d	500 IU/d: -1.9 ± 3.6 1000 IU/d: 1.3 ± 4.3	500 IU/d: 1.3 ± 4.3 1000 IU/d: 1.3 ± 4.3	
Ala-Houhala et al., 1985 (18)	92	—	Finland	From delivery to 20 wk p.p.	Vitamin D3 1000 IU/d vs. no treatment	Infants in control group were given 400 IU/d of vitamin D3	No	Serum 25(OH)D, P, & Ca	Serum 25(OH)D, P, & Ca	11.9 ± 6.0 Summer: 19.5 ± 11.6 Winter: 8.7 ± 3.6	8.9 ± 7.1 Summer: 7 ± 6.7 Winter: 9.7 ± 6.0	2.8 ± 3.7 Summer: -2.4 ± 7.5 Winter: 1.9 ± 5.2	0.5 ± 5.56 Summer: -6.2 ± 4.1 Winter: 1.9 ± 5.2	
Ala-Houhala et al., 1986 (19)	49	—	Finland	From delivery to 15 wk p.p.	Vitamin D3 1000 or 2000 IU/d vs. no treatment	Infants in control group were given 400 IU/d of vitamin D2	No	Serum vitamin D metabolites, total Ca, Ca ²⁺ & PTH	Serum vitamin D metabolites, total Ca, Ca ²⁺ & PTH	10.1 ± 11.0 2000 IU/d: 12.9 ± 6.0 16.9 ± 0.8	5.3 ± 6.4 1000 IU/d: 5.3 ± 6.4	18.9 ± 8.0 2000 IU/d: 25.1 ± 9.6 21.0 ± 0.73	9.5 ± 3.6 2000 IU/d: 18.0 ± 7.7 —	
Takeuchi et al., 1989 (36)	30	28.5 ± 5.7	Japan	From 1 to 5 wk p.p.	Vitamin D2 1200 IU/d vs. no treatment	No	No	Vitamin D3 and its metabolites in plasma and breast milk	—	—	—	—	—	
Wagner et al., 2006 (24)	19	29.3 ± 4.6	USA	From 1 to 6 mo p.p.	Vitamin D3 6400 vs. 400 IU/d	Infants in control group were given 300 IU/d of vitamin D3	No	Serum 25(OH)D, Ca, & breast milk activity	Serum 25(OH)D, Ca, & breast milk	34.0 ± 13.0	14.0 ± 28.2	24.8 ± 11.5	32.0 ± 17.4	
Zhang et al., 2011 (38)	100	—	Ireland	From 6 to 18 wk p.p.	Vitamin D3 800 IU/d vs. placebo	No	No	Serum 25(OH)D	—	18.3 ± 1.6	—	13.57 ± 1.6	—	
Buğrul et al., 2013 (44)	90	—	Turkey	From 1 to 6 mo p.p.	Vitamin D3 400 IU/d vs. no treatment	Infants in both intervention and control groups were given 400 IU/d of vitamin D	No	Serum 25(OH)D	Serum 25(OH)D	13.5 ± 8.6	—	0.41 ± 9.9	—	
Czech-Kowalska et al., 2014 (20)	137	30 ± 2.7	Poland	From delivery to 6 mo p.p.	Vitamin D3 1200 vs. 400 IU/d	All infants in both control and intervention groups were given 400 IU/d of vitamin D3	200 mg Ca/d	Serum 25(OH)D, iPTH, & Ca	Serum 25(OH)D, iPTH, & Ca	13.7 ± 6.5	17.9 ± 8.6	10.8 ± 9.4	15.6 ± 5.4	
Hollis et al., 2015 (23)	226	33.6 ± 6.1	USA	From 4–6 wk to 6 mo p.p.	Vitamin D3 2400 or 6400 vs. 400 IU/d	Infants in control group were given 400 IU/d of vitamin D3	No	Serum 25(OH)D, iPTH, Ca, & P	Serum 25(OH)D, iPTH, Ca, & P	39.7 ± 13.3	16.4 ± 10.2	20.8 ± 12.7	27.0 ± 9.3	
March et al., 2015 (39)	226	33.7 ± 4.4	Canada	From 13–24 wk GA to 8 wk p.p.	Vitamin D3 1000 or 2000 vs. 400 IU/d	No	No	Serum 25(OH)D & Ca	Serum & cord 25(OH)D	1000 IU/d: 25.5 ± 0.6 2000 IU/d: 26.6 ± 0.4	1000 IU/d: 29.6 ± 1.8 2000 IU/d: 38.4 ± 1.6	1000 IU/d: 6.1 ± 0.36 2000 IU/d: 9.8 ± 0.32	1000 IU/d: -8.2 ± 1.08 2000 IU/d: -7.3 ± 0.98	
Chandry et al., 2016 (37)	152	—	India	From delivery to 3.5 mo p.p.	Vitamin D3 120,000 IU/mo vs. placebo	No	500 mg Ca/d	Serum 25(OH)D	Serum 25(OH)D, Ca, P & PTH	8.9 ± 1.8	8.9 ± 1.8	17.3 ± 2.1	15.5 ± 2.7	

(Continued)

TABLE 1 (Continued)

Authors, year	n	Maternal age, year (mean $\pm$ SD)	Country	Intervention period	Intervention		Cointerventions		Outcomes		Circulating 25(OH)D, ng/mL (mean $\pm$ SD)			
					Mothers	Infants	Mothers	Infants	Mothers	Infants	Baseline	Changes over time in intervention group		
Naik et al., 2016 (45)	115	24.4 $\pm$ 3.1	India	From delivery to 6 mo p.p.	Vitamin D3 60,000 IU/d during 10 d following delivery vs. placebo	No	No	No	Serum 25(OH)D	Serum 25(OH)D	16.3 $\pm$ 9.4	9.9 $\pm$ 5.8	24.1 $\pm$ 15.2	19.2 $\pm$ 10.6
Thiele et al., 2017 (42)	13	26.5 $\pm$ 6.0	USA	From 24–28 wk GA to 4–6 wk p.p.	Vitamin D3 3800 vs. 400 IU/d	No	No	No	Serum 25(OH)D, Ca, & PTH	Serum 25(OH)D	31.4 $\pm$ 4.3	32.6 $\pm$ 2.2	46 $\pm$ 2.8	–7.68 $\pm$ 3.0
Wheeler et al., 2016 (25)	87	32.1 $\pm$ 5.0	New Zealand	From 4 to 20 wk p.p.	Vitamin D3 50,000 or 100,000 IU/mo vs. placebo monthly	No	No	No	Serum 25(OH)D, PTH, P, & Ca	Serum 25(OH)D, PTH, P, & Ca	50,000 IU/mo: 20.7 $\pm$ 10.0 100,000 IU/mo: 20.2 $\pm$ 9.2	50,000 IU/mo: 14.4 $\pm$ 7.2 100,000 IU/mo: 16.0 $\pm$ 8.0	50,000 IU/mo: 12.1 $\pm$ 10.4 100,000 IU/mo: 16.0 $\pm$ 11.7	50,000 IU/mo: 17.88 100,000 IU/mo: 21.1 $\pm$ 18.7
Niramitmahapanya et al., 2017 (40)	68	27.2 $\pm$ 5.0	Thailand	From delivery to 6 wk p.p.	Vitamin D3 1800 IU/d vs. placebo	No	No	No	Serum 25(OH)D, iPTH, Ca, & breast milk	Serum 25(OH)D	21.5 $\pm$ 5.7	—	5.78 $\pm$ 6.3	—
Roth et al., 2018 (46)	1298	25.5 $\pm$ 3.3	Bangladesh	From 17–24 wk GA to 26 wk p.p.	Vitamin D3 28,000 IU/wk vs. placebo	No	500 mg Ca, 66 mg iron & 350 μ g folic acid/d	No	Serum 25(OH)D, Ca, & iPTH	Serum 25(OH)D, Ca, & venous cord vitamin D	10.6 $\pm$ 5.3	28.0 $\pm$ 6.6	30.8 $\pm$ 6.1	4.2 $\pm$ 5.3
Stoutjesdijk et al., 2019 (41)	36	31.0 $\pm$ 3.5	The Netherlands	From 20 wk GA to 4 wk p.p.	Vitamin D3 1400 or 2400 or 3200 vs. 400 IU/d	No	Multivitamin supplement including 400 IU vitamin D + fish oil supplement/d	No	Plasma 25(OH)D, Ca, P, & breast milk vitamin D profile	—	1400 IU/d: 35.8 $\pm$ 8.8	—	1400 IU/d: –0.5 $\pm$ 6.41	—
Dawodu et al., 2019 (21)	104	29.6 $\pm$ 4.8	Qatar	From 4 wk to 7 mo p.p.	Vitamin D3 6000 IU/d vs. 600 IU/d	Infants in control group were given 400 IU/d of vitamin D	No	No	Serum 25(OH)D, Ca, PTH, & breast milk vit D	Serum 25(OH)D, Ca, & PTH	40.1 $\pm$ 5.2 14.0 $\pm$ 6.5	12.8 $\pm$ 8.7	0.7 $\pm$ 3.14 25.2 $\pm$ 10.2	24.1 $\pm$ 8.9
Trivedi et al., 2020 (43)	115	—	India	From delivery to 14 wk p.p.	Vitamin D3 60,000 IU after delivery, and at 6, 10, and 14 wk postpartum vs. placebo	No	No	No	Serum 25(OH)D	Serum 25(OH)D	5.6 $\pm$ 4.2	6.0 $\pm$ 4.5	—	12.9 $\pm$ 3.1

