Effect of vitamin D supplementation or fortification on bone turnover markers in women: A systematic review and meta-analysis

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Abbreviations: RCT: Randomized Clinical Trial; sCTX: serum C-terminal Telopeptide of type-I collagen; uNTX: urinary type I collagen cross-linked N-telopeptide; OC: Osteocalcin; BALP: Bone Alkaline Phosphatase; P1NP: Procollagen type-1 intact N-terminal Propeptide; MD: Mean difference; CI: Confidence Interval; BMD: Bone Mineral Density; 25(OH)D: 25-hydroxy vitamin D; PTH: Parathyroid Hormone; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; GRADE: Grading of Recommendation Assessment, Development, and Evaluation: RoB2: Revised Cochrane Risk of Bias tool for randomized trials; SE: standard error.

Conclusions

In conclusion, this study found a favorable effect of vitamin D consumption on bone turnover markers in women, lementation or fortificationindicating that vitamin D supp might be an effective nutritional strategy for improving bone health. The meta-analysis showed that vitamin D intake significantly reduced bone resorption markers including sCTX, uNTX. A significant reduction in levels of OC, a bone formation marker, was also observed. However, there was no significant effect of vitamin D supplementation or fortification on P1NP and BALP levels. This meta-analysis suggested that age, sample size, dose, duration, baseline vitamin D level, study region, and quality of studies might be sources of heterogeneity. Lastly, this meta-analysis did not find a consistent dose-response relationship between vitamin D and bone turnover markers in women.

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Abstract

Vitamin D is a vital indicator of musculoskeletal health, as it plays an important role through the regulation of bone and mineral metabolism. This meta-analysis was performed to investigate the effects of vitamin D supplementation/fortification on bone turnover markers in women. All human randomized clinical trials (RCTs) reported changes in bone resorption markers (serum C-terminal Telopeptide of type-I collagen (sCTX) and urinary type I collagen cross-linked N-telopeptide (uNTX)) or bone formation factors (osteocalcin (OC), bone alkaline phosphatase (BALP), and Procollagen type-1 intact N-terminal Propeptide (P1NP)) following vitamin D administration in women (aged ≥18 years old) were considered. Mean differences (MDs) and their respective 95% confidence intervals (CIs) were calculated based on fixed or random effects models according to the heterogeneity status. Subgroup analyses, meta-regression models, sensitivity analysis, risk of bias, publication bias, and the quality of the included studies were also evaluated. We found that vitamin D supplementation had considerable effect on sCTX (MD: -0.038, n= 22) and OC (MD: -0.610, n=24) with high heterogeneity and uNTX (MD: -8.188, n= 6) without heterogeneity. Our results showed that age, sample size, dose, duration, baseline vitamin D level, study region, and quality of studies might be sources of heterogeneity in this meta-analysis. Subgroup analysis also revealed significant reductions in P1NP level in dose less than 600IU/day and larger study sample size (>100 participants). Moreover, no significant change was found in BALP level. Vitamin D supplementation/fortification significantly reduced bone resorption markers in women. However, results were inconsistent for bone formation markers.

Keywords: Vitamin D, Bone turnover, CTX, NTX, Osteocalcin, P1NP, BALP

Registration number: PROSPERO, CRD42022304099

1. Introduction

Low vitamin D status has been associated with decreased bone mineral density (BMD), increased bone turnover markers, falls and bone fracture, immune dysfunction, and increasing mortality [1]. Vitamin D deficiency is a global health concern and an epidemic not only in older populations, but in young people as well [2; 3].

Vitamin D plays a key role in the regulation of bone metabolism [4]. Vitamin D deficiency can stimulate bone deposition and turnover, which may increase the risk of bone loss, fractures, and osteoporosis [5; 6]. Serum 25-hydroxy vitamin D (25(OH)D) level deficiency results in decreased concentrations of ionized calcium, immediately recognized by the parathyroid glands. To maintain calcium homeostasis, parathyroid hormone (PTH) increases, which consequently results in elevated bone turnover due to PTH interacting with osteoblasts to release calcium [7].

Considering the high prevalence of vitamin D insufficiency and deficiency, vitamin D supplementation and food fortification have been identified as dietary strategies to promote bone homeostasis [8; 9; 10; 11]. A meta-analysis conducted in 2017 on 20 randomized clinical trials (RCTs) showed that consumption of vitamin-fortified foods can significantly increase serum 25(OH)D and BMD levels and decrease PTH concentrations, without having a beneficial effect on bone turnover markers [12]. In another meta-analysis of 40 RCTs in 2020, vitamin D fortification resulted in significant reductions in serum levels of PTH and C-terminal telopeptide of type-I collagen (CTX), with no impact on BMD [13]. These two meta-analyses evaluated the effect of vitamin D fortification on bone markers regardless of biological differences in the two sexes.

Given the discrepancies in previous findings and lack of a comprehensive meta-analysis evaluating the effect of vitamin D supplementation or fortification on bone turnover markers in women, the present study was conducted to summarize the evidence of related RCTs.

2. Methods

This systematic review and meta-analysis was developed according to the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions [14] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) report [15]. The study protocol was

registered in PROSPERO, an international prospective register of systematic reviews (registration number: CRD42022304099).

2.1 Search strategy

A comprehensive literature search was conducted for RCTs evaluating the effects of vitamin D supplementation or fortification on bone turnover markers up to January 2023. Electronic databases, including PubMed, Web of Science, and Scopus were searched to find eligible articles. Endnote X8 software was used to screen the references and remove duplicates.

2.2 Eligibility criteria

The inclusion criteria for the studies were an open-label or single-, double-, or triple-blind RCT comparing interventions that differed only in vitamin D content and measured at least one bone turnover marker in women (aged ≥ 18 years old). The control groups could take a placebo, without any intervention or other dosage of vitamin D. Any other combinations of supplements and/or drug treatments were excluded unless administered in both the control and intervention groups. In this meta-analysis, bone resorption factors (including sCTX and urinary type I collagen cross-linked N-telopeptide (uNTX)) and bone formation factors (including osteocalcin (OC), bone alkaline phosphatase (BALP), and procollagen type-1 N propeptide (P1NP) were considered as the primary outcomes.

Furthermore, non-randomized clinical trials, non-human studies (animal, in-vitro, and in-vivo studies), review articles, observational studies, proceedings, case studies, case reports, grey literature, book chapters, abstracts in conferences, editorials, letters, and seminars were excluded. No restriction was placed on the type of vitamin D, administration form (supplementation or fortification), or its dosage, as well as the participants' baseline vitamin D levels and duration of the intervention.

2.3 Screening and data extraction

Studies were first screened for eligibility criteria based on titles and abstracts. The full texts of the included articles were subsequently reviewed for final decision by AA and NZ independently. Any disagreements were resolved by consultation with another reviewer (ShF).

Quantitative data were extracted from eligible articles by two investigators (AA and NZ). Information was collected on study identifications (first author's name, year of publication, country in which the study was conducted), study design (type of study, duration of intervention,

dosage of vitamin D supplements or fortified foods, and baseline serum 25(OH)D concentrations), participants (age and comorbidities), intervention and comparator details (sample size for each treatment group, blinding, attrition), and numerical data for the outcomes of sCTX, uNTX, OC, P1NP, and BALP. Furthermore, the Get Data Graph Digitizer (http://getdata-graph-digitizer.com/) was used to extract data from the figures when needed. Biochemical methods for bone turnover markers were harmonized and differences were calculated based on the same unit for each outcome. Data extraction was verified by a third author (NN).

