

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

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ABSTRACT

Objective Osteoporosis is a common disease in postmenopausal women. Several studies have analysed 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 randomised 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 (standardised mean difference, SMD=0.27, 95% CI 0.09 to 0.44) than in control. There was no difference between probiotic supplements and BMD in hips (SMD=0.22, 95% CI -0.07 to 0.52). Collagen type 1 cross-linked C-telopeptide levels in the treatment groups were significantly lower than those of the placebo group (SMD=-0.34, 95% CI -0.60 to -0.09). In subgroup meta-analysis, levels of bone-specific alkaline phosphatase, osteoprotegerin, osteocalcin and tumour necrosis factor did not differ between the probiotic and placebo groups.

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

INTRODUCTION

Osteoporosis is characterised 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 oestrogen, reducing BMD (on average 2%–5% BMD/year)² and adverse effects on bone microarchitecture.

Currently, many medications are used in osteoporosis to decrease bone resorption or increase bone formation. Large

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 randomised 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.

randomised controlled trials (RCTs) showed that oestrogen 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 antiresorptive 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 health⁸; 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 favourable 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 Organisation/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, analysing 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 Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.¹² The keywords were as follows: 'probiotics', 'probiotic supplement', 'bone', 'osteoporosis', 'osteopenia', 'BMD', 'bone turnover' and 'postmenopausal' (search queries available in online supplemental table 1). References of retrieved articles were also scanned to identify any additional relevant studies. Two independent reviewers (JY and GC) conducted this work. Discrepancies were resolved by consensus of the two reviewers. If required, the final disposition was determined by MC.

Inclusion and exclusion criteria

Inclusion criteria are as follows: (1) RCTs 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 (JY and GC) 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 tool¹³ 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 RCTs. 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 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 SD, we converted the data using a common method.^{14 15} The pooled effects of included studies were expressed in terms of standardised mean difference (SMD) with 95% CI. Q test and I² index were

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

Study	Year	Area	Age	Blinding	Type of probiotic supplement	No of treatment	No 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 bacteria 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

**Lactobacillus paracasei* DSM 13434, *L. plantarum* DSM 15312 and *L. plantarum* DSM 15313.

†*L. casei*, *Bifidobacterium longum*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *Bifidobacterium breve* and *Streptococcus thermophilus*.

BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; BTM, bone turnover marker; CTX, collagen type 1 cross-linked C-telopeptide; N/A, not available; OC, osteocalcin; OPG, osteoprotegerin; TNF, tumour necrosis factor.

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 analyses were performed using STATA V.12.0 (StataCorp).

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 RCTs^{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 online supplemental 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 Iran²⁰ and the other three were in Europe (two in Sweden,^{19 22} the last one in Denmark).²¹ All trials were randomised 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 tumour necrosis factor (TNF) were used as BTMs. Details of the characteristics are displayed in table 1 and online supplemental table 3.

