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Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials

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Abstract

Objective: Osteoporosis is a common disease in postmenopausal women. Several studies have analyzed the associations between dietary supplement of probiotics and bone health in postmenopausal women, but the results are still controversial. We conducted this meta-analysis to assess the effects of probiotics supplement on bone mineral density (BMD) and bone turnover markers for postmenopausal women.

Design: systematic review and meta-analysis.

Methods: We systematically searched PubMed, EMBASE and the Cochrane Library from their inception to May 2019 for randomized controlled trials (RCTs) assessing probiotic supplements and osteoporosis in postmenopausal women. Study-specific risk estimates were combined using fixed-effect or random-effect models.

Results: Four RCTs (n = 218) were included. Probiotic supplements were associated with a significantly higher BMD in both hips (SMD (standardized mean difference) = 0.37, 95% CI: 0.12–0.62) and lumbar spine (SMD = 0.28, 95% CI: 0.03–0.53) than in the control (*P* = 0.004, 0.029 respectively). Collagen type 1 cross-linked C-telopeptide (CTX) levels in the treatment groups were significantly lower than those of the placebo group (SMD = -0.34, 95% CI: -0.60–- 0.09). In subgroup meta-analysis, levels of bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC) and tumor necrosis factor (TNF) did not differ between the probiotic and placebo groups.

Conclusions: Supplementation with probiotics can increase lumbar and hip BMD, and reduce bone resorption. Probiotics retard osteoporosis in postmenopausal women.

Strengths and limitations of this study

1.We conduct a systematic review and meta-analysis of high-quality randomized controlled trials. We find probiotics could retard osteoporosis in postmenopausal women.

2.To our knowledge, this is the first meta-analysis describing the evidence of the association of probiotic supplements and bone status in postmenopausal women.

3.There is little heterogeneity between included articles and fixed-effects model used

to calculate the results.

4.Only four randomized controlled trials satisfied our inclusion criteria. The limited number of reports prevented us from conducting subgroup analysis. Furthermore, insufficient number of estimates inflate the impact of the results of a particular study.

Keywords: probiotics supplement; bone mineral density; bone turnover markers; postmenopausal; meta-analysis

Introduction

Osteoporosis is characterized by low bone mineral density (BMD) and deteriorated bone microstructure, leading to reduced bone strength and increased susceptibility to fractures [1]. Osteoporosis and fracture are particularly common in postmenopausal women, who experience a natural decline in endogenous estrogen, reducing BMD (on average 2%–5% BMD/y) [2] and leading to adverse effects on bone microarchitecture.

Currently, many medications are used in osteoporosis to decrease bone resorption or increase bone formation. Large randomized controlled trials (RCTs) showed that estrogen therapy was effective for the prevention and treatment of osteoporosis in postmenopausal women[3-5]. However, this remains controversial because of the increased risk of cancer, including endometrial, breast and ovarian cancer [6]. Nevertheless, other anti-resorptive agents are not widely used because of their side-effects, high prices and poor compliance on the part of patients; these include bisphosphonates, calcitonin and raloxifene. Therefore, complementary and dietary therapies are more acceptable to some patients. It was shown that calcium and vitamin D supplement effectively improved bone microarchitecture and health [7]; however, supplementation with calcium and vitamin alone is not sufficient to halt menopausal bone loss [8].

Therefore, alternative ways to prevent and/or treat osteoporosis are sought. Probiotics are popular dietary therapies that have favorable effects on the skeletal system.[9] Probiotics are "live microorganisms that when administered in adequate amounts will confer a health benefit on the host" defined by the Food and Agricultural Organization/World Health Organization (FAO/WHO) [10]. They are affordable and have fewer side-effects.

To our knowledge, there has been no systematic review or meta-analysis of RCTs with probiotics in the treatment arms, analyzing the effect of probiotics in postmenopausal-related osteoporosis. Therefore, this systematic review and meta-analysis was performed to provide an overview of the effects of dietary probiotic supplements in postmenopausal related bone resorption in women and to inform researchers of new potential sources of bias to be addressed in future clinical

trials.

Methods and analysis

Data sources and search strategies

A literature search of relevant studies was performed in PubMed, EMBASE and the Cochrane Library. A comprehensive search strategy was developed. The key words were as follows: 'probiotics,' 'bone,' 'osteoporosis,' 'osteopenia,' 'bone mineral density,' 'bone turnover,' 'menopause,' 'postmenopausal,' and 'post-menopause.' References of retrieved articles were also scanned to identify and additional relevant studies. Two independent reviewers (Jiawei Yu and Gaoyang Cao) conducted this work. Discrepancies were resolved by consensus of the two reviewers. If required, final disposition was determined by Ming Cai.

Inclusion and exclusion criteria

Inclusion criteria are as follows: (1) randomized controlled trials; (2) consideration of dietary probiotic supplement as baseline exposure, and bone status (BMD and bone turnover markers) as outcomes; (3) postmenopausal women administered probiotics for more than 6 months; and (4) English language original articles indexed up to May 2020.

Exclusion criteria are as follows: (1) absence of key data for meta-analysis; and (2) low-quality articles according to Cochrane checklist.

Data extraction and quality assessment

The characteristics of the relevant articles were extracted and recorded independently by two reviewers (Jiawei Yu and Gaoyang Cao) as follows: first author's name, year, area, age (mean or range), type of probiotic supplement, dose design, course of treatment, number of cases, number of controls, and bone status (as shown in Table 1). The Cochrane Collaboration's tool [11] was used for assessing risk of bias, and results were displayed as low risk, unclear risk or high risk of bias.

Statistical analysis

Meta-regression was conducted to verify whether different types of probiotic supplement would introduce sources of heterogeneity. The mean relative change from baseline to the end of course and standard deviation (SD) were used to express the

effect of probiotic supplement on bone status in postmenopausal women. If the original studies did not provide the mean relative change and standard deviation, we converted the data using a common method [12-13]. The pooled effects of included studies were expressed in terms of standardized mean difference (SMD) with 95% confidence interval (CI). Q test and I^2 index were used to evaluate heterogeneity among the included results. If the Q test and I^2 index did not show heterogeneity ($P > 0.05$ and $I^2 \leq 50\%$), a fixed-effects model was used; otherwise, a random-effects model was used. Forest plots and funnel plots were produced and publication bias was tested using Begg's test and the weighted Egger test [14-15]. Sensitivity analysis was conducted to verify the impact of each individual study on the pooled results. All analysis was performed using STATA 12.0 (StataCorp LP, College Station, TX, USA).

Results

Search results and characteristics of identified studies

A total of 524 articles were identified from the initial search in PubMed and EMBASE, 468 articles were removed because of no relevance to the topic. Then, 8 articles were retained after reviewing the abstract according to the exclusion criteria. Finally, 4 randomized controlled trials [16-19] satisfied the inclusion criteria and entered this meta-analysis after full-text review. A detailed overview of the selection process is outlined in Figure 1.

A total of 218 postmenopausal women completed these trials. Among the four trials, half of the trials were conducted in Asia (one in Japan [16], the other in Iran [18]), and the other two trials were in Europe (one in Sweden [17], the other in Denmark [19]). All trials were randomized with the double-blinded method. Each trial identified the type of probiotic supplements used and described the dosage design. Three studies had treatment with probiotics only [16-18], while another study included treatment with combined isoflavone and probiotics [19]. All studies provided BMD data from DXA scans at the lumbar spine and total hip. Collagen type 1 cross-linked C-telopeptide (CTX), bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC) and tumor necrosis factor (TNF) were used as bone turnover markers. Details of the characteristics are displayed in Table 1.

Probiotics supplements and total hip BMD

Overall, four estimates of the association between probiotics supplement and hip BMD were included in the meta-analysis. The results of meta-regression revealed that various types of probiotics were not a source of heterogeneity ($P = 0.927$). Therefore, we brought the four estimates into the pooled analysis. We found that probiotic supplements gave higher hip BMD of the supplementary group than did the placebo group (SMD = 0.37, 95% CI: 0.12–0.62), with no heterogeneity ($P = 0.404$; $I^2 = 0.0$) (Figure 2). The funnel plot is shown in Supplementary Figure 1a; it was symmetrical, excluding publication bias (Begg's test $z_c = 1.02$, $P = 0.308$; Egger's test $t = -1.42$, $P = 0.291$). Sensitivity analyses indicated that the positive result was affected by the Lambert trial [19] (Supplementary Figure 2a).

Probiotic supplements and lumbar spine BMD

A total of four estimates were included in the meta-analysis. The results of meta-regression also showed no source of heterogeneity from various types of probiotics ($P = 0.813$). Therefore, the four estimates were incorporated into the pooled analysis. Compared to the placebo group, the lumbar spine BMD level of the supplementary group was higher (SMD = 0.28, 95% CI: 0.03–0.53), with no heterogeneity ($P = 0.661$; $I^2 = 0.0$) (Figure 3). The funnel plot was symmetrical (Supplementary Figure 1b) and excluded publication bias (Begg's test $z_c = 1.02$, $P = 0.308$; Egger's test $t = -2.07$, $P = 0.174$). Sensitivity analyses indicated that the positive result was affected by the Takimoto [16] and Lambert trials [19] (Supplementary Figure 2b).

Probiotic supplements and bone turnover markers

Four estimates of CTX, and two estimates of BALP, OPG, OC and TNF were incorporated into the pooled analysis. The results suggested that probiotic supplements help decrease body CTX level of the supplementary group when compared with the placebo group (SMD = -0.34, 95% CI: -0.60 – -0.09), with substantial heterogeneity. There was no evidence that probiotic supplements were associated with the levels of BALP, OPG, OC and TNF(Figure 4).

Discussion

This meta-analysis provides evidence that dietary probiotics supplement can slow bone resorption in postmenopausal women. Daily supplementation with probiotics for 24 weeks to 12 months significantly decreased levels of bone turnover marker CTX (compared to placebo) in postmenopausal women. BMD loss at total hip and lumbar spine was significantly lower in the treatment group.

Bone loss occurs throughout life following maturation, and is accelerated following menopause in women [20]. Postmenopausal women have an increased risk of fragility fractures. Using a naturally-occurring bacterium to significantly reduce the annual bone loss in this group of patients is a new concept that could lead to a paradigm shift in osteoporosis prevention. Previous studies in rodents have demonstrated that supplementation with specific bacterial strains decreased bone loss and improved bone mineral density [21-23]. Kim et al. reported that the administration of *Lactobacillus casei* 393 significantly increased BMD in ovariectomized rats [24]. For the first time, the present meta-analysis systemically demonstrated that this probiotic also works in humans.

The vertebrae and metaphyses of long bones, rich in trabecular bone, have a higher turnover rate than do cortical bones in the axis of long bones. Therefore, medications and diseases affecting lumbar spine and hip are identified earlier than in other skeletal segments [25]. The vertebrae and hips are easily accessible for measuring BMD. Therefore, the lumbar spine and hip BMD were suitable primary outcome variables in the present studies. McCabe et al. [26] showed that oral administration of *Lactobacillus* probiotics identified a 45% increase in hip and vertebral trabecular bone volume fraction in male mice. In another study, the administration of *Lactobacillus plantarum* and *Lactobacillus paracasei* to ovariectomized mice showed increased trabecular number compared to sham-ovariectomized control groups [27]. Our meta-analysis showed, in the probiotics group, both total hip and lumbar vertebrae BMD were at a significantly high levels than those of the control.

Because BMD depends on the dynamic balance of bone formation and resorption, bone turnover markers are also very important parameters analyzed in our meta-analysis. The measurement of CTX has been taken as a marker of bone

resorption; it is produced by osteoclasts during bone resorption [28]. Therefore, the increased levels of serum CTX indicated increased bone resorption. Subgroup included 3 RCT studies, suggesting that ingestion of probiotic supplements significantly reduced the bone resorption marker CTX. Another study from Japan [16] showed that the probiotics group had significantly lower uNTx (urinary type I collagen cross-linked N-telopeptide) levels than did the placebo group at 12 weeks of treatment. uNTx is another fragment of type I collagen generated during resorption detected in urine; therefore, this also suggested that probiotics inhibit bone resorption by suppressing osteoclast activity. BALP is another well-known bone turnover marker, an indicator of osteoblast proliferation that is thought to be a marker of bone formation [29]. However, the present meta-analysis showed no significant changes in BALP. Similarly, no differences were detected in levels of biochemical markers for bone metabolic indices (OPG, OC).

Probiotics have many functional properties in humans. They function in the gastrointestinal system by modifying the microbiota composition, intestinal barrier function, and the immune system which feeds back systemic benefits to the host, including bone health. Some can be used in intestinal infections and treatment of diarrhea, because they not only tolerate low PH environment but also colonize the human colon, adhering to the gastrointestinal tract, with antimicrobial effects [30]. Moreover, probiotic function modifying physiological homeostasis of the intestinal flora can also benefit bone metabolism [31]. Many studies have looked at changes of gastrointestinal flora during aging, which may alter mineral absorption. Gastrointestinal inflammation and systemic inflammation are closed related to enhanced generation of potent osteoclastogenic cytokines as the main cause of bone loss [32-33]. Probiotics can restore balance of the gut microbiota, preventing or moderating gut and systemic inflammation and allowing absorption of nutrients, especially in elderly people [34]. Probiotics may restore microbiota composition through several mechanisms. They act in the gastrointestinal tract simply by proliferation, as well as by ability inhibiting other flora. Furthermore, probiotics turn complex carbohydrates to oligosaccharides [35], which can be used by other bacteria, indirectly

improving the balance of microflora. Furthermore, probiotics decrease the levels of inflammatory mediators and cytokines in the gut and bone marrow [36]. These changes give signals to bone cells, including osteoblasts, osteoclasts and stem cells, significantly affecting bone homeostasis. Endocrine factors (such as serotonin and incretins) secreted by intestine also remarkably affect bone cells [37].

Anti-inflammatory effects are among the underlying mechanisms by which probiotics benefit bone metabolism. There is evidence that arginine deiminase, produced by the probiotic Lactobacillus brevis CD2, has an anti-inflammatory effect [38]. Supplementation of probiotics may reduce expression of pro-inflammatory and osteolytic cytokines, including TNF-α. These cytokines alter anti-osteoclastogenic cytokine expression, leading to enhanced osteoclast formation and inhibited osteoblast activity [39]. Some studies found that probiotic supplementation reduces TNFα, IL-17, and RANKL expression levels in ovariectomized mice [40]. These changes give signals to bone cells, such as osteoblasts, osteoclasts and stem cells, which significantly affect bone homeostasis. In this meta-analysis, TNF-αwas reported by two RCTs. One reported [17] that serum levels of TNF-were significantly lower in the probiotic-treated group; however, another study [18] showed there was no differences between probiotic and control groups. More clinical trials are needed in the future to elucidate the relationship between administration of probiotics and anti-inflammatory effects.