2.4 Quality assessment and risk of bias

The overall assessment of evidence was done using the Grading of Recommendation Assessment, Development, and Evaluation (GRADE) system. The GRADE criteria included risk of bias, inconsistency, indirectness, imprecision, publication bias, and effect size, and its rating summary was generated using the GRADEpro platform (https://gdt.gradepro.org/).

The risk of bias was evaluated using a Revised Cochrane Risk of Bias tool for randomized trials (RoB2) and ROBVIS-1 framework [16]. All included studies were assessed for sources of bias in selection, performance, detection, attrition, and selective reporting. Each study was classified into one of three categories of bias: low; some concerns; or high risk of bias.

2.5 Statistical analysis

Mean difference (MD) of change, as the effect size, for bone turnover markers along with the corresponding 95% Confidence Interval (CI) were calculated for each study. The pooled effect sizes were estimated thorough a random-effects model using restricted maximum likelihood method. The forest plots of the variables were sorted according to the effect size of the studies.

Cochran's Q test and I^2 statistic ($I^2 > 50\%$ indicates moderate to high heterogeneity) were used to assess the extent of heterogeneity between the included studies. The I^2 statistic was evaluated as 0–40% unimportant heterogeneity, 30–60% moderate heterogeneity, and 50–90% substantial heterogeneity [17].

Subgroup analysis and meta-regression models were carried out to explore the potential sources of heterogeneity. Subgroup analysis was conducted according to the dose of supplementation/fortification (≤600 IU/day and >600 IU/day)[18], intervention duration (≤12 weeks and >12 weeks), and participants' baseline vitamin D levels (≤20 ng/dL and >20 ng/dL), age of participants (≤60 years old and >60 years old), publication year (<2010 and ≥2010), study

sample size (≤100 participants and >100 participants), bone health (healthy postmenopausal women and postmenopausal women with osteoporosis), country region (Asia, Europe, America, and South America and Australia), and study quality score (high, some concerns, and low risk of bias).

Linear meta-regression model was performed based on dosage of vitamin D administered, duration of intervention, baseline vitamin D level, participants age, publication year, and study sample size.

meta-regression model was also done based on the classifications created for subgroup analysis. In addition, fractional polynomial regression model was applied to investigate non-linear relationships between these factors and the study outcomes.

Sensitivity analysis was also conducted using the leave-one-out method to examine whether the results were robust. Publication bias evaluated using Egger's linear regression test, and visual assessment thorough the funnel plots. In Egger's test, P-value< 0.1 was considered as a significant level [19]. All analyses were performed using the STATA software (Stata Crop, College Station Texas, USA) version 17 and P-value less than 0.05 was considered as statistically significant.

3. Results

3.1 Literature search and study selection

The initial search identified 2424 relevant publications and after removing duplicates (n= 678), 1746 records were screened. Based on title and abstract screening, review articles, conference abstracts, animal studies, and studies using multi-ingredient nutritional supplements were excluded (n=1626). After assessing the eligibility of full texts, 88 records were excluded due to the gender of study participants (both sexes, n=22; male, n=6), presentation of results as percentage changes (n=16), or lack of eligibility criteria (n= 44). Consequently, 32 RCTs were included in the meta-analysis. The selection process is presented in *Figure 1*.

3.2 Study characteristics for randomized controlled trials

Detailed characteristics of the 32 included studies are shown in *Table 1*. All studies were performed exclusively on women, with mean ages ranging from 24.75 to 80.35 years. The studies were conducted between 1995 and 2022 in South Korea [9; 20; 21; 22], Poland [23], China [24; 25; 26], Denmark [27; 28], Japan [29; 30; 31; 32; 33; 34; 35; 36; 37], Brazil [38], Finland [10], Spain [11; 39], United

Kingdom [40; 41], United States [42], New Zealand [43], Greece [44], Australia [45; 46], Germany [47], and the Netherlands [48]. The dosage of vitamin D supplementation/fortification varied from 10 IU/day to 200000 IU/3 months, and the intervention period ranged from 4 to 96 weeks. All studies had a parallel randomized design, and were conducted on postmenopausal women with osteopenia/osteoporosis [9; 20; 21; 22; 24; 29; 30; 31; 32; 33; 34; 36; 39], healthy postmenopausal women [10; 23; 25; 26; 28; 35; 37; 38; 40; 41; 42; 44; 45; 46; 47; 48], and healthy women aged 18-49 years [11; 27; 43]. Of the 32 included studies, 15 provided data on sCTX [9; 10; 20; 21; 24; 25; 26; 27; 28; 38; 39; 40; 42; 43; 44] and 4 reported uNTX [11; 29; 30; 32]. Moreover, changes in OC [22; 23; 25; 26; 27; 28; 32; 33; 34; 35; 36; 37; 41; 43; 44; 46; 47; 48], P1NP [10; 11; 24; 26; 27; 28; 39; 40; 42; 45], and BALP [20; 21; 31; 36; 48] were extracted from 18, 10, and 5 studies, respectively.

3.3 Meta-analysis

3.3.1 Effect of vitamin D supplementation/fortification on sCTX

Pooling 22 effect sizes indicated that vitamin D consumption had a significant effect on sCTX level (MD: -0.038, 95% CI: -0.057, -0.019, n= 22, *Table 2*, *Figure 2*), with considerable heterogeneity (I^2= 64.2%, and P<0.001).

Based on the findings of subgroup analyses and meta-regression models, duration, baseline level of vitamin D, age, sample size, region, and quality of the studies could be the potential sources of heterogeneity. Accordingly, in duration less than 12 weeks, the heterogeneity was very low (I^2= 0%, P= *0.285*), and no significant difference was observed in mean level of sCTX between the treatments. Besides, 10 weeks increase in duration was found to be associated with 0.01 significant decrease in MD of sCTX level which is in favor of the intervention group (MD: -0.000916, 95%CI: -0.0014727, -0.0003592), P= *0.001*, *Figure S11*). Regarding the baseline vitamin D level, no significant decrease was observed in sCTX level in the subgroup with low heterogeneity (≤ 20 ng/ml). However, the meta-regression model result showed that reduction in sCTX level was more pronounced in participants with sufficient levels of vitamin D compared to participant with vitamin D deficiency (MD= -0.0362, 95%CI (-0.0720, -0.0004), P= *0.047*). Moreover, sub-group analysis showed that vitamin D significantly reduced sCTX level in participants younger than 60 years (MD= -0.031, 95% CI (-0.059, -0.003), P=0.027, *Figure S1*) with low heterogeneity (I^2 = 36.3%, P=0.15). Also, significant differences were observed in studies conducted in Europe (MD= -0.016, 95%CI (-0.030, -0.001), P=*0.034*, *Figure S2)* and

America (US) (MD= -0.050, 95%CI (-0.091, -0.009), P= *0.016, Figure S2)*, and studies with some concerns about risk of bias (MD= -0.037, 95%CI (-0.064, -0.011), P= 0.005, *Figure S3)* which all had very low heterogeneity (I^2= 0.01%, 0.02%, and 0.01%, respectively). Furthermore, meta-regression model showed that the MD for sCTX was significantly reduced in studies with high risk of bias compared to studies with low risk of bias (MD= -0.0788, 95%CI (-0.1248, -0.0327), P= 0.001), confirming that studies with high risk of bias might be a source of heterogeneity. Moreover, linear meta-regression revealed that 100 participants increase in sample size was associated with a significant reduction of 0.02 in MD level of sCTX which is in favor of the intervention group (MD= -0.0002, 95%CI (-0.0003, -0.00003), P= *0.020, Figure S12*). Finally, the sample size was found to have a non-linear effect on the pooled effect size (*Figure S14*).