¹Ca, calcium; GA, gestational age; iPTH, intact parathyroid hormone; P, phosphate; Pi, inorganic phosphate; PTH, parathyroid hormone; p.p., postpartum; 25(OH)D, 25-hydroxy vitamin D.

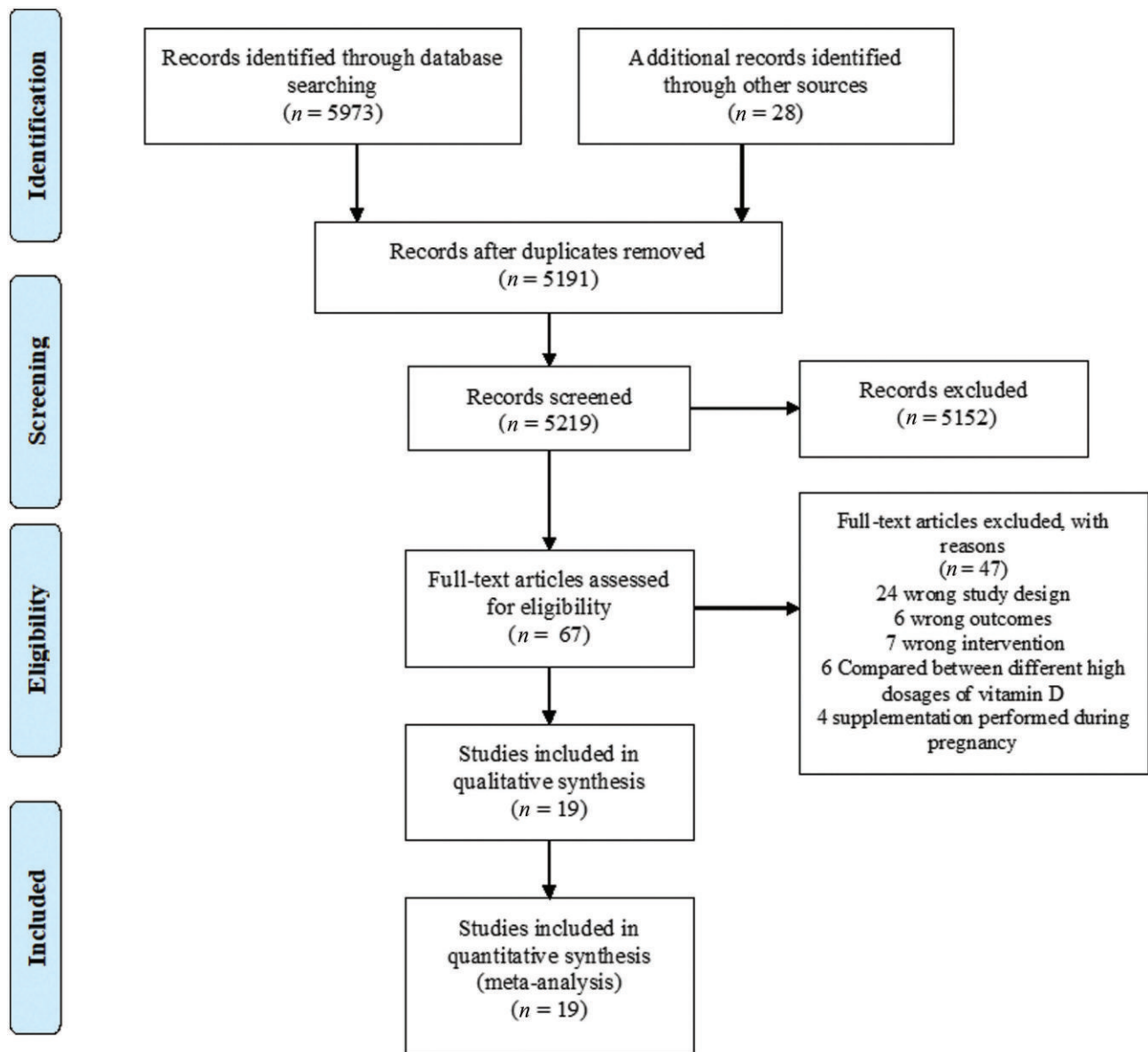


FIGURE 1 Flow diagram of the literature search process for the systematic review and meta-analysis of the effect of maternal vitamin D supplementation on the circulating 25(OH)D changes in breastfeeding women and their nursing infants. 25(OH)D, 25-hydroxy vitamin D.

measuring baseline 25(OH)D concentration in infants, 5 (35.7%) reported concentrations to be <10 ng/mL (19, 35, 37, 43, 45); 7 (50.0%) 10–20 ng/mL (18, 20, 21, 23–25, 46); and 2 (14.2%) >20 ng/mL (39, 42). Among studies with infant baseline 25(OH)D concentrations above 20 ng/mL, maternal vitamin D supplementation (400–3800 IU/d) had been implemented from 13 to 28 weeks of gestation (39, 42). Among studies showing significant improvement in infants' 25(OH)D concentrations in the intervention group (35, 37, 39, 43, 45), mothers in almost all trials were treated with vitamin D $\geq$ 1000 IU/d (37, 39, 43, 45). Infants in control groups in 5 out of 8 trials demonstrating decreases (18, 19, 46) or nonsignificant changes (20, 21, 23–25, 42) in 25(OH)D concentration were directly supplemented with vitamin D (18, 19, 21, 23, 24).

Risk of bias assessments

We applied a review manager (version 5.4) to assess the quality of the included studies. About half of the included studies were at unclear risk of selection bias due to lack of information given on random sequence generation (18, 19, 25, 35, 36, 38, 39, 41) and allocation concealment domain (18, 19, 25, 35, 36, 38, 39, 41, 44). A total of 3 studies (15%) were considered to have a low risk of performance bias because the participants were not blinded (18, 19, 41). Among the 19 trials included in the meta-analysis, 2 (10%) trials had a high risk of selection bias (other bias) due to imbalance between treatment and control groups at baseline (19, 42) and we could not determine the risk of detection bias in 7 (50%) studies (18, 19, 23, 35, 36, 41, 44) (Figure 2).

