British Journal of Nutrition (2024), 131, 567–580 doi:10.1017/S0007114523002362

Effect of probiotics on postmenopausal bone health: a preclinical meta-analysis

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(Submitted 6 September 2023 – Final revision received 12 October 2023 – Accepted 18 October 2023 – First published online 23 October 2023)

Abstract

Postmenopausal osteoporosis is a major concern for women worldwide due to increased risk of fractures and diminished bone quality. Recent research on gut microbiota has suggested that probiotics can combat various diseases, including postmenopausal bone loss. Although several preclinical studies have explored the potential of probiotics in improving postmenopausal bone loss, the results have been inconsistent and the mechanism of action remains unclear. To address this, a meta-analysis was conducted to determine the effect of probiotics on animal models of postmenopausal osteoporosis. The bone parameters studied were bone mineral density (BMD), bone volume fractions (BV/TV), and hallmarks of bone formation and resorption. Pooled analysis showed that probiotic treatment significantly improves BMD and BV/TV of the ovariectomised animals. Probiotics, while not statistically significant, exhibited a tendency towards enhancing bone formation and reducing bone resorption. Next, we compared the effects of *Lactobacillus* sp. and *Bifidobacterium* sp. on osteoporotic bone. Both probiotics improved BMD and BV/TV compared with control, but *Lactobacillus* sp. had a larger effect size. In conclusion, our findings suggest that probiotics have the potential to improve bone health and prevent postmenopausal osteoporosis. However, further studies are required to investigate the effect of probiotics on postmenopausal bone health in humans.

Keywords: **Postmenopausal osteoporosis: Bone health: Preclinical meta-analysis: Probiotics: *Lactobacillus*: *Bifidobacterium***

Osteoporosis is a metabolic bone disorder characterised by skeletal fragility and fractures even with minor trauma. It affects both sexes, but women are more prone to developing osteoporosis than men. Postmenopausal osteoporosis is the most common type of osteoporosis, caused by the loss of oestrogen after menopause. Roughly 50 % of the postmenopausal women suffer from osteoporosis.

Bone tissue is subjected to continuous cycles of modelling and remodelling where homoeostasis is maintained by a balance between bone formation by osteoblasts and bone resorption by osteoclasts. However, this balance is disrupted after menopause with oestrogen deficiency favouring bone resorption over bone formation. Macroscopically, bone can be classified into two types: cortical bone and trabecular bone. Cortical bone is compact, dense and solid bone, while trabecular bone is a lace-like structure of interconnected trabecular plates and bars surrounding marrow-filled cavities[1]. In osteoporotic conditions, there is a disruption of trabecular continuity due to trabecular perforation, leading to increased bone fragility. In addition, thinning and increased porosity of the cortices occur, with the conversion of plate-like trabeculae into thinner rod-like structures[2].

Current treatments for osteoporosis aim to improve bone quality and strength by increasing bone formation through anabolic drugs or decreasing bone resorption by antiresorptive agents. Teriparatide and abaloparatide are the only two FDA (Food and Drug Administration)-approved anabolic agents for the treatment of osteoporosis. Both have been shown to reduce the incidence of vertebral and non-vertebral fractures[3,4]. Antiresorptive agents include oestrogen, bisphosphonates (e.g. alendronate), selective oestrogen receptor modulators (e.g. raloxifene), human monoclonal antibody against RANKL (denosumab) and strontium ranelate (SR). However, these treatments can cause side effects such as joint and muscle pain, heartburn and urinary tract infections. Additionally, they may have lengthy treatment duration and high cost, which can limit their use.

Recent research on the influence of gut microbiota on a person's health is providing exciting new insights into the crosstalk between the homoeostasis of bone metabolism and the intestinal flora[5–10] and could help in developing new treatment strategies. Probiotic gut bacteria, such as *Lactobacillus* and *Bifidobacterium,* have been shown to promote the absorption of minerals such as Ca, Mg and P and thus increase bone mineral

Abbreviations: ALP, alkaline phosphatase; BFR, bone formation rate; BMD, bone mineral density; BV/TV, bone volume fractions; CTX-1, C-terminal telopeptide; Oc.S/B.S, osteoclast surface by bone surface; SMD, standardised mean difference.