Our study has some limitations. First, only four randomized controlled trials satisfied our inclusion criteria. The limited number of reports focusing on the association between probiotic supplement and BMD and bone turnover markers prevented us from conducting subgroup analysis and drawing conclusive summaries. Furthermore, insufficient number of estimates inflate the impact of the results of a particular study. Second, although, meta-regression was used to determine that various types of probiotic supplement did not have an impact on the pooled results, dosage design and course of treatment could also introduce bias. Third, in Lambert's study [19]， probiotics plus soflavones were used as a treatment regimen， rather than probiotics alone. This may cause some bias; however, we did not want to ignore this valuable study. Third, the units describing BMD change were inconsistent among the

four reports. Nilsson's study [17] applied T score to describe BMD change, while other three studies used g/cm^2 instead. We could only calculate SMD rather than weighted mean difference (WMD). Thus, our results of meta-analysis should be interpreted with caution.

Our research also has some strengths. First, to our knowledge, this is the first meta-analysis describing the evidence of the association of probiotic supplements and bone status in postmenopausal women. Second, there is little heterogeneity between included articles and fixed-effects model used to calculate the results. Third, all included randomized controlled trials were of high quality for analysis.

Conclusion

Our systematic review and meta-analysis showed that probiotic supplementations in postmenopausal women were associated with preserving BMD and attenuating bone resorption. Appropriate supplement of probiotic could be recommended to improve bone status in postmenopausal women.

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Contributors

M Cai and J Yu conceived and designed the meta analysis; J Wu, G Cao, S Yuan and Cong Luo searched the literature; J Yu, G Cao, and S Yuan analysed the data; X Cai contributed analysis tools; J Yu and G Cao wrote the paper; X Cai and M Cai revised the manuscript.

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Competing interests

The authors declare no conflicts of interest. This study does not contain human participants or animals.

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No patient involved

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Table 1. Characteristics of included randomized controlled trials in the meta-analysis

Study	Year	Area	Age (year)	Blingding	Type of probiotic supplement	Number of T	Number of P	Course of treatment (months)	dose design	Minerals intake	BMD	BTM
TAKIMOTO	2018	Japan	T: 57.5 P: 57.8	double-bind	bacillus subtilis C-3102	31	30	6	3.4×10^9 CFU /d	Estimated by BDHQ	hip lumbar spine	CTX
Nilsson	2018	Sweden	T: 76.4 P: 76.3	double-bind	lactobacillus reuteri 6475	32	36	12	$5x10^9$ CFU twice/d	Estimated by astandardized questionnaire	hip lumbar spine	CTX BALP TNF
Jafarnejad	2017	Iran	T: 58.9 P: 57.3	double-bind	seven probiotic bacteria species#	20	21	6	one Gerilact capsule /d	500 mg Ca plus 200 IU vitamin D daily	hip lumbar spine	CTX BALP OPG OC TNF
Lambert	2017	Denmark	T: 60.8 P: 62.9	double-bind	lactic acid bacteria and soflavones	38	40	12	60mg isoflavone and probiotics/d	1200 mg Ca, 550 mg Mg, and 25mg calcitriol daily	hip lumbar spine	CTX OPG OC

BDHQ: a brief-type self-administered diet history questionnaire; BMD: bone mineral density; BTM: bone turnover marker; CFU: colony-forming unit; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone-specific alkaline phosphatase; OPG: osteoprotegerin; OC: osteocalcin; P: placebo group; T: treat group; TNF: tumor necrosis factor; RCE: red clover extract which is rich in isoflavone aglycones and probiotics; # Lactobacillus casei 1.3 x 10^{10} colony-forming units[CFU], Bifidobacterium longum 5 x 10^{10} CFU, Lactobacillus acidophilus 1.5 x 10^{10} CFU, Lactobacillus rhamnosus 3.5 x 10^9 CFU, Lactobacillus bulgaricus 2.5 x 10^8

CFU, Bifidobacterium breve 1 x 10^{10} CFU, and Streptococcus thermophilus 1.5 x 10^{8} CFU per 500 mg.

Figure 1. Flow diagram of the studies search process

Figure 2. Forest plots of meta-analysis on probiotics supplements and total hip BMD

Figure 3. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers

Supplementary Figure 1. Funnel plots of meta-analysis on probiotics supplements and BMD: A. total hip BMD; B. lumbar spine BMD.

Supplementary Figure 2. Sensitivity analyses of meta-analysis on probiotics supplements and BMD: A. total hip BMD; B. lumbar spine BMD.

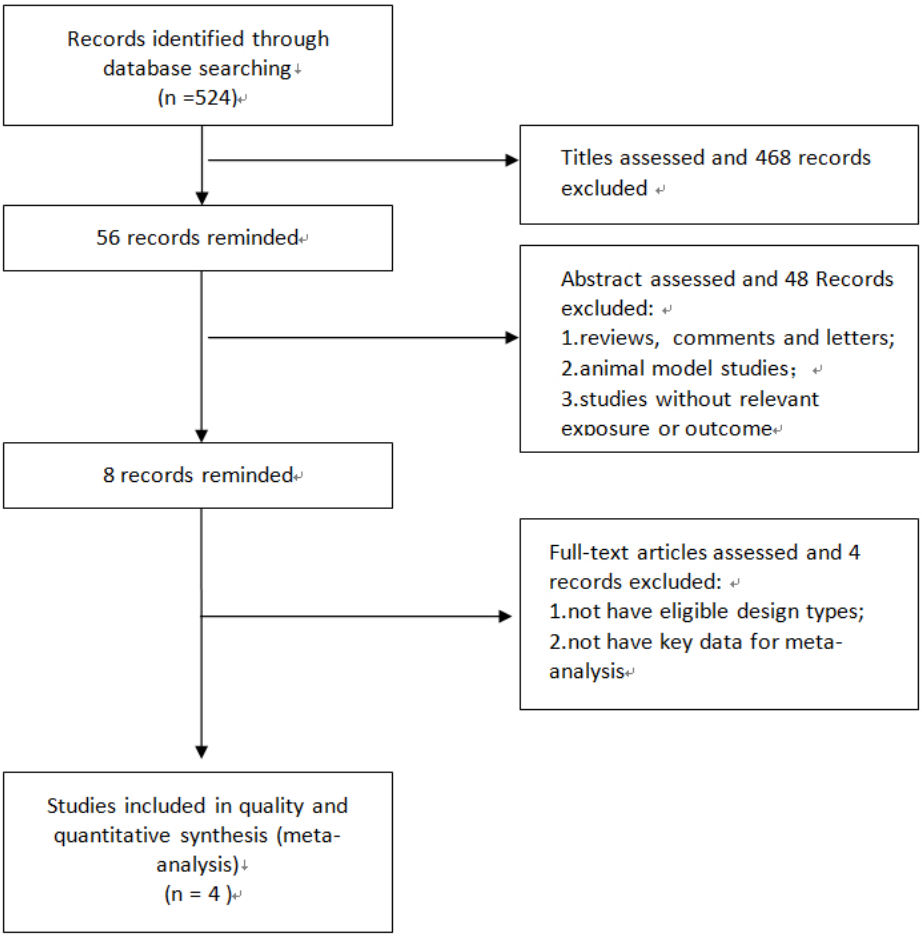

Figure 1. Flow diagram of the studies search process

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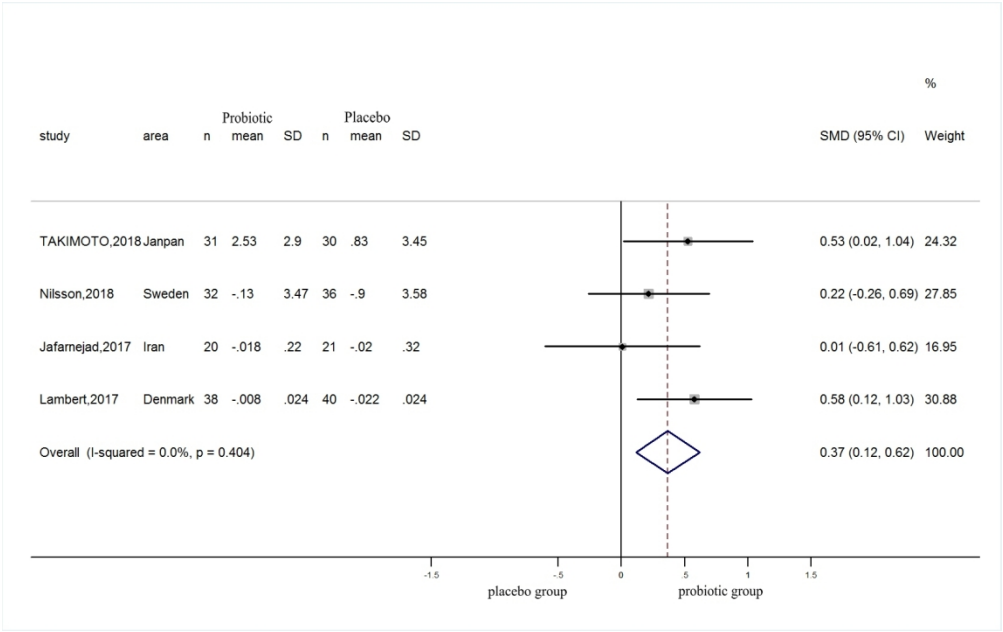


Figure 2. Forest plots of meta-analysis on probiotics supplements and total hip BMD

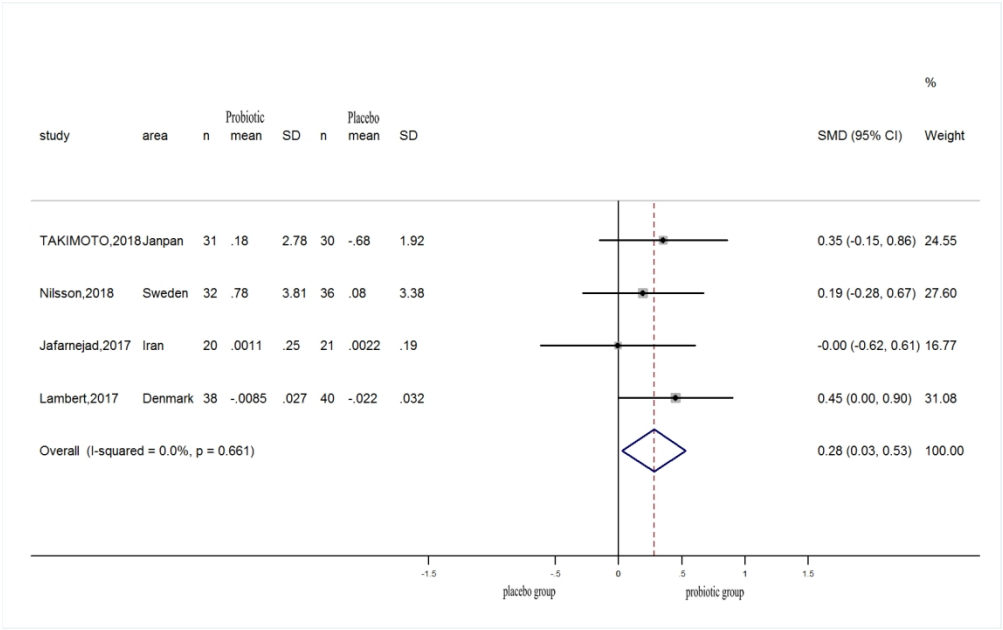


Figure 3. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

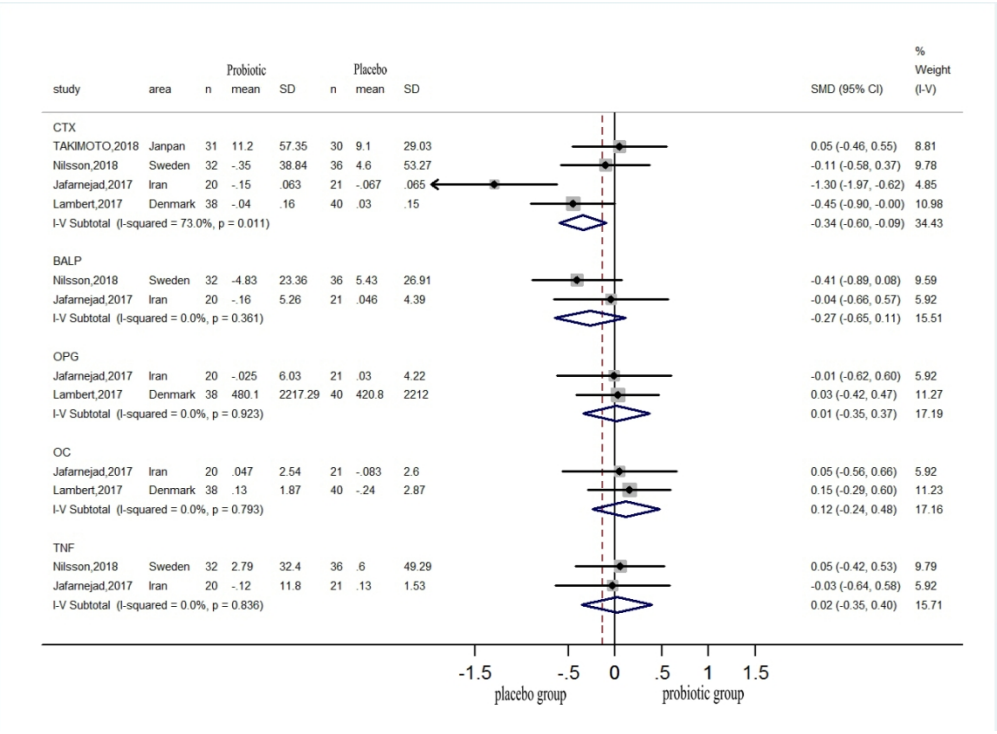
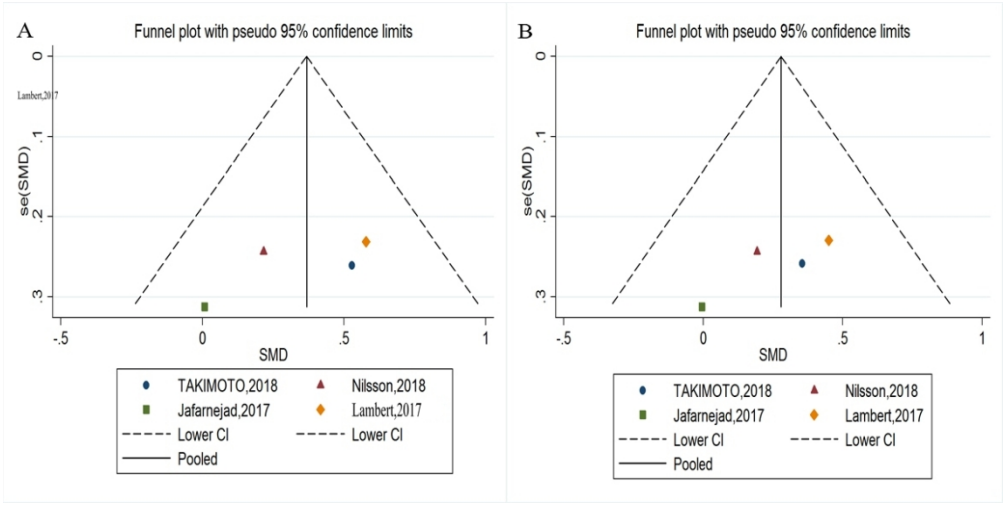


Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers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Lambert,2017
Lower CI
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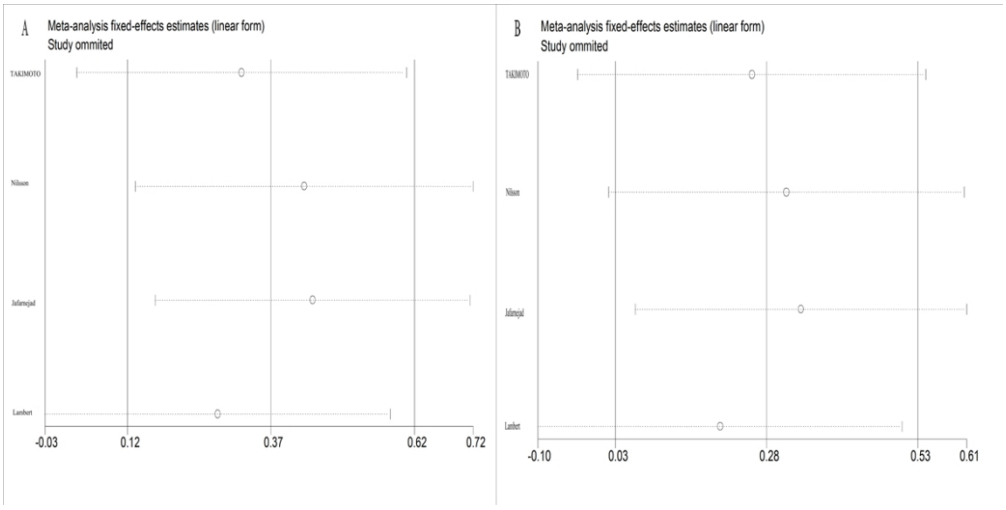
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Study ommited
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Jofarnejad
Lambert
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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	http://www.crd.york.ac.uk/ PROSPERO/
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4-5

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4-5

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4-5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	4-5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	5-6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	5-6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	5-6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	5-6
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	5-6
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	6-10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	6-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	6-10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	n/a

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials

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Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials

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Abstract

Objective: Osteoporosis is a common disease in postmenopausal women. Several studies have analyzed the associations between dietary supplementation with probiotics and bone health in postmenopausal women, but the results are still controversial. We conducted this meta-analysis to assess the effects of probiotics supplement on bone mineral density (BMD) and bone turnover markers for postmenopausal women.

Design: systematic review and meta-analysis.

Methods: We systematically searched PubMed, EMBASE, and the Cochrane Library from their inception to November 2020 for randomized controlled trials (RCTs) assessing probiotic supplements and osteoporosis in postmenopausal women. Study-specific risk estimates were combined using random-effect models.

Results: Five RCTs (n = 497) were included. Probiotic supplements were associated with a significantly higher BMD in the lumbar spine (standardized mean difference, SMD = 0.27, 95% CI: 0.09–0.44) than in control. There was no difference between probiotic supplements and BMD in hips (SMD = 0.22, 95% CI: -0.07 – 0.52). Collagen type 1 cross-linked C-telopeptide (CTX) levels in the treatment groups were significantly lower than those of the placebo group (SMD = -0.34, 95% CI: -0.60 – -0.09). In subgroup meta-analysis, levels of bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC), and tumor necrosis factor (TNF) did not differ between the probiotic and placebo groups.

Conclusions: Supplementation with probiotics increases lumbar BMD and reduces bone resorption. More randomized controlled trials are recommended to validate these results.

Strengths and limitations of this study

This is the first meta-analysis on the effectiveness of probiotic supplements on bone status in postmenopausal women.

We included only high-quality randomized controlled trials to improve the level of evidence.

These results provide new insights into the association between probiotic supplements lumbar spine bone mineral density

The limited number of reports prevented us from conducting subgroup analysis and made it difficult to draw firm conclusions.

Keywords: probiotics supplement; bone mineral density; bone turnover markers; postmenopausal; meta-analysis

Introduction

Osteoporosis is characterized by low bone mineral density (BMD) and deteriorated bone microstructure, leading to reduced bone strength and increased susceptibility to fractures[1]. Osteoporosis and fracture occur commonly in postmenopausal women, who experience a natural decline in endogenous estrogen, reducing BMD (on average 2%–5% BMD/y) [2] and adverse effects on bone microarchitecture.

Currently, many medications are used in osteoporosis to decrease bone resorption or increase bone formation. Large randomized controlled trials (RCTs) showed that estrogen therapy (such as red clover isoflavone supplementation) was effective for preventing and treating osteoporosis in postmenopausal women[3-5]. However, this remains controversial because of the increased risk of cancer, including endometrial, breast, and ovarian cancer [6]. Nevertheless, other anti-resorptive agents are not widely used because of their side-effects, high prices, and poor compliance on the part of patients; these include bisphosphonates, calcitonin, and raloxifene. Therefore, complementary and dietary therapies are more acceptable to some patients. Also, natural treatments are increasingly requested by patients.[7] It was shown that calcium and vitamin D supplements effectively improved bone microarchitecture and health [8]; however, supplementation with calcium and vitamin alone is not sufficient to halt menopausal bone loss [9].

Therefore, alternative ways to prevent and treat osteoporosis are sought. Probiotics are popular dietary therapies that have favorable effects on the skeletal system.[10] Probiotics are "live microorganisms that when administered in adequate amounts will confer a health benefit on the host" defined by the Food and Agricultural Organization/World Health Organization (FAO/WHO) [11], such as bacillus subtilis, lactobacillus, and other mixed strains. They are affordable and have fewer side-effects.

To our knowledge, there has been no systematic review or meta-analysis of RCTs with probiotics in the treatment arms, analyzing the effect of probiotics in postmenopausal-related osteoporosis. Therefore, this systematic review and meta-analysis were performed to provide an overview of the effects of dietary

probiotic supplements in postmenopausal related bone resorption in women and to inform researchers of new potential sources of bias to be addressed in future clinical trials.

Methods and analysis

Data sources and search strategies

A literature search of relevant studies was performed in PubMed, EMBASE, and the Cochrane Library. A comprehensive search strategy was developed. The protocol was drafted according to the PRISMA statement[12]. The keywords were as follows: 'probiotics', 'probiotic supplement', 'bone,' 'osteoporosis', 'osteopenia', 'bone mineral density', 'bone turnover', and 'postmenopausal' (search queries available in Supplementary Table 1). References of retrieved articles were also scanned to identify any additional relevant studies. Two independent reviewers (Jiawei Yu and Gaoyang Cao) conducted this work. Discrepancies were resolved by consensus of the two reviewers. If required, the final disposition was determined by Ming Cai.

Inclusion and exclusion criteria

Inclusion criteria are as follows: (1) randomized controlled trials and prospective cohort studies; (2) consideration of postmenopausal women as patients, consideration of probiotic supplement as interventions, consideration of placebo as a comparison, and consideration of the change of BMD and bone turnover markers (BTM) as outcomes; (3) BMD was measured by dual-energy X-ray absorptiometry (DXA) and BTM was measured using blood tests at baseline, and the end of trial; (4) administered probiotics for more than 6 months; and (5) English language original articles indexed up to November 2020.

Exclusion criteria are as follows: (1) absence of critical data for meta-analysis; and (2) low-quality articles according to Cochrane checklist.

Data extraction and quality assessment

The characteristics of the relevant articles were extracted and recorded independently by two reviewers (Jiawei Yu and Gaoyang Cao) as follows: first author's name, year, area, age (mean or range), type of probiotic supplement, dose design, course of treatment, number of cases, number of controls, and bone status (as

shown in Table 1). The Cochrane Collaboration's tool [13] was used for assessing the risk of bias. Six domain-based evaluations (selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias) were used in the tool to assess the possible bias of randomized controlled trials. The results were displayed as low risk, unclear risk, or high risk of bias (available in Supplementary Table 2).

Statistical analysis

The mean relative change from baseline to the end of the course and standard deviation (SD) were used to express the effect of the probiotic supplement on bone status in postmenopausal women. If the original studies did not provide the mean relative change and standard deviation, we converted the data using a common method [14-15]. The pooled effects of included studies were expressed in terms of standardized mean difference (SMD) with 95% confidence interval (CI). Q test and I^2 index were used to evaluate heterogeneity among the included results. Meta-regression was conducted to determine whether different types of probiotic supplements would introduce sources of heterogeneity. Random-effects model and subgroup analysis were used in the face of heterogeneity. Forest plots and funnel plots were produced, and publication bias was tested using Begg's test and the weighted Egger test [16-17]. Sensitivity analysis was conducted to verify the impact of each study on the pooled results. In the sensitivity analyses, each study was omitted to recalculate the pooled estimates. All analysis was performed using STATA 12.0 (StataCorp LP, College Station, TX, USA).

Patient and public involvement

Patient and public involvement is not applicable for this meta-analysis.

Results

Search results and characteristics of identified studies

A total of 604 articles were identified from the initial searches of PubMed and EMBASE, and 547 articles were removed because of absence of relevance. Nine articles were retained after reviewing the abstract according to the exclusion criteria. Finally, five randomized controlled trials[18-22] satisfied the inclusion criteria and entered this meta-analysis after full-text review. A detailed overview of the selection

process is outlined in Figure 1.

A total of 497 postmenopausal women completed these trials. Among the five trials, two were conducted in Asia (one in Japan [18], the other in Iran [20]), and the other three were in Europe (two in Sweden [19 22], the last one in Denmark [21]). All trials were randomized using the double-blinded method. Each trial identified the type of probiotic supplements used and described the dosage design. Three studies considered treatment with probiotics only [18-20], while the other two studies included treatment with combined isoflavone and probiotics [21 22]. All studies provided BMD data from DXA scans at the lumbar spine and total hip. Collagen type 1 cross-linked C-telopeptide (CTX), bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC), and tumor necrosis factor (TNF) were used as bone turnover markers. Details of the characteristics are displayed in Table 1 and Supplementary Table 3.

Probiotic supplements and lumbar spine BMD

A total of five estimates were included in the meta-analysis. The meta-regression results also showed no source of heterogeneity from various types of probiotics (P = 0.987). Therefore, the five estimates were incorporated into the pooled analysis. Compared to the placebo group, the lumbar spine BMD level of the supplementary group was higher (SMD = 0.27, 95% CI: 0.09 – 0.44), with no heterogeneity (P = 0.805; I^2 = 0.0) (Figure 2). The funnel plot was symmetrical (Supplementary Figure 1) and excluded publication bias (Begg's test z_c = 0.73, P = 0.462; Egger's test t = -0.22, P = 0.843). Sensitivity analyses indicated that the positive result was robust. (Supplementary Figure 2).

Probiotics supplements and total hip BMD

Overall, five estimates of the association between probiotics supplement and hip BMD were included in the meta-analysis. The meta-regression results revealed that various types of probiotics were not a source of heterogeneity (P = 0.237). Therefore, we brought the five estimates into the pooled analysis. There was no difference between probiotic supplements and BMD in hips (SMD = 0.22, 95% CI: -0.07 – 0.52), with no heterogeneity (P = 0.055; I^2 = 56.8) (Figure 3). The funnel plot is

shown in Supplementary Figure 3; it was symmetrical, excluding publication bias (Begg's test z_c = -0.24, P = 1.00; Egger's test t = 1.59, P = 0.209). Sensitivity analyses indicated that the positive result was affected by the Jansson trial (Supplementary Figure 4).

Probiotic supplements and bone turnover markers

Four estimates of CTX and two estimates of BALP, OPG, OC, and TNF were incorporated into the pooled analysis. The results suggested that probiotic supplements help decrease the supplementary group's body CTX level compared with the placebo group (SMD = -0.34, 95% CI: -0.60 – -0.09) with substantial heterogeneity. There was no evidence that probiotic supplements were associated with BALP, OPG, OC, and TNF (Figure 4).

Discussion

Main findings

This meta-analysis provides evidence that dietary probiotics supplement can slow bone resorption in postmenopausal women. Daily supplementation with probiotics for 24 weeks to 12 months significantly decreased bone turnover marker CTX (compared to placebo) in postmenopausal women. BMD loss at the lumbar spine was significantly lower in the treatment group.

Bone loss occurs throughout life following maturation and is accelerated following menopause in women [23]. Postmenopausal women have an increased risk of fragility fractures. Using a naturally-occurring bacterium to significantly reduce the annual bone loss in this group of patients is a new concept that could lead to a paradigm shift in osteoporosis prevention. Previous studies in animals demonstrated that supplementation with specific bacterial strains increases bone density and protect against osteoporosis [24-26]. Kim et al. reported that the administration of *Lactobacillus casei* 393 significantly increased BMD in ovariectomized rats [27]. For the first time, the present meta-analysis systemically demonstrated that this probiotic also works in humans.

The lumbar spine and hip are the most suitable organs to assess bone metabolism. The vertebrae and metaphyses of long bones, rich in trabecular bone, have a higher

turnover rate than cortical bones in the axis of long bones. Therefore, medications and diseases affecting the lumbar spine and hip are identified earlier than in other skeletal segments [28]. The vertebrae and hips are easily accessible for measuring BMD. Therefore, the lumbar spine and hip BMD were suitable primary outcome variables in the present studies. McCabe et al [29] showed that oral administration of *Lactobacillus* probiotics identified a 45% increase in hip and vertebral trabecular bone volume fraction in male mice. In another study, the administration of *Lactobacillus plantarum* and *Lactobacillus paracasei* to ovariectomized mice showed increased trabecular number compared to sham-ovariectomized control groups [30]. Our meta-analysis showed, in the probiotics group, both total hip and lumbar vertebrae BMD were at significantly higher levels than those of the control.