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Ala-Houhala 1985 (18)	?	?	-	?	?	+	?
Ala-Houhala 1986 (19)	?	?	-	?	-	+	-
Buğrul 2013 (44)	-	?	?	?	+	+	+
Chandy 2016 (37)	+	+	+	+	+	+	?
Czech-Kowalska 2014 (20)	+	+	+	+	+	+	+
Dawodu 2019 (21)	+	+	+	+	+	+	+
Hollis 2015 (23)	+	+	?	?	+	+	+
March 2015 (39)	?	?	+	+	+	+	+
Naik 2017 (45)	+	+	+	+	+	+	+
Nirarnitmahapanya 2017 (40)	+	+	+	+	+	+	+
Roth 2018 (46)	+	+	+	+	+	+	+
Rothberg 1982 (35)	?	?	?	?	+	+	?
Stoutjesdijk 2019 (41)	?	?	-	?	+	+	+
Takeuchi 1989 (36)	?	?	?	?	?	+	?
Thiele 2017 (42)	+	+	+	+	+	+	-
Trivedi 2020 (43)	+	+	+	+	-	+	+
Wagner 2006 (24)	+	+	+	+	?	+	?
Wheeler 2016 (25)	?	+	+	+	+	+	+
Zhang 2011 (38)	?	?	+	+	+	-	?

FIGURE 2 Risk of bias summary of included studies in the systematic review and meta-analysis of the effect of maternal vitamin D supplementation on the circulating 25(OH)D changes in breastfeeding women and their nursing infants. 25(OH)D, 25-hydroxy vitamin D.

Primary outcome

Serum 25(OH)D concentration in mothers.

Pooled data from 26 trials and 1994 breastfeeding women (18–21, 23–25, 35–42, 44–46) revealed significant differences in serum 25(OH)D between the intervention groups

supplemented with 400–6400 IU/d vitamin D and control groups receiving between 0 and 400 IU/d vitamin D [SMD (ng/mL): 3.39; 95% CI: 2.52, 4.26; $P < 0.001$] (Figure 3).

Serum 25(OH)D concentration in infants.

The pooled data from 19 trials including 1400 infants (18–21, 23–25, 35, 37, 39, 42, 43, 45, 47) indicated no significant difference between the serum 25(OH)D concentration of infants in intervention groups whose mothers supplemented with 400–6400 IU/d vitamin D compared with infants in the control group who received placebo or direct vitamin D supplementation ≤ 600 IU/d [SMD (ng/mL): 0.49; 95% CI: –0.37, 1.34; $P = 0.26$] (Figure 4).

Secondary outcomes

Milk antirachitic activity (ARA).

We found a significant increase in milk ARA in women supplemented with vitamin D compared with their counterparts in control groups [including 4 trials and 261 breastfeeding women (21, 24, 36, 40) – SMD (IU/L): 1.21; 95% CI: 0.56, 1.86; $P < 0.001$] (Figure 5). However, only 1 study (milk ARA of 791 IU/L in the intervention group) (24) reached the IOM recommended dose of 400 IU/d for infants aged 0–6 mo, which translates to a milk ARA of 513 IU/L (41). We also observed that maternal supplementation with 6000 IU/d vitamin D supplied breast milk with 564 IU/L ARA. The prediction equation was as follows: milk ARA (IU/L) = $0.099 \times \text{vitamin D3 dosage (IU/d)} + (-30.0)$ ($R^2 = 0.59$).

Serum PTH, phosphorous, and calcium in mothers.

Overall, we observed no significant changes in PTH concentrations of mothers supplemented with vitamin D compared with controls [including 9 trials and 886 breastfeeding women (19–21, 23, 25, 42, 46) – SMD (pmol/L): –0.61; 95% CI: –1.60, 0.38; $P = 0.22$] (Supplemental Figure 1A). However, we found a significant reduction in serum PTH concentration of breastfeeding women who were given ≥ 4000 IU/d [including 2 trials and 322 breastfeeding women (21, 23) – SMD (pmol/L): 0.30; 95% CI: –0.57, –0.04; $P = 0.02$] (Supplemental Figure 1B). Vitamin D supplementation did not display statistically significant effects on serum phosphorous [12 trials including 669 women (18, 19, 23, 25, 41) – SMD (mmol/L): –0.08; 95% CI: –0.32, 0.15; $P = 0.47$] (Supplemental Figure 2) and calcium concentrations [17 trials including 1309 women (18, 19, 23–25, 40–42, 46, 48) – SMD (mmol/L): 0.03; 95% CI: –0.28, 0.33; $P = 0.85$] between supplemented and nonsupplemented women (Supplemental Figure 3).

Serum PTH, phosphorous, and calcium in infants.

Overall, serum PTH concentrations in infants whose mothers were treated with vitamin D did not change compared with the control group [7 trials including 635 breastfed infants (19–21, 23, 25) – SMD (pmol/L): –0.11; 95% CI: –0.27, 0.04; $P = 0.15$] (Supplemental Figure 4). In contrast, a significant reduction of serum PTH concentration in infants whose mothers received vitamin D supplement

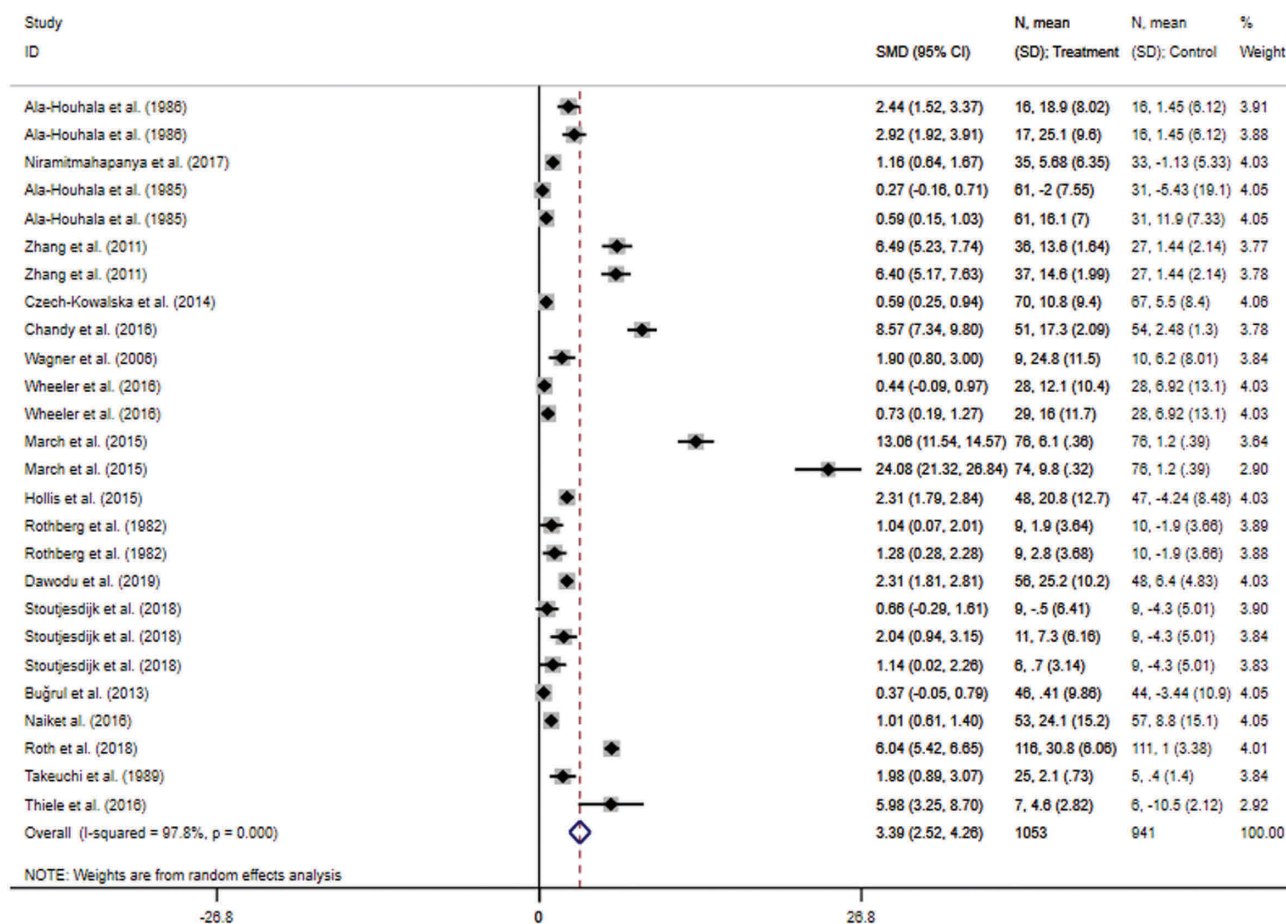


FIGURE 3 Forest plot of the effect of vitamin D supplementation on serum 25-hydroxy vitamin D (25(OH)D) (ng/mL) concentration in mothers. Values are the standardized mean differences (SMDs) and 95% CI of serum 25(OH)D concentration between treatment and control groups.