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density (BMD)[11]. Probiotics are live micro-organisms that confer health benefits on the host when consumed in adequate amounts[12]. *Lactobacillus*, *Bifidobacterium*, *Saccharomyces* and *Clostridium* are few examples of probiotics. The gut microbiome also plays a vital role in the synthesis of vitamin B and K and metabolising bile acids[13], which play a key role in bone health and Ca absorption[14,15]. These studies point towards the idea that manipulation of the microbiome or its metabolites by the consumption of probiotics may improve bone health and thus prevent or treat osteoporosis. Probiotics function by manipulating the intestinal microbiota and stimulating the proliferation and differentiation of epithelial cells, which can lead to a stronger immune system. They have also been shown to have inhibitory effects on osteoclastic bone resorption[16,17] and properties of osteoblastic bone formation[18,19], which is why probiotics are now being considered as an alternative osteoporosis treatment.

There have been only ten clinical studies investigating the effect of probiotics on bone health, with only five focusing on postmenopausal loss of bone. Among these studies, only one reported a significant improvement in bone mass content following probiotic supplementation, while the others showed smaller effects or no change at all. Several preclinical studies have explored the potential of probiotics in improving bone health, with most conducted on bone loss associated with diseases like diabetes or in the presence of certain drugs. Only few studies have specifically focused on postmenopausal bone loss. Additionally, the results of these studies are contradictory, and the exact mechanisms by which probiotics improve bone health are not well understood. While some studies suggest that probiotics may increase Ca absorption or have a direct effect on bone formation, others propose that they may inhibit bone resorption. Thus, a meta-analysis could help to synthesise the available evidence and determine the overall effect of probiotics on postmenopausal osteoporotic bones. However, there is currently insufficient data from human studies to conduct a meta-analysis, so our analysis was conducted on preclinical studies instead. We also aimed to check which of the two genera of probiotics, *Lactobacillus* or *Bifidobacterium*, could have a more positive effect on bone health. Finally, we evaluated whether probiotics exert their positive effect on bone by decreasing bone resorption or increasing bone formation.

Materials and methods

The protocol of this meta-analysis was registered in the systematic review trials registry 'PROSPERO' with the registration number – CRD42023445290.

Search strategy

To identify the studies that assessed the effect of probiotics on bone, we conducted a literature search on three electronic databases: PubMed, Google Scholar and Web of Science, until June 2023. We used various combinations of the keywords 'probiotics', 'bone' and 'osteoporosis' in our electronic search. The search was not restricted by language. The process of study identification and selection is represented as a flow chart in Fig. 1.

Inclusion and exclusion

Inclusion and exclusion criteria were defined, and studies were screened and selected strictly according to that. Inclusion criteria were (i) original and full-length research articles, (ii) studies on primary osteoporosis of the long bone and vertebra, (iii) studies on laboratory animals, (iv) studies where ovariectomy was used to mimic postmenopausal osteoporotic conditions, (v) studies where animals were treated with single probiotic species or a mixture of them and (vi) studies published in English language. Exclusion criteria were (i) review articles/meta-analytical reviews/systematic reviews, (ii) clinical reports and/or trials, (iii) books, (iv) studies on osteoporosis of the jaw bone, (v) studies on secondary osteoporosis, (vi) studies where animals were treated with GMO (Genetically Modified Organism) species or cell-free culture supernatant, (vii) studies on knockout models, (viii) articles where *in vitro* effects were studied and (ix) studies that failed to provide the required information. There were no restrictions regarding species and duration of probiotic treatment.