3.3.2 Effect of vitamin D supplementation/fortification on uNTX

Results of the analysis on 6 effect sizes revealed a significant reduction in uNTX following vitamin D supplementation/fortification (MD: -8.138, 95% CI: -12.864, -3.413, n= 6, *Table S1*, *Figure S4*), with low heterogeneity (I^2= 0.00%, and P= 0.627). Furthermore, a significant reduction in uNTX levels was observed in both study duration subgroups (*Figure S5*). Meta-regression analysis did not indicate any effect of possible sources of heterogeneity on the estimated effect sizes.

3.3.3 Effect of vitamin D supplementation/fortification on OC

Among all studies, vitamin D supplementation or fortification had a significant effect on OC level (MD: -0.614, 95% CI: -1.146, -0.081, n=24, *Table 3*, *Figure 3*), with high heterogeneity (I^2= 79.9%, and P< 0.001).

Subgroup analysis yielded that a decrease in OC level was significant in the subgroup with larger sample size (MD=0.343, 95%CI (-0.686, -0.001), P=*0.049, Figure S6*). Although there was very low heterogeneity in subgroups including sufficient baseline vitamin D level and studies with some concerns risk of bias, no significant difference was found in mean level of OC between the treatments. Meta-regression analyses indicated the pooled estimate was independent of potential sources of heterogeneity, but the dose was found to have a non-linear effect on the pooled effect size (*Figure S15*).

3.3.4 Effect of vitamin D supplementation/fortification on P1NP

The analysis found no change in P1NP marker with vitamin D consumption (n=17, *Table 4, Figure S7*), with considerable heterogeneity (I^2= 60.8%, and P= 0.007). However, a significant reduction was detected in P1NP level in the vitamin D dosage ≤ 600 IU/d subgroup (MD: -3.068, 95% CI: -5.894, -0.242, P=*0.033, Figure S8*) and sample size more than 100 participants (MD= -2.339, 95% CI (-4.414, -0.264), P= 0.027) without any heterogeneity (*Figure S9*).

Our analysis presented that dose, sample size, region, quality of the studies, and baseline vitamin D level could be the potential sources of heterogeneity. Linear meta-regression showed that 100 IU/day increase in dose associated with 0.18 significant decrease in MD of OC level which is in favor of the intervention group (MD= 0.0018, 95%CI (0.0008, 0.0029), P<*0.001, Figure S13*). Furthermore, meta-regression model revealed that the MD for OC was significantly decreased in studies with larger sample sizes compared to those with lower sample sizes (MD= -3.9261, 95%CI (-7.604, -0.247), P=0.036). In addition, subgroup analysis showed that all subgroups of region except America and all subgroups of study quality except some concerns risk of bias had non- significant heterogeneity and the results of classified meta-regression were completely consistent with the results of subgroup analysis. Finally, the baseline vitamin D level was found to have a non-linear effect on the pooled effect size (*Figure S16*).

3.3.5 Effect of vitamin D supplementation/fortification on BALP

According to the pooled effect size of 6 studies, vitamin D intake did not significantly alter BALP levels (n=6, *Table S2, Figure S10*), with high heterogeneity (I^2= 95.3%, and P< 0.001). No significant effect was observed in any of the subgroups. In addition, meta-regression analysis showed the pooled estimate was independent of possible confounding factors.

3.4 Risk of bias and publication bias of the included studies

A summary of the risk of bias analysis and traffic light figure of each domain of the risk of bias assessment are presented in *Figures S17 a, b*. One study had a high risk of bias and 10 studies were categorized as “some concerns” due to improper randomization procedure. Nineteen studies were scored as having some concerns of deviation from intended interventions. Moreover, one study had a high risk of missing outcome data, whereas 6 studies had some concerns of detection

bias. Measurement details were sufficiently reported in all studies. Furthermore, only one study reported some concerns regarding the selection of the results.

Funnel plots for the effects of vitamin D supplementation/fortification on bone turnover markers (including sCTX, uNTX, OC, P1NP, and BALP) are presented in *Figures S18-S22*. Based on visual inspection of the funnel plots, there was no evidence of publication bias. These findings were also confirmed by Egger's test for sCTX (P= 0.691), uNTX (P= 0.847), OC (P= 0.675), P1NP (P= 0.251), and BALP (P= 0.946).

3.5 Sensitivity analysis and Quality of evidence

Based on the results, the estimated effect size of each outcome was not affected by any single study.

The GRADE-Pro evidence profile rating results for changes in bone turnover markers are shown in *Table S3*. The GRADE rating was found to be high for sCTX, uNTX, and P1NP variables. However, results showed the GRADE rating was moderate and low for OC and BALP, respectively.

4. Discussion

The primary objective of this systematic review and meta-analysis was to investigate the probable effect of vitamin D supplementation or fortification on bone turnover markers. In summary, the results of this meta-analysis suggest that vitamin D consumption benefits bone resorption markers, including sCTX and uNTX. Moreover, vitamin D significantly reduced OC (a bone formation marker), but not P1NP or BALP.

Vitamin D intake was found to significantly reduce sCTX, particularly at age younger than 60 years, studies conducted on Europe and America, and studies with some concerns for risk of bias. Consistent with our results, Von Hurst et al. found that vitamin D supplementation could modify bone turnover, suppress bone resorption, and prevent deterioration in quality of life related to aging [(43)]. Conversely, Valimaki and colleagues did not find a significant effect of supplementing with 100,000 or 200,000 IU of vitamin D every 3 months on sCTX levels [(10)], which may be at least partly related to such large doses of vitamin D being administered far

apart. The placebo group also received calcium supplements, which may have affected the results.

There are different pathways that could explain the beneficial effects of vitamin D on bone turnover markers. In the absence of sufficient concentrations of serum 25(OH)D (< 20ng/ml), calcium absorption from the intestine is decreased, resulting in increased PTH to compensate calcium reabsorption from the kidney, stimulating osteoclasts to release calcium into blood circulation [7; 49], and deteriorating osteoblasts function due to low 25(OH)D levels [50]. Moreover, available evidence suggested that insufficient vitamin D level may cause secondary hyperparathyroidism, leading to increased bone loss, bone turnover, and consequently, greater risk of osteoporosis [51]. Recent evidence has shown that the risk of vitamin D deficiency is considerably higher in the older adults and in the Middle East, China, Mongolia, and India [52], for instance it has estimated that 490 million Indians have vitamin D deficiency [53]. On the other hand, prevalence rate of vitamin D deficiency have reported 24% in America (US), and 40% in Europe [53]. These results are consistent with those obtained from this meta-analysis, which showed that groups with participants who had presumably normal baseline vitamin D levels had significantly lower sCTX levels.