compared with infants given placebo was noted [1 trial including 124 breastfed infants (25) – SMD (pmol/L): –0.37; 95% CI: –0.75, 0.00; $P = 0.05$] (data not shown). There were no statistically significant differences in serum phosphorus [9 trials including 667 breastfed infants (18, 19, 23, 25, 35, 37) – SMD (mmol/L): 0.07; 95% CI: –0.43, 0.57; $P = 0.77$] (**Supplemental Figure 5**) and calcium [12 trials including 1048 breastfed infants (18, 19, 21, 23–25, 35, 46) – SMD (mmol/L): –0.24; 95% CI: –0.66, 0.19; $P = 0.27$] (**Supplemental Figure 6**) concentrations between the intervention and control groups.

Dose-response analysis

A 1-stage random-effects dose-response model using restricted cubic splines in studies in which placebos or no treatment groups were compared with individuals taking vitamin D supplements (range: 400 to 6400 IU/d) showed a statistically significant nonlinear relation between vitamin D supplement dosages and circulating 25(OH)D concentrations in breastfeeding mothers, with an estimate in the correlation matrix of 0.0086 and the estimated 95% CI of 0.0041 and 0.012 across studies ($P < 0.001$). We observed that 1000 IU vitamin D supplementation raised the serum

25(OH)D concentration by 7.74 ng/mL, whereas the rate of 25(OH)D elevation was lower at vitamin D dosages of ≥ 2000 IU/d (**Figure 6A**) (18, 19, 25, 35–38, 40, 44–46). Also, the 1-stage random-effects dose-response analysis exhibited a linear association between maternal vitamin D supplement doses and circulating 25(OH)D concentrations in infants when studies were limited to those in which infants in control groups were given a placebo and the intervention commenced at postpartum (parameter estimate from the mixed-effects regression model: 2.73×10^{-3} , 95% CI: 1.19×10^{-3} , 4.26×10^{-3} , $P = 0.0005$) (20, 25, 37, 39, 43, 45). We observed that each 1000 IU of vitamin D supplementation in breastfeeding mothers was accompanied by a 2.73 ng/mL increase in serum 25(OH)D concentration in their infants (**Figure 6B**). Additionally, there was a positive linear association between maternal vitamin D dosages and breast milk ARA (correlation coefficient: 3.94×10^{-2} , 95% CI: 2.25×10^{-3} , 7.66×10^{-2} , $P = 0.03$) (21, 24, 36, 40), as 6000 IU/d of vitamin D increased breast milk ARA by 236.51 IU/L (95% CI: 13.55–459.47) (**Figure 6C**).

Subgroup analyses

Subgroup analyses were performed based on vitamin D supplement dosages (≤ 4000 or > 4000 IU/d in mothers,

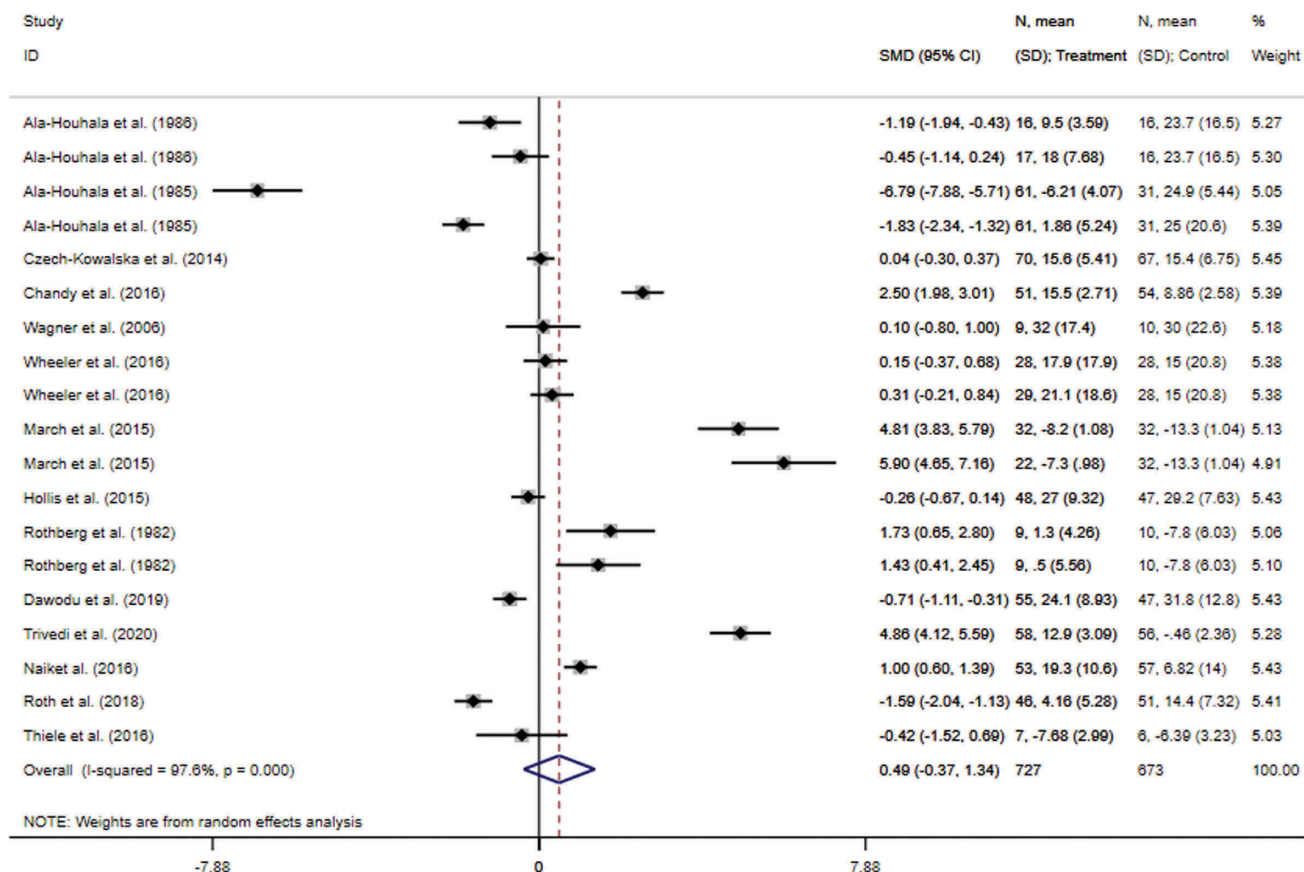


FIGURE 4 Forest plot of the effect of vitamin D supplementation on serum 25-hydroxy vitamin D (25(OH)D) (ng/mL) concentration in infants. Values are the standardized mean differences (SMDs) and 95% CI of serum 25(OH)D concentration between treatment and control groups.

and ≤ 1000 or > 1000 IU/d in infants); if breastfeeding women and infants in the control group received a lower dosage of vitamin D instead of placebo; trial duration (≤ 10 or > 10 wk in mothers, and ≤ 20 or > 20 wk in infants); baseline 25(OH)D concentration (< 20 or ≥ 20 ng/mL); year of study publication (< 2011 or ≥ 2011); the season of intervention (winter or summer or all seasons); study sample size (< 100 or ≥ 100); ethnicity (white or East Indian or other ethnicities); if vitamin D supplementation was started during pregnancy and continued to postpartum; maternal BMI (< 25 or ≥ 25 kg/m²); type of assay used to measure 25(OH)D concentration; quality of studies (low or high or unclear risk of bias); and the vitamin D supplementation frequency (daily or weekly/monthly) (Table 2).