CTX and BALP were chosen as critical bone turnover markers. Because BMD depends on the dynamic balance of bone formation and resorption, bone turnover markers are also important parameters analyzed in our meta-analysis. The measurement of CTX has been taken as a marker of bone resorption; osteoclasts produce it during bone resorption [31]. Therefore, the increased levels of serum CTX indicated increased bone resorption. Subgroup included 3 RCT studies, suggesting that probiotic supplements' ingestion significantly reduced the bone resorption marker CTX. Another study from Japan [18] showed that the probiotics group had significantly lower uNTx (urinary type I collagen cross-linked N-telopeptide) levels than the placebo group at 12 weeks of treatment. uNTx is another fragment of type I collagen generated during resorption detected in urine; therefore, this also suggested that probiotics inhibit bone resorption by suppressing osteoclast activity. BALP is another well-known bone turnover marker, an indicator of osteoblast proliferation that is thought to be a bone formation marker [32]. However, the present meta-analysis showed no significant changes in BALP. Similarly, no differences were detected in levels of biochemical markers for bone metabolic indices (OPG, OC).

The mechanism of action

The mechanisms of action of probiotics are as follows. Probiotics have many functional properties in humans. They function in the gastrointestinal system by

modifying the microbiota composition, intestinal barrier function, and the immune system, which feeds back systemic benefits to the host, including bone health. Moreover, probiotic function modifying physiological homeostasis of the intestinal flora can also benefit bone metabolism [33]. Gastrointestinal inflammation and systemic inflammation are close to enhanced generation of potent osteoclastogenic cytokines as the leading cause of bone loss [34-35]. Probiotics can restore the balance of the gut microbiota, preventing or moderating gut and systemic inflammation and allowing absorption of nutrients, especially in older adults [36].

Furthermore, probiotics decrease levels of inflammatory mediators and cytokines in the gut and bone marrow [37]. These changes give bone cell signals, including osteoblasts, osteoclasts, and stem cells, significantly affecting bone homeostasis. Endocrine factors (such as serotonin and incretins) secreted by the intestine also remarkably affect bone cells [38]. Anti-inflammatory effects are among the underlying mechanisms by which probiotics benefit bone metabolism. There is evidence that arginine deiminase, produced by the probiotic *Lactobacillus brevis* CD2, has an anti-inflammatory effect [39]. Supplementation of probiotics may reduce the expression of pro-inflammatory and osteolytic cytokines, including TNF-α. These cytokines alter anti-osteoclastogenic cytokine expression, leading to enhanced osteoclast formation and inhibited osteoblast activity [40]. Some studies found that probiotic supplementation reduces TNFα, IL-17, and RANKL expression levels in ovariectomized mice [41]. These changes give bone cell signals, such as osteoblasts, osteoclasts, and stem cells, significantly affecting bone homeostasis. More clinical trials are needed in the future to elucidate the relationship between the administration of probiotics and anti-inflammatory effects.

Limitations and Strengths

Our study has some limitations. First, only five randomized controlled trials with specific population groups satisfied our inclusion criteria. The limited number of reports and specific population groups focusing on the association between the probiotic supplement and BMD and bone turnover markers prevented us from conducting subgroup analysis and drawing conclusive summaries. Furthermore, the

insufficient number of estimates inflates the impact of the results of a particular study. Second, although meta-regression was used to determine that various types of probiotic supplements did not impact the pooled results, dosage design and course of treatment could also introduce bias. Third, in Lambert's study [21], probiotics plus isoflavones were used as a treatment regimen， rather than probiotics alone. This may cause some bias; however, we did not want to ignore this valuable study. Third, the units describing BMD change were inconsistent among the five reports. Nilsson's study [19] and Jansson's study [22] applied T score to describe BMD change, while the other three studies used g/cm^2 instead. We could only calculate SMD rather than the weighted mean difference (WMD). Fifth, unfortunately, we did not find a relevant prospective cohort for this meta-analysis. Thus, our results of a meta-analysis should be interpreted with caution.

Our research also has some strengths. First, to our knowledge, this is the first meta-analysis describing the evidence of the association of probiotic supplements and bone status in postmenopausal women. Second, there is little heterogeneity between the included articles and the fixed-effects model used to calculate the results. Third, all included randomized controlled trials were of high quality.

Implications and future research

This systematic review and meta-analysis are useful for multidisciplinary clinicians to evaluate their practices and make a proper clinical decision. The beneficial effects of probiotic supplements may infect probiotic indication in postmenopausal women with osteoporosis. More RCT studies from different regions are needed to validate our argument and help answer research questions about probiotic supplements, dose, and the optimal duration.

Conclusion

Our systematic review and meta-analysis showed that probiotic supplementations in postmenopausal women were associated with preserving **lumbar spine** BMD and attenuating bone resorption. An appropriate supplement of probiotics could be recommended to improve bone status in postmenopausal women.

Acknowledgments

The authors thank the researchers of the included articles for information relevant to the analysis.

Contributors

MC and JY(Jiawei Yu) conceived and designed the meta-analysis; GC, SY and CL searched the literature; JY(Jiawei Yu), GC, and SY analyzed the data; JY(Jiawei Yu) contributed analysis tools; JY(Jiawei Yu) and GC wrote the paper; JY(Jiafeng Yu) and MC revised the manuscript.

Funding

None

Competing interests

The authors declare no conflicts of interest. This study does not contain human participants or animals.

Patient consent for publication

Not required

Provenance and peer review

Not commissioned; externally peer-reviewed

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information. No additional data are available.

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Table 1. Characteristics of included randomized controlled trials in the meta-analysis

Study	Year	Area	Age	Blinding	Type of probiotic supplement	Number of Treatment	Number of Placebo	Course of treatment	BMD	BTM
Jansson	2019	Sweden	T: 59.1 P: 58.1	double blind	three Lactobacillus strains*	126	123	12 months	lumbar spine hip	N/A
Takimoto	2018	Japan	T: 57.5 P: 57.8	double blind	bacillus subtilis C-3102	31	30	6 months	lumbar spine hip	CTX
Nilsson	2018	Sweden	T: 76.4 P: 76.3	double blind	lactobacillus reuteri 6475	32	36	12 months	lumbar spine hip	CTX BALP TNF
Jafarnejad	2017	Iran	T: 58.9 P: 57.3	double blind	seven probiotic b acteria species#	20	21	6 months	lumbar spine hip	CTX BALP OPG OC TNF
Lambert	2017	Denmark	T: 60.8 P: 62.9	double blind	lactic acid bacteria and soflavones	38	40	12 months	lumbar spine hip	CTX OPG OC

BMD: bone mineral density; BTM: bone turnover marker; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone-specific alkaline phosphatase; OPG: osteoprotegerin; OC: osteocalcin; TNF: tumor necrosis factor; N/A: not available; * Lactobacillus paracasei DSM 13434, Lactobacillus plantarum DSM 15312, and Lactobacillus plantarum DSM 15313; # Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, and Streptococcus thermophilus.

Figure 1. Flow diagram of the studies search process

Figure 2. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

Figure 3. Forest plots of meta-analysis on probiotics supplements and hip BMD

Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers

Supplementary Figure 1. Funnel plots of meta-analysis on probiotics supplements and lumbar spine BMD

Supplementary Figure 2. Sensitivity analyses of meta-analysis on probiotics supplements and lumbar spine BMD

Supplementary Figure 3. Funnel plots of meta-analysis on probiotics supplements and hip BMD

Supplementary Figure 4. Sensitivity analyses of meta-analysis on probiotics supplements and hip BMD