Analyzing all included RCTs showed a significant reduction in uNTX level after vitamin D intake. Similar to our findings, intake of the vitamin D-fortified skimmed milk with 200 IU/day content of vitamin D_3 for 16 weeks reduced both uNTX and P1NP, as well as increased circulating 25(OH)D in young healthy women [11]. However, findings from another study did not support the mentioned effect of vitamin D supplementation on uNTX during 48 weeks [31], which may be due to the low dosage (40 IU/day) of alfacalcidol treatment.

This meta-analysis also found that vitamin D consumption significantly reduced OC particularly in studies with larger sample size (more than 100 participants). According to another meta-analysis, simultaneous administration of vitamins D and K could significantly improve the total BMD and reduce undercarboxylated OC [54]. Additionally, a beneficial effect of vitamin D on OC is supported by Shiraki et al., where OC was reduced after alfacalcidol treatment for 6 months [32]. Another study conducted in Emirati women indicated that serum 25(OH)D concentration is negatively associated with PTH and OC levels ($r = -0.13$, $P < 0.05$) [55]. Moreover, vitamin D3 supplementation in diabetic individuals also reduces undercarboxylated

OC level and the undercarboxylated to carboxylated OC index (uOC/cOC) [56]. However, Bislev et al. found no significant change in bone turnover markers (CTX, P1NP), and OC levels indicated a borderline elevation due to vitamin D3 intake [28]. In addition to the PTH pathway involved in bone turnover, there is evidence that the promoter region of the OC gene has a vitamin D receptor (VDR) binding site, although the exact relationship is not apparent [57].

Results of our meta-analysis revealed that lower vitamin D administration (≤ 600 IU/day) and larger sample size (> 100 participants) significantly decreased P1NP levels, but this effect was not found in the overall analysis. Zhue et. al. [45] showed that although P1NP levels significantly decreased due to vitamin D intakes of 1000 IU/day during 48 weeks, no difference was observed between the treatment and placebo groups. Moreover, Gronborg et. al. did not declare any significant changes in bone turnover markers including P1NP, OC, BALP, and sCTX following daily intake of 1200 IU vitamin D for 12 weeks [27]. Similarly, Madar et. al. investigated the effect of 10 and 25 μg/day vitamin D treatment in healthy individuals and discovered significant acceleration in 25(OH)D and reduction in PTH levels, but no changes in serum P1NP [58]. Gao et al. found that simultaneous intake of calcium and calcitriol significantly decreased P1NP, while no significant effect was seen in individuals receiving cholecalciferol [26]. Consistent with these results, no extra effect of vitamin D was seen on P1NP when comparing a calcium group and calcium plus vitamin D group [45]. We hypothesize that in subjects with adequate calcium intake, the effect of vitamin D on P1NP levels is not evident.

One reason why the role of vitamin D supplementation and its dosage is still debated might be due to limitations in trial design as most studies did not meet the basic requirements of a nutrient intervention study, including vitamin D-replete populations, too small sample sizes, and inconsistent intervention methods regarding dose and metabolites [53]. Our meta-analysis results clearly showed the role of larger sample size in studies.

The effect of vitamin D supplementation on BALP marker was not significant in the overall analysis nor in the subgroup, which is inconsistent with the findings of Ooms et. al. and Chung et. al. [21; 48]. In agreement with our results, a recent meta-analysis showed no significant changes in BALP levels following consumption of vitamin D fortified foods [12]. The limited number of studies included in this section of our analysis might prevent meaningful evaluation of BALP.

A strength of our analysis was the comprehensive assessment of the effect of vitamin D supplementation or fortification on several key bone turnover markers (including bone resorption and formation factors) in women, as recent evidence supports sex differences in vitamin D serum levels and metabolism [59]. However, this meta-analysis has some limitations. Firstly, moderate to high heterogeneity was found in some variables although we attempted to identify the source of heterogeneity. Furthermore, there are limitations to the general use of the results obtained for uNTX and BALP markers, as few studies have examined these markers (n=6) and when performing subgroup analyses, some domains had few or no studies. Moreover, Considering the various types of vitamin D in form of supplements or fortified foods, it was not possible to sub-group based on the type of vitamin D prescribed.

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

Studies	Country	Study population	Mean age (y)	Intervention group	Vitamin D dose	Control group	duration	Outcomes
Bin Lee et al. (2022) [9]	South Korea	Postmenopausal osteopenia/osteoporosis women	63.3	Vitamin D3 + raloxifene + calcium	800 IU/ day	Raloxifene + calcium	16 weeks	sCTX
Rodziewicz-Flis et al. (2022) [23]	Poland	1) Elderly women with sufficient vitamin D level 2) Elderly women with insufficient vitamin D level	72.85 72.85	Exercise + vitamin D3	2000 IU/ day	Exercise + no placebo	12 weeks	OC
Zhang et al. (2020) [24]	China	1) Postmenopausal osteoporosis women with Serum VitD < 10 ng/ml 2) Postmenopausal osteoporosis women with Serum VitD > 10 ng/ml	65.68 62.74	Calcitriol/oral + calcium	10 IU/ day	Calcium	24 weeks	sCTX P1NP
Gronborg et al. (2019) [27]	Denmark	1) Healthy Danish women 2) Healthy Pakistani women	33 36	Fortified food with vitamin D3	1200 IU/ day	Placebo	12 weeks	OC sCTX P1NP
Bislev et al. (2019) [28]	Denmark	Postmenopausal women (60-79 years)		Vitamin D3/oral	2800 IU/ day	Placebo	12 weeks	OC sCTX P1NP
Uenishi et al. (2018) [29]	Japan	Post-menopausal osteoporosis women	74.4 74.8 73.75	1) Eldecalcitol/oral + calcium 2) 1α-hydroxyl	30 IU/ day	Calcium	4 weeks	uNTX

				calcidiol/oral + calcium 3) Plain vitamin D3 /oral + calcium				
Nahas-Neto et al. (2018) [38]	Brazil	Postmenopausal women	59	Vitamin D3/oral	1000 IU/ day	Placebo	36 weeks	sCTX P1NP
Cheng et al. (2018) [25]	China	Postmenopausal women	57.65	Calcitriol/oral	20 IU/ day	Placebo	12 weeks	OC sCTX
Välimäki et al. (2016) [10]	Finland	Older adults	74.95 75.75	Cholecalciferol + calcium	1) 200000 IU /every 3 months 2) 100000 IU /every 3 months	Placebo + calcium	48 weeks	sCTX P1NP
Studies	**Country**	**Study population**	**Mean age (y)**	**Intervention group**	**Vitamin D dose**	**Control group**	**duration**	**Outcomes**
Gao et al. (2015) [26]	China	Postmenopausal women	63.78 63.28	Cholecalciferol + Caltrate	1) 800 IU/ day 2) 10 IU/ day	Caltrate	96 weeks	OC sCTX P1NP
Cho et al. (2015) [20]	South Korea	Postmenopausal osteoporosis women	64	Cholecalciferol/oral + ibandronate + calcium	24000 IU/month	Ibandronate + calcium	16 weeks	sCTX BALP