Data extraction

Literature was screened independently by the authors, and disagreements, if any, were resolved by discussion. WebPlotDigitizer-4 software was used to extract data in numeric form from bar graphs of the selected research articles. Data were also noted down directly from the tables of the articles. The data were presented in a Microsoft Excel spreadsheet where we recorded PubMed Identifier (PMID) and authors of the study, species and strains used, age and sample size of the control and treated groups, probiotic species used for treatment, treatment method along with its duration and the bone parameters that were measured, that is, bone mineral density (BMD), bone volume fraction (BV/TV), bone formation rate (BFR), serum osteocalcin (OCN), serum C-terminal telopeptide (CTX-1), serum alkaline phosphatase (ALP), serum calcium (Ca) and osteoclast surface by bone surface (Oc.S/B.S).

Outcome assessment

The studies were categorised into two groups: probiotic and control group. BMD and BV/TV data were primarily categorised into three groups based on the type of bone: femur, tibia or vertebra. Under BMD, wherever applicable, each group was further divided into three based on the region considered, that is, total, trabecular or cortical bone. Further, analysis was done on BFR, bone formation markers (like serum osteocalcin, serum ALP and serum Ca) and bone resorption markers (serum CTX-1). Effect of probiotics on Oc.S/B.S was also analysed.

Quantitative data analysis

Pooled data analysis was conducted using Review Manager 5.4 software (RevMan) and Jeffreys's Amazing Statistics Program 0.16.3.0 (JASP). Studies where more than one species of probiotic group was involved were split to include only one probiotic group per analysis. The effect size chosen was standardised mean difference (SMD) also known as Cohen's d. Heterogeneity index (I^2 statistic) was used for assessing

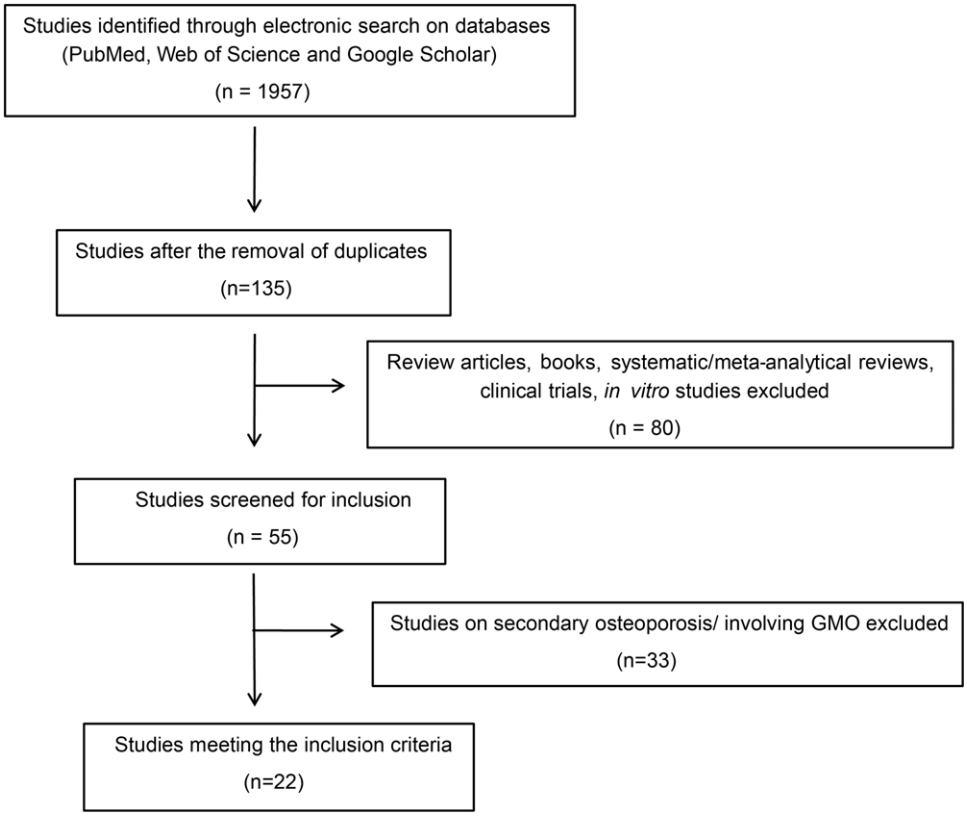

Fig. 1. Flow chart showing the process of study identification and selection.

