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The combination effect of vitamin K and vitamin D on human bone quality: a meta-analysis of randomized controlled trials

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Abstract

Background

Previous studies did not draw a consistent conclusion about the effects of vitamin K combined with vitamin D on human skeletal quality.

Method and Findings

A comprehensive search on Web of Science, PubMed, Embase and Cochrane Library (from 1950 to February 2020) and bibliographies of relevant articles was undertaken, with eight randomized controlled trials (RCTs) with a total of 971 subjects included in the meta-analysis. Vitamin K combined with D significantly increased the total bone mineral density (BMD): the pooled effect size was 0.316 [95% CI (confidence interval) 0.031 to 0.601]. A significant decrease of undercarboxylated osteocalcin (-0.945, -1.113 to -0.778) can be witnessed with the combination of vitamin K and D. Simultaneously, subgroup analysis showed that K_2 or vitamin K (not specified) supplement was less than 500 μg/d, which combined with vitamin D can significantly increase the total BMD compared with control group on normal diet or the group with no treatment (0.479, 0.101 to 0.858 and 0.570, 0.196 to 0.945).

Conclusions

The combination of vitamin K and D can significantly increase total BMD and significantly decrease undercarboxylated osteocalcin, and a more favorable effect is expected when vitamin K_2 is used.

Keywords

Vitamin K; Vitamin D; Bone Mineral Density; Undercarboxylated osteocalcin

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Introduction

Bone-related diseases, especially osteoporosis and fractures, are increasing worldwide. As life expectancy rises, skeletal diseases are becoming a major public health problem, especially for postmenopausal women. The osteoporosis prevalence was 4% [95% CI (confidence interval), 3.84 to 4.13], 1.19% in men and 6.84% for women in a cross-sectional study with 45,990 participants.[1] Nutrition plays a vital role in the prevention and treatment of bone-related diseases. For example, calcium supplementation can prevent osteoporotic fractures.[2] In addition to calcium, vitamin D has long been paid most attention. Vitamin D_3 is made from a derivative of cholesterol, 7-dehydrocholesterol, which is stored under the skin and converted under ultraviolet light. Vitamin D can also be obtained from food. Natural food sources of vitamin D are mainly fatty fish, cod liver oil and the fats of fish-eating animals. In countries such as northern Europe, fatty fish are a major part of the diet.[3] It is estimated that women consume up to 236 international units (5.9 mg) of vitamin D per day and men 272 international units (6.8 mg).[4] Foods fortified with vitamin D are the main dietary source of it in countries teemed with it. Many previous studies have shown that long term vitamin D supplementation[5] and appropriate dose[6] is of great importance to bone quality of the elderly,[7] postmenopausal women[8,9] and other high-risk groups such as teenagers,[10] and can improve bone density parameters.[8,9] The reason is that vitamin D stimulates bone matrix formation and bone maturation, which also enhances osteoclast activity and affect osteoclast differentiation.[3]

In addition to calcium and vitamin D, recent studies have shown that vitamin K supplementation also has a beneficial effect on bone metabolism and bone quality maintenance, especially in improving the density of vertebrae.[11,12] Vitamin K exists in two forms that share a methylated naphthoquinone nucleus (menadione) having a variable aliphatic side chain at the 3′ position. Green vegetables, which is rich in lutein (vitamin K_1), such as kale, spinach, cauliflower and some fruits and herbs, is one of the most important foods in people's diet. Certain types of oils, such as soy and canola oils, also contain high amounts of vitamin K_1.[11] Vitamin K_2 is endogenous and

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synthesized by intestinal bacteria, so its distribution in diet is more limited than vitamin K_1, and it is found in some cheeses, eggs, meat, and natto (the most abundant source of fermented soybeans commonly found in Japan, at 775 μg /100g).[13-15] Vitamin K is involved in regulating calcium utilization in the body. Although vitamin K prevents vascular and soft tissue calcification, it promotes the integration of calcium into bone. There are 3 vitamin K-dependent proteins in bone: osteocalcin [bone gamma-carboxyglutamic acid (GLA) protein], matrix GLA protein, and protein S.[16] The effect of vitamin K on osteocalcin is perhaps the best understood among the 3 proteins.

Vitamin D and K have different effects on bone microenvironment. The active form of vitamin D, $1,25(OH)_2D_3$, can increase the activity of alkaline phosphatase and the expression of bone calcification gene in osteoblasts, so as to maintain the concentration of calcium and phosphorus and promote ossification.[17] Vitamin K, as an auxiliary factor of carboxylase activity, can promote osteocalcin carboxylation and promote bone formation.[18] Therefore, many experiments have investigated the effect of the combination of vitamin D and K on bone health. The combination of vitamin D and K seems to be more beneficial to lumbar vertebral density in postmenopausal women with osteoporosis than that of vitamin D and calcium.[19] However, it has been reported that when taking the recommended amount of vitamin D, dietary supplements of vitamin K did not lead to any additional benefits to the bone health of the spine or buttocks.[20]

The lack of consistency in results among different studies leads to a poor understanding of the effect of combined vitamin D and K on bone health. Therefore, we conducted a meta-analysis to evaluate the effects of this combination on bone health.

Methods

Literature search

Article searching was conducted by two investigators independently. Relevant articles were identified though the search among PubMed, Embase, Web of Science and the Cochrane Library databases from their starting dates through February 2020. The following format of keywords were used in the structured search strategies: (VK and VD OR vitamin K and vitamin D OR vitamin K plus vitamin D OR vitamin K_1 and vitamin D OR vitamin K_2 and vitamin D) AND (bone OR osteoporosis OR osteoporotic OR osteopenia OR fracture OR spinal cord injury). The references of relevant articles were also checked to identify suitable publications with this systematic review conducted in adherence to the standards of the Preferred Reporting Items for Systematic Review and Meta-Analysis statement.[21]

Eligibility criteria

Trials were selected based on the following inclusion criteria: (1) randomized controlled trials (RCTs) be conducted to compare the combination effect of vitamin K and D supplements with a control group on with only vitamin D or vitamin K alone or even not treated; (2) trials providing bone related data on total bone mineral density (BMD), neck BMD, lumbar BMD or undercarboxylated osteocalcin (UcOC). Exclusion criteria were: (1) trials without a control group; (2) trials of participants with corticosteroid-induced secondary osteoporosis.[21]

Data extraction

The following information was extracted by two researchers independently from each study: name of the first author; publication year; country of origin; participant characteristics, including body mass index (BMI) and age; doses of vitamin K and vitamin D; the type of vitamin K; trial duration; mean and standard deviation (SD) of total BMD, neck BMD, lumbar BMD (measured by dualenergy X-ray absorptiometer (DEXA)) and UcOC at baseline and endpoint or their changes from baseline to endpoint, with the disagreements resolved by consensus. If there were more than 2 groups, factorial designs or permitted multiple comparisons of trials, the information and data of interest reported in the original articles could be extracted.[22]

The reason why the total BMD, the lumbar BMD, the neck BMD and UcOC were considered as the main result concerning bone mass is that the number of bone is mainly expressed as BMD, which determines the ability of the bones to recover.[23] It has been reported that UcOC could well predict bone mineral density,[24,25] which is considered to be an accurate indicator of bone quality.[26] Therefore, it could be used as an indirect biomarker to evaluate bone quality.

Quality assessment and risk of bias

Risk of bias of the included studies were assessed using the Jadad score criteria, including five items, which were used for quality assessment, namely random sequence generation, randomization, allocation concealment, double blinding and reason of dropout.[27] The trial scored one point for each area reported, and a trial with Jadad score ≥ 4 shall be classified as high quality.

Statistical analysis

Meta-analysis was performed for total BMD, neck BMD, lumbar BMD and UcOC to determine the pooled effect of vitamin K combined D. For the parallel test, the changes from baseline to endpoint and their corresponding SDs in trial group and control group were used to calculate the effect size, namely the standard mean difference (SMD). If SD for change was not reported, it was imputed based on SDs at baseline and endpoint through previous study.[22] For studies with a crossover design, mean changes between the total BMD, the lumbar BMD, the neck BMD and UcOC and corresponding SDs at the end of two intervention periods were used in data analysis, as suggested by Cochrane handbook[28] for Systematic Reviews of Intervention. For studies with two or more intervention groups involved in one control group, the shared control group could be separated into two or more groups (the number was the same as intervention groups) and these comparisons into meta-analysis as if they were from different studies.[22] The overall effect size (standard mean difference) was calculated by a random effect model using Stata/SE 11.0 software (Stata Corporation, College Station, TX). Heterogeneity between the studies was assessed by I^2 statics, with values of 25%, 50% and 75% regarded as low,

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moderate and high degree of heterogeneity, respectively.[29] The fixed-effects model was used for data synthesis when there was no obvious heterogeneity. To identify the source of heterogeneity, subgroup and meta-regression analyses were conducted to focus on the information of the trials: the dose of vitamin K supplement, the dose of vitamin D supplement, gender and age.

To evaluate whether the overall effect was steady, sensitivity analysis was performed with deleting one trail at a time, and the effect size was re-calculated. Publication bias was conducted by using Begg's rank correlation test (Significant level at $P < 0.1$).[30] The trim-and-fill method was conducted to correct the potential publication bias, if the Begg's test was significant. Statistical analysis was conducted with STATA 11.0 for windows (Stata CORP, College station, TX). A P value of ≤ 0.05 was regarded as statistically significant.

Results

Literature search

The search strategy retrieved 780 unique citations: 187 from PubMed, 128 from Web of Science, 348 from Embase, 117 from the Cochrane Library, among which 125 articles were about vitamin K_1, 153 articles vitamin K_2, 332 articles vitamin K_1 and vitamin K_2. Ultimately, 8 studies (including 971 participants) were included in quantitative synthesis,[20,31-37] with the details of the screening steps and reasons for exclusion shown in Figure 1.

Characteristics of included studies

Intervention duration ranged from 6 months to 3 years, participants' age ranged from 3 to 80 years old in included studies. Among all these, eight described women, while six of them were conducted exclusively in women (Table 1). Four trials from 4 studies were conducted with vitamin K_2, while the other eight conducted with vitamin K_1. Two studies did not specify the type of vitamin D, while the other six studies were conducted with vitamin D_3. The number of related studies reporting on outcomes of interest were as follows: the total BMD (n = 8), the neck BMD (n = 4), the lumbar

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BMD (n = 7), and UcOC (n = 8) respectively.