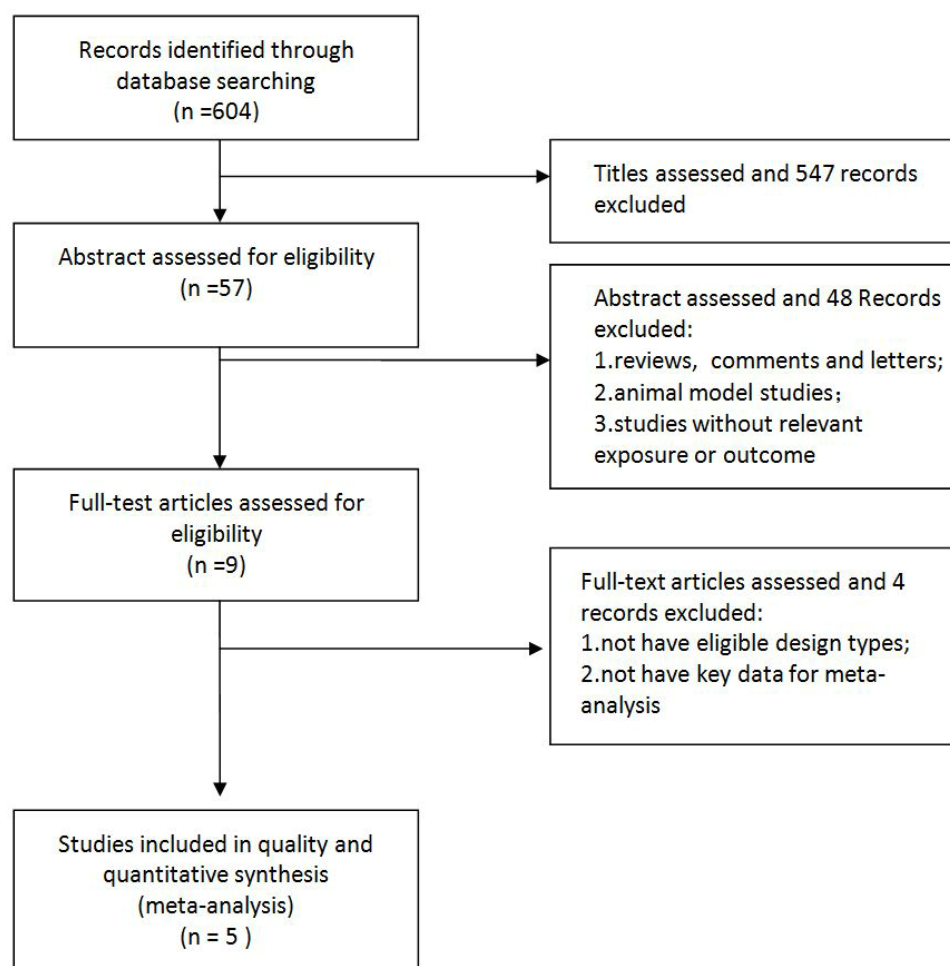


Figure 1 Flow diagram of the studies search process.