Mothers.

There were no significant interactions for the effect of vitamin D supplements on mothers' serum 25(OH)D in terms of mothers' baseline 25(OH)D (P -interaction = 0.09), ethnicity (P -interaction = 0.17), and BMI (P -interaction = 0.31) across studies. Conversely, vitamin D dosage (P -interaction = 0.01), control group vitamin D supplementation status (P -interaction = 0.02), trial duration (P -interaction < 0.001), year of study publication (P -

interaction < 0.001), season of intervention (P -interaction < 0.001), study sample size (P -interaction < 0.001), intervention period (P -interaction = 0.01), type of assay used to measure 25(OH)D concentration (P -interaction < 0.001), and quality of studies (P -interaction < 0.001) interacted with the effects of vitamin D supplementation in modifying serum 25(OH)D concentrations in breastfeeding women. In studies with longer duration, women in the intervention group showed a greater increase in serum 25(OH)D concentrations compared with controls (18–21, 23–25, 37–39, 41, 42, 44–46) [22 trials, 1744 breastfeeding women – SMD (ng/mL): 3.80, 95% CI: 2.79, 4.80; $P < 0.001$]. Further, the largest increase in maternal 25(OH)D concentration was observed in the studies with a duration of ≥ 8 wk and using weekly or monthly dosing schemes (18–21, 23, 24, 38, 39, 41, 42, 44, 45) (P -interaction < 0.001) [18 trials, 445 breastfeeding women – SMD (ng/mL): 3.91, 95% CI: 0.57, 7.25; $P = 0.02$]. In the trials conducted after or in 2011 (20, 21, 23, 25, 37–42, 44–46), mothers in the intervention group had a greater serum 25(OH)D concentration compared with the control group [18 trials, 1544 women – SMD (ng/mL): 4.31, 95% CI: 3.12, 4.94; $P < 0.001$]. Among studies in which vitamin D supplementation was done during all seasons (20, 21, 23, 25, 37, 39, 41,

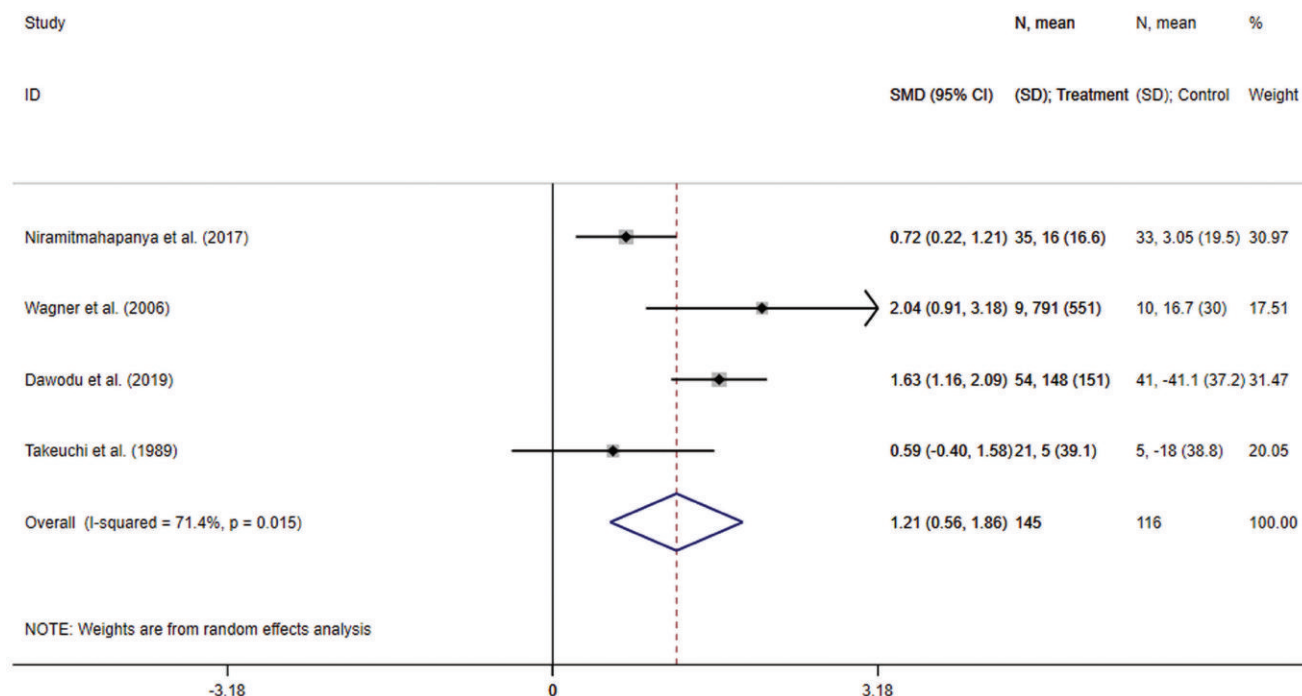


FIGURE 5 Forest plot of the effect of vitamin D supplementation on milk antirachitic activity (IU/L). Values are the standardized mean differences (SMDs) and 95% CI of milk antirachitic activity between treatment and control groups.

42, 44–46), women in the intervention groups showed a greater increase in circulating 25(OH)D concentrations [15 trials, 1349 women – SMD (ng/mL): 4.25, 95% CI: 2.93, 5.58; $P < 0.001$]. Moreover, among studies with a larger sample size (≥ 100) (20, 21, 23, 37–39, 45, 46) [10 trials, 1207 breastfeeding women – SMD (ng/mL): 6.78, 95% CI: 4.75–8.81; $P < 0.001$], a greater increase in serum 25(OH)D concentrations among mothers in the treatment compared with the control group was observed. Breastfeeding mothers of white ethnicity (18–20, 23–25, 38, 39, 41, 42) [17 trials, 1108 breastfeeding women – SMD (ng/mL): 3.87, 95% CI: 2.70, 5.05; $P < 0.001$] and those whose treatment started from gestation and continued to postpartum (5, 11, 13, 15) [7 trials, 595 breastfeeding women – SMD (ng/mL): 7.43, 95% CI: 3.58, 11.28; $P < 0.001$] tended to have higher serum 25(OH)D concentrations in the treatment groups. With respect to study quality, vitamin D supplementation led to a larger increase in maternal 25(OH)D concentration among trials with a low risk of bias (20, 21, 23–25, 36, 37, 39, 40, 42, 45, 46) [14 trials, 1313 breastfeeding women – SMD (ng/mL): 4.64, 95% CI: 3.25, 6.03; $P < 0.001$]. Moreover, studies with a low risk of performance, detection, and attrition bias showed a greater increase in mothers' serum 25(OH)D concentration in the intervention than control groups, and the differences between groups were also significant (data not shown). No significant interaction was observed across studies in terms of supplementation frequency (data not shown).

Infants.