Effect of combination of vitamin K and D on total bone mineral density

A significant increasing effect of combination of vitamin K and D on total BMD was observed: the pooled effect size was 0.316 (95% CI 0.031 to 0.601, P = 0.03) (Figure 2). Moderate heterogeneity was observed in the study: the I^2 value were 63.3%. Subgroup analysis showed that the dosage of vitamin K and the type of vitamin K had a significant influence on the effect of the combination on total BMD: significant increasing effect was observed only when the dose of vitamin K supplement was less than 500 μg/d, or the vitamin K was vitamin K_2 (P for subgroup difference were 0.013 and 0.03, respectively) (Figure 3) (Figure 4); and the pooled effect size in the two subgroups were 0.479 (0.101, 0.858) and 0.570 (0.196, 0.945), respectively. The pooled effect showed that combined vitamin K with D supplementation significantly increased total BMD (0.41; 95% CI: 0.07, 0.74, P = 0.019) in female subjects (Figure 5). The variation of vitamin K supplement ≥ 500 μg/d (as categorical variable) could help explain 70.9% heterogeneity. The combined vitamin K with D supplementation indicated that no significant differences were discerned on total BMD of the trials stratified by vitamin K supplementation (P for meta-regression = 0.971) and vitamin D supplementation (P for meta-regression = 0.845) with meta-regression analysis, respectively (Supplementary figure S1-S2).

Effect of combination of vitamin K and D on neck bone mineral density

No significant effect of the combination of vitamin K and D on total BMD was observed (-0.03, -0.198 to 0.137, P = 0.724) (Figure 6). No heterogeneity was observed among this group of studies (I^2 = 0.0%).

Effect of combination of vitamin K and D on lumbar bone mineral density

All the articles have shown the effect of combination of vitamin K and D on second-fourth lumbar BMD, only one study has also investigated effect of combination of vitamin K and D on first lumbar BMD,[35] the pooled effect (SMD_1) was 0.137, -0.022 to 0.295; P = 0.092 (Figure 7). For the second lumbar BMD, the third BMD, the forth BMD, the corresponding pooled effect were as follows: SMD_2=

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0.245, -0.038 to 0.528; P = 0.09 (Figure 8), SMD_3 = 0.322, 0.039 to 0.606; P = 0.026 (Figure 9), SMD_4 = 0.265, -0.010 to 0.539; P = 0.059 (Figure 10), which means that the combination of these two elements can significantly increase the third lumbar BMD. However, it did not significantly increase BMD in the first, second and fourth lumbar segments. Moderate heterogeneity was observed in the third lumbar BMD group: the I^2 value was 60.9%.

Subgroup analysis was performed based on dosage and type of vitamin K supplementation: significant increasing effect on the third lumbar BMD was observed when there was vitamin K supplement ≤ 100 μg/d, or the type of vitamin K was vitamin K_2 (P for subgroup difference were 0.002 and 0.011, respectively) (Figure 11) (Figure 12); and the pooled effect size in the two subgroups were 0.537 (0.206 to 0.869) and 0.475 (0.110 to 0.840) respectively. The pooled effect showed that supplementation with combination of vitamin K and D significantly increased the third lumbar BMD (0.41; 95% CI: 0.06, 0.76, P = 0.023) in female subjects (Figure 13). However, the variation of vitamin K supplement = 100 μg/d (as categorical variable) could explain 32.8% of heterogeneity. Supplemental vitamin K combined with D indicated that no significant differences were discerned on the third lumbar BMD of the trials stratified by vitamin K supplementation (P for meta-regression = 0.967) with meta-regression analysis (Supplementary figure S3).

Effect of combination of vitamin K and D on undercarboxylated osteocalcin

A significant decreasing of combination of vitamin K and D on UcOC was observed (-1.191, -1.714 to -0.668; P = 0.000; I^2 = 87.7%) (Figure 14). In order to find the source of heterogeneity, sensitivity analyses were conducted. Sensitivity analysis was performed by studying whether effect size changes significantly after excluding special trials one by one. Some trials were not consistent with others in the age of the subjects or in the type of intervention: Panis et al.[33] studied subjects between the ages of 3 and 17; in Braam et al. study[32] treatment group received additional minerals such as calcium, magnesium and zinc, while the control group did not. However, the sensitivity analysis of UcOC showed that when excluded the study by Braam et al.,[32]

the combined effect remains significant (Figure 15). The pooled effect size of vitamin combination on UcOC was -0.943, -1.117 to -0.770; P = 0.000; I^2 = 0.0% (Figure 16). The pooled effect showed that a significant decrease can be witnessed on the UcOC in both female subjects (-0.948; 95% CI: -1.153, -0.742, P = 0.0) and male subjects (-0.932; 95% CI: -1.255, -0.610, P = 0.0) (Figure 17) with supplementation of vitamin K combined with D, which also showed a significant reduction in UcOC in subjects whose mean age was ≥ 60 years (-0.941; 95% CI: -1.120, -0.762, P=0.0) , and in subjects whose mean age was < 60 years (-0.976; 95% CI: -1.640, -0.312, P=0.004) (Figure 18). Also, no significant differences were discerned on that UcOC of the trials stratified by vitamin K supplementation (P for meta-regression = 0.995) with meta-regression analysis (Supplementary figure S4) added supplemental vitamin K combined with D. The reason of heterogeneity change may due to Braam et al. study,[32] in which treatment group received additional minerals such as calcium, magnesium and zinc, which is well known that these minerals can have an impact on bone quality, as confounding factors for the study outcome. Thus this article[32] is considered as the source of the heterogeneity, and therefore exclude it.

Quality assessment, sensitivity analysis and publication bias

According to the Jadad score criteria, four trials were regarded as high-quality studies[20,32-34] (Table 1), with the remaining 4 trials were classified as low-quality ones,[31,35-37] for the lack of relevant information, including randomization, generation of random sequence, binding, allocation concealment or reasons for dropout.

Sensitivity analysis showed that there was no apparent difference for the effect of vitamin K combined with D on total BMD, neck BMD or lumbar BMD before and after any one study was removed (Supplementary Figure S5–S9).

The results of Begg's rank correlation test and Egger's linear regression test indicated that there was no obvious publishing bias detected in the meta-analysis of total BMD, neck BMD or UcOC. Publication bias may exist for third lumbar BMD according to Egger's linear regression test (P = 0.028) and Begg's rank correlation test (P = 0.016)

(Figure 19). The funnel plot revealed an apparent asymmetry, suggesting the presence of a potential publication bias. What should be mentioned is that statistical power of Begg's test lowered when there was limited number (less than 25) of studies. After the trim and fill method was employed, three theoretically missing studies were added. The pooled analysis incorporating the hypothetical studies showed a statistically insignificant association between vitamin K combined D and the third lumbar BMD (0.063, -0.254 to 0.380) (Figure 20). Hence, the conclusions from the meta-analysis of the should be interpreted with caution due to potential publication bias.

Discussion

As far as we know, this is the first meta-analysis to systematically examine the effects of vitamin K (not specified) combined with D on bone quality, which provides a basis for the proper use of the combination of vitamins to improve the quality of bones and thus prevent skeletal diseases. This meta-analysis of 8 RCTs suggests that the combination of vitamin K and D had the total BMD and the third lumbar BMD increased significantly and UcOC decreased significantly. However, no remarkable effect was detected on neck BMD.

Although a number of reviews have shown that combination of vitamin K (not specified) and D had promoting effect on BMD, a few studies have found that vitamin K did not seem to have that good effect on it.[38,39] However, a meta-analysis revealed that the modest overall effect of vitamin K on BMD may be biased and should be interpreted with caution.[40] Present study only focused on the combined effect of vitamin K and D, but Fang et al.[40] focused on the vitamin K alone, which suggested that vitamin D combined with K may be more effective in promoting BMD than vitamin K alone. There may be some interaction between vitamin D and K, which have osteoinduction properties (Figure 21).[12,41] Some studies have shown that vitamin D can increase the concentration of vitamin K-dependent bone protein by stimulating the expression of osteoblast-specific genes,[17] and induce bone formation *in vitro*.[42-44] Vitamin D_3, for example, increases the expression of BMP6 mRNA in human bone

marrow mesenchymal stem cells 12-fold.[45] Vitamin K exists in the endoplasmic reticulum as the coenzyme of gamma-glutamyl carboxylase (GGCX), which catalyzes the carboxylation of glutamate residues (GLU) into gamma-carboxylated glutamate (GLA), affecting the activity of vitamin k-dependent protein (VKDPs). The most relevant VKDP is bone GLA protein (BGP, or osteocalcin).[41] Physiological 1,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] can increase the rate of bone GLA protein biosynthesis, thus promoting bone mineralization.[44] Vitamin K may stimulate the expression of 1,25 $(OH)_2D_3$-induced osteocalcin mRNA to enhance the mineralization of osteoblasts.[42] Osteoblast-specific expression of osteocalcin is controlled at the transcriptional level by 25-hydroxyvitamin D [25(OH)D] through the 1,25(OH)D-responsive element within the promoter of the osteocalcin gene.[17] The addition of vitamin K enhanced the accumulation of 1,25$(OH)_2D_3$-induced osteocalcin in the intracellular and extracellular matrix (cell layer), thereby promoting bone mineralization.[46] In rats, 1,25(OH)D receptor binding can undergo gamma-carboxylation in the presence of vitamin K, which means that 1,25(OH)D receptor carboxylation can potentially modify the intrinsic biochemical properties of the nuclear receptors and modulates its binding to DNA.[47] Human intervention studies also shown that vitamin D_3 is necessary before activating vitamin K_2 of vitamin D_3 to carboxylation osteocalcin, because osteocalcin is induced by this effect. Vitamin K_2 distinctively enhances activated vitamin D_3-induced mineralization by osteoblasts when there is administration of activated vitamin D_3, which can be denoted as that vitamin K_2 administration enhances the carboxylation of highly produced osteocalcin by vitamin D_3 administration, resulting in a vigorous increase in mineralization in bone tissue.[19] These evidences help to explained the mechanism by synergetic effect of vitamin K and D on BMD.