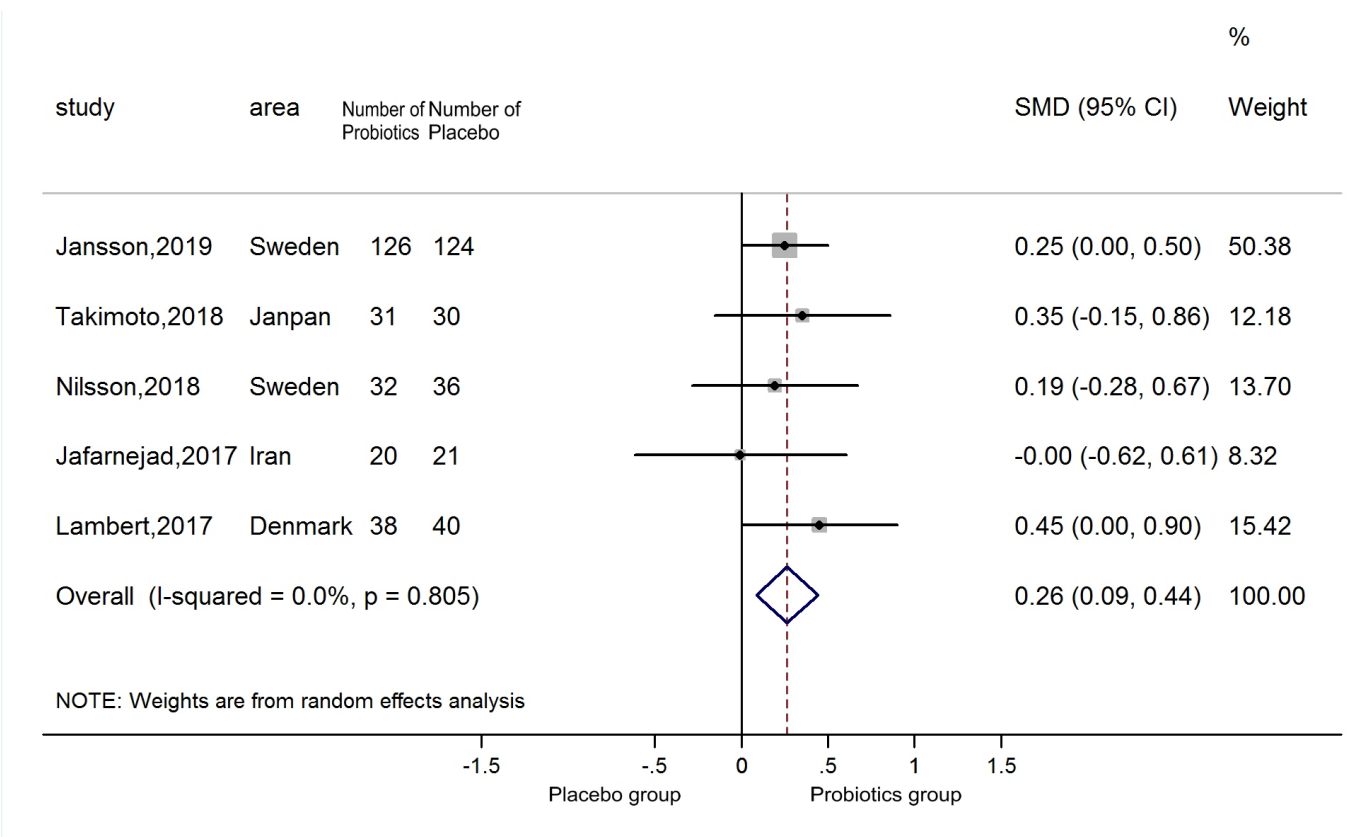


Figure 2 Forest plots of meta-analysis on probiotics supplements and lumbar spine BMD. BMD, bone mineral density.

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 with the placebo group, the lumbar spine BMD level of the supplementary group was higher (SMD=0.26, 95% CI 0.09 to 0.44), with no heterogeneity ($p=0.805$; $I^2=0.0$) (figure 2). The funnel plot was symmetrical (online supplemental 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 (online supplemental 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 online supplemental 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 (online supplemental figure 4).

Probiotic supplements and BTM

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 to -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 RCTs 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 BTM CTX (compared with 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