The serum 25(OH)D concentrations of infants whose mothers received vitamin D dosages of > 1000 IU/d were trending

higher than those in the control group (19–21, 23–25, 37, 39, 42, 43, 45, 46) [13 trials, 992 infants – SMD (ng/mL): 0.83, 95% CI: -0.02, 1.68; $P = 0.05$]. Moreover, among the trials in which infants in the control groups were not treated with vitamin D, infant 25(OH)D concentrations were higher in the intervention compared with nonintervention groups (25, 37, 39, 42, 43, 45, 46) [9 trials, 670 infants – SMD (ng/mL): 1.91, 95% CI: 0.49, 3.33; $P = 0.008$]. Likewise, in trials with follow-up duration > 20 wk, a significant increase in infants' serum 25(OH)D concentrations in the intervention group compared with controls was noted (20, 21, 39, 43, 45, 46) [7 trials, 678 infants – SMD (ng/mL): 1.98, 95% CI: 0.39, 3.56; $P = 0.01$]. Studies published after 2010 (20, 21, 23, 25, 37, 39, 42, 43, 45, 46) showed significant differences between supplemented and control groups in infant 25(OH)D concentrations [12 trials, 1004 infants – SMD (ng/mL): 1.33, 95% CI: 0.34, 2.31; $P = 0.008$]. Infants in the treatment groups where maternal supplementation was carried out during all seasons (20, 21, 23, 25, 37, 39, 42, 43, 45, 46) exhibited a significant increase in serum 25(OH)D concentration compared with the nontreatment group [12 trials, 1004 infants – SMD (ng/mL): 1.33, 95% CI: 0.34, 2.31; $P = 0.008$]. Additionally, in trials with larger sample sizes (20, 21, 23, 37, 39, 43, 45, 46) [9 trials, 878 infants – SMD (ng/mL): 1.77, 95% CI: 0.50, 3.04; $P = 0.006$] and those involving East Indian participants (37, 43, 45) [4 trials, 329 infants – SMD (ng/mL): 2.76, 95% CI: 0.76, 4.77; $P < 0.001$], increases in infant circulating 25(OH)D concentrations were higher in the intervention group. Further, for trials in which maternal BMI was < 25 kg/m², a significant increase in infant serum 25(OH)D concentrations in intervention groups compared with controls was observed (20, 37, 43, 45) [4 trials, 466

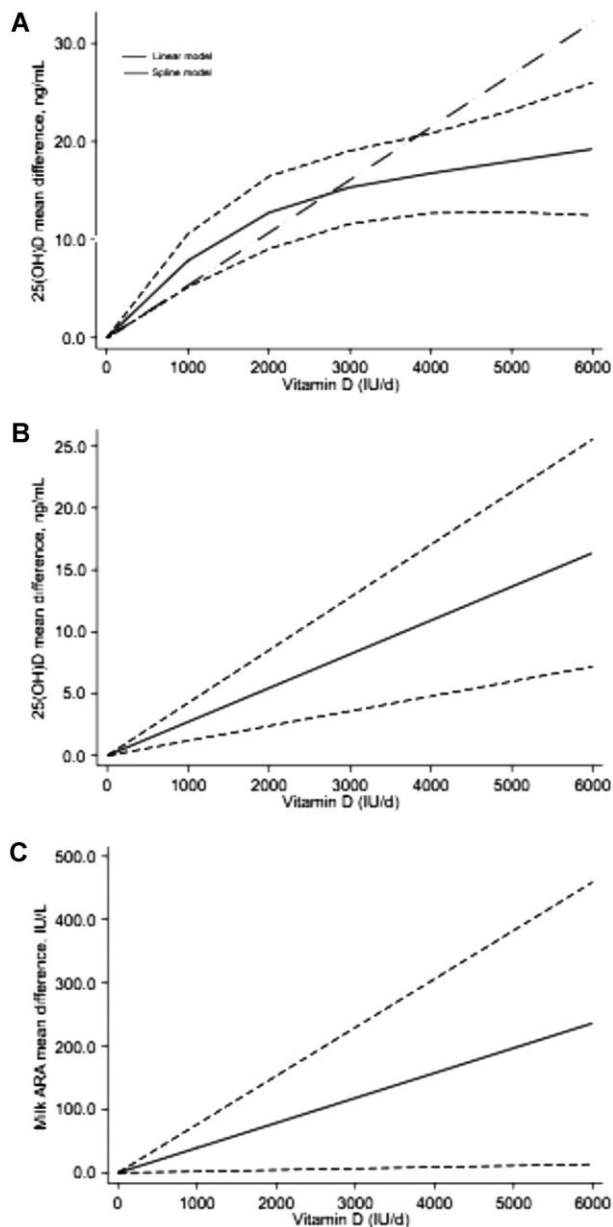


FIGURE 6 Dose-response effects of vitamin D supplementation on A) circulating 25-hydroxy vitamin D (25(OH)D) concentration (ng/mL) in breastfeeding mothers, B) infants, and C) milk antirachitic activity (ARA) (IU/L). Continuous line indicated linear model and dotted line indicated 95% confidence.

infants – SMD (ng/mL): 2.07, 95% CI: 0.36, 3.78; $P = 0.01$]. Finally, in studies with an overall low risk of bias, infants in the intervention groups showed a significant increase in serum 25(OH)D concentration compared with controls (20, 21, 23–25, 37, 39, 42, 43, 45, 46) [13 trials, 1023 infants – SMD (ng/mL): 1.23, 95% CI: 0.30, 2.17; $P = 0.01$], as well as studies with a low risk of detection, performance, and attrition bias (data not shown).

The tests for interactions of trial duration (P -interaction = 0.01), date of study publication (P -interaction = 0.02), season of intervention (P -interaction <

0.001), study sample size (P -interaction < 0.001), type of assay used to measure 25(OH)D concentration (P -interaction = 0.01), and quality of studies (P -interaction < 0.001) investigating the effect of maternal vitamin D supplementation on infants' serum 25(OH)D concentrations were statistically significant. Likewise, we found a significant interaction between studies in which infants in control groups received a vitamin D supplement or not (P -interaction = 0.01). In contrast, there were no interactions between maternal vitamin D supplement dosages (P -interaction = 0.42), infants' baseline 25(OH)D concentrations (P -interaction = 0.08), and ethnicity (P -interaction = 0.05) on circulating 25(OH)D concentrations in infants (Table 2). No significant interactions were found between studies regarding supplementation frequency and if intervention spanned from pregnancy to postpartum (data not shown).

Discussion

Our dose-response analyses indicated a nonlinear relation between vitamin D supplement dosage and circulating 25(OH)D concentration in breastfeeding mothers; the increases in serum 25(OH)D concentration were attenuated from lower to higher supplemental doses. This dose-dependent manner was also supported by the findings of previous reviews suggesting a biphasic relation between vitamin D supplementation dose and 25(OH)D concentration, as the increases in 25(OH)D concentration reaches a plateau with progressively higher doses (49–52). In the subgroup analysis of breastfeeding mothers, trial duration and maternal vitamin D supplementation spanning both pregnancy and postpartum were significant predictors of achieved 25(OH)D concentrations. In line with our analysis, evidence from previous studies also indicated that sustained long-term, lower dose (<1500 IU/d) vitamin D regimens were more effective than short-term, higher dose (>3000 IU/d) supplements to maintain adequate vitamin D status (53). This finding suggests that vitamin D supplementation should begin during pregnancy to ensure a sufficient vitamin D status to prevent rickets in infants. The largest increase in maternal 25(OH)D concentration was observed among individuals who received weekly or monthly dosing schemes. Following a daily vitamin D intake regimen can be challenging for long periods, and a single large dose of vitamin D may improve adherence, especially in children and the elderly (54). However, the compliance rate among breastfeeding mothers and their infants appears to be higher (55). Furthermore, it is suggested that daily vitamin D supplementation leads to a more sustained elevation in serum 25(OH)D concentration, which is preferable for breastfeeding women (56).