heterogeneity across studies, and *P*-values of $< 0{\cdot}1$ were considered to be statistically significant due to the low stringency of this test[20]. I^2 value of $< 25\,\%$ was considered as low heterogeneity, 50 % as moderate heterogeneity and $> 75\,\%$ as high heterogeneity[21]. Based on the level of heterogeneity, the summary/pooled effect size was calculated either using random effects model or fixed effects model in RevMan[22]. In addition to this, data obtained from RevMan, that is, effect size and CI of the individual studies, were entered in Microsoft Excel, standard error of effect size was calculated and csv files were created. These were loaded in JASP, and classical meta-analysis was performed to obtain pooled effect sizes again. Here also, based on heterogeneity, fixed or random effects model was used. The method for meta-analysis used in the case of random effects model was restricted maximum likelihood method.

Assessment of risk of bias

Risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) bias risk tool[23]. This tool consists of ten entries covering various biases like selection bias, performance bias, outcome bias, detection bias, attrition bias, reporting bias and other bias. For each entry, studies were categorised as high, low or unclear risk of bias by both the authors independently taking the help of signalling questions provided in the tool for assisting judgement. Disagreements, if any, were resolved by discussion.

Publication bias analysis

Publication bias analysis was carried out qualitatively on the basis of funnel plot asymmetry and quantitatively on the basis of Egger's intercept test using the JASP program. In the case of publication bias, the unbiased estimates were computed using Duval and Tweedie's Trim and Fill method using the JASP program.

Results

Design of the study and parameters measured

Literature search revealed that the effect of probiotics on postmenopausal bone health has been studied majorly on animal models, that is, mouse and rat. In contrast, there were only ten studies conducted on human subjects. After the removal of duplicates, we found a total of 135 hits in our initial search which included fifty-five research articles and the rest were clinical trials, books, review articles, systematic reviews and meta-analytical reviews. After screening the title and abstract of the articles according to our inclusion/exclusion criteria, we selected twenty-two research articles that were relevant to our meta-analytical study. Of these, eleven studies each were conducted on mice and rats. All the eleven studies on rats used the strain Sprague Dawley[24–34]. A total of 238 ovariectomised rats were used; 95 in control and 143 in experimental group. Among the eleven studies on mice, four studies used Balb/c mice[35–38], one used Institute of Cancer Research (ICR) mice[39], five used C57Bl/6 mice[16,40–43] and one used *ddy* mouse strain[44]. A total of 215 ovariectomised mice were

used, of which 88 were in control group and 127 in experimental group. Fifteen studies used *Lactobacillus* as the probiotic of choice[16,24–29,33–37,39–41], three used *Bifidobacterium*[31,38,43], and *Bacillus*[32] and *Saccharomyces*[44] were used by one study each. In one study, treatment groups included *Lactobacillus*, *Bacillus* or *Bifidobacterium* supplementation[30], and in another study, treatment groups included *Lactobacillus*, *Bifidobacterium* or a mixture of *Lactobacillus* and *Bifidobacterium*[42]. Probiotic supplementation to the animals was carried out in three different ways. In six studies, animals were treated with the probiotic in the form of its fermented product/extract[24,26–28,34,41]. In one study, diet of the animals was supplemented with the probiotic[44]. In the remaining fifteen studies, probiotics were orally gavaged to the animals[16,25,29–33,35–40,42,43]. The duration of the treatment varied from study to study, ranging from 4 weeks to 3 months. A flow diagram for the search, screening, eligibility and inclusion/exclusion of the studies is given in Fig. 1. Table 1 provides the general characteristics of the included studies, while Table 2 shows the parameters measured in each study for the meta-analysis.