A meta-analysis of the effects of vitamin D on bones health showed that single vitamin D did not significantly promote total BMD.[48] Another meta-analysis did not use total BMD to study the effect of vitamin K on bone health, but lumbar spine BMD

(significant without considering heterogeneity and publication bias) and neck BMD (insignificant) as indicators.[40] The present results suggest that vitamin K (not specified) combined with D has a significant effect on the promotion of total bone mineral density, which may be superior to that of vitamin K or vitamin D alone. Vitamin K_2 combined with vitamin D_3 significantly increased vertebral bone mass compared with vitamin K2 or vitamin D3 alone in Japanese women with bone loss and osteoporosis.[31] Ninety-two osteoporotic women who were more than 5 years after menopause were randomly divided into four groups (vitamin D_3 group, vitamin K_2 group, vitamin D_3 + vitamin K_2 group and calcium group). After two years of supplement, compared with vitamin D_3 and K_2 alone, the density of lumbar vertebrae in vitamin D_3 combined with K_2 group was significantly increased ($P < 0.05$, $P < 0.001$).[19] A randomized controlled trial divided postmenopausal women into three experimental groups (calcium + vitamin D + K_1 fortified dairy products, calcium + vitamin D + K_2 fortified dairy products, calcium + vitamin D fortified dairy products) and a control group (normal diet). After 12 months of supplement, compared with the single vitamin D fortified milk group, the favorable changes in the two vitamin K + vitamin D groups were as long as they were shown as the inhibition of serum bone remodeling indexes and the positive changes in lumbar spine density.[37] Another RCT also divided postmenopausal women into four groups the same as the above study, intervening in fortified milk and yogurt, with the additional benefits of L2-L4 bone mineral density were observed in the combined vitamin K and D group compared with the single vitamin D fortified dairy group.[36] These findings indicated that the combined administration of vitamin D and K have a synergistic effect in increasing BMD. However, it has been found that the combination of vitamin K and D had no significant effect on bone mineral density among healthy women as compared with vitamin D alone, which is possibly due to the shorter duration of the study (1 year) and the normal bone mineral density of healthy people, which could be illustrated as a healthy physiological state.[49]

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The results of meta-analysis showed that vitamin K (not specified) combined with D increased the lumbar BMD. However, it did not significantly promote that of the neck BMD. Some studies also found that vitamin D_3 combined with K_2, compared with calcium, significantly increased the density of lumbar bone in postmenopausal women with osteoporosis.[19] Thus it can be said that the combination of vitamin K and D can increase the bone density of the lumbar spine, the left femoral neck, and the left hip, but not neck BMD.[50] It has been found that when serum 25(OH)D concentration was > 40 nmol/L, the contribution of serum 25(OH)D on BMD at lumbar spine and femoral neck BMD was different: 5.9% and 2.7%, respectively.[51] The present meta-analysis showed that vitamin K combined with D did not significantly increase the second lumbar BMD, fourth lumbar BMD and neck BMD, and there was significant publication bias among studies concerning the effect of vitamin K combined D on the third lumbar BMD. After the correction of trim and fill analysis, the effect size became insignificant. Therefore, this result should be considered carefully. Present conclusion will help us to further study the different effects of vitamin K combined D on different parts of the body (lumbar vertebrae, neck, femur, hip, etc.).

Present evidences suggested that vitamin K (not specified) combined with D can reduce serum UcOC, which is consistent with the results of many studies. Prospective studies found that older women with elevated serum UcOC had a six times higher risk of hip fracture during follow-up than elderly women with normal serum UcOC.[24] An increase in the amount of UcOC was observed in patients with osteoporosis.[33,52] The UcOC was negatively correlate with BMD.[53] However, another study found that decreased UcOC is positively associated with stress fractures.[54] UcOC can promote osteoblast activity[55,56] and regulate insulin secretion and sensitivity.[57] Therefore, more experiments are needed to prove the relationship between UcOC and bone mass in the future.

Subgroup analyses indicated that when the vitamin K (not specified) supplementation was less than 500 μg/d, the combination of vitamin K and D has a significant positive

effect on total BMD and third lumbar vertebral BMD. The daily consumption of 400 IU vitamin D_3 and 80 μg vitamin K_1 of postmenopausal women could prevent 25(OH)D from falling in winter.[44] The meta-analysis also showed that a daily increase of 50 μg of vitamin K intake would reduce the fracture risk by 3 percent,[45] which is consistent with the results of present subgroup analysis, which proves that when vitamin K intake is less than 500 μg/d, the combined effect of vitamin D_3 and K_1 on bone quality is better than that of when vitamin K intake is great than 500 μg/d. The review by Shah et al.[58] and Hamidi et al.[16] also suggest that once the vitamin K for bone health is satisfied, there may be no additional benefit to the extra intake, but the appropriate dose of vitamin K for bone health in people with low bone density is still unknown. More trials are needed to investigate the relationship between vitamin K supplementation and the combined effect of vitamin K and D on BMD in patients with low bone density or osteoporosis.

Subgroup analyses also showed that when the type of vitamin K was vitamin K_2, the combined effect of vitamin K and D can significantly increase the total BMD and third-lumbar BMD. The meta-analysis has also proved that, vitamin K_2 may play a role in maintaining bone density and reducing the incidence of fractures in postmenopausal women with osteoporosis.[59] A meta-analysis summarized the efficacy of vitamin K_2 in the treatment of non-spinal and hip fractures, suggesting that compared with vitamin K_1, vitamin K_2 may have a better effect in improving bone density and bone quality.[48] It has been proved that bone mineralizing inducer in human osteoblasts can increase the production of osteocalcin.[60] It has been proved that vitamin K_2 can inhibit osteoclast *in vitro* studies.[61] More studies are needed to investigate the detailed mechanism of combined effect of vitamin K and D on bone quality.

The results of the present meta-analysis suggested that the combined effect of vitamin K (not specified) and D seems to be more effective than that of mono-vitamin, and that a reasonable diet and nutritional pattern may be more important than the intake of a single nutrient. This provides evidence that healthy diet includes a variety of foods

to promote bone health. For example, Kanellakis et al.[37] and Moschonis et al.[36] used vitamin K and D fortified dairy products rather than simply using vitamin tablets, their effect on bone mineral density were better than that of the control group with normal diet. Those who need to pay attention to bone health, especially postmenopausal women, should eating foods rich in vitamin K and D to adjust their diet except taking appropriate vitamin supplements.

Although the present meta-analysis may provide some useful information, several potential limitations should be considered. Firstly, as a meta-analysis, the residual confounding factors are always of concern in the included studies, and the possibility that other risk factors may impact the observed association between vitamin K (not specified) combined D on bone quality. However, most of the studies were adjusted to a wide range of potential confounding factors, including age, BMI and nationality. Secondly, in the present meta-analysis, 2 studies included men and women and 6 studies included only women. We could not examine the association between vitamin K combined with D and bone quality in men. Thirdly, the sample size was relatively small (5 subjects in each group were less than 50). Fourthly, the quality of the included studies was not always optimal. However, after excluding low-quality studies, the results have not been changed. Furthermore, Begg's rank correlation test showed some asymmetry suggesting the possibility of publication bias within the included studies on the effect of vitamin K combined D on BMD at the third lumbar spine. After trim and fill analysis correction, the combined effect becomes insignificant, suggested that we should be cautious the result.

Conclusions

This meta-analysis showed that the combination of vitamin K and D can improve bone quality via increasing total bone mineral density and decreasing undercarboxylated osteocalcin, and a more favorable effect is expected when vitamin K_2 is used. These findings have important public health implications with regard to the improvement of bone quality, especially in women, via a combination of vitamin K and D supplement. Further well designed RCTs are required to investigate the

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mechanism of the effect of combined vitamin K and D on bone quality.

Conflict of interest

There are no identified conflicts of interest with any of the authors.

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Fig. 1 Flow chart for article selection process. RCT, randomized controlled trial.
Fig. 2 Pooled effect size of vitamin K combined with vitamin D on total BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 3 Effect of vitamin K dose on the effect size of vitamin K combined with vitamin D on total BMD (1: ≥ 500 μg/d or 2: < 500 μg/d). BMD, bone mineral density. SMD, standard mean difference.
Fig. 4 Effect of vitamin K types on the effect size of vitamin K combined with vitamin D on total BMD (1: vitamin K_1 or 2: vitamin K_2). BMD, bone mineral density. SMD, standard mean difference.
Fig. 5 Effect of gender on the effect size of vitamin K combined with vitamin D on total BMD (1: male or 2: female). BMD, bone mineral density. SMD, standard mean difference.
Fig. 6 Pooled effect size of vitamin K combined with vitamin D on neck BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 7 Pooled effect size of vitamin K combined with vitamin D on first lumbar BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 8 Pooled effect size of vitamin K combined with vitamin D on second lumbar BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 9 Pooled effect size of vitamin K combined with vitamin D on third lumbar BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 10 Pooled effect size of vitamin K combined with vitamin D on forth lumbar BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 11 Pooled effect size of Pooled effect size of vitamin K combined with vitamin D on the third lumbar BMD including vitamin K supplement (1: 500 μg/d or 2: 100 μg/d). BMD, bone mineral density. SMD, standard mean difference.
Fig. 12 Pooled effect size of Pooled effect size of vitamin K combined with vitamin D on the third lumbar BMD including the type of vitamin K (1: vitamin K_1 or 2: vitamin K_2). BMD, bone mineral density. SMD, standard mean difference.
Fig. 13 Effect of gender on the effect size of vitamin K combined with vitamin D on the third lumbar BMD (1: male or 2: female). BMD, bone mineral density. SMD, standard mean difference.
Fig. 14 Pooled effect size of vitamin K combined with vitamin D on UcOC. UcOC, undercarboxylated osteocalcin. SMD, standard mean difference.
Fig. 15 Sensitivity analysis of vitamin K combined with vitamin D on UcOC. UcOC, undercarboxylated osteocalcin. SMD, standard mean difference.
Fig. 16 Pooled effect size of vitamin K combined with vitamin D on UcOC after excluded one article. UcOC, undercarboxylated osteocalcin. SMD, standard mean difference.
Fig. 17 Effect of gender on the effect size of vitamin K combined with vitamin D on the UcOC (1: male or 2: female). UcOC, undercarboxylated osteocalcin. SMD, standard mean difference.
Fig. 18 Effect of age on the effect size of vitamin K combined with vitamin D on the UcOC (1: ≥ 60 years or 2: < 60 years). UcOC, undercarboxylated osteocalcin. SMD, standard mean difference.
Fig. 19 Begg's funnel plot of vitamin K combined with vitamin D on third lumbar BMD. BMD, bone mineral density. SMD, standard mean difference.
Fig. 20 Filled funnel plots for the third lumbar BMD. The square represents theoretically missing studies. The funnel plots were achieved using trim and fill method. BMD, bone mineral density.
Fig. 21 Potential synergistic mechanisms between vitamin D and K and bone. $1,25(OH)_2D_3$:

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1,25-Dihydroxyvitamin D3; Glu: glutamate residues; GLA: gamma-carboxylated glutamate; OC: osteocalcin.