Toxqui et al. (2014) [11]	Spain	Healthy adults	24.75	Fe + D3 fortified milk	200 IU/ day	Fe fortified milk	16 weeks	uNTX P1NP
Macdonald et al. (2013) [40]	United Kingdom	Healthy postmenopausal women	64.4 64.75	Vitamin D3/oral	1) 400 IU/ day 2) 1000 IU/ day	Placebo	48 weeks	sCTX P1NP
Chung et al. (2013) [21]	South Korea	Postmenopausal osteoporosis women	65.4	Cholecalciferol/oral + calcium + risedronate	30000 IU/month	Calcium + risedronate	16 weeks	sCTX BALP
Aloia et al. (2013) [42]	United State	Healthy postmenopausal women	58.8 59.15	1) Vitamin D3/oral + calcium 2) Vitamin D3/oral + placebo calcium	4000 IU/ day	1) Placebo + calcium 2) Placebo D3 + placebo calcium	24 weeks	sCTX P1NP
Gorai et al. (2012) [30]	Japan	Postmenopausal osteopenia/osteoporosis women	64.5	Alfacalcidol/oral + raloxifene	40 IU/ day	Raloxifene	96 weeks	uNTX BALP
Olmos et al. (2012) [39]	Spain	Postmenopausal osteoporotic women	68	Cholecalciferol + alendronate	10640 IU/week	Alendronate	12 weeks	sCTX P1NP
von Hurst et al. (2010) [43]	New Zealand	1) Premenopausal and <49 years women 2) Postmenopausal and/or ≥49 years women		Vitamin D3/oral	4000 IU/daily	placebo	24 weeks	OC sCTX

Manios et al. (2009) [44]	Greece	Postmenopausal women	61	Vitamin D3 + calcium	300 IU/ day	Calcium	20 weeks	OC sCTX
Majima et al. (2008) [31]	Japan	Postmenopausal Osteoporosis women	71.04	Alfacalcidol + raloxifene	40 IU/ day	Raloxifene	48 weeks	BALP
Zhu et al. (2008) [45]	Australia	Older Adults	76.85	Vitamin D2 + calcium	1000 IU/ day	Placebo + calcium	48 weeks	P1NP
Shiraki et al. (2004) [32]	Japan	Osteoporosis elderly	77.7	Alfacalcidol + calcium aspartate	40 IU/ day	Calcium aspartate	24 weeks	OC uNTX
Studies	**Country**	**Study population**	**Mean age (y)**	**Intervention group**	**Vitamin D dose**	**Control group**	**duration**	**Outcomes**
Cooper et al. (2003) [46]	Australia	Postmenopausal women	56.3	Vitamin D2/oral + calcium	10000 IU/week	Placebo + calcium	96 weeks	OC
Ushiroyama et al. (2002) [33]	Japan	Postmenopausal osteopenia and osteoporosis women	53.7	1-αhydroxycholecalciferol + K2	40 IU/ day	K2	96 weeks	OC
Ushiroyama et al. (2001) [34]	Japan	Postmenopausal osteopenia and osteoporosis women	51.85 52.15	1) 1α-hydroxycholecalciferol + calcitonin 2) 1α-hydroxycholecalciferol	40 IU/ day	1) Calcitonin 2) No placebo	96 weeks	OC
Son et al. (2001) [22]	South Korea	Osteopenic elderly	72	Alphacalcidol/oral	20 IU/ day	Placebo	40 wee	OC

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Pfeifer et al. (2000) [47]	Germany	Healthy older adults	74.75	Cholecalciferol + calcium	800 IU/ day	Calcium	8 weeks	OC
Hunter et al. (2000) [41]	United Kingdom	Monozygotic postmenopausal women	58.7	Cholecalciferol/oral	800 IU/ day	Placebo	24 weeks	OC
Gorai et al. (1999) [35]	Japan	Postmenopausal women	51.3 51.9	1) 1α(OH)D3 /oral 2) 1α(OH)D3 /oral + estrogen	40 IU/ day	1) No placebo 2) Estrogen	96 weeks	OC
Shiraki et al. (1996) [36]	Japan	Osteoporosis women	72.4	1α(OH)D3 + calcium	30 IU/ day	Placebo + calcium	96 weeks	OC BALP
Ushiroyama et al. (1995) [37]	Japan	Postmenopausal and ovariectomized women	50.85 52.15	1) Alfacalcidol/oral + Ipriflavone 2) Alfacalcidol/oral	40 IU/day	1) Ipriflavone 2) No placebo	72 weeks	OC
Ooms et al. (1995) [48]	Netherlands	Older adults	80.35	Vitamin D3/oral	400 IU/day	Placebo	48 weeks	OC BALP

Abbreviations: sCTX; serum C-terminal crosslinked telopeptide of type 1 collagen, OC; Osteocalcin, P1NP; Procollagen type 1 amino-terminal propeptide, uNTX; urinary N-terminal telopeptides of type 1 collagen, BALP; Bone specific Alkaline Phosphatase.

Table 2. Summary findings of comparison of sCTX between the study treatments.

Outcomes	**N**	**Subgroup Analysis**		**Meta-Regression 1**	**Meta-Regression 2**	**Non-linear Meta Regression**
		MD (95%CI), *P-value*	**I^2, P-value**	**B (95% CI), *P-value*, Res I^2**	**B (95% CI), *P-value*, Res I^2**	**S or NS**
Total	22	- 0.038 (- 0.057, - 0.019), *<0.001*	64.2%, *<0.001*			
Dose ≤ 600 IU/day	6	-0.044 (- 0.100, 0.012), *0.121*	81.2%, *<0.001*	Ref	<0.001 (-0.0000142, 0.0000132), *0.947*, 64.91%	NS
Dose > 600 IU/day	16	-0.035 (- 0.052, -0.018), *<0.001*	47.7%, *0.019*	0.0174 (-0.0268, 0.0618), *0.439*, 63.10%		
Duration ≤ 12weeks	5	- 0.011 (-0.028, 0.005), *0.174*	**0.0%**, *0.285*	Ref	-0.000916 (-0.0014727, -0.0003592), ***0.001***, 44.86%	NS
Duration > 12weeks	17	- 0.047 (-0.068, -0.025), *<0.001*	59.8%, *0.001*	-0.0345 (-0.0757, 0.0065), *0.099*, 56.66%		
Baseline vitD ≤ 20ng/ml	9	- 0.017 (- 0.035, 0.001), *0.072*	**16.3%**, *0.394*	Ref	-0.0006057 (-0.0036108, 0.0023994), *0.693*, 63.33%	NS
Baseline vitD > 20ng/ml	13	- 0.052 (- 0.077, - 0.027), *<0.001*	64.0%, *<0.001*	-0.0362 (-0.0720, -0.0004),***0.047***,55.92%		
Age ≤ 60 years	6	-0.031 (-0.059, -0.003), **0.027**	**36.3%**, *0.15*	Ref	-0.0006303 (-0.0027073, 0.0014467), *0.552*, 57.67%	NS
Age > 60 years	13	-0.042 (-0.071, -0.014), 0.004	61.3%, *<0.001*	-0.0133 (-0.0579, 0.0313), *0.558*, 57.90%		
Publication year < 2010		NA		NA	0.0037076 (-0.0018287, 0.009244), *0.178*, 61.14%	NS
Publication year ≥ 2010						