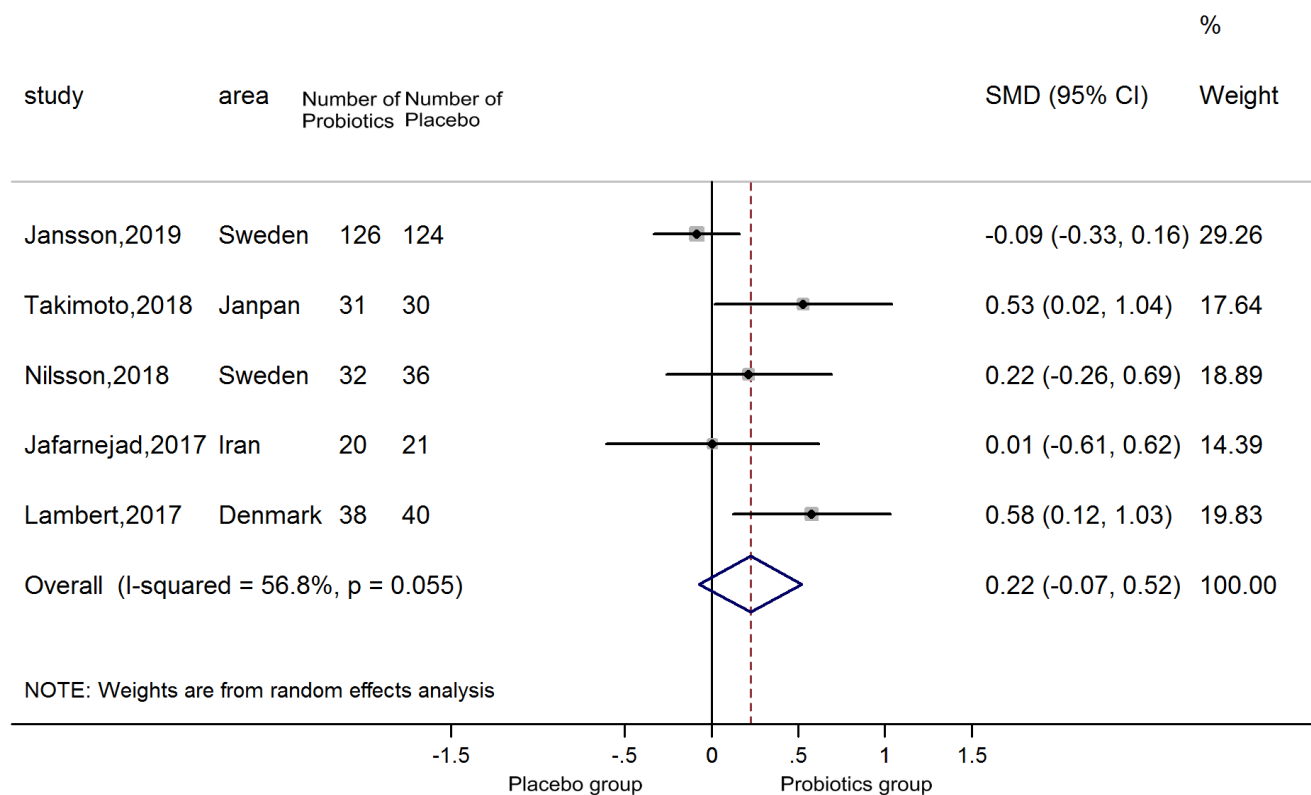


Figure 3 Forest plots of meta-analysis on probiotics supplements and hip BMD. BMD, bone mineral density.

in animals demonstrated that supplementation with specific bacterial strains increases bone density and protect against osteoporosis.^{24–26} Kim *et al*²⁷ reported that the administration of *Lactobacillus casei* 393 significantly increased BMD in ovariectomised rats. 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 *L. probiotics* identified a 45% increase in hip and vertebral trabecular bone volume fraction in male mice. In another study, the administration of *L. plantarum* and *L. paracasei* to ovariectomised mice showed increased trabecular number compared with sham-ovariectomised 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 BTMs. Because BMD depends on the dynamic balance of bone formation and resorption, bone turnover markers are also

important parameters analysed 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 three RCT studies, suggesting that probiotic supplements' ingestion significantly reduced the bone resorption marker CTX. Another study from Japan¹⁸ showed that the probiotics group had significantly lower uNTx (urinary type I collagen cross-linked N-te-lopeptide) 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 BTM, 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,