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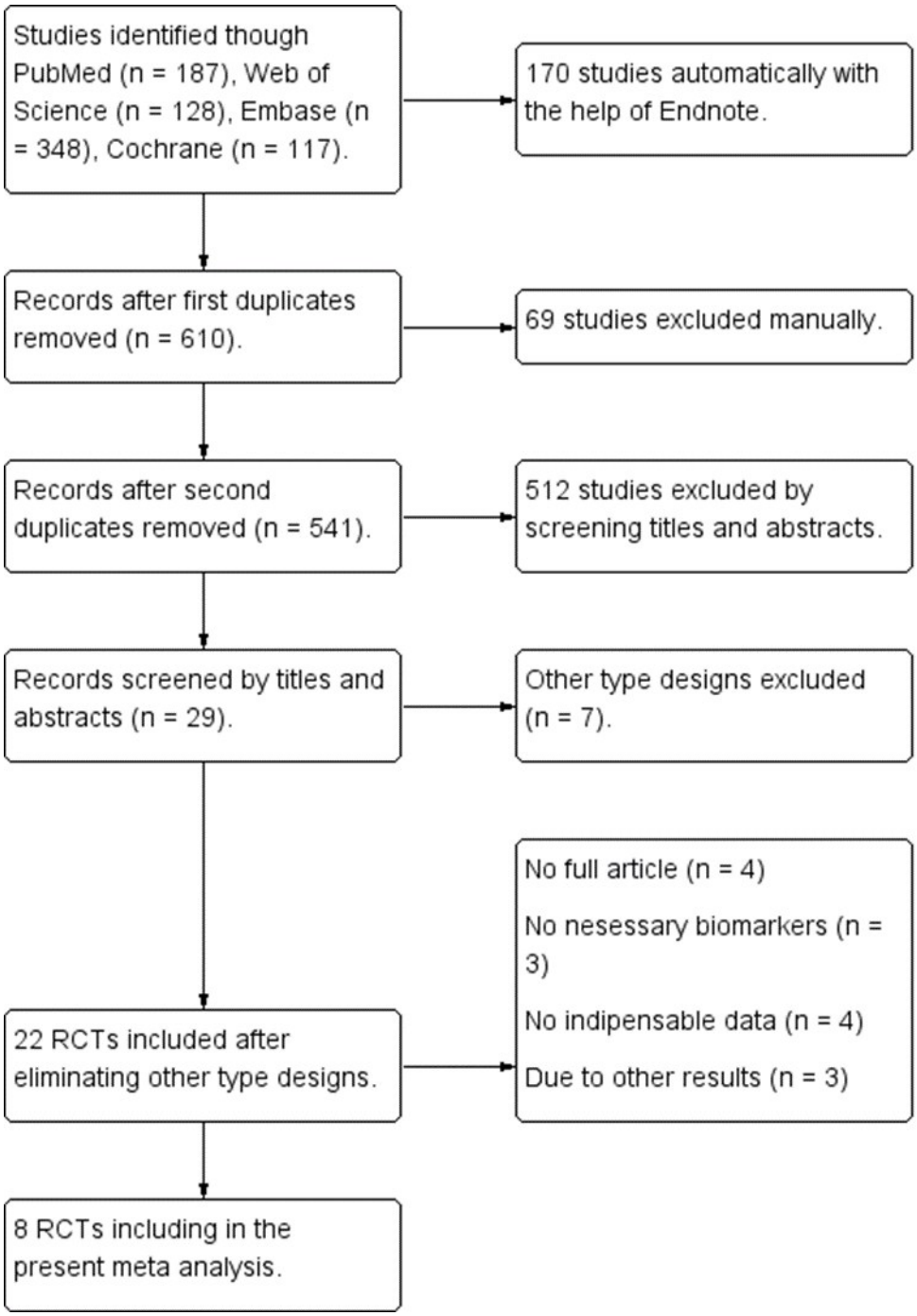

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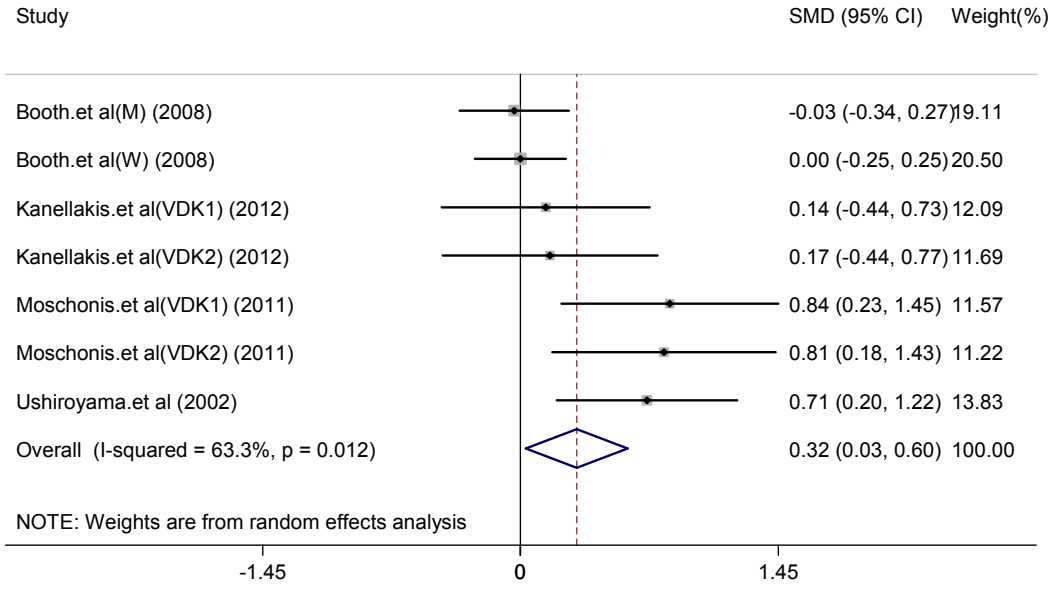

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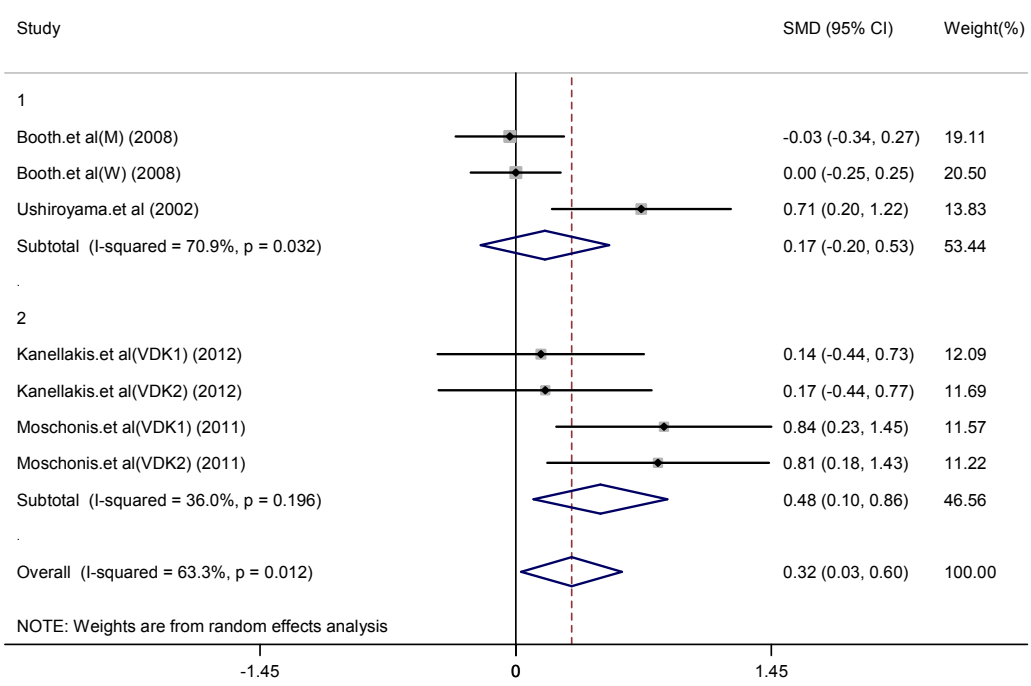

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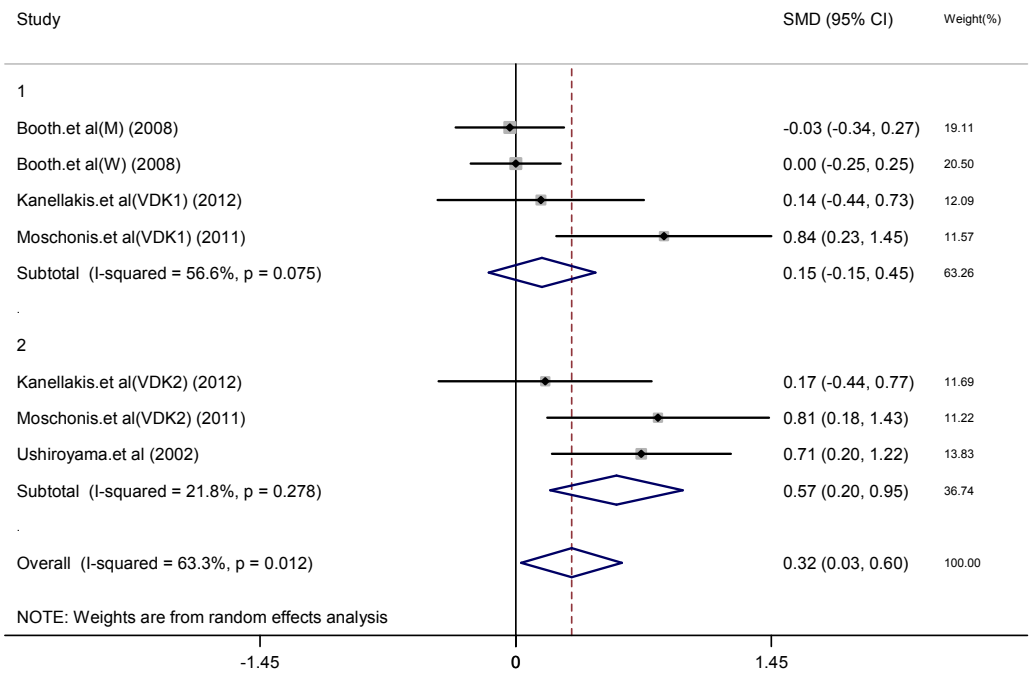