In general, we observed no significant difference in circulating 25(OH)D concentration between infants in the intervention group whose mothers were given vitamin D supplements (ranging from 400 to 6400 IU/d) and those infants in the control groups who received a placebo or vitamin D supplements of ≤ 600 IU/d. The subgroup analysis did

TABLE 2 Subgroup analyses for the effect of maternal vitamin D supplementation on the circulating 25(OH)D changes in breastfeeding women and their nursing infants¹

	Subgroup	Participants, n	Studies, n	References	SMD ² (95% CI), ng/mL	P value for overall effect	I ²	P value for subgroup difference
Mothers	Dosage, IU/d	1662	23	(18–20, 25, 35–42, 44–46)	3.59 (2.60, 4.58)	<0.001	98.0%	0.01
	>4000 IU/d	218	3	(21, 23, 24)	2.27 (1.93, 2.62)	<0.001	0.0%	
	Control groups supplemented with vitamin D	723	10	(20, 21, 23, 24, 39, 41, 42)	5.05 (3.14, 6.96)	<0.001	97.4%	0.02
	Duration of trial, wk	1157	16	(18, 19, 25, 35–38, 40, 44–46)	2.54 (1.57, 3.52)	<0.001	98.3%	
	≤10 wk	136	4	(35, 36, 40)	1.26 (0.87, 1.65)	<0.001	0.0%	<0.001
	>10 wk	1744	22	(18–21, 23–25, 37–39, 41, 42, 44–46)	3.80 (2.79, 4.80)	<0.001	98.1%	
	Duration of trial, wk/frequency of supplementation	1299	18	(18–21, 23, 24, 38, 39, 41, 42, 44, 45)	3.73 (2.70, 4.76)	<0.001	97.7%	<0.001
	<8 wk/daily	136	4	(35, 36, 40)	1.26 (0.87, 1.65)	<0.001	0.0%	
	≥8 wk/weekly or monthly	445	4	(25, 37, 46)	3.91 (0.57, 7.25)	0.02	99.1%	
	Baseline 25(OH)D, ng/mL	1217	15	(18–21, 35–38, 44–46)	2.76 (1.75, 3.76)	<0.001	97.6%	0.09
Year of study publication	<20 ng/mL	663	11	(23–25, 39–42)	4.50 (2.72, 6.28)	<0.001	98.1%	
	≥20 ng/mL	336	8	(18, 19, 24, 35, 36)	1.48 (0.81, 2.16)	<0.001	83.9%	<0.001
	<2011	1544	18	(20, 21, 23, 25, 37–42, 44–46)	4.31 (3.12, 5.51)	<0.001	98.4%	
	≥2011	195	5	(18, 19, 35)	1.62 (0.68, 2.55)	0.001	84.5%	<0.001
	Winter	92	1	(18)	0.27 (–0.16, 0.71)	0.22	—	
	Summer	1349	15	(20, 21, 23, 25, 37, 39, 41, 42, 44–46)	4.25 (2.93, 5.58)	<0.001	98.5%	
	All seasons	673	16	(18, 19, 24, 25, 35, 36, 40–42, 44)	1.25 (0.85–1.65)	<0.001	79.1%	<0.001
	<100	1207	10	(20, 21, 23, 37–39, 45, 46)	6.78 (4.75–8.81)	<0.001	99.0%	
	≥100	1108	17	(18–20, 23–25, 38, 39, 41, 42)	3.87 (2.70, 5.05)	<0.001	97.8%	0.17
	White	772	9	(21, 35–37, 40, 41, 44–46)	2.14 (1.50, 2.78)	<0.001	97.8%	
Intervention period	Others	595	7	(39, 41, 42, 46)	7.43 (3.58, 11.28)	<0.001	98.7%	0.01
	Pregnancy and lactation							
	Lactation	1285	19	(18–21, 23–25, 35–38, 40, 44, 45)	2.11 (1.43, 2.80)	<0.001	95.5%	
	<25 kg/m ²	352	3	(20, 37, 45)	3.27 (0.78, 5.75)	0.01	98.7%	0.31
	≥25 kg/m ²	325	5	(21, 23, 25, 42)	1.86 (0.83, 2.89)	<0.001	92.5%	
	CPBA	306	7	(18, 19, 24, 35)	1.42 (0.70, 2.14)	0.01	85.0%	<0.001
	HLPC	120	2	(36, 44)	1.09 (–0.48, 2.66)	<0.001	86.3%	
	Immunoassay	993	10	(20, 21, 23, 37–39, 42, 45)	6.78 (4.76, 8.79)	0.01	98.8%	
	LC-MS/MS	461	8	(25, 40, 41, 46)	1.75 (0.14, 3.36)	<0.001	97.4%	
	Low risk of bias	1313	14	(20, 21, 23–25, 36, 37, 39, 40, 42, 45, 46)	4.64 (3.25, 6.03)	<0.001	98.5%	<0.001
Quality of studies	High risk of bias	208	6	(19, 41, 44)	1.56 (0.62, 2.50)	0.001	86.2%	
	Unclear risk of bias	349	6	(18, 35, 38)	2.60 (0.92, 4.29)	0.002	96.9%	

(Continued)

TABLE 2 (Continued)

Subgroup	Participants, n	Studies, n	References	SMD ² (95% CI), ng/mL	P value for overall effect	I ²	P value for subgroup difference
Infants							
Dosage, IU/d	318	6	(18, 19, 35, 39)	-0.31 (-2.98, 2.36)	0.82	96.6%	0.42
Control groups supplemented with vitamin D	992	13	(19-21, 23-25, 37, 39, 42, 43, 45, 46)	0.83 (-0.02, 1.68)	0.05	98.5%	
	503	9	(18, 19, 21, 23, 24, 35)	-0.88 (-1.91, 0.16)	0.09	95.7%	0.01
Duration of trial, wk	670	9	(25, 37, 39, 42, 43, 45, 46)	1.91 (0.49, 3.33)	0.008	98.0%	
	632	12	(18, 19, 23-25, 35, 37, 42)	-0.38 (-1.40, 0.65)	0.47	96.6%	0.01
Duration of trial, wk/frequency of supplementation	678	7	(20, 21, 39, 43, 45, 46)	1.98 (0.39, 3.56)	0.01	98.5%	
	843	18	(18-21, 23, 24, 39, 42, 45)	-0.01 (-1.04, 1.02)	0.98	97.4%	0.05
	38	4	(35)	1.57 (0.83, 2.31)	<0.001	0.0%	
Baseline 25(OH)D, ng/mL	429	4	(25, 37, 43, 46)	1.24 (-0.74, 3.21)	0.22	98.5%	
Year of study publication	1179	16	(19-21, 24, 25, 35, 37, 43, 45, 46, 68)	-0.03 (-0.87, 0.81)	0.94	97.4%	0.08
Season of intervention	131	3	(39, 42)	3.43 (-0.33, 7.19)	0.07	97.1%	
	306	7	(18, 19, 24, 35)	-1.00 (-2.64, 0.64)	0.23	96.6%	0.02
	1004	12	(20, 21, 23, 25, 37, 39, 42, 43, 45, 46)	1.33 (0.34, 2.31)	0.008	97.7%	
	195	5	(18, 19, 35)	-0.12 (-1.41, 1.17)	0.85	93.0%	<0.001
	82	1	(18)	-6.79 (-7.88, -5.71)	<0.001	—	
Study sample size, n	1004	12	(20, 21, 23, 25, 37, 39, 42, 43, 45, 46)	1.33 (0.34, 2.31)	0.008	97.7%	
	432	10	(18, 19, 24, 25, 42)	-0.69 (-1.80, 0.42)	0.22	95.6	<0.001
	878	9	(20, 21, 23, 37, 39, 43, 45, 46)	1.77 (0.50, 3.04)	0.006	98.3	
Ethnicity	744	12	(18-20, 23-25, 39, 42)	0.01 (-1.05, 1.06)	0.98	97.2%	0.05
	329	4	(37, 43, 45)	2.76 (0.76, 4.77)	0.007	97.7%	
	237	4	(21, 35, 46)	0.12 (-1.17, 1.41)	0.85	94.1%	
Maternal BMI, kg/m ²	466	4	(20, 37, 43, 45)	2.07 (0.36, 3.78)	0.01	98.2%	0.01
	323	5	(21, 23, 25, 42)	-0.18 (-0.58, 0.22)	0.38	65.9%	
Assay type	306	7	(18, 19, 24, 35)	-1.00 (-2.64, 0.64)	0.23	96.6%	0.01
	794	10	(20, 21, 23, 37, 39, 42, 43, 45)	1.92 (0.69, 3.14)	0.002	98.0%	
	210	3	(16, 46)	-0.38 (-1.62, 0.86)	0.54	94.6%	
Quality of studies	1023	13	(20, 21, 23-25, 37, 39, 42, 43, 45, 46)	1.23 (0.30, 2.17)	0.01	97.5%	<0.001
	65	2	(19)	-0.80 (-1.53, -0.08)	0.02	49.8%	