Outcomes

Probiotics significantly increase bone mineral density of ovariectomised animals. To understand the effect of probiotics on the BMD, we analysed data from all the studies done on probiotics in the ovariectomy model irrespective of the type of bacteria. There were seventeen studies that had reported effect of probiotics on BMD. The studies were divided into five groups based on the type of bone and region included: femur total BMD[25,30], femur trabecular BMD[16,26–29,31,32,34,35,37,39–41], tibia trabecular BMD[24,32,37], tibia cortical BMD[24,32,37] and vertebral BMD[30,32,35,37,43]. A pooled analysis was conducted for all groups except tibia cortical BMD using a random effects model since there was high heterogeneity. Pooled analysis revealed that probiotic treatment favours an increase in BMD significantly at all the sites, with the highest effect seen in femur total BMD (SMD = 5·24, 95 % CI (3·04, 7·43); $P < 0·001$). The result is summarised in Fig. 2 and Table 3.

Probiotics significantly improve bone volume fractions in the bones of ovariectomised animals. Next, we investigated whether probiotics are able to improve bone health by increasing the bone volume in ovariectomised animals. Out of twenty-two selected studies, sixteen reported the effects of probiotics on bone volume. Again, we grouped the studies based on the type of bone: femur BV/TV[16,25–28,31,32,35–37,39–41,43], tibia BV/TV[24,36,37,43] and vertebra BV/TV[35–37,43,44]. We conducted a pooled effect analysis for the three groups using a random effects model and found that probiotic treatment significantly increased bone volume compared with the control (Fig. 3).

Table 3 shows the summary of the results of the pooled analysis conducted on BV/TV data. For femur BV/TV, the model yielded a SMD of 1·399 with a 95 % CI of (0·83, 1·96) and *P*-value of < 0·001. The I^2 statistic showed a high degree of heterogeneity (75·98 %, *P*-value < 0·001). For tibia BV/TV, the model yielded an SMD of 2·76 with a 95 % CI of (0·43, 5·09) and *P*-value of 0·02. The I^2 statistic showed a very high degree of heterogeneity (90·7 %, *P*-value < 0·001). For vertebral BV/TV, the model yielded an SMD of 2·26 with a 95 % CI of (0·75, 3·77) and *P*-value of 0·003. The I^2 statistic showed a high degree of heterogeneity (85·76 %, *P*-value = 0·001).

In summary, our findings demonstrate that probiotics significantly improve BV/TV in ovariectomised animals, with the most significant effects observed in the tibia and vertebra.

Probiotics show potential to increase serum bone formation markers. To further investigate the effect of probiotic treatment on bone formation, we examined the data from twenty-two selected articles that reported serum bone formation markers levels. Of these, six studies reported on serum osteocalcin[16,25,31,33,34,42], seven studies on serum Ca[16,25,27,30,31,41,42] and six studies on serum ALP[27,29,30,34,40,41]. Pooled effect analysis was carried out using random effects model due to sufficient heterogeneity among studies. Results showed that probiotic treatment favoured a trend towards increasing all three serum bone formation markers (Fig. 4). However, the results were not statistically significant. A summary of the results is depicted in Table 4.

Probiotics show potential to decrease bone resorption marker – serum C-terminal telopeptide levels. Of the twenty-two selected articles, we identified five studies that reported on serum CTX-1 levels after probiotic treatment[16,25,31,34,38]. Two of these were split to make two studies each[16,34], since they showed the effect of two different probiotic groups on CTX-1 levels. While five studies showed a decrease in serum CTX-1 levels[25,31,34,38] in the probiotic-treated group, two studies showed an increase[16]. Random effects model analysis revealed that overall, probiotic treatment tended to decrease serum CTX-1 levels, but the effect was not significant (Fig. 4, Table 4), possibly due to high heterogeneity ($I^2 = 93$ %).

Probiotic treatment reduced bone formation rate, but not significantly. Next, we aimed to investigate if treatment with probiotics had any effect on the BFR. We found only two studies[16,35] among the twenty-two selected studies that reported the impact of probiotics on BFR. The heterogeneity between these two studies was sufficiently low, and thus we conducted a meta-analysis using fixed effects model. Pooled effect analysis revealed a reduction in BFR upon probiotic treatment (SMD = −0·66). However, the effect was not statistically significant (CI (−1·34, 0·02); $P = 0·056$) (Fig. 5, Table 4).