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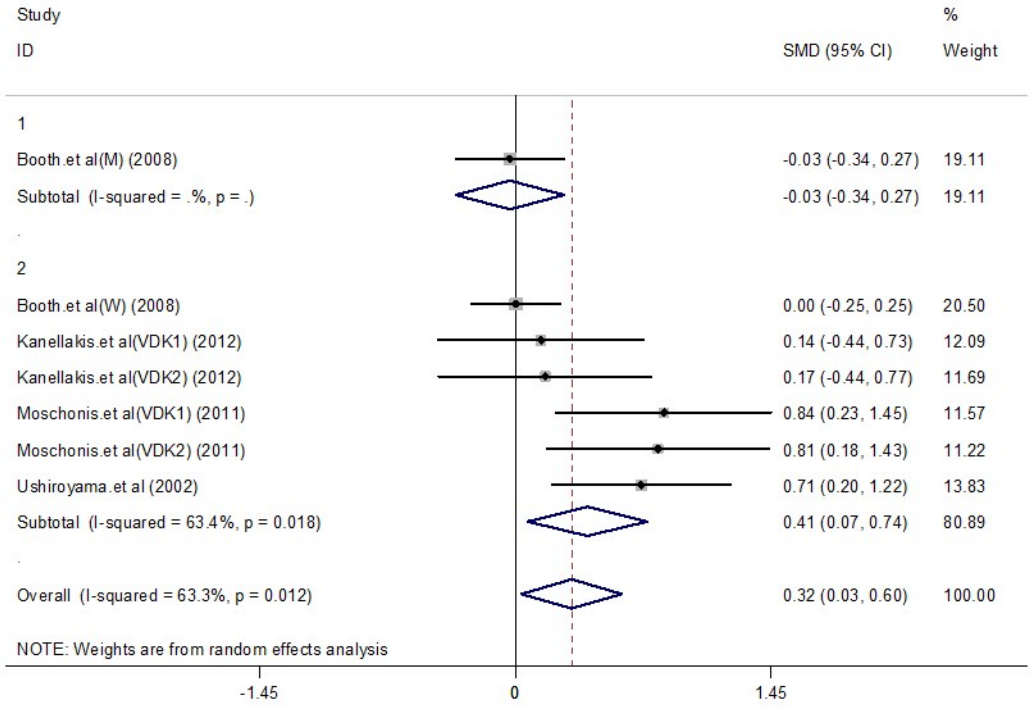

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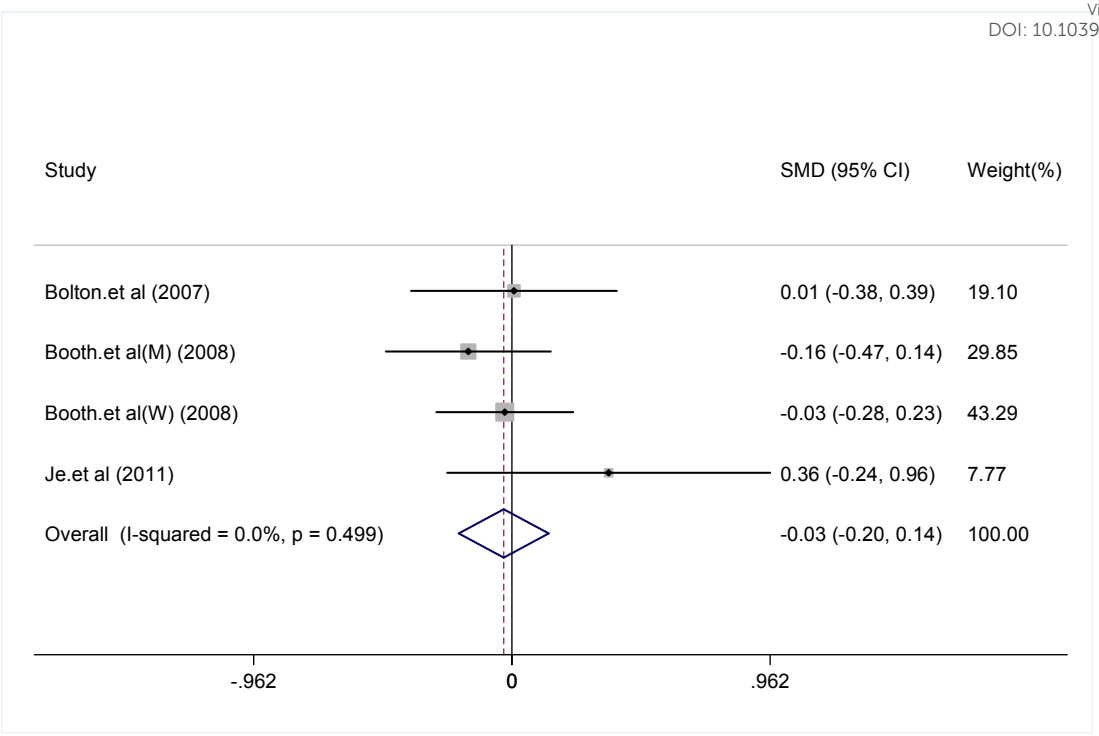

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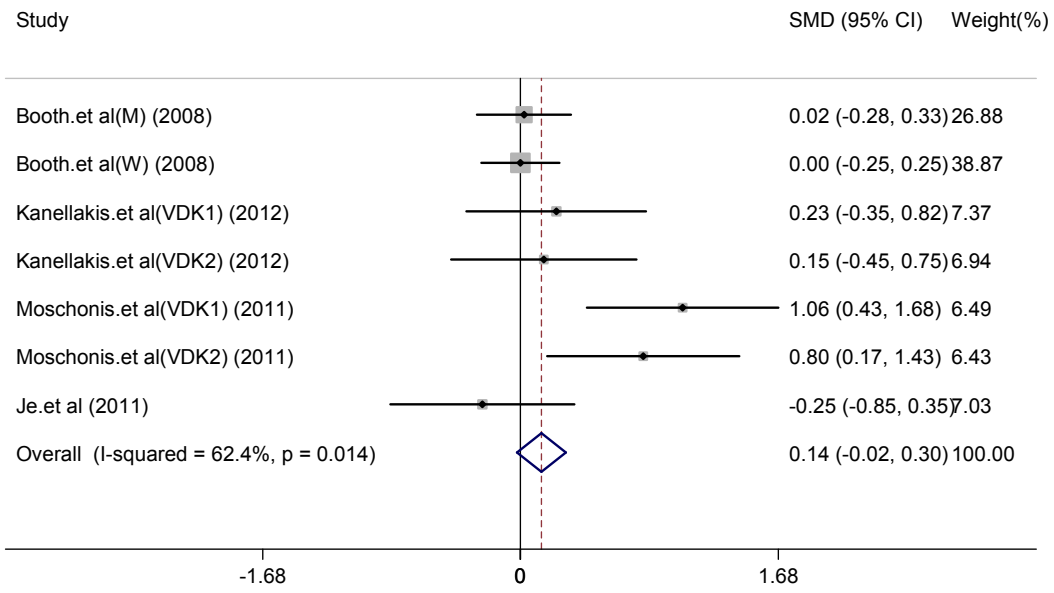

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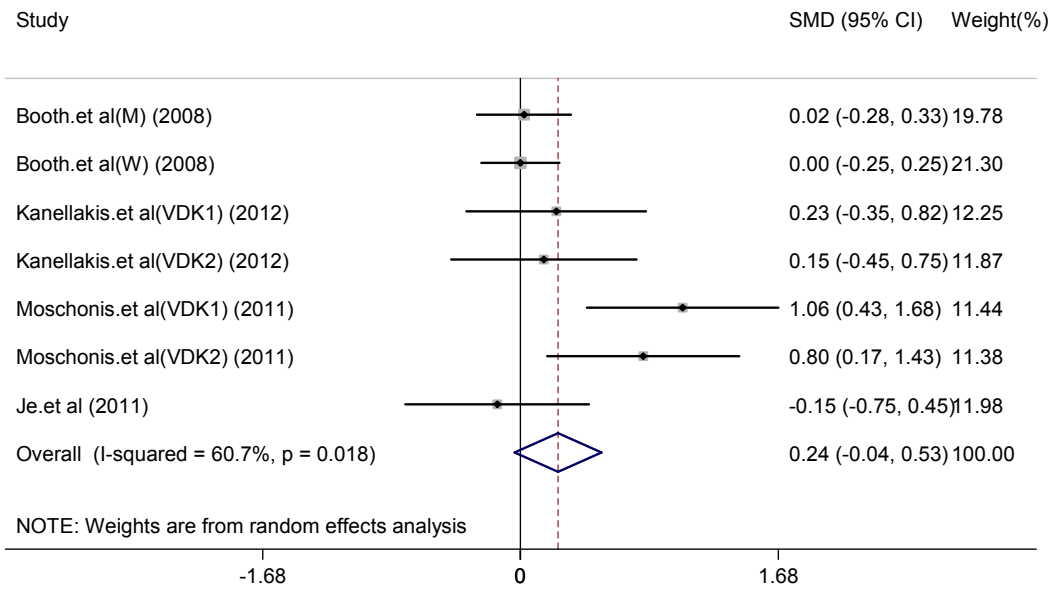