Sample Size ≤ 100	13	-0.031 (-0.053, -0.010), 0.004	46.5%, *0.051*	Ref	-0.000204 (-0.0003765, -0.0000315), ***0.020***, 54.98%	**S**
Sample Size > 100	9	-0.045 (-0.079, -0.012), 0.008	74.6%, *<0.001*	-0.0166 (-0.0546, 0.0214), *0.392*, 62.81%		
Healthy postmeno	13	-0.048 (-0.074, -0.022), *<0.001*	74.7%, *<0.001*	Ref	NA	NA
Postmeno osteoporosis	6	-0.023 (-0.053, 0.006), 0.115	**0.01%**, *0.89*	0.0251 (-0.0234, 0.0738), *0.311*, 64.63%		
Region				Res I^2 = 60.32%	NA	NA
Asia	8	-0.040 (-0.084, 0.003), *0.071*	75.7%, *<0.001*	0.0105 (-0.0445, 0.0656), *0.708*		
Europe	9	-0.016 (-0.030, -0.001), ***0.034***	**0.01%**, *0.704*	0.0358 (-0.0177, 0.0894), *0.189*		
America	2	-0.050 (-0.091, -0.009), ***0.016***	**0.02%**, *>9.999*	0.0067 (-0.0685, 0.0820), *0.860*		
South America, Australia	3	-0.058 (-0.097, -0.018), *0.004*	59.3%, *0.088*	Ref		
Risk of bias				Res I^2 = 46.27%	NA	NA
High	2	-0.105 (-0.174, -0.036), *0.003*	86.1%, *0.007*	-0.0788 (-0.1248, -0.0327), ***0.001***		
Some Concerns	9	-0.037 (-0.064, -0.011), ***0.005***	**0.01%**, *0.917*	-0.0097 (-0.0472, 0.0277), *0.611*		
Low	11	-0.026 (-0.046, -0.006), *0.011*	55.9%, *0.009*	Ref		

Meta-Regression 1: the subgrouping variable was included into the model as a categorized variable. **Meta-Regression 2**: the subgrouping variable was included into the model as a continuous variable. **Abbreviations**: N; Number of included interventions, B; Beta coefficient reflecting the effect of the subgrouping variable on the pooled effect size. vit; vitamin, postmeno; post-menopausal, CI; confidence interval, Res $I^{2;}$ Residual I^2, NA; Not Applicable, S; Singnificant, NS; Non-significant. *Italic*; P-values; **Bold**; significant P-value.

Table 3. Summary findings of comparison of OC between the study treatments.

Outcomes	**N**	**Subgroup Analysis**		**Meta-Regression 1**	**Meta-Regression 2**	**Non-linear Meta Regression**
		MD (95%CI), *P-value*	**I^2, *P-value***	**B (95% CI), *P-value*, Res I^2**	**B (95% CI), *P-value*, Res I^2**	**S or NS**
Total	24	- 0.614 (-1.146, -0.081), *0.024*	79.9%, *<0.001*			
Dose ≤ 600 IU/day	13	- 0.727 (- 1.442, - 0.012), *0.046*	83.9%, *<0.001*	Ref	-0.00003 (0.0005, 0.0004), *0.894*, 80.45%	**S**
Dose > 600 IU/day	11	- 0.481 (- 1.315, 0.353), *0.259*	69.7%, *<0.001*	0.2545 (-0.8391, 1.3482), *0.648*, 73.83%		
Duration ≤ 12weeks	7	0.052 (- 1.200, 1.303), *0.935*	57.6%, *0.018*	Ref	-0.0010 (-0.0164, 0.0142), *0.889*, 80.10%	NS
Duration > 12weeks	17	- 0.791 (- 1.344, - 0.238), *0.005*	79.3%, *<0.001*	-0.9333 (-2.170, 0.304), *0.139*, 76.41%		
Baseline vitD ≤ 20ng/ml	8	- 1.121 (- 2.517, 0.275), *0.116*	69.7%, *<0.001*	Ref	0.0082 (-0.0818, 0.0983), *0.858*, 77.35%	NS
Baseline vitD > 20ng/ml	9	- 0.234 (- 0.478, 0.011), *0.061*	**0.0%**, *0.066*	0.4048 (-0.9701, 1.7798), *0.564*, 77.29%		

Age ≤ 60 years	12	-0.660 (-1.342, 0.022), *0.058*	72.7%, *<0.001*	Ref	0.0069 (-0.0348, 0.0488), *0.744*, 68.47%	NS
Age > 60 years	9	-0.435 (-1.058, 0.189), *0.172*	52.2%, *0.059*	0.1340 (-.9148, 1.1829), *0.802*, 70.61%		
Publication year < 2010	15	-0.508 (-1.068, 0.053), *0.076*	79.5%, *<0.001*	Ref	0.0119 (-0.052, 0.075), *0.715*, 78.79	NS
Publication year ≥ 2010	9	-1.057 (-2.388, 0.273), *0.119*	73.2%, *<0.001*	-0.3984 (-1.6205, 0.8237), 0.523, 80.80%		
Sample Size ≤ 100	18	-0.657 (-1.370, 0.055), *0.071*	80.2%, *<0.001*	Ref	0.0002 (-0.0060, 0.0064), *0.943*, 77.53%	NS
Sample Size > 100	6	-0.343 (-0.686, -0.001), ***0.049***	**9.03%**, *0.099*	0.0644 (-1.118, 1.246), *0.915*, 78.30%		
Healthy postmeno	15	-0.582 (-1.268, 0.105), *0.097*	86.9%, *<0.001*	Ref	NA	NA
Postmeno osteoporosis	6	-0.531 (-1.786, 0.724), *0.407*	56.0%, *0.060*	0.0738 (-1.3220, 1.4697), *0.917*, 84.30%		
Region				Res I^2 = 69.73%	NA	NA
Asia	12	-1.019 (-1.822, -0.217), *0.013*	72.8%, *<0.001*	-0.0455 (-1.3519, 1.2609), *0.946*		
Europe	8	0.280 (-0.382, 0.942), *0.407*	51.3%, *0.063*	1.2102 (-0.1753, 2.5958), *0.087*		
America		---	---	---		

South America, Australia	4	-1.017 (-2.173, 0.139), *0.085*	80.8%, *0.034*	Ref		
Risk of bias				Res I^2 = 76.32%	NA	NA
High	7	-0.973 (-2.010, 0.064), *0.066*	85.5%, *<0.001*	-0.3116 (-1.5916, 0.9684), *0.633*		
Some Concerns	9	-0.121 (-0.391, 0.149), *0.380*	**0.0%**, *0.757*	0.5526 (-0.7937, 1.8990), *0.421*		
Low	8	-0.798 (-1.877, 0.281), *0.147*	79.6%, *<0.001*	Ref		

Meta-Regression 1: the subgrouping variable was included into the model as a categorized variable. **Meta-Regression 2**: the subgrouping variable was included into the model as a continuous variable. **Abbreviations**: N; Number of included interventions, B; Beta coefficient reflecting the effect of the subgrouping variable on the pooled effect size. vit; vitamin, postmeno; post-menopausal, CI; confidence interval, Res $I^{2;}$ Residual I^2, NA; Not Applicable, S; Singnificant, NS; Non-significant. *Italic*; P-values; **Bold**; significant P-value.

Table 4. Summary findings of comparison of P1NP between the study treatments.