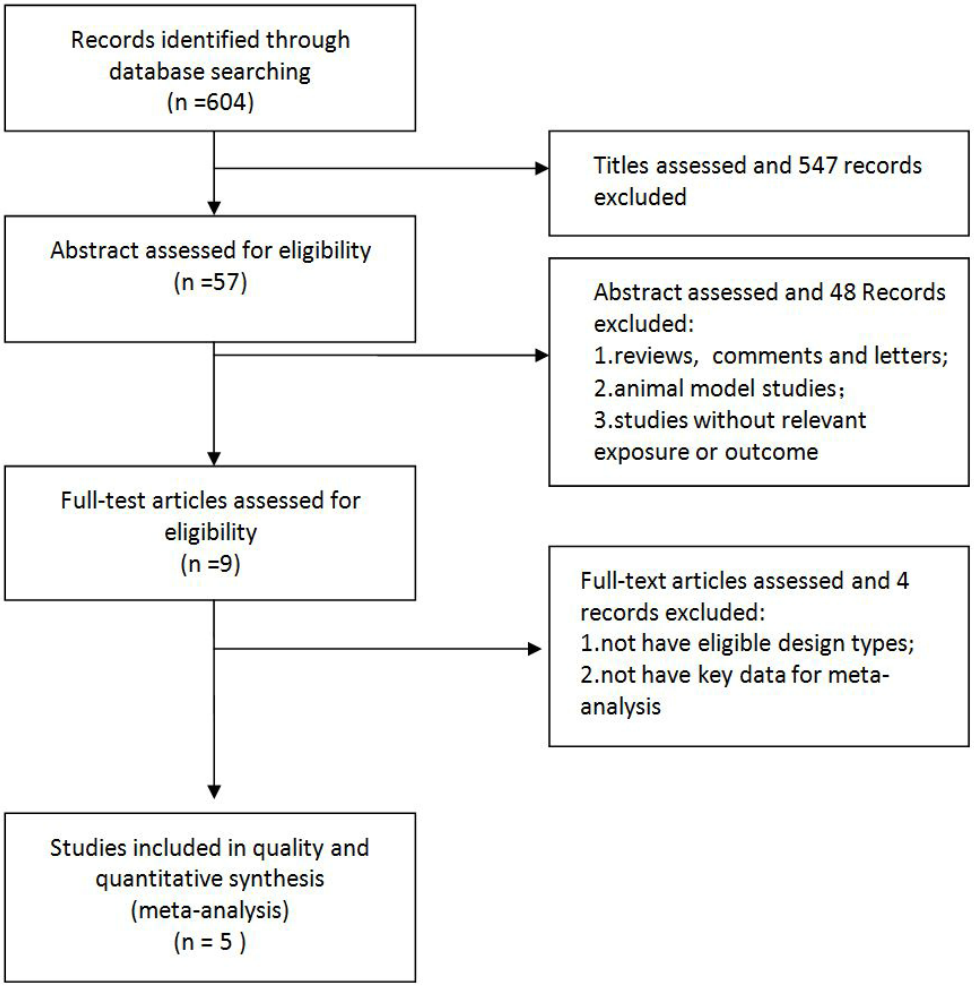


Figure 1. Flow diagram of the studies search process

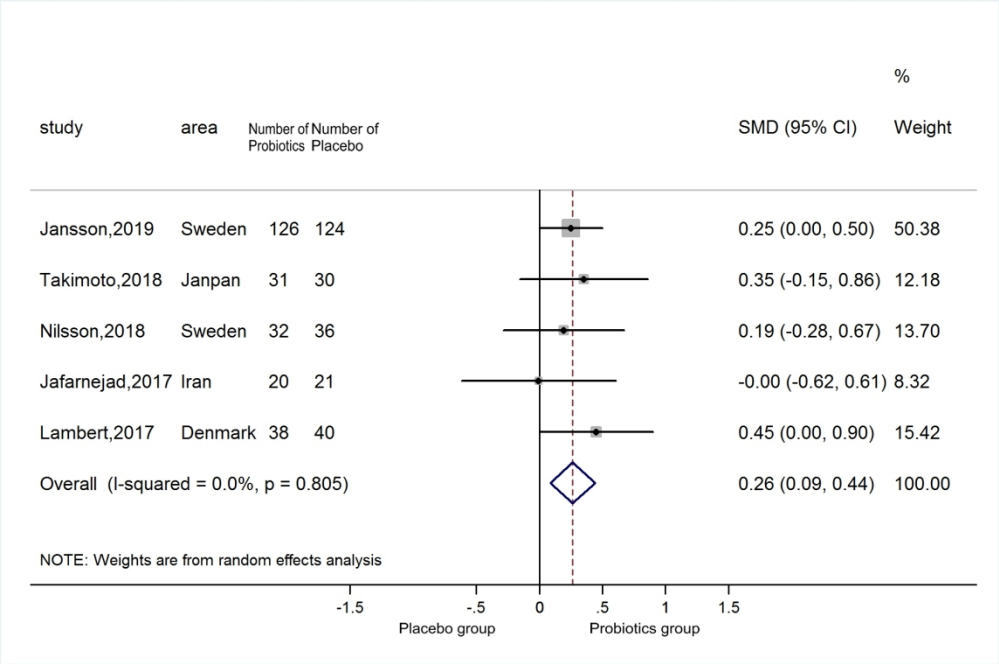


Figure 2. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

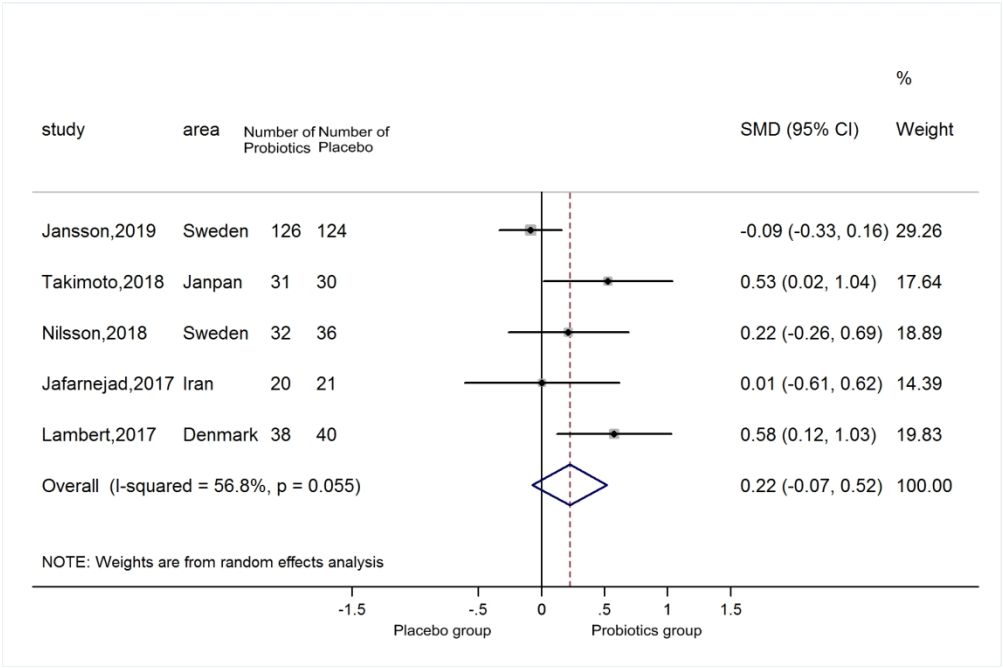


Figure 3. Forest plots of meta-analysis on probiotics supplements and hip BMD

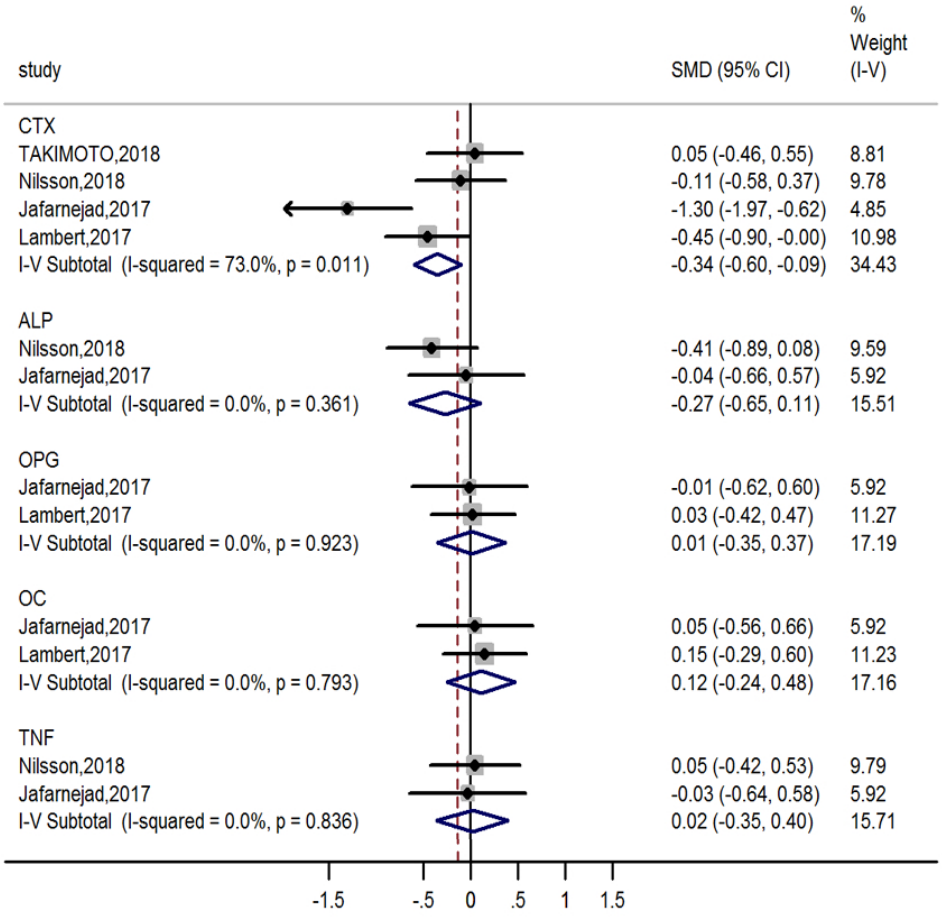


Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers

Supplementary Table 1 Search strategy of Medline

#	Searches
1	Probiotics
2	Probiotic supplement
3	Bone
4	Osteoporosis
5.	Osteopenia
6.	Bone mineral density
7.	Bone turnover
8.	Postmenopausal
9.	1 and 3 and 8
10.	1 and 4 and 8
11.	1 and 5 and 8
12.	1 and 6 and 8
13.	1 and 7 and 8
14.	2 and 3 and 8
15.	2 and 4 and 8
16.	2 and 5 and 8
17.	2 and 6 and 8
18.	2 and 7 and 8

The same strategy for other databases

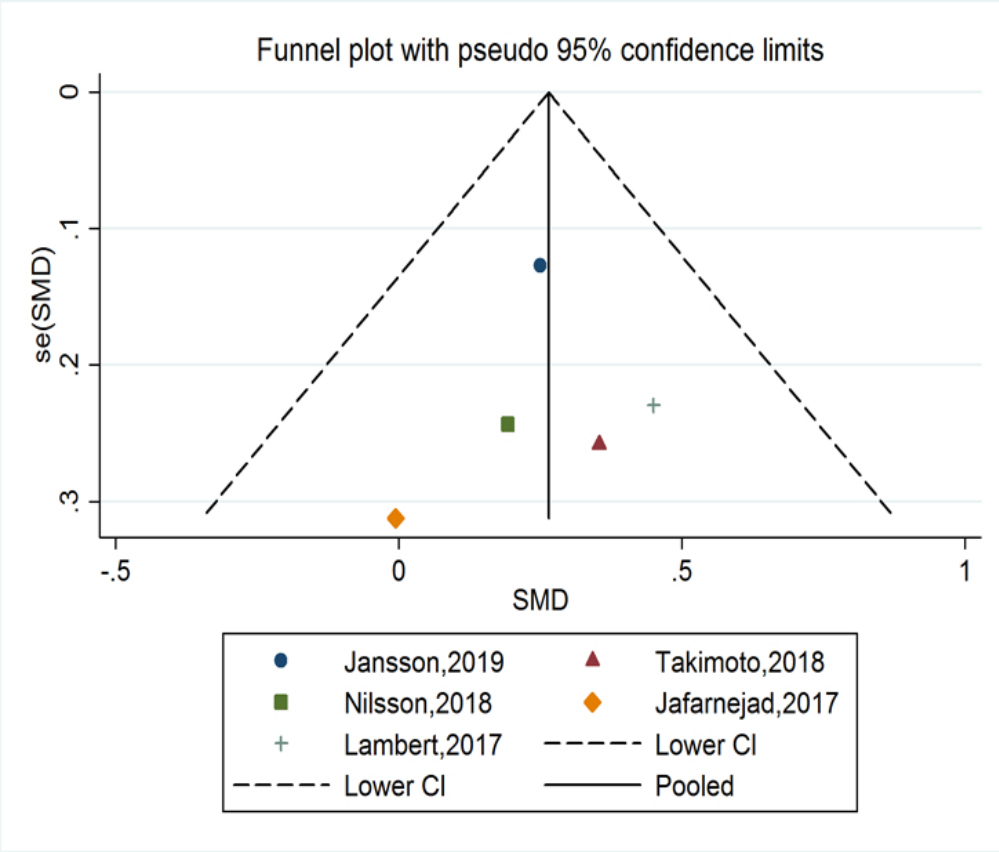
Supplementary Table 2. Assessment of risk bias of the studies included in the meta-analysis

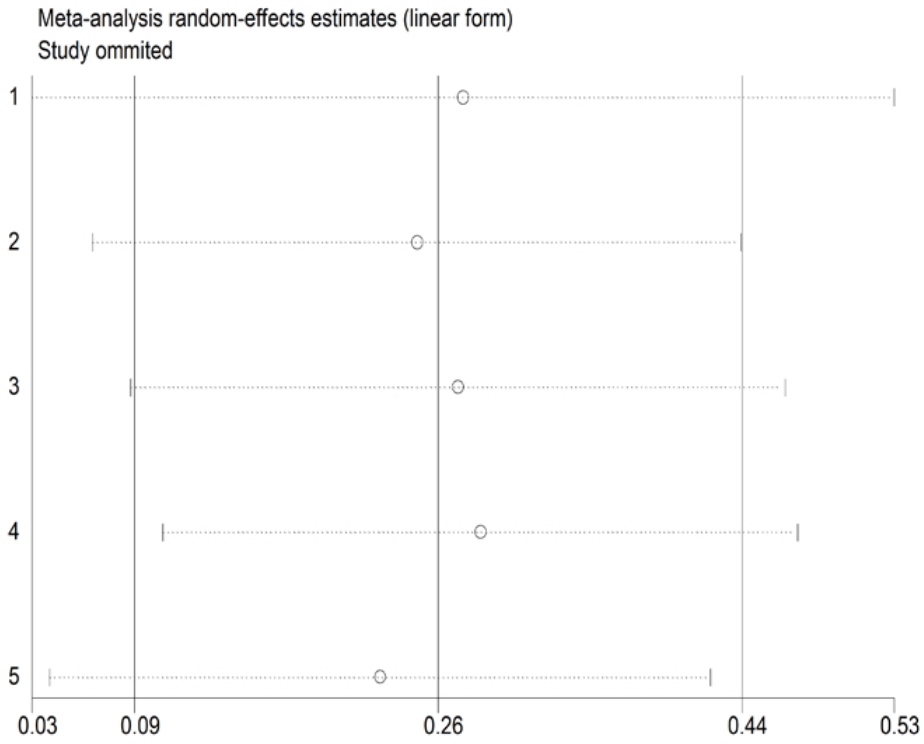
Study	Selection bias	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall
Jansson	Low	Low	Low	Low	Low	Low	Low
Takimoto	Low	Low	Low	Low	Low	Low	Low
Nilsson	Low	Unclear	Low	Low	Low	Low	Low
Jafarnejad	Low	Low	Low	Low	Low	Low	Low
Lambert	Low	Unclear	Low	Low	Low	Low	Low

Supplementary Table 3. Other characteristics of included randomized controlled trials in the meta-analysis

Study	Year	Area	dose design	Minerals intake	Measurement of outcome
Jansson	2019	Sweden	1 x10^{10} CFU/d	N/A	After 12 months
Takimoto	2018	Japan	3.4×10^{9} CFU /d	BDHQ	After 6 months
Nilsson	2018	Sweden	5x10^{9} CFU twice/d	A standardized questionnaire	After 12 months
Jafarnejad	2017	Iran	one Gerilact capsule /d*	500 mg Ca plus 200 IU vitamin D daily	After 6 months
Lambert	2017	Denmark	60mg isoflavone and probiotics/d	1200 mg Ca, 550 mg Mg, and 25mg calcitriol daily	After 12 months

BDHQ: a brief-type self-administered diet history questionnaire; CFU: colony-forming unit; *Lactobacillus casei 1.3 x 10^{10} CFU, Bifidobacterium longum 5 x 10^{10} CFU, Lactobacillus acidophilus 1.5 x 10^{10} CFU, Lactobacillus rhamnosus 3.5 x 10^{9} CFU, Lactobacillus bulgaricus 2.5 x 10^{8} CFU, Bifidobacterium breve 1 x 10^{10} CFU, and Streptococcus thermophilus 1.5 x 10^{8} CFU per 500 mg.


Funnel plot with pseudo 95% confidence limits
se(SMD)
0
.1
.2
.3
-.5
0
.5
1
SMD
Jansson,2019
Takimoto,2018
Nilsson,2018
Jafarnejad,2017
Lambert,2017
Lower CI
Lower CI
Pooled


Meta-analysis random-effects estimates (linear form)
Study ommited
1
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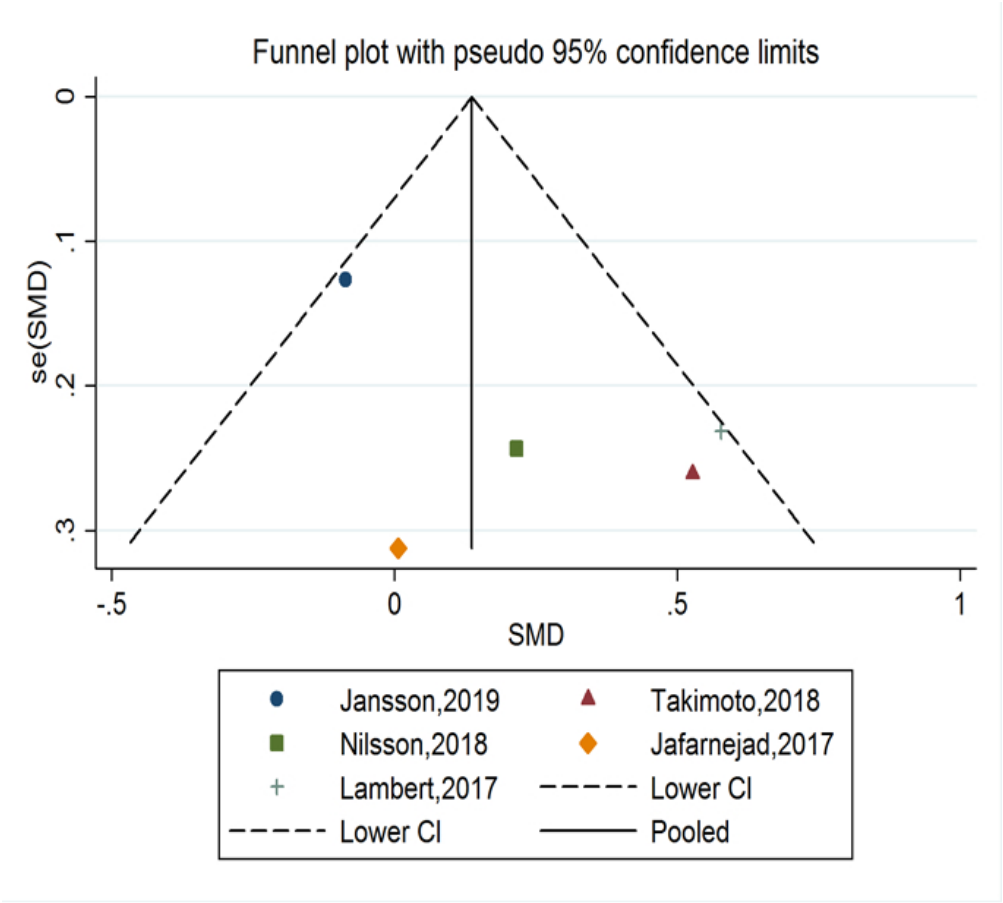
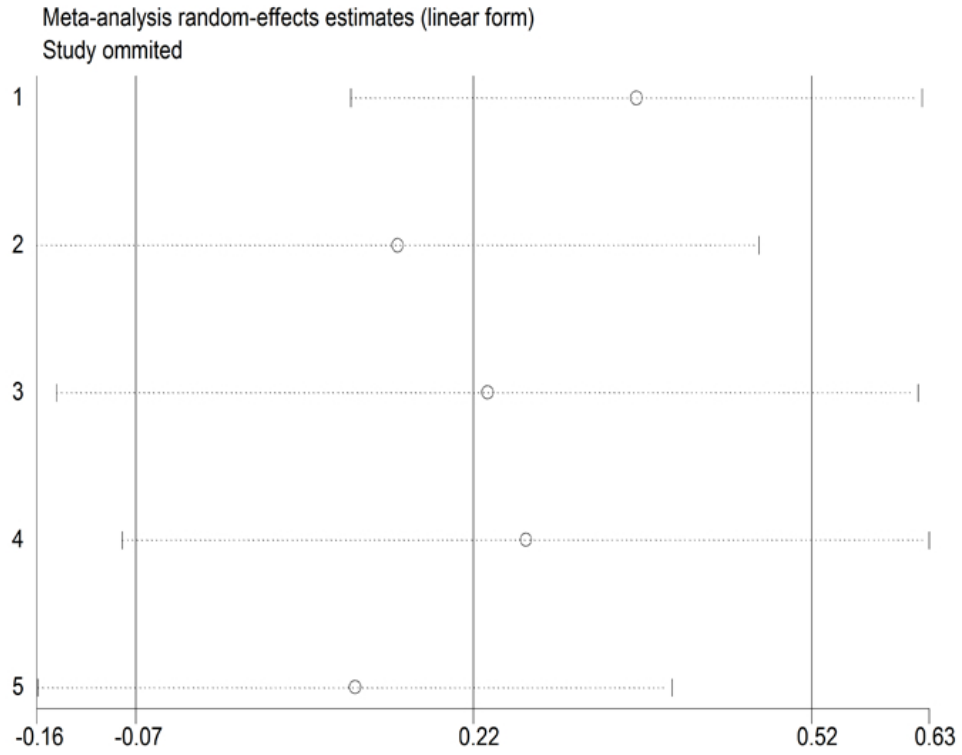
3
4
5
0.03
0.09
0.26
0.44
0.53


Funnel plot with pseudo 95% confidence limits
se(SMD)
0
.1
.2
.3
-.5
0
.5
1
SMD
Jansson,2019
Takimoto,2018
Nilsson,2018
Jafarnejad,2017
Lambert,2017
Lower CI
Lower CI
Pooled


Meta-analysis random-effects estimates (linear form)
Study ommited
1
2
3
4
5
-0.16
-0.07
0.22
0.52
0.63

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	http://www.crd.york.ac.uk/ PROSPERO/
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4-5

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4-5

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4-5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	4-5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	5-6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	5-6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	5-6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	5-6
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	5-6
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	6-10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	6-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	6-10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	n/a

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Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials

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Abstract

Objective: Osteoporosis is a common disease in postmenopausal women. Several studies have analyzed the associations between dietary supplementation with probiotics and bone health in postmenopausal women, but the results are still controversial. We conducted this meta-analysis to assess the effects of probiotics supplement on bone mineral density (BMD) and bone turnover markers for postmenopausal women.