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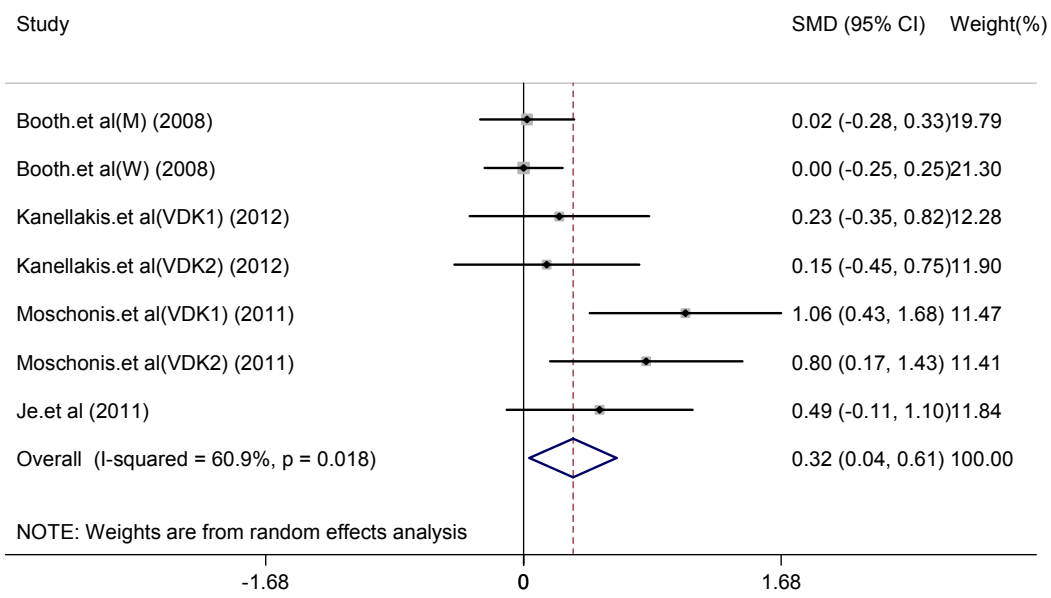

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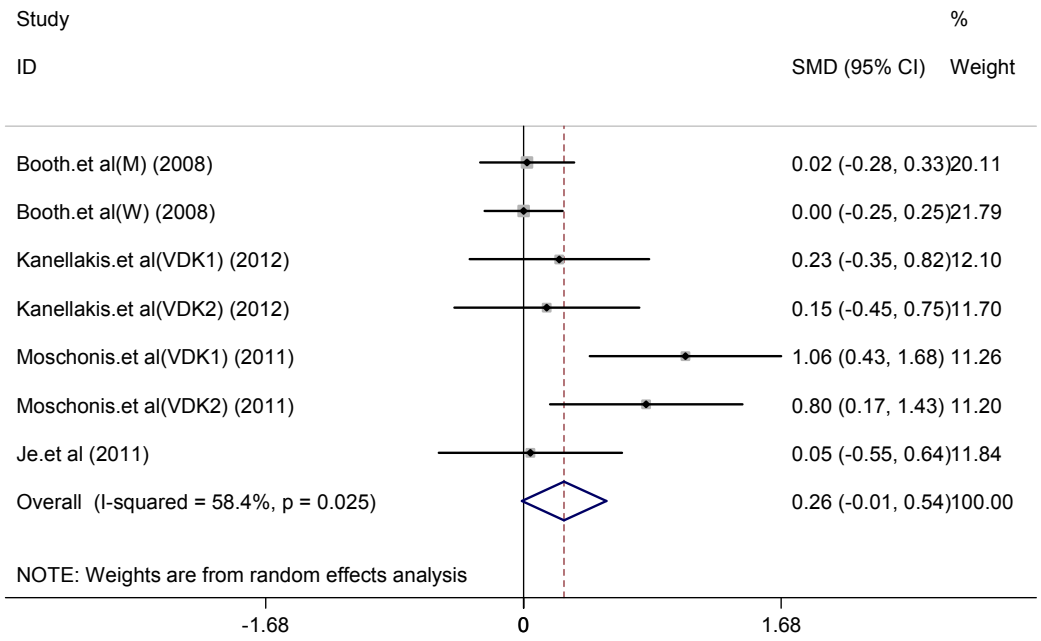

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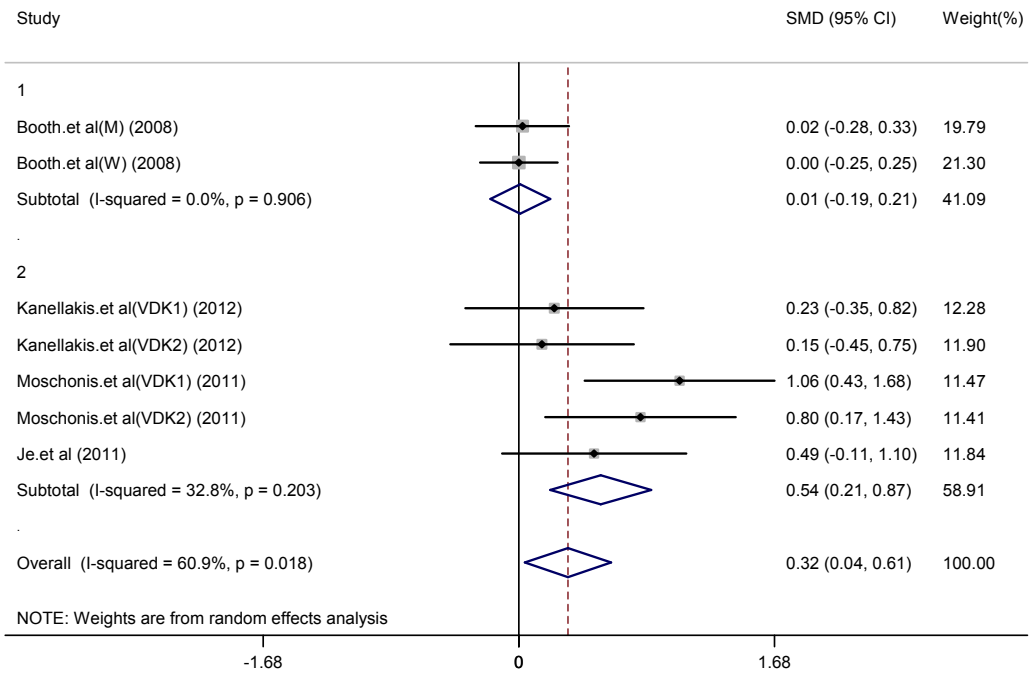

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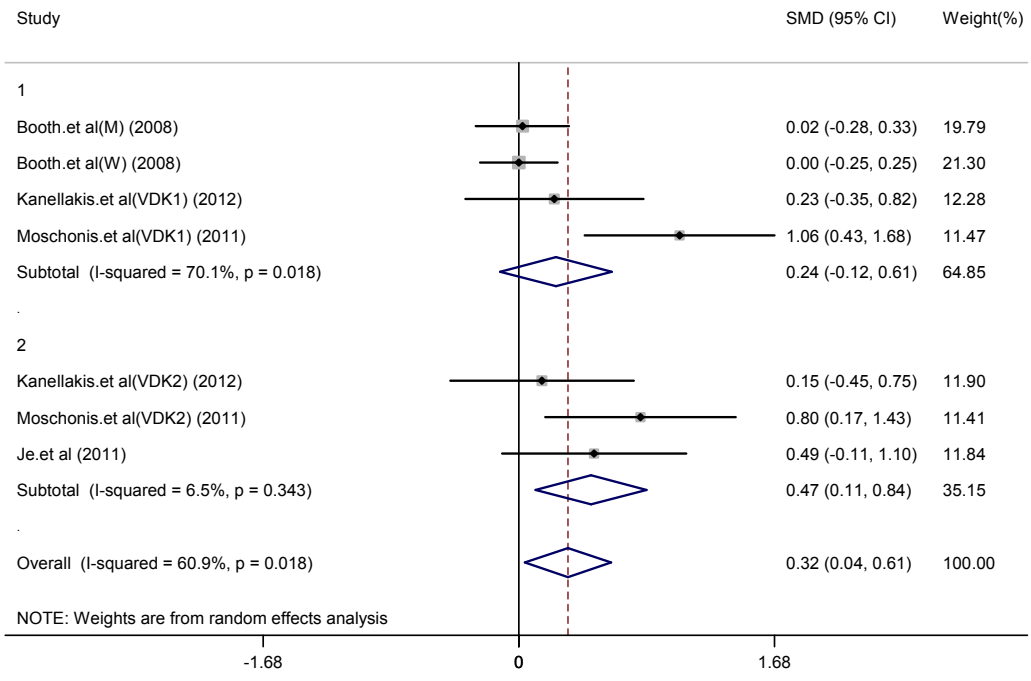

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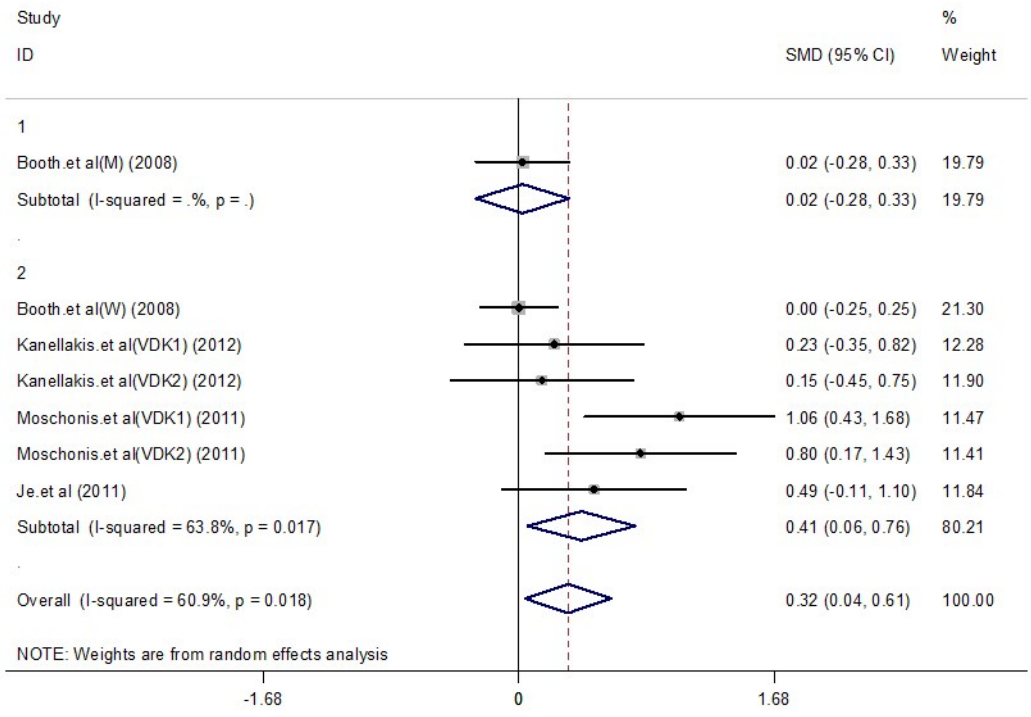