Outcomes		**Subgroup Analysis**		**Meta-Regression 1**	**Meta-Regression 2**	**Non-linear Meta Regression**
		MD (95%CI), *P-value*	**I^2, *P-value***	**B (95% CI), *P-value*, Res I^2**	**B (95% CI), *P-value*, Res I^2**	**S or NS**
Total	17	- 0.201 (- 2.206, 1.804), *0.844*	60.8%, *0.007*			
Dose ≤ 600 IU/day	5	- 3.068 (- 5.894, - 0.242), ***0.033***	**0.0%**, *0.974*	Ref	0.0018 (0.0008, 0.0029), ***<0.001***, 30.70%	NS
Dose > 600 IU/day	12	0.668 (- 1.797, 3.134), *0.595*	68.8%, *0.001*	3.6031 (-0.7986, 8.004), *0.109*, 55.69%		
Duration ≤ 12weeks	4	- 0.503 (-2.823, 1.818), *0.671*	**24.7%**, *0.352*	Ref	-0.0178 (-0.0916, 0.0558), *0.635*, 61.03%	NS
Duration > 12weeks	13	0.097 (- 2.552, 2.746), *0.943*	63.8%, *<0.001*	1.2527 (-3.3426, 5.8480), *0.593*, 59.95%		
Baseline vitD ≤ 20ng/ml	5	- 1.168 (- 4.078, 1.742), *0.431*	32.6%, *0.255*	Ref	0.0595 (-0.2070, 0.3260), *0.662*, 60.81%	**S**
Baseline vitD > 20ng/ml	12	0.395 (- 2.203, 2.993), *0.766*	64.8%, *<0.001*	2.1164 (-2.4227, 6.6556), *0.361*, 59.37%		
Age ≤ 60 years	6	-0.149 (-4.367, 4.070), *0.945*	79.7%, *<0.001*	Ref	0.0532 (-0.0683, 0.1748), *0.391*, 59.72%	NS
Age > 60 years	10	-1.189 (-3.182, 0.805), *0.243*	**0.0%**, *0.296*	-0.8665 (-5.378, 3.645), *0.707*, 58.97%		
Publication year < 2010		NA		NA	-0.2701 (-0.8847, 0.3444), *0.389*, 59.01%	NS
Publication year ≥ 2010						

Sample Size ≤ 100	9	1.531 (-1.513, 4.575), *0.324*	74.0%, *0.001*	Ref	-0.0144 (-0.0330, 0.0042), *0.130*, 56.71%	NS
Sample Size > 100	8	-2.339 (-4.414, -0.264), ***0.027***	**0.0%**, *0.839*	-3.9261 (-7.604, -0.247), ***0.036***, 51.47%		
Healthy postmeno	11	0.738 (-2.001, 3.477), *0.597*	73.6%, *0.000*	Ref	NA	NA
Postmeno osteoporosis	3	-2.911 (-7.408, 1.585), *0.204*	**0.0%**, *0.999*	3.6596 (-2.748, 10.067), *0.263*, 66.57%		
Region				Res I^2 = 42.24%	NA	NA
Asia	4	-2.590 (-5.366, 0.187), *0.068*	**0.0%**, *0.752*	0.5250 (-6.0560, 7.1061), *0.876*		
Europe	9	0.032 (-1.634, 1.697), *0.970*	**5.38%**, *0.234*	2.9418 (-3.0551, 8.9389), *0.336*		
America	2	4.429 (-2.568, 11.426), *0.215*	89.6%, *0.002*	7.4353 (0.7589, 14.1116), ***0.029***		
South America, Australia	2	-3.283 (-9.829, 3.264), *0.326*	50.5%, *0.155*	Ref		
Risk of bias				Res I^2 = 48.46%	NA	NA
High	2	-2.474 (-5.986, 1.037), *0.167*	**16.0%**, *0.275*	-0.9788 (-6.3670, 4.4092), *0.722*		
Some Concerns	7	2.378 (-1.805, 6.560), *0.265*	70.2%, *0.002*	4.0831 (0.1214, 8.0448), ***0.043***		
Low	8	-0.942 (-2.803, 0.919), *0.321*	**17.7%**, *0.473*	Ref		

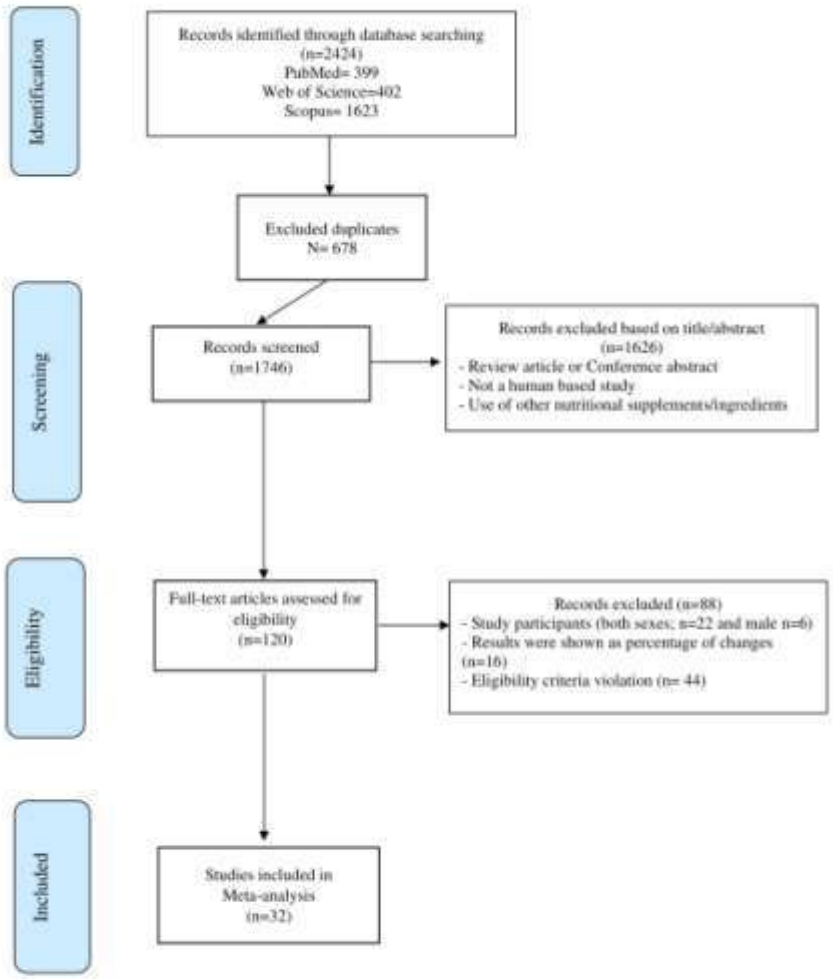

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart of the study selection process.