Probiotic treatment shows a trend towards reduced osteoclast surface/bone surface, but not significantly. To investigate the effects of probiotics on bone-forming and bone-resorbing cells, specifically osteoblasts and osteoclasts, we conducted a literature search and identified two studies that reported the impact of probiotics on osteoblast surface (Ob.S/B.S)[25,31] and three studies that reported the impact on Oc.S/B.S[24,25,31]. However, due to high heterogeneity between the two studies, a meta-analysis could not be performed on Ob.S/B.S data[45]. Among the three studies reporting Oc.S/B.S data, two studies showed a decrease in osteoclast surface upon probiotic treatment[25,31], while one

Table 1. Characteristics of the included studies that reported impact of probiotic consumption on bone health in animal models

Author	Target species	Sample size	Control group	Experimental group	Probiotic species used	Probiotic dose	Duration
Parvaneh et al. 2019	Rat – Sprague Dawley	*n* 8/group	OVX + distilled water	OVX + *L. helveticus*	*Lactobacillus helveticus*	10^8–10^9 CFU daily	16 weeks
Narva et al. 2007	Rat – Sprague Dawley	*n* 10/group	OVX + 30 mg/l VPP peptide in water	OVX + *L. helveticus*	*Lactobacillus helveticus*		12 weeks
Shim et al. 2012	Rat – Sprague Dawley	*n* 8/group	OVX + 1 g/kg of HRT	OVX + L. casei fermented HRT (fHRT)	*Lactobacillus casei* KFRI – 127		12 weeks
Lee et al. 2019	Rat – Sprague Dawley	*n* 10/group	OVX + distilled water	OVX + ethanol extract of *Lactobacillus casei* fermented black rice	*Lactobacillus casei*		8 weeks
Britton et al. 2014	Mouse – Balb/c	*n* 8/group	OVX + MRS broth	OVX + *L. reuteri*	*Lactobacillus reuteri* 6475	300 ul of 10^9 CFU/ml thrice a week	4 weeks
Shim et al. 2013	Rat – Sprague Dawley	*n* 8/group	OVX + 0·3 g/kg HRT	OVX + 0·3 g/kg *L. curvatus* fermented HRT (fHRT)	*Lactobacillus curvatus* KFRI 166		12 weeks
Yu et al. 2022	Mouse – C57Bl/6	*n* 8/group	OVX + saline	OVX + *L. brevis*	*Lactobacillus brevis* AR281	10^9 CFU/ml daily	7 weeks
Sapra et al. 2021	Mouse – Balb/c	*n* 6/group	OVX + drinking water	OVX + *L. rhamnosus*	*Lactobacillus rhamnosus*	400 ul of 10^9 CFU/ml daily	6 weeks
Dar et al. 2018	Mouse – Balb/c	*n* 6/group	OVX + drinking water	OVX + *L. acidophilus*	*Lactobacillus acidophilus* ATCC 4356	200 ul of 10^9 CFU/ml daily	6 weeks
Lim et al. 2021	Rat – Sprague Dawley	*n* 10–11 / group	OVX + PBS	1. OVX + *L. intestinalis* 2. OVX + *L. intestinalis* YT2	1. *Lactobacillus intestinalis* type strain 2. *Lactobacillus intestinalis* YT2	10^9 CFU/ml thrice a week	18 weeks
Lee et al. 2021	Rat – Sprague Dawley	*n* 8/group	OVX + PBS	OVX + *L. gasseri*	*Lactobacillus gasseri*	100 ul of 10^{10} CFU/ml twice/d	14 weeks
Chiang et al. 2011	Mouse – C57BL/6J	*n* 8/group	OVX + soya skim milk	1. OVX + *L. paracasei* fermented soya skim milk 2. OVX + *L. plantarum* fermented soya skim milk	1. *Lactobacillus paracasei subsp. paracasei* NTU 101 2. *Lactobacillus plantarum* NTU 102		8 weeks
Ohlsson et al. 2014	Mouse – C57BL/6N	*n* 10/group	OVX + vehicle (tap water with glycerol)	1. OVX + *L. paracasei* 2. OVX + L. mix	1. *Lactobacillus paracasei* DSM13434 2. Mixture of *Lactobacillus paracasei* DSM13434, *Lactobacillus plantarum DSM 15 312 and Lactobacillus plantarum DSM 15 313*	10^9 CFU/ml in drinking water	6 weeks
Yang et al. 2020	Mouse – ICR	*n* 9/group	OVX + CMC	1. OVX + *L. plantarum* 2. OVX + *L. paracasei*	1. *Lactobacillus plantarum* GKM3 2. *Lactobacillus paracasei* GKS6	20·5 mg/kg	4 weeks
Lee et al. 2020	Rat – Sprague Dawley	*n* 8/group	OVX + skimmed milk product	1. OVX + *L. plantarum* fermented milk product 2. OVX + *L. fermentum* fermented milk product	1. *Lactobacillus plantarum A41* 2. *Lactobacillus fermentum SRK414*	10^{10} CFU/ml	8 weeks
H Y Dar et al. 2018	Rat – Sprague Dawley	*n* 10/group	OVX + drinking water	OVX + *Bacillus clausii*	*Bacillus clausii*	200 µl of 10^9 cfu/ml/d in drinking water	6 weeks
Parvaneh et al. 2015	Rat – Sprague Dawley	*n* 8/group	OVX + demineralised water	OVX + *B. longum*	*Bifidobacterium longum*	1 ml of 10^8–10^9 CFU/ml daily	16 weeks
Wallimann et al. 2021	Mouse – Balb/c	*n* 6/group	OVX + saline	OVX + *B. longum*	*Bifidobacterium longum* subsp. *longum* 35 624	10^9 CFU in 200 ul of 0·9 % saline five times a week	4 weeks