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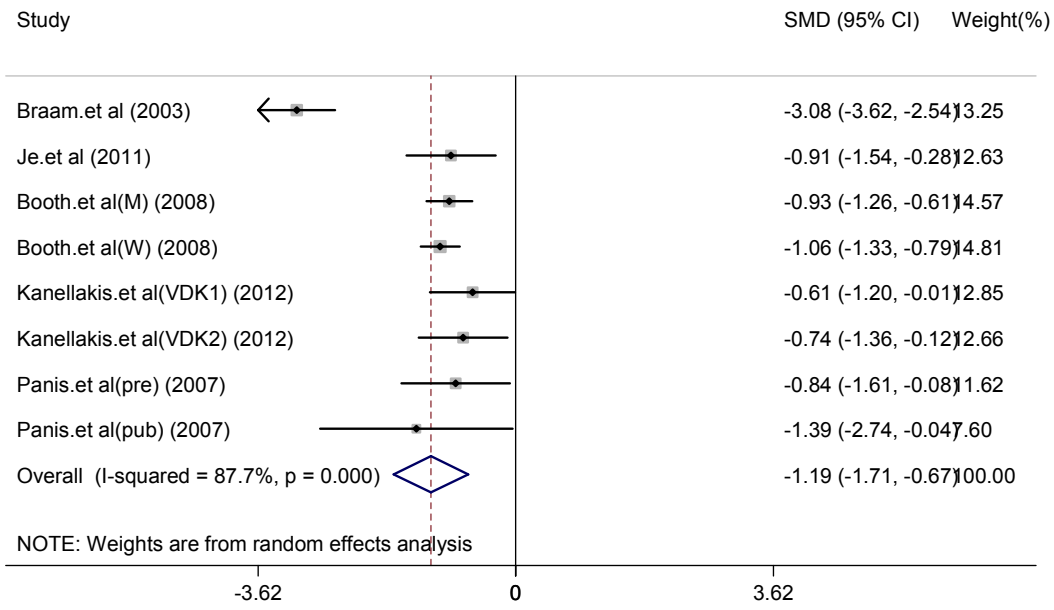

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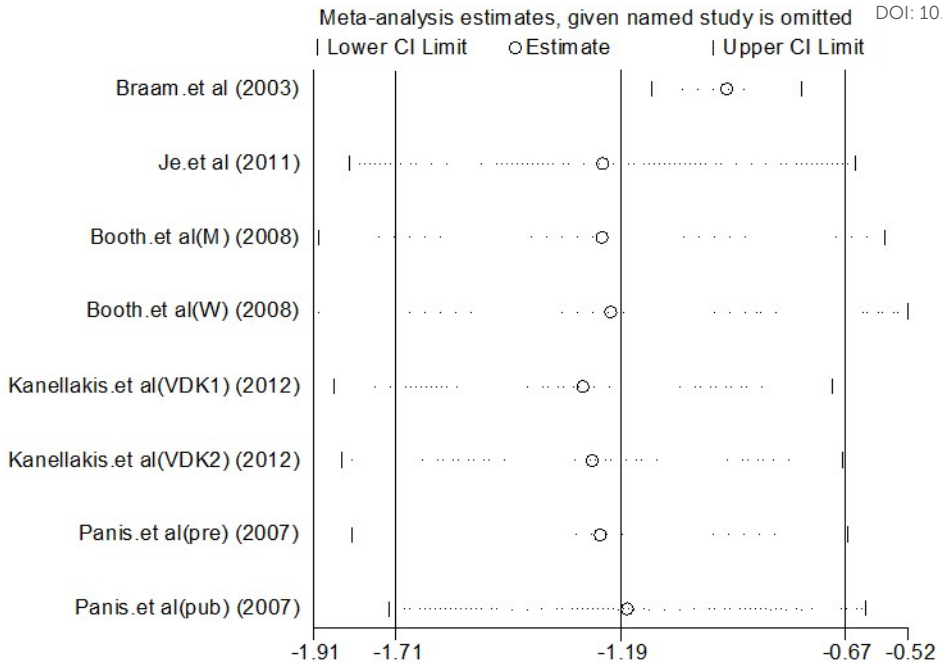

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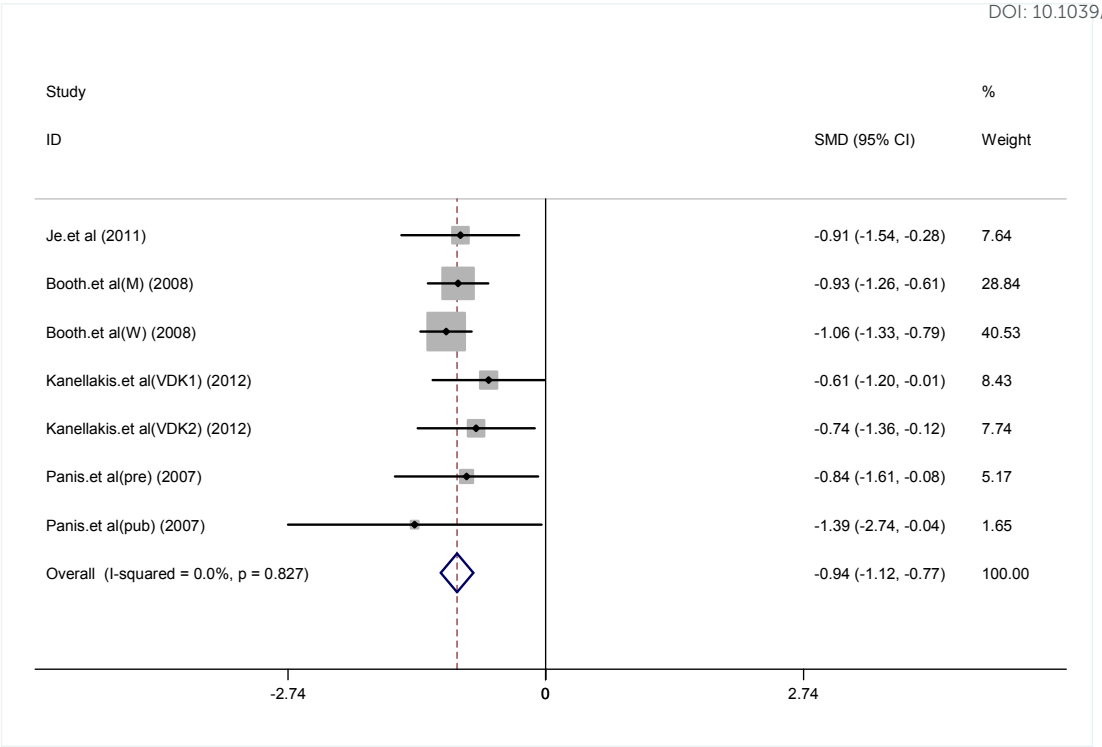

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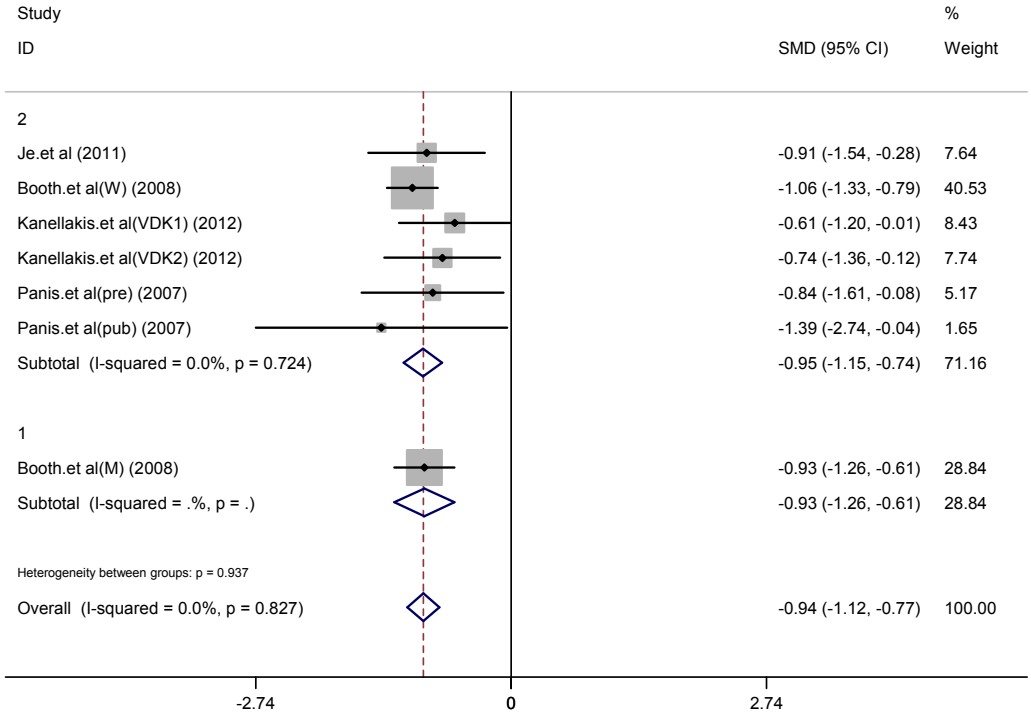