Design: systematic review and meta-analysis.

Methods: We systematically searched PubMed, EMBASE, and the Cochrane Library from their inception to November 2020 for randomized controlled trials (RCTs) assessing probiotic supplements and osteoporosis in postmenopausal women. Study-specific risk estimates were combined using random-effect models.

Results: Five RCTs (n = 497) were included. Probiotic supplements were associated with a significantly higher BMD in the lumbar spine (standardized mean difference, SMD = 0.27, 95% CI: 0.09–0.44) than in control. There was no difference between probiotic supplements and BMD in hips (SMD = 0.22, 95% CI: -0.07 – 0.52). Collagen type 1 cross-linked C-telopeptide (CTX) levels in the treatment groups were significantly lower than those of the placebo group (SMD = -0.34, 95% CI: -0.60 – -0.09). In subgroup meta-analysis, levels of bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC), and tumor necrosis factor (TNF) did not differ between the probiotic and placebo groups.

Conclusions: We conclude cautiously that supplementation with probiotics could increase lumbar BMD. More randomized controlled trials are recommended to validate or update these results.

Strengths and limitations of this study

This is the first meta-analysis on the effectiveness of probiotic supplements on bone status in postmenopausal women.

We included only high-quality randomized controlled trials to improve the level of evidence.

The limited number of reports prevented us from conducting subgroup analysis and made it difficult to draw firm conclusions.

Keywords: probiotics supplement; bone mineral density; bone turnover markers; postmenopausal; meta-analysis

Introduction

Osteoporosis is characterized by low bone mineral density (BMD) and deteriorated bone microstructure, leading to reduced bone strength and increased susceptibility to fractures[1]. Osteoporosis and fracture occur commonly in postmenopausal women, who experience a natural decline in endogenous estrogen, reducing BMD (on average 2%–5% BMD/y) [2] and adverse effects on bone microarchitecture.

Currently, many medications are used in osteoporosis to decrease bone resorption or increase bone formation. Large randomized controlled trials (RCTs) showed that estrogen therapy (such as red clover isoflavone supplementation) was effective for preventing and treating osteoporosis in postmenopausal women[3-5]. However, this remains controversial because of the increased risk of cancer, including endometrial, breast, and ovarian cancer [6]. Nevertheless, other anti-resorptive agents are not widely used because of their side-effects, high prices, and poor compliance on the part of patients; these include bisphosphonates, calcitonin, and raloxifene. Therefore, complementary and dietary therapies are more acceptable to some patients. Also, natural treatments are increasingly requested by patients.[7] It was shown that calcium and vitamin D supplements effectively improved bone microarchitecture and health [8]; however, supplementation with calcium and vitamin alone is not sufficient to halt menopausal bone loss [9].

Therefore, alternative ways to prevent and treat osteoporosis are sought. Probiotics are popular dietary therapies that have favorable effects on the skeletal system.[10] Probiotics are "live microorganisms that when administered in adequate amounts will confer a health benefit on the host" defined by the Food and Agricultural Organization/World Health Organization (FAO/WHO) [11], such as bacillus subtilis, lactobacillus, and other mixed strains. They are affordable and have fewer side-effects.

To our knowledge, there has been no systematic review or meta-analysis of RCTs with probiotics in the treatment arms, analyzing the effect of probiotics in postmenopausal-related osteoporosis. Therefore, this systematic review and meta-analysis were performed to provide an overview of the effects of dietary

probiotic supplements in postmenopausal related bone resorption in women and to inform researchers of new potential sources of bias to be addressed in future clinical trials.

Methods and analysis

Data sources and search strategies

A literature search of relevant studies was performed in PubMed, EMBASE, and the Cochrane Library. A comprehensive search strategy was developed. The protocol was drafted according to the PRISMA statement[12]. The keywords were as follows: 'probiotics', 'probiotic supplement', 'bone,' 'osteoporosis', 'osteopenia', 'bone mineral density', 'bone turnover', and 'postmenopausal' (search queries available in Supplementary Table 1). References of retrieved articles were also scanned to identify any additional relevant studies. Two independent reviewers (Jiawei Yu and Gaoyang Cao) conducted this work. Discrepancies were resolved by consensus of the two reviewers. If required, the final disposition was determined by Ming Cai.

Inclusion and exclusion criteria

Inclusion criteria are as follows: (1) randomized controlled trials and prospective cohort studies; (2) consideration of postmenopausal women as patients, consideration of probiotic supplement as interventions, consideration of placebo as a comparison, and consideration of the change of BMD and bone turnover markers (BTM) as outcomes; (3) BMD was measured by dual-energy X-ray absorptiometry (DXA) and BTM was measured using blood tests at baseline, and the end of trial; (4) administered probiotics for more than 6 months; and (5) English language original articles indexed up to November 2020.

Exclusion criteria are as follows: (1) absence of critical data for meta-analysis; and (2) low-quality articles according to Cochrane checklist.

Data extraction and quality assessment

The characteristics of the relevant articles were extracted and recorded independently by two reviewers (Jiawei Yu and Gaoyang Cao) as follows: first author's name, year, area, age (mean or range), type of probiotic supplement, dose design, course of treatment, number of cases, number of controls, and bone status (as

shown in Table 1). The Cochrane Collaboration's tool [13] was used for assessing the risk of bias. Six domain-based evaluations (selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias) were used in the tool to assess the possible bias of randomized controlled trials. The results were displayed as low risk, unclear risk, or high risk of bias.

Statistical analysis

The mean relative change from baseline to the end of the course and standard deviation (SD) were used to express the effect of the probiotic supplement on bone status in postmenopausal women. If the original studies did not provide the mean relative change and standard deviation, we converted the data using a common method [14-15]. The pooled effects of included studies were expressed in terms of standardized mean difference (SMD) with 95% confidence interval (CI). Q test and I^2 index were used to evaluate heterogeneity among the included results. Meta-regression was conducted to determine whether different types of probiotic supplements would introduce sources of heterogeneity. Random-effects model and subgroup analysis were used in the face of heterogeneity. Forest plots and funnel plots were produced, and publication bias was tested using Begg's test and the weighted Egger test [16-17]. Sensitivity analysis was conducted to verify the impact of each study on the pooled results. In the sensitivity analyses, each study was omitted to recalculate the pooled estimates. All analysis was performed using STATA 12.0 (StataCorp LP, College Station, TX, USA).

Patient and public involvement

Patient and public involvement is not applicable for this meta-analysis.

Results

Search results and characteristics of identified studies

A total of 604 articles were identified from the initial searches of PubMed and EMBASE, and 547 articles were removed because of absence of relevance. Nine articles were retained after reviewing the abstract according to the exclusion criteria. Finally, five randomized controlled trials[18-22] satisfied the inclusion criteria and entered this meta-analysis after full-text review. All the five RCTs had low risk of

bias (available in Supplementary Table 2).

A detailed overview of the selection process is outlined in Figure 1.

A total of 497 postmenopausal women completed these trials. Among the five trials, two were conducted in Asia (one in Japan [18], the other in Iran [20]), and the other three were in Europe (two in Sweden [19 22], the last one in Denmark [21]). All trials were randomized using the double-blinded method. Each trial identified the type of probiotic supplements used and described the dosage design. Three studies considered treatment with probiotics only [18-20], while the other two studies included treatment with combined isoflavone and probiotics [21 22]. All studies provided BMD data from DXA scans at the lumbar spine and total hip. Collagen type 1 cross-linked C-telopeptide (CTX), bone-specific alkaline phosphatase (BALP), osteoprotegerin (OPG), osteocalcin (OC), and tumor necrosis factor (TNF) were used as bone turnover markers. Details of the characteristics are displayed in Table 1 and Supplementary Table 3.

Probiotic supplements and lumbar spine BMD

A total of five estimates were included in the meta-analysis. The meta-regression results also showed no source of heterogeneity from various types of probiotics (P = 0.987). Therefore, the five estimates were incorporated into the pooled analysis. Compared to the placebo group, the lumbar spine BMD level of the supplementary group was higher (SMD = 0.26, 95% CI: 0.09 – 0.44), with no heterogeneity (P = 0.805; I^2 = 0.0) (Figure 2). The funnel plot was symmetrical (Supplementary Figure 1) and excluded publication bias (Begg's test z_c = 0.73, P = 0.462; Egger's test t = -0.22, P = 0.843). Sensitivity analyses indicated that the positive result was robust. (Supplementary Figure 2).

Probiotics supplements and total hip BMD

Overall, five estimates of the association between probiotics supplement and hip BMD were included in the meta-analysis. The meta-regression results revealed that various types of probiotics were not a source of heterogeneity (P = 0.237). Therefore, we brought the five estimates into the pooled analysis. There was no difference between probiotic supplements and BMD in hips (SMD = 0.22, 95% CI: -0.07 –

0.52), with no heterogeneity (P = 0.055; I^2 = 56.8) (Figure 3). The funnel plot is shown in Supplementary Figure 3; it was symmetrical, excluding publication bias (Begg's test z_c = -0.24, P = 1.00; Egger's test t = 1.59, P = 0.209). Sensitivity analyses indicated that the positive result was affected by the Jansson trial (Supplementary Figure 4).

Probiotic supplements and bone turnover markers

Four estimates of CTX and two estimates of BALP, OPG, OC, and TNF were incorporated into the pooled analysis. The results suggested that probiotic supplements help decrease the supplementary group's body CTX level compared with the placebo group (SMD = -0.34, 95% CI: -0.60 – -0.09) with substantial heterogeneity. There was no evidence that probiotic supplements were associated with BALP, OPG, OC, and TNF (Figure 4).

Discussion

Main findings

This meta-analysis included five randomized controlled trials with low risk of bias and 497 postmenopausal women. The results provides evidence that dietary probiotics supplement can slow bone resorption in postmenopausal women. Daily supplementation with probiotics for 24 weeks to 12 months significantly decreased bone turnover marker CTX (compared to placebo) in postmenopausal women. BMD loss at the lumbar spine was significantly lower in the treatment group.

Bone loss occurs throughout life following maturation and is accelerated following menopause in women [23]. Postmenopausal women have an increased risk of fragility fractures. Using a naturally-occurring bacterium to significantly reduce the annual bone loss in this group of patients is a new concept that could lead to a paradigm shift in osteoporosis prevention. Previous studies in animals demonstrated that supplementation with specific bacterial strains increases bone density and protect against osteoporosis [24-26]. Kim et al. reported that the administration of *Lactobacillus casei* 393 significantly increased BMD in ovariectomized rats [27]. For the first time, the present meta-analysis systemically demonstrated that this probiotic also works in humans.

The lumbar spine and hip are the most suitable organs to assess bone metabolism. The vertebrae and metaphyses of long bones, rich in trabecular bone, have a higher turnover rate than cortical bones in the axis of long bones. Therefore, medications and diseases affecting the lumbar spine and hip are identified earlier than in other skeletal segments [28]. The vertebrae and hips are easily accessible for measuring BMD. Therefore, the lumbar spine and hip BMD were suitable primary outcome variables in the present studies. McCabe et al [29] showed that oral administration of *Lactobacillus* probiotics identified a 45% increase in hip and vertebral trabecular bone volume fraction in male mice. In another study, the administration of *Lactobacillus plantarum* and *Lactobacillus paracasei* to ovariectomized mice showed increased trabecular number compared to sham-ovariectomized control groups [30]. Our meta-analysis showed, in the probiotics group, both total hip and lumbar vertebrae BMD were at significantly higher levels than those of the control.

CTX and BALP were chosen as critical bone turnover markers. Because BMD depends on the dynamic balance of bone formation and resorption, bone turnover markers are also important parameters analyzed in our meta-analysis. The measurement of CTX has been taken as a marker of bone resorption; osteoclasts produce it during bone resorption [31]. Therefore, the increased levels of serum CTX indicated increased bone resorption. Subgroup included 3 RCT studies, suggesting that probiotic supplements' ingestion significantly reduced the bone resorption marker CTX. Another study from Japan [18] showed that the probiotics group had significantly lower uNTx (urinary type I collagen cross-linked N-telopeptide) levels than the placebo group at 12 weeks of treatment. uNTx is another fragment of type I collagen generated during resorption detected in urine; therefore, this also suggested that probiotics inhibit bone resorption by suppressing osteoclast activity. BALP is another well-known bone turnover marker, an indicator of osteoblast proliferation that is thought to be a bone formation marker [32]. However, the present meta-analysis showed no significant changes in BALP. Similarly, no differences were detected in levels of biochemical markers for bone metabolic indices (OPG, OC).

The mechanism of action

The mechanisms of action of probiotics are as follows. Probiotics have many functional properties in humans. They function in the gastrointestinal system by modifying the microbiota composition, intestinal barrier function, and the immune system, which feeds back systemic benefits to the host, including bone health. Moreover, probiotic function modifying physiological homeostasis of the intestinal flora can also benefit bone metabolism [33]. Gastrointestinal inflammation and systemic inflammation are close to enhanced generation of potent osteoclastogenic cytokines as the leading cause of bone loss [34-35]. Probiotics can restore the balance of the gut microbiota, preventing or moderating gut and systemic inflammation and allowing absorption of nutrients, especially in older adults [36].