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Table 1. (*Continued*)

Author	Target species	Sample size	Control group	Experimental group	Probiotic species used	Probiotic dose	Duration
Sapra et al. 2022	Mouse – C57BL/6	*n* 6/group	OVX + drinking water	OVX + *B. longum*	*Bifidobacterium longum*	400 ul of 10^9 CFU in drinking water daily	6 weeks
Yamada et al. 2019	Mouse – ddY	*n* 10/group	OVX + chow diet	OVX + high polyamine yeast diet	*Saccharomyces cerevisiae* S631	Diet containing 5 % heat sterilized S. cerevisiae with high concentration of polyamine	28 d
Montazery – Najafabady et al. 2019	Rat – Sprague Dawley	*n* 7/group	OVX + saline	1. OVX + *L. acidophilus* 2. OVX + *L. reuteri* 3. OVX + *L. casei* 4. OVX + *B. coagulans* 5. OVX + *B. longum*	1. *Lactobacillus acidophilus* 2. *Lactobacillus reuteri* 3. *Lactobacillus casei* 4. *Bacillus coagulans* 5.*Bifidobacterium longum*	1 ml of 10^9 CFU/ml/d	4 weeks
Kim et al. 2019	Mouse – C57Bl/6	*n* 6/group	OVX + 1 % maltose	1. OVX + L. plantarum 2. OVX + B. longum 3. OVX + 1:1 Mixture of *L. plantarum* and *B. longum*	1. *Lactobacillus plantarum* 2.*Bifidobacterium longum*	10^9 CFU/mouse/d	4 weeks

OVX, ovariectomised; CFU, Colony Forming Unit; VPP, Valyl-Prolyl-Proline; HRT, Hwangryun-haedok-tang; KFRI, Korea Food Research Institute; CMC, carboxymethylcellulose.

showed an increase[24]. Pooled analysis results using random effects model showed that probiotic treatment favoured a decrease in Oc.S/B.S, but the effect was not significant (Fig. 5). A summary of the results is depicted in Table 4.

Comparative analysis of Lactobacillus *and* Bifidobacterium *probiotics on bone health.* From all the twenty-two articles selected for this meta-analysis, the majority of studies used either *Lactobacillus* or *Bifidobacterium*, which are also the major genera of gut microbiota. We therefore aimed to determine which of these probiotic genera was more effective in improving bone health. Thus, we segregated the studies based on these two probiotic genera and carried out meta-analysis. The parameters that we took into consideration were BMD, BV/TV and bone formation marker, that is, serum Ca.