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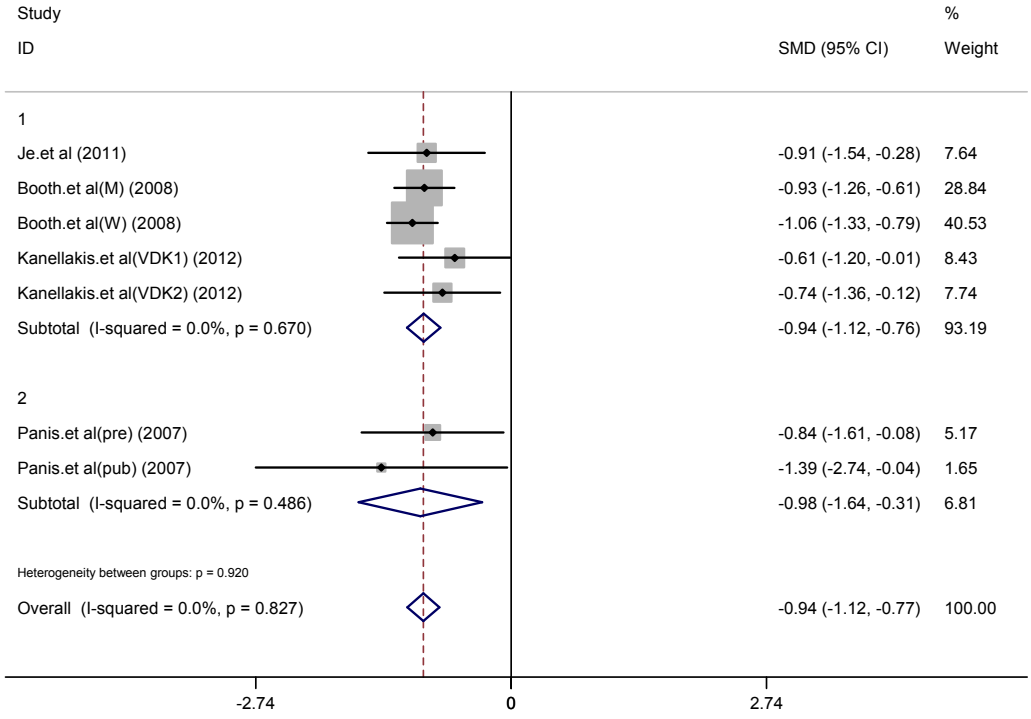

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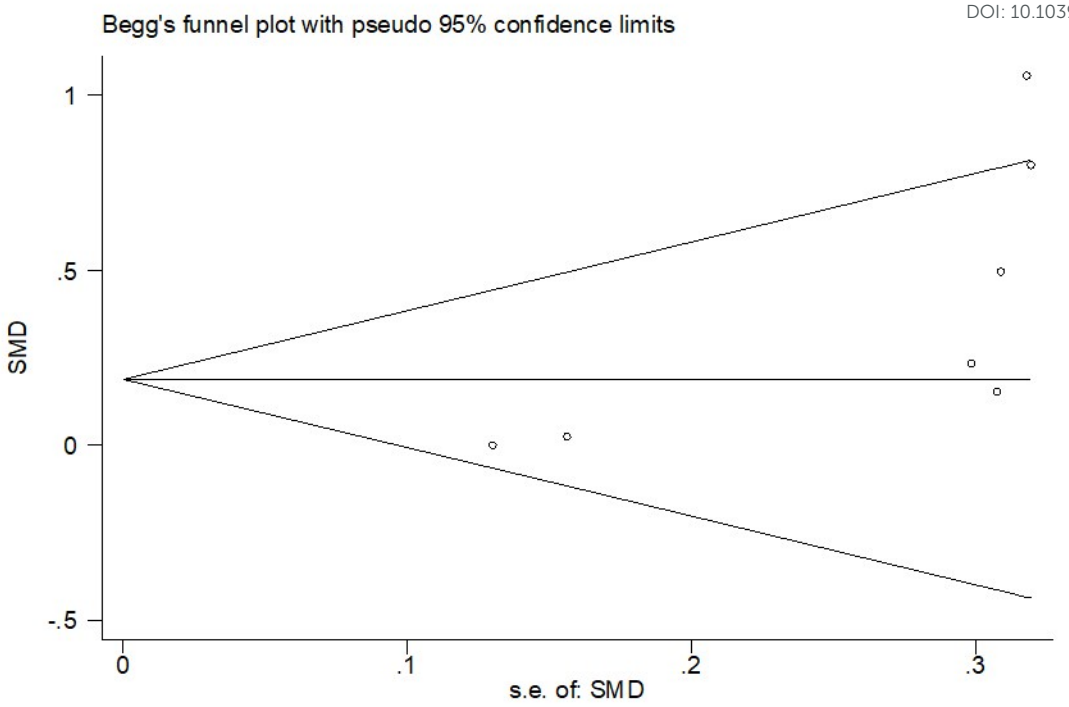

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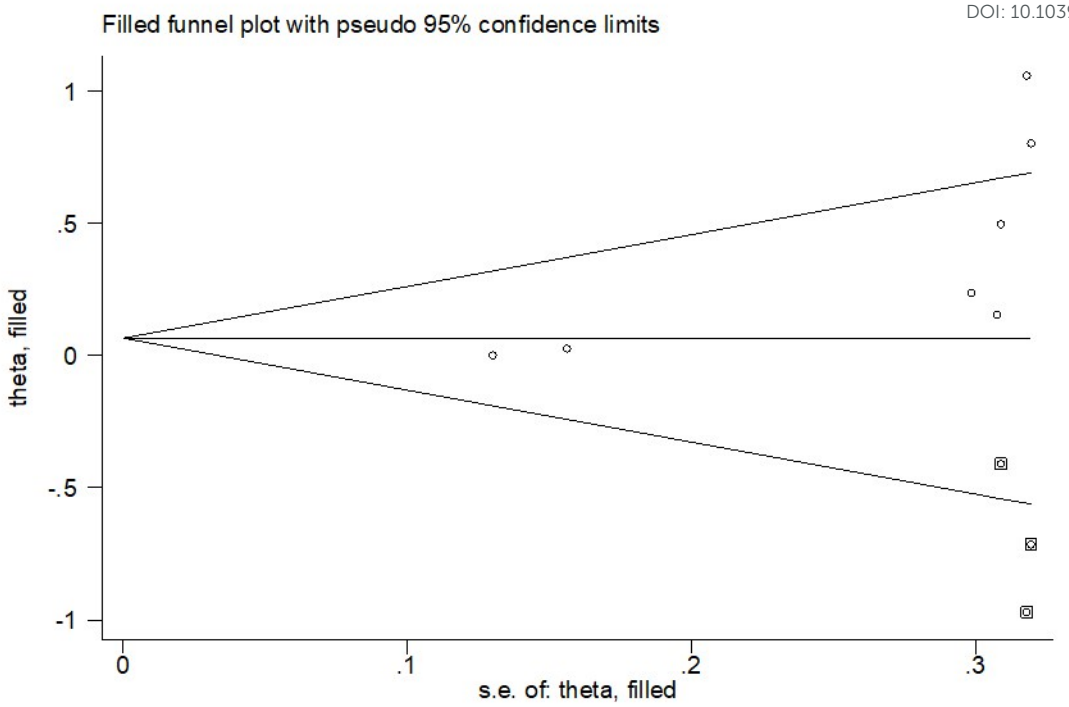

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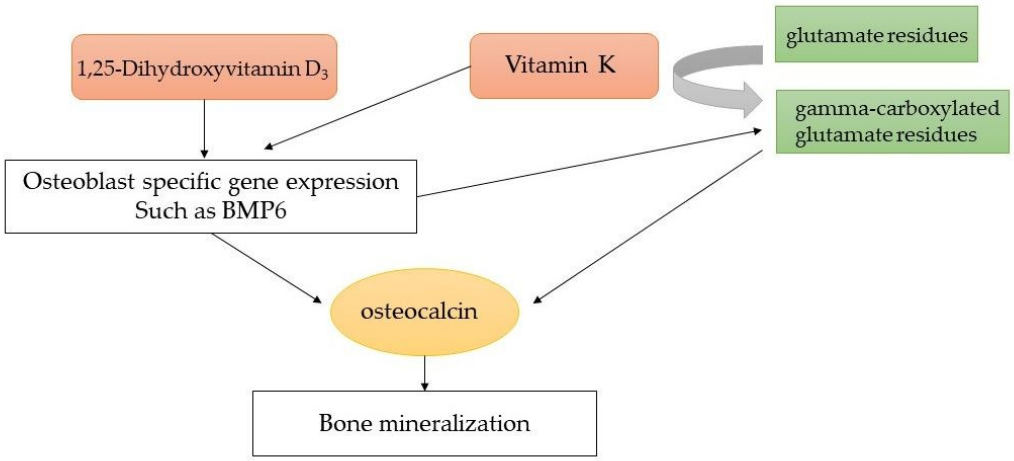

Table 1. Characteristics of the eligible RCTs

Author	Publication year	Duration of follow-up	No. (intervention/control, subjects)	Country	BMI (kg/m^2)	Age (mean± SD)	Vitamin D	Vitamin K	Type of vitamin K	Jadad score
Ushiroyama.et al.	2002	2 years	31/33	Japan	22.3	53.5±6.0	1 μg/d	45 mg/d	K_2	3
Braam.et al.	2003	3 years	63/60	Netherland	25.6	55.3±2.8	10 μg/d	1 mg/d	K_1	6
Panis.et al. (Pre)	2006	2 years	14/15	Netherland	NR	6.7±1.9	10 μg/d	1 mg/d	K_1	6
Panis.et al. (Pub)	2006	2 years	5/6	Netherland	NR	14.1±2.7	10 μg/d	1 mg/d	K_1	6
Bolton-Smith.et al.	2007	2 years	49/56	UK	26.15	67.8±5.4	10 μg/d	200 μg/d	K_1	5
Booth.et al. (M)	2008	3 years	84/80	USA	NR	69.0±5.0	10 μg/d	500 μg/d	K_1	4
Booth.et al. (W)	2008	3 years	120/117	USA	NR	68.0±5.4	10 μg/d	500 μg/d	K_1	4
Je.et al.	2011	6 months	38/40	Korean	24.15	68.1±6.3	10 μg/d	45 mg/d	K_2	3
Moschonis.et al. (VDK_1)	2011	1 year	26/20	Greece	30.00	60.9±5.3	10 μg/d	100 μg/d	K_1	2
Moschonis.et al. (VDK_2)	2011	1 year	24/19	Greece	29.65	62.0±6.4	10 μg/d	100 μg/d	K_2	2
Kanellakis.et al. (VDK_1)	2012	1 year	26/20	Greece	30.00	62.0±5.8	10 μg/d	100 μg/d	K_1	2
Kanellakis.et al. (VDK_2)	2012	1 year	24/19	Greece	29.69	62.0±5.8	10 μg/d	100 μg/d	K_2	2

Abbreviations: Pre, prepubertals; Pub, Pubertals; M, men; W, women; VDK_1, vitamin D and vitamin K_1; VDK_2, vitamin D and vitamin K_2; NR, not reported.