¹BMI; Body mass index; SMD, standardized mean difference; 25(OH)D, 25-hydroxy vitamin D.²Values are the standardized mean differences and 95% CI of serum 25(OH)D concentration between treatment and control groups in mother-infant dyads.

not show any interaction between either maternal vitamin D supplement dosages or infants' 25(OH)D concentrations at baseline with circulating 25(OH)D concentrations at the final visit. However, we found a significant difference in 25(OH)D concentrations across studies in which infants in the control group received a placebo or vitamin D supplement, i.e. maternal vitamin D supplementation led to a significant increase in infant circulating 25(OH)D concentration in the subgroup of trials where infants in the control groups were given a placebo. It suggests that the direct supplementation of vitamin D to infants may be more effective in improving their vitamin D status than maternal supplementation with vitamin D <4000 IU/d. Likewise, a dose-response analysis of trials where infants in the control group received a placebo revealed a significant linear relation between maternal vitamin D supplementation dosages and infants' circulating 25(OH)D concentrations. A dose-response analysis indicated that maternal vitamin D supplementation with 6000 IU/d increased infants' 25(OH)D concentration by 16.4 ng/mL. This increase is almost comparable to the values achieved when infants were directly supplemented with vitamin D3 at a dose of 300–400 IU/d (~12 ng/mL) (23, 24). According to Tan et al., direct supplementation with 400 IU of vitamin D/d increased infant 25(OH)D concentrations by 22.60 nmol/L, comparable to 24.60 nmol/L from maternal supplementation (57). Additionally, it has been shown that infants who received maternal vitamin D supplements at dosages of ≥ 6000 IU/d (21, 23, 24) or a bolus dose [120,000 IU/mo (37)] attained serum 25(OH)D concentrations of ≥ 20 ng/mL, equivalent to daily 300–400 IU of oral vitamin D3 for infants. Nevertheless, it should be noted that maternal supplementation with >6000 IU/d is recommended when it is the sole source of vitamin D for the infants to overcome vitamin D deficiency.

Another promising finding was a significant rise in milk ARA in women supplemented with vitamin D compared with controls. A dose-response analysis indicated a linear relation between maternal vitamin D doses and milk ARA, which corresponded to a 237 IU/L increase for each 6000 IU of vitamin D. However, none of these studies reached the IOM (58) recommended dose of 400 IU/d for infants aged 0–6 mo, which translates to a milk ARA of 513 IU/L, except in a trial by Wagner, et al. (24). Together, the present findings suggest that either maternal dosages of ≥ 2000 IU/d combined with infant supplementation (59) or maternal supplementation with a vitamin D dose >6000 IU/d for ≥ 20 wk in the early lactation period could be interchangeably used to build vitamin D store in mother-infant dyads.

When performing the test for subgroup differences of serum 25(OH)D concentrations, statistically significant differences were found when comparing trials published before and during 2010 compared with 2011 and later. A possible reason for these differences may be due to the various measurement methods applied for estimating circulating 25(OH)D concentration during these 2 decades (60, 61), as shown in our subgroup analysis (Table 2).

Therefore, the results of this review are potentially challenged by different laboratory methods used to quantify 25(OH)D concentration, contributing to the high level of heterogeneity in our analyses (62, 63). Hence, 25(OH)D data cannot be interpreted accurately and precisely without standardization of the assay (64, 65).

Further, we performed subgroup analyses based on the quality of studies to determine sources of heterogeneity. Among studies with a low risk of bias, infants' circulating 25(OH)D concentration increased significantly following maternal vitamin D supplementation, whereas this effect was not noted in studies with a high or unclear risk of bias. Similarly, we found a greater increase in 25(OH)D concentrations in breastfeeding mothers in the intervention than the control group in trials with a low risk of bias. It should be taken into account that the quality of studies is a multidimensional concept including external and internal validity, which may distort findings of systematic review and meta-analyses, as the findings of studies with low or unclear risk of bias may be invalid (66).

Strong evidence of heterogeneity was observed in the current analyses. The asymmetrical funnel plot in our analysis suggested either a selective reporting bias or a publication bias, or both. In addition, the results of Begg's and Egger's tests confirmed that publication bias might be responsible for some effects observed in our analyses. The Trim and Fill method was employed to reduce the bias in pooled estimates (Supplemental Figure 7). The corrected effect size resulting from imputed studies on the left and right side of the funnel plot remained unchanged or increased, respectively.

To our knowledge, this is the first study to combine data from 19 published studies, including 3337 breastfeeding women, to estimate the dose-response treatment effects of vitamin D supplements on 25(OH)D concentration and other biomarkers related to vitamin D in mothers and their infants. We also examined how participant and intervention characteristics, as well as the timing of outcome measurements, may affect 25(OH)D concentration. However, our work has some limitations. The main limitation of the current analysis is substantial unexplained heterogeneity which limits our meta-analytic results. We sought to determine the reason behind heterogeneity among the results of included studies by performing a quality assessment, random-effects meta-analysis, and subgroup or a meta-regression analysis. The result of subgroup analysis implies substantial heterogeneity in the current review would arise partly from differences in intervention characteristics (e.g. dose and duration). However, we were not able to explain the other source of heterogeneities across included studies. Given this limitation, the findings of this pooled analysis should be interpreted with caution since it could cause inaccurate summary effects and associated conclusions (67).

In this investigation, we focused on clinical and statistical heterogeneity, contributing to modifying the magnitude of the intervention effect. Nonetheless, factors such as variability in recruitment and measurement instruments, chance,

and/or analytical methods, which may not be discernible from published results, could also influence findings. Our analyses are further limited by the low methodological quality of some primary studies, limited data regarding vitamin D content of breast milk, different racial and ethnic groups, and other personal and lifestyle factors (e.g. exposure to sunlight, skin pigmentation). A gap in the vitamin D dosages used in primary studies should also be noted. For example, most primary studies administered vitamin D at doses of 2000, 4000, or 6400 IU/d, whereas a few studies used dosages between those amounts. Last, but not least, few studies have been conducted early enough to identify the effect of low doses of vitamin D over a long period since supplementation during pregnancy would lead to achieving adequate maternal status, and the infant would still be protected at lower maternal doses.

Additional high-quality trials on the effects of maternal vitamin D supplementation on the antirachitic content of human milk and vitamin D status of breastfed infants based on different doses during pregnancy and lactation are required. Further, studies comparing the effect of infant oral vitamin D supplementation compared with breastfeeding mothers' supplementation in achieving desirable vitamin D status in both mothers and infants are also required.

In conclusion, our findings suggested that maternal vitamin D supplementation doses had a nonlinear association with circulating 25(OH)D concentrations in breastfeeding mothers and a linear relation with infants' serum 25(OH)D concentration in placebo-controlled trials. Our data proposed that either maternal supplementation with ≥ 2000 IU/d combined with infant supplementation or solely maternal supplementation with vitamin D doses of > 6000 IU/d for ≥ 20 wk could be interchangeably recommended to build vitamin D stores in mother-infant dyads with vitamin D insufficiency. It appears that direct infant supplementation with vitamin D may be more effective in improving their vitamin D status than maternal supplementation with vitamin D < 4000 IU/d. However, it should be noted that when infants have 25(OH)D values > 20 ng/mL, the maternal dose needed to maintain vitamin D sufficiency is not as high and may be in alignment with the RDA and AI for infants. However, there is still insufficient evidence to suggest a policy change, and more trials need to be carried out.

Acknowledgments

The authors' responsibilities were as follows—EK, and AA: conceived the current analysis; EK and AA: performed data collection and statistical analysis; EK, AA, and CLW: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript; PS: contributed to data collection; SHD: assisted in manuscript preparation and interpretation of results, reviewed, and revised the manuscript; WBP: contributed to the interpretation of results and revision of the manuscript; and all authors: read and approved the final manuscript and agree to be accountable for all aspects of the work.

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