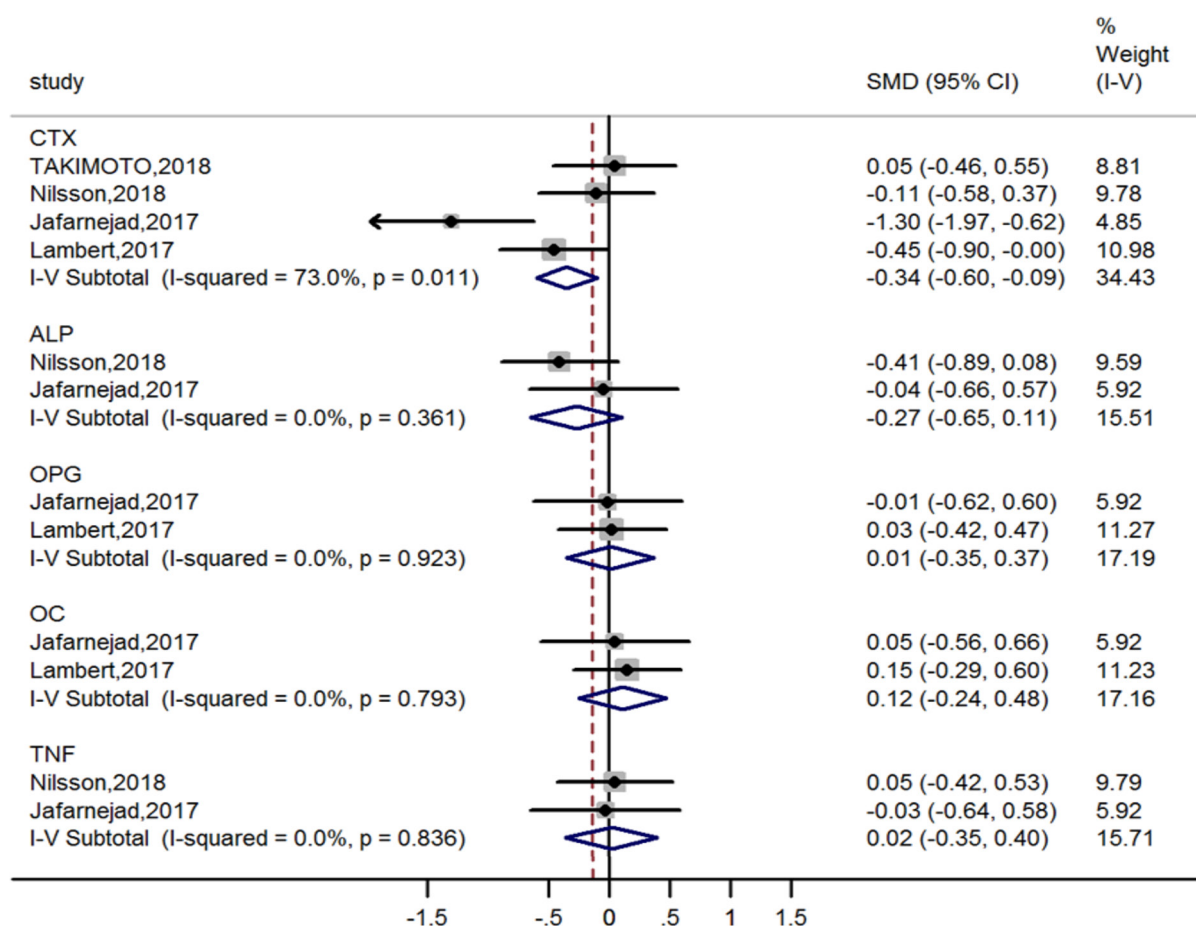


Figure 4 Forest plots of meta-analysis on probiotics supplements and bone turnover markers. ALP, alkaline phosphatase; CTX; cross-linked C-telopeptide; OC, osteocalcin; OPG, osteoprotegerin; SMD, standardised mean difference; TNF, tumour necrosis factor.

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.³³ Anti-inflammatory effects are among the underlying mechanisms by which probiotics benefit bone metabolism. There is evidence that arginine deiminase, produced by the probiotic *L. brevis* CD2, has an anti-inflammatory effect.³⁸ Supplementation of probiotics may reduce the expression of proinflammatory and osteolytic cytokines, including TNF- α . These cytokines alter antiosteoclastogenic cytokine expression, leading to enhanced osteoclast formation and inhibited osteoblast activity.³⁹ Some studies found that probiotic

supplementation reduces TNF α , IL - 17(interferon 17) and RANKL (Receptor activator of nuclear factor-kappa ligand) expression levels in ovariectomised mice.⁴⁰ 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 RCTs 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 BTMs 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 *et al*'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 *et al*'s study¹⁹ and Jansson *et al*'s study²² applied T score to describe BMD change, while the other three studies used g/cm² instead. We could only calculate SMD rather than the weighted mean difference. 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 RCTs 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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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	Overall
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	
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 10¹⁰ CFU, Bifidobacterium longum 5 x 10¹⁰ CFU, Lactobacillus acidophilus 1.5 x 10¹⁰ CFU, Lactobacillus rhamnosus 3.5 x 10⁹ CFU, Lactobacillus bulgaricus 2.5 x 10⁸ CFU, Bifidobacterium breve 1 x 10¹⁰ CFU, and Streptococcus thermophilus 1.5 x 10⁸ CFU per 500 mg.