Furthermore, probiotics decrease levels of inflammatory mediators and cytokines in the gut and bone marrow [37]. These changes give bone cell signals, including osteoblasts, osteoclasts, and stem cells, significantly affecting bone homeostasis. Endocrine factors (such as serotonin and incretins) secreted by the intestine also remarkably affect bone cells [38]. Anti-inflammatory effects are among the underlying mechanisms by which probiotics benefit bone metabolism. There is evidence that arginine deiminase, produced by the probiotic *Lactobacillus brevis* CD2, has an anti-inflammatory effect [39]. Supplementation of probiotics may reduce the expression of pro-inflammatory and osteolytic cytokines, including TNF-α. These cytokines alter anti-osteoclastogenic cytokine expression, leading to enhanced osteoclast formation and inhibited osteoblast activity [40]. Some studies found that probiotic supplementation reduces TNFα, IL-17, and RANKL expression levels in ovariectomized mice [41]. These changes give bone cell signals, such as osteoblasts, osteoclasts, and stem cells, significantly affecting bone homeostasis. More clinical trials are needed in the future to elucidate the relationship between the administration of probiotics and anti-inflammatory effects.

Limitations and Strengths

Our study has some limitations. First, only five randomized controlled trials with specific population groups satisfied our inclusion criteria. The limited number of reports and specific population groups focusing on the association between the

probiotic supplement and BMD and bone turnover markers prevented us from conducting subgroup analysis and drawing conclusive summaries. Furthermore, the insufficient number of estimates inflates the impact of the results of a particular study and the conclusions may change on the publication of future studies. Second, although meta-regression was used to determine that various types of probiotic supplements did not impact the pooled results, dosage design and course of treatment could also introduce bias. Third, in Lambert's study [21], probiotics plus isoflavones were used as a treatment regimen，rather than probiotics alone. This may cause some bias; however, we did not want to ignore this valuable study. Third, the units describing BMD change were inconsistent among the five reports. Nilsson's study [19] and Jansson's study [22] applied T score to describe BMD change, while the other three studies used g/cm^2 instead. We could only calculate SMD rather than the weighted mean difference (WMD). Fifth, unfortunately, we did not find a relevant prospective cohort for this meta-analysis. Thus, our results of a meta-analysis should be interpreted with caution.

Our research also has some strengths. First, to our knowledge, this is the first meta-analysis describing the evidence of the association of probiotic supplements and bone status in postmenopausal women. Second, there is little heterogeneity between the included articles and the fixed-effects model used to calculate the results. Third, all included randomized controlled trials were of high quality.

Implications and future research

This systematic review and meta-analysis are useful for multidisciplinary clinicians to evaluate their practices and make a proper clinical decision. The beneficial effects of probiotic supplements may infect probiotic indication in postmenopausal women with osteoporosis. More RCT studies from different regions are needed to validate our argument and help answer research questions about probiotic supplements, dose, and the optimal duration.

Conclusion

Our systematic review and meta-analysis showed that probiotic supplementations in postmenopausal women were associated with preserving lumbar spine BMD. The

results should be interpreted with caution and more high quality RCTs are needed to validate or update these results. An appropriate supplement of probiotics could be recommended to improve bone status in postmenopausal women.

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Contributors

MC and JY(Jiawei Yu) conceived and designed the meta-analysis; GC, SY and CL searched the literature; JY(Jiawei Yu), GC, and SY analyzed the data; JY(Jiawei Yu) contributed analysis tools; JY(Jiawei Yu) and GC wrote the paper; JY(Jiafeng Yu) and MC revised the manuscript.

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All data relevant to the study are included in the article or uploaded as supplementary information. No additional data are available.

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microbiota dependent and prevented by probiotics. *J CLIN INVEST* 2016;126(6):2049-63. doi: 10.1172/JCI86062 pmid:271112322016-06-01].

Table 1. Characteristics of included randomized controlled trials in the meta-analysis

Study	Year	Area	Age	Blinding	Type of probiotic supplement	Number of Treatment	Number of Placebo	Course of treatment	BMD	BTM
Jansson	2019	Sweden	T: 59.1 P: 58.1	double blind	three Lactobacillus strains*	126	123	12 months	lumbar spine hip	N/A
Takimoto	2018	Japan	T: 57.5 P: 57.8	double blind	bacillus subtilis C-3102	31	30	6 months	lumbar spine hip	CTX
Nilsson	2018	Sweden	T: 76.4 P: 76.3	double blind	lactobacillus reuteri 6475	32	36	12 months	lumbar spine hip	CTX BALP TNF
Jafarnejad	2017	Iran	T: 58.9 P: 57.3	double blind	seven probiotic b acteria species#	20	21	6 months	lumbar spine hip	CTX BALP OPG OC TNF
Lambert	2017	Denmark	T: 60.8 P: 62.9	double blind	lactic acid bacteria and soflavones	38	40	12 months	lumbar spine hip	CTX OPG OC

BMD: bone mineral density; BTM: bone turnover marker; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone-specific alkaline phosphatase; OPG: osteoprotegerin; OC: osteocalcin; TNF: tumor necrosis factor; N/A: not available; * Lactobacillus paracasei DSM 13434, Lactobacillus plantarum DSM 15312, and Lactobacillus plantarum DSM 15313; # Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, and Streptococcus thermophilus.

Figure 1. Flow diagram of the studies search process

Figure 2. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

Figure 3. Forest plots of meta-analysis on probiotics supplements and hip BMD

Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers

Supplementary Figure 1. Funnel plots of meta-analysis on probiotics supplements and lumbar spine BMD

Supplementary Figure 2. Sensitivity analyses of meta-analysis on probiotics supplements and lumbar spine BMD

Supplementary Figure 3. Funnel plots of meta-analysis on probiotics supplements and hip BMD

Supplementary Figure 4. Sensitivity analyses of meta-analysis on probiotics supplements and hip BMD

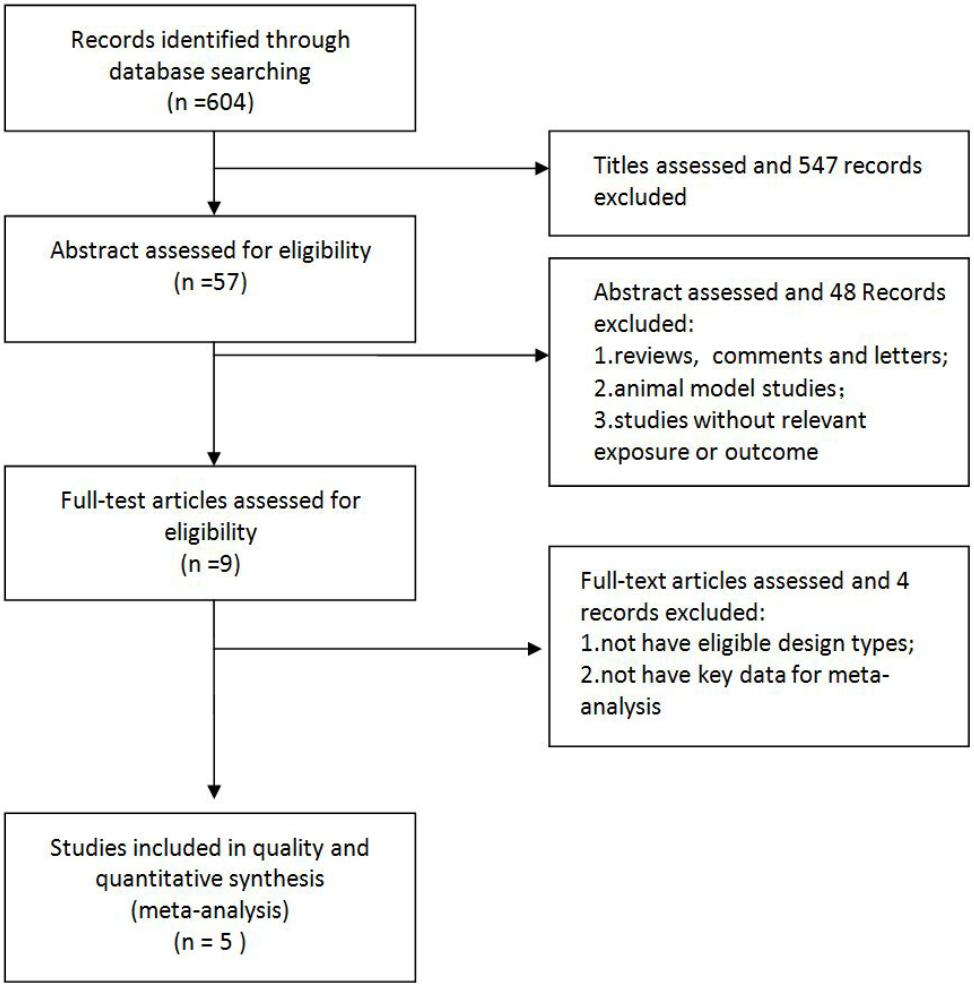


Figure 1. Flow diagram of the studies search process

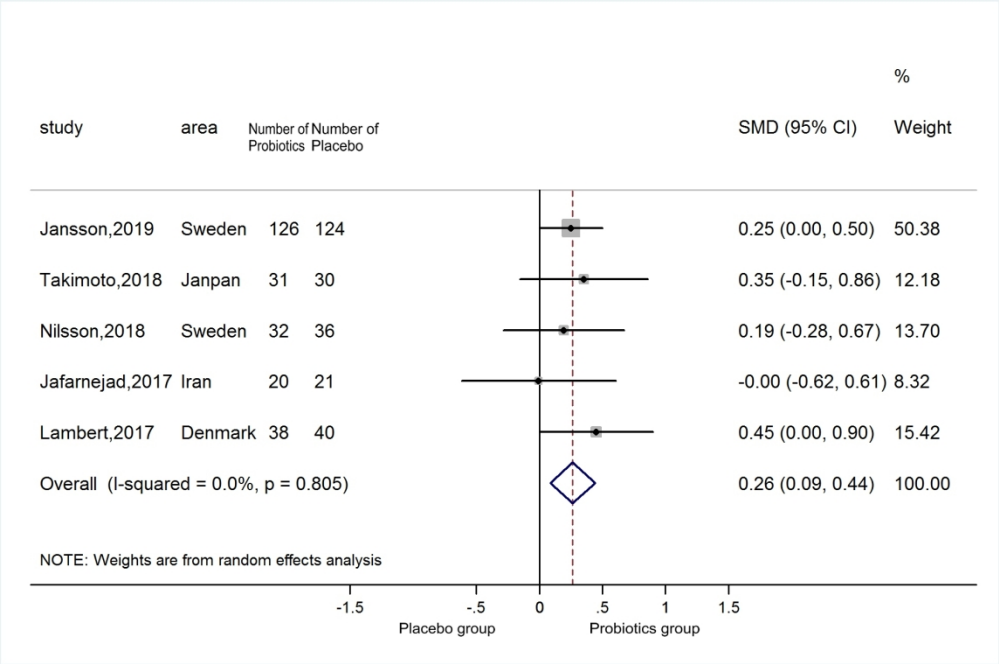


Figure 2. Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD

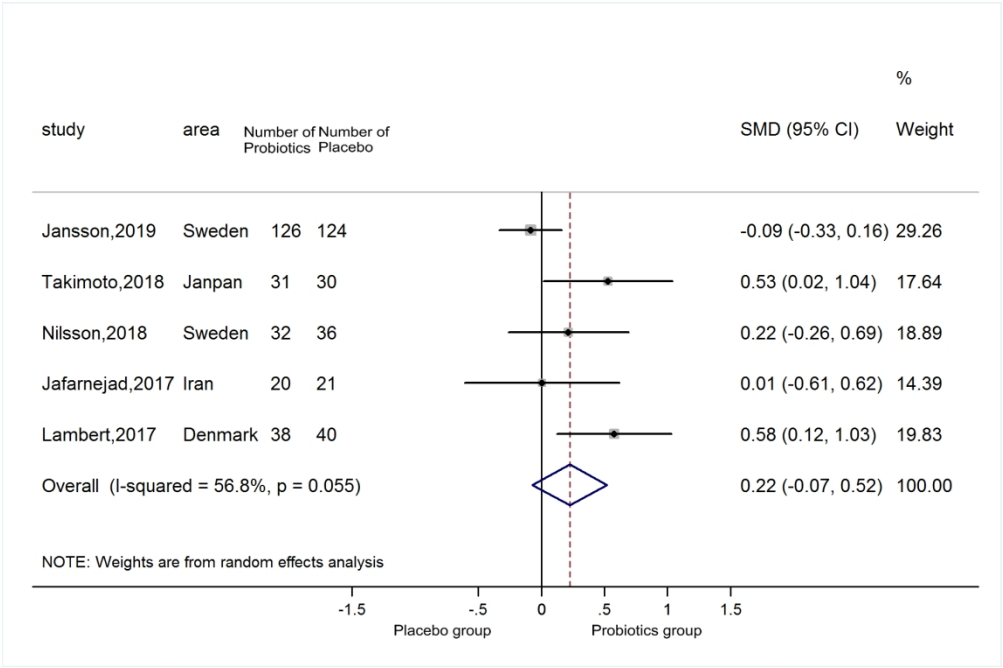


Figure 3. Forest plots of meta-analysis on probiotics supplements and hip BMD

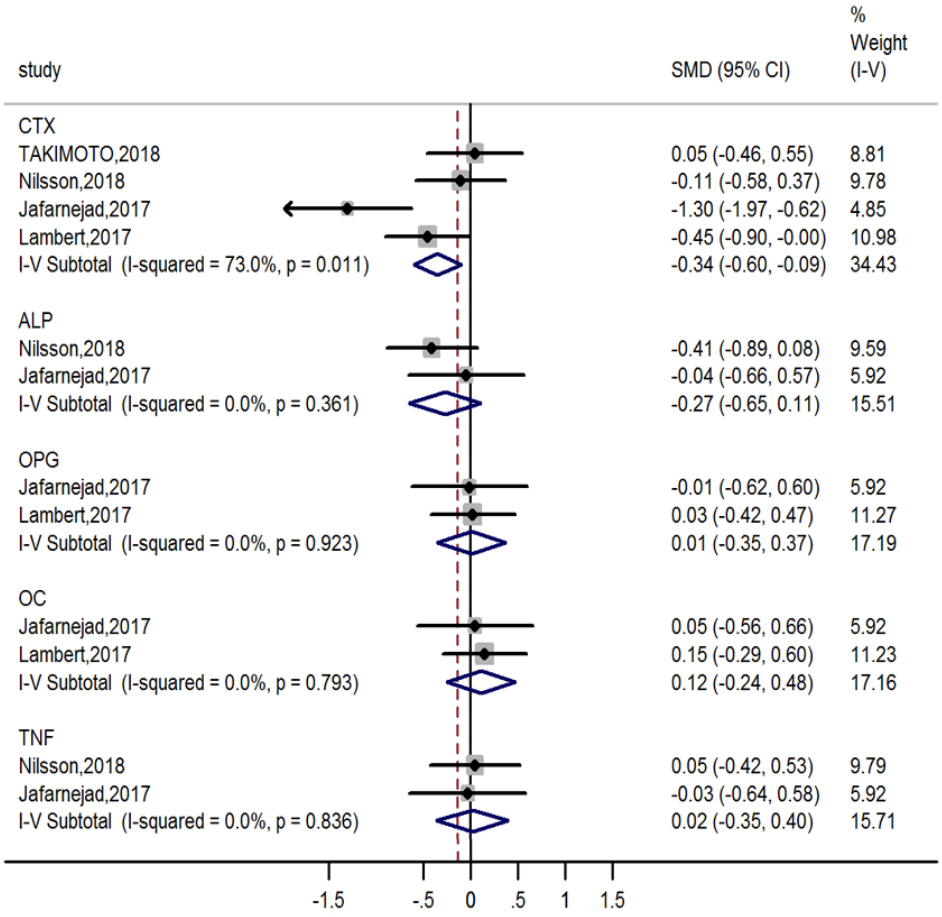


Figure 4. Forest plots of meta-analysis on probiotics supplements and bone turnover markers