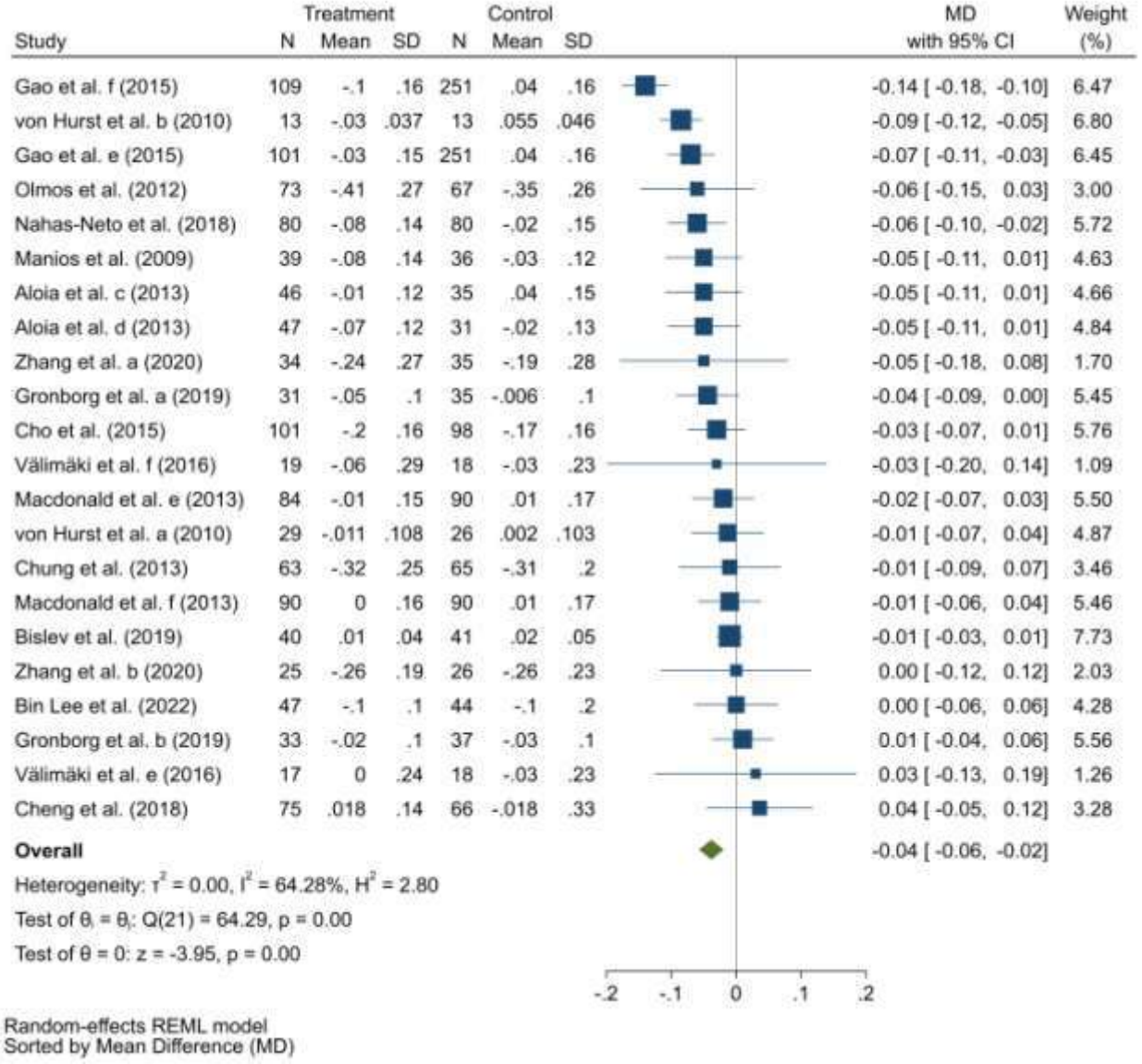

Figure 2. Forest plot of the Randomized Clinical Trials (RCTs) examining the effect of vitamin D supplementation on sCTX. Data have been expressed as mean differences (MDs) between intervention and control groups with a 95% confidence interval (CI). Estimates were pooled using the random effects model. Letters between parentheses represent: a, b: different participant groups; c, d: different intervention/ control groups; e, f: different dose of vitamin D.

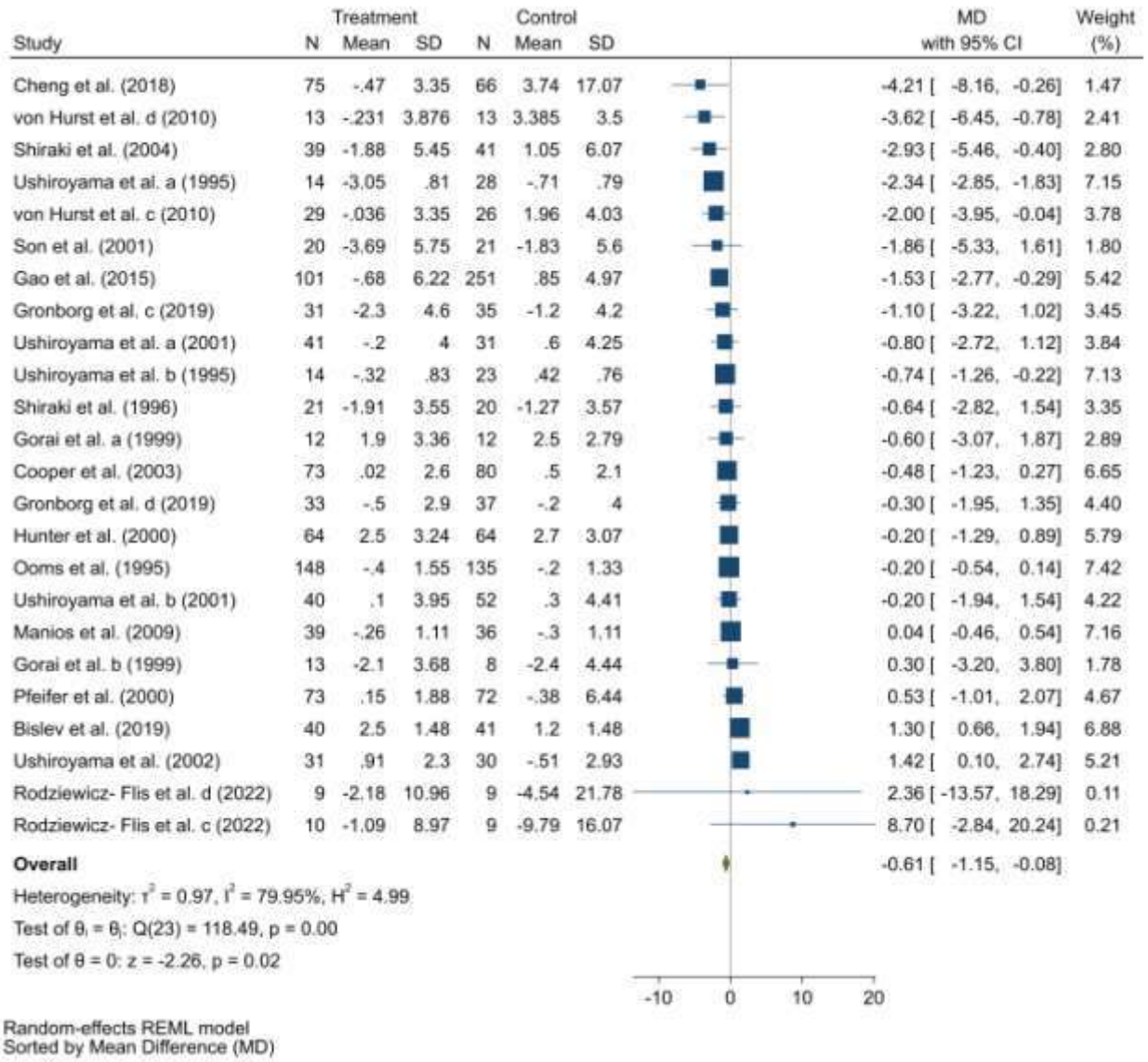

Figure 3. Forest plot of the Randomized Clinical Trials (RCTs) examining the effect of vitamin D supplementation on OC. Data have been expressed as mean differences (MDs) between intervention and control groups with 95% confidence interval (CI). Estimates were pooled using the random effects model. Letters between parentheses represent: a, b: different intervention/ control groups; c, d: different participant groups.