Supplementary Table 1 Search strategy of Medline

#	Searches
1	Probiotics
2	Probiotic supplement
3	Bone
4	Osteoporosis
5.	Osteopenia
6.	Bone mineral density
7.	Bone turnover
8.	Postmenopausal
9.	1 and 3 and 8
10.	1 and 4 and 8
11.	1 and 5 and 8
12.	1 and 6 and 8
13.	1 and 7 and 8
14.	2 and 3 and 8
15.	2 and 4 and 8
16.	2 and 5 and 8
17.	2 and 6 and 8
18.	2 and 7 and 8

The same strategy for other databases

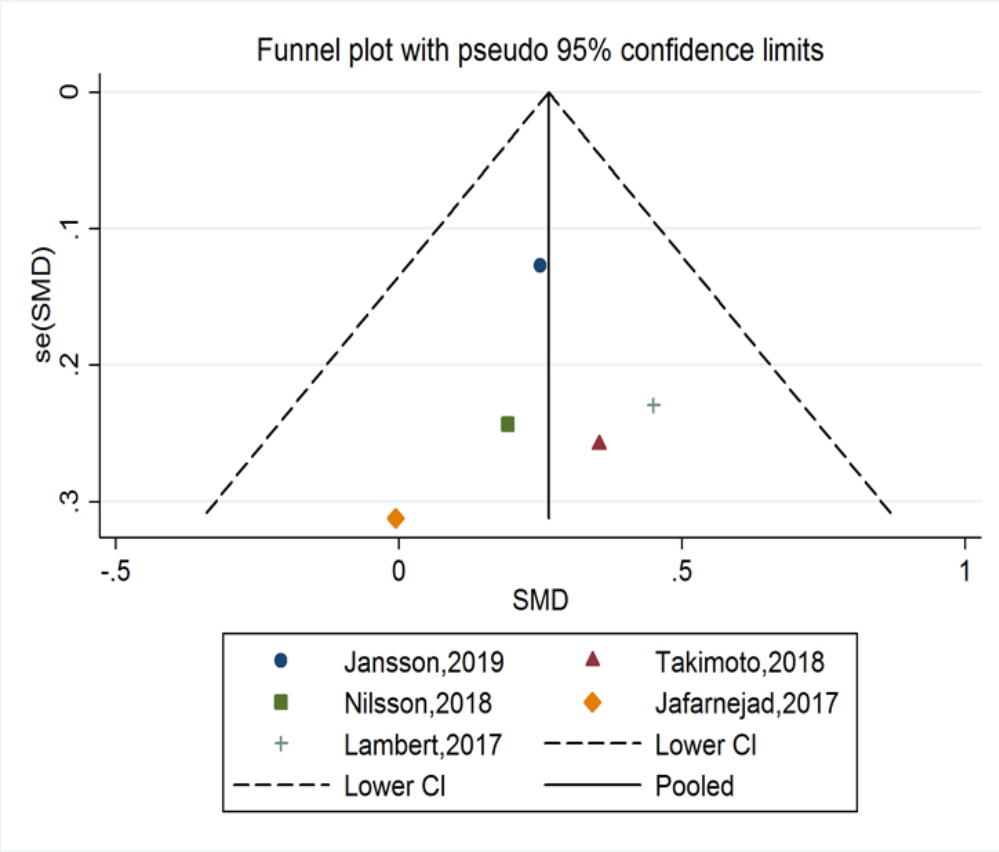
Supplementary Table 2. Assessment of risk bias of the studies included in the meta-analysis

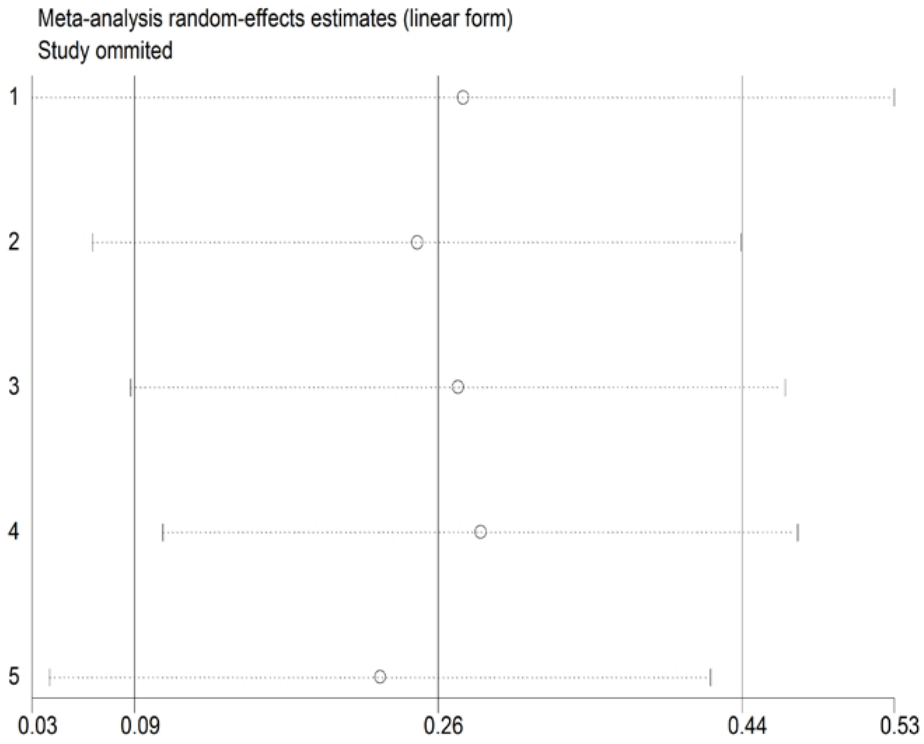
Study	Selection bias	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall
Jansson	Low	Low	Low	Low	Low	Low	Low
Takimoto	Low	Low	Low	Low	Low	Low	Low
Nilsson	Low	Unclear	Low	Low	Low	Low	Low
Jafarnejad	Low	Low	Low	Low	Low	Low	Low
Lambert	Low	Unclear	Low	Low	Low	Low	Low

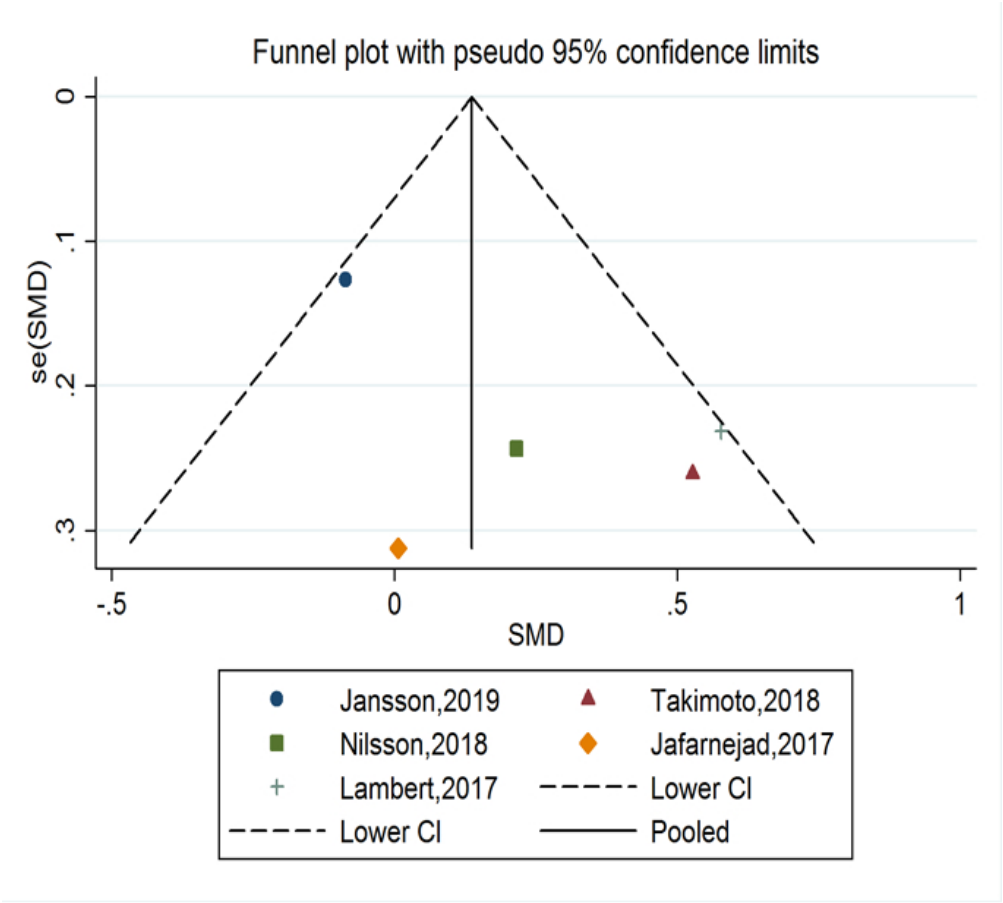
Supplementary Table 3. Other characteristics of included randomized controlled trials in the meta-analysis

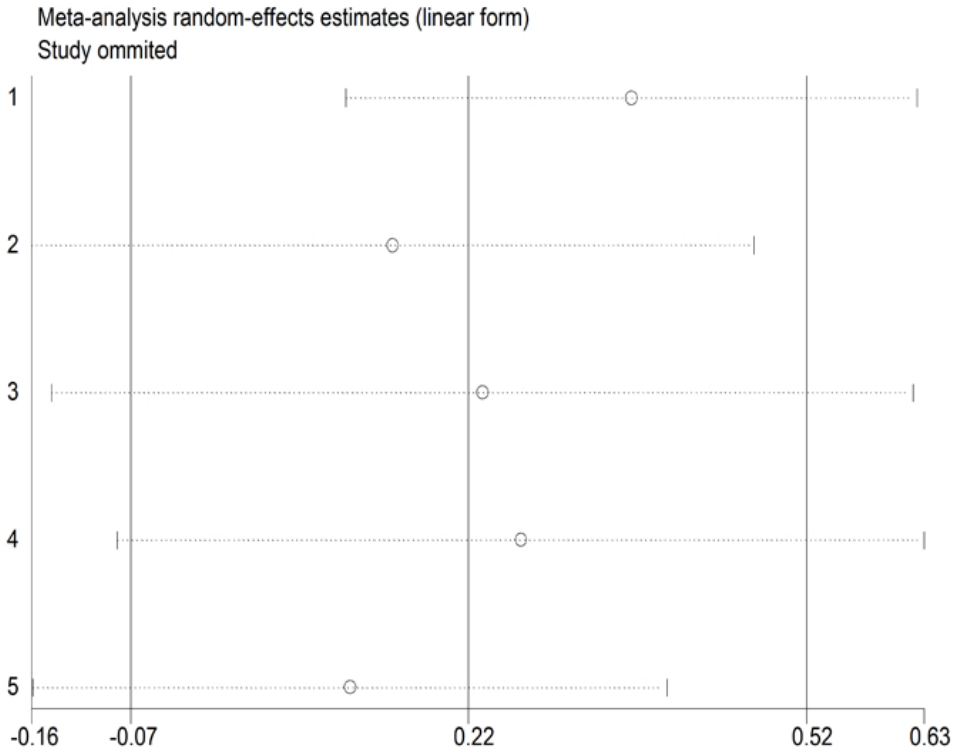
Study	Year	Area	dose design	Minerals intake	Measurement of outcome
Jansson	2019	Sweden	1 x10^{10} CFU/d	N/A	After 12 months
Takimoto	2018	Japan	3.4×10^{9} CFU /d	BDHQ	After 6 months
Nilsson	2018	Sweden	5x10^{9} CFU twice/d	A standardized questionnaire	After 12 months
Jafarnejad	2017	Iran	one Gerilact capsule /d*	500 mg Ca plus 200 IU vitamin D daily	After 6 months
Lambert	2017	Denmark	60mg isoflavone and probiotics/d	1200 mg Ca, 550 mg Mg, and 25mg calcitriol daily	After 12 months

BDHQ: a brief-type self-administered diet history questionnaire; CFU: colony-forming unit; *Lactobacillus casei 1.3 x 10^{10} CFU, Bifidobacterium longum 5 x 10^{10} CFU, Lactobacillus acidophilus 1.5 x 10^{10} CFU, Lactobacillus rhamnosus 3.5 x 10^{9} CFU, Lactobacillus bulgaricus 2.5 x 10^{8} CFU, Bifidobacterium breve 1 x 10^{10} CFU, and Streptococcus thermophilus 1.5 x 10^{8} CFU per 500 mg.


Funnel plot with pseudo 95% confidence limits
se(SMD)
0
.1
.2
.3
-.5
0
.5
1
SMD
Jansson,2019
Takimoto,2018
Nilsson,2018
Jafarnejad,2017
Lambert,2017
Lower CI
Lower CI
Pooled


Meta-analysis random-effects estimates (linear form)
Study ommited
1
2
3
4
5
0.03
0.09
0.26
0.44
0.53


Funnel plot with pseudo 95% confidence limits
se(SMD)
0
.1
.2
.3
-.5
0
.5
1
SMD
Jansson,2019
Takimoto,2018
Nilsson,2018
Jafarnejad,2017
Lambert,2017
Lower CI
Lower CI
Pooled


Meta-analysis random-effects estimates (linear form)
Study ommited
1
2
3
4
5
-0.16
-0.07
0.22
0.52
0.63

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5-6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6-7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6-7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7-8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7-8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7-8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7-8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	8-11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	8-11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	n/a

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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