Bone mineral density;. We found three articles reporting on vertebral BMD among those using *Lactobacillus* as the probiotic[30,35,37] and two articles using *Bifidobacterium* as the probiotic[30,43]. Unfortunately, due to a lack of sufficient studies or homogeneity, we were unable to include BMD of other regions such as femur trabecular, femur cortical and tibia trabecular. As usual, articles involving more than one species were split such that each study included only one species. Pooled effect analysis for BMD studies on *Lactobacillus* was carried out using random effects model and that on *Bifidobacterium* was carried out using fixed effects model. In both the cases, pooled analysis results showed that probiotics increased the BMD over control (Fig. 6). However, the effect size was larger for *Lactobacillus* (SMD = 2·643) compared with *Bifidobacterium* (SMD = 1·38). Results are summarised in Table 5.

Bone volume fractions. Femur BV/TV was reported by ten articles using *Lactobacillus*[16,25–28,35–37,39,41] and by two articles using *Bifidobacterium* as the probiotic[31,43]. Analysis on BV/TV of tibia and vertebra could not be conducted due to the insufficient number of studies using *Bifidobacterium*. Random effects model was used to carry out analysis on *Lactobacillus* group and fixed effects model was used on *Bifidobacterium* group. Similar to that of BMD data, here too pooled analysis results showed that both *Lactobacillus*- and *Bifidobacterium*-treated groups favoured an increase in BV/TV over control (Fig. 6). However, the effect size for *Lactobacillus* was larger than that for *Bifidobacterium*. Results are summarised in Table 5.

Bone formation marker. Among the previously selected articles, serum osteocalcin was reported in five articles using *Lactobacillus* species[16,25,33,34,42] and two articles using *Bifidobacterium* species[31,42]. However, due to high heterogeneity, meta-analysis could not be performed on *Bifidobacterium*. Similarly, serum ALP was reported in six articles using *Lactobacillus*[27,29,30,34,40,41] and only one article using *Bifidobacterium*[30], preventing a comparison of the two species.

Serum Ca was reported in six articles using *Lactobacillus* species[16,25,27,30,41,42]. These were split into separate studies for each species, resulting in a total of ten studies. Of these, six

Table 2. Bone-related parameters reported by the studies included in our meta-analysis

	BMD			BV/TV									
	Femur	Tibia	Vertebra	Femur	Tibia	Vertebra	BFR	Serum OCN	Serum ALP	Serum Ca	Serum CTX-1	Ob.S/B.S	Oc.S/B.S
Parvaneh 2019	✓			✓				✓		✓	✓	✓	✓
Narva 2007		✓			✓		✓						✓
Shim 2012	✓			✓									
Lee 2019	✓			✓					✓	✓			
Britton 2014	✓		✓	✓		✓	✓						
Shim 2013	✓			✓									
Yu 2022	✓			✓					✓				
Sapra 2021				✓	✓	✓							
Dar 2018	✓	✓	✓	✓	✓	✓							
Lim 2021	✓								✓				
Lee 2021								✓					
Chiang 2011	✓			✓					✓	✓			
Ohlsson 2014	✓			✓				✓		✓	✓		
Yang 2020	✓			✓									
Lee 2020	✓							✓	✓		✓		
H Y Dar 2018	✓	✓	✓	✓	✓	✓							
Parvaneh 2015	✓			✓				✓		✓	✓	✓	✓
Wallimann 2021											✓		
Sapra 2022			✓	✓	✓	✓							
Yamada 2019						✓	✓						✓
Montazery-Najafabady 2019	✓	✓	✓						✓	✓			
Kim 2019								✓		✓			

BMD, bone mineral density; BV/TV, bone volume fractions; BFR, bone formation rate; OCN, Osteocalcin; ALP, alkaline phosphatase; Ca, calcium; CTX-1, C-terminal telopeptide; Ob.S/B.S osteoblast surface by bone surface; Oc.S/B.S, osteoclast surface by bone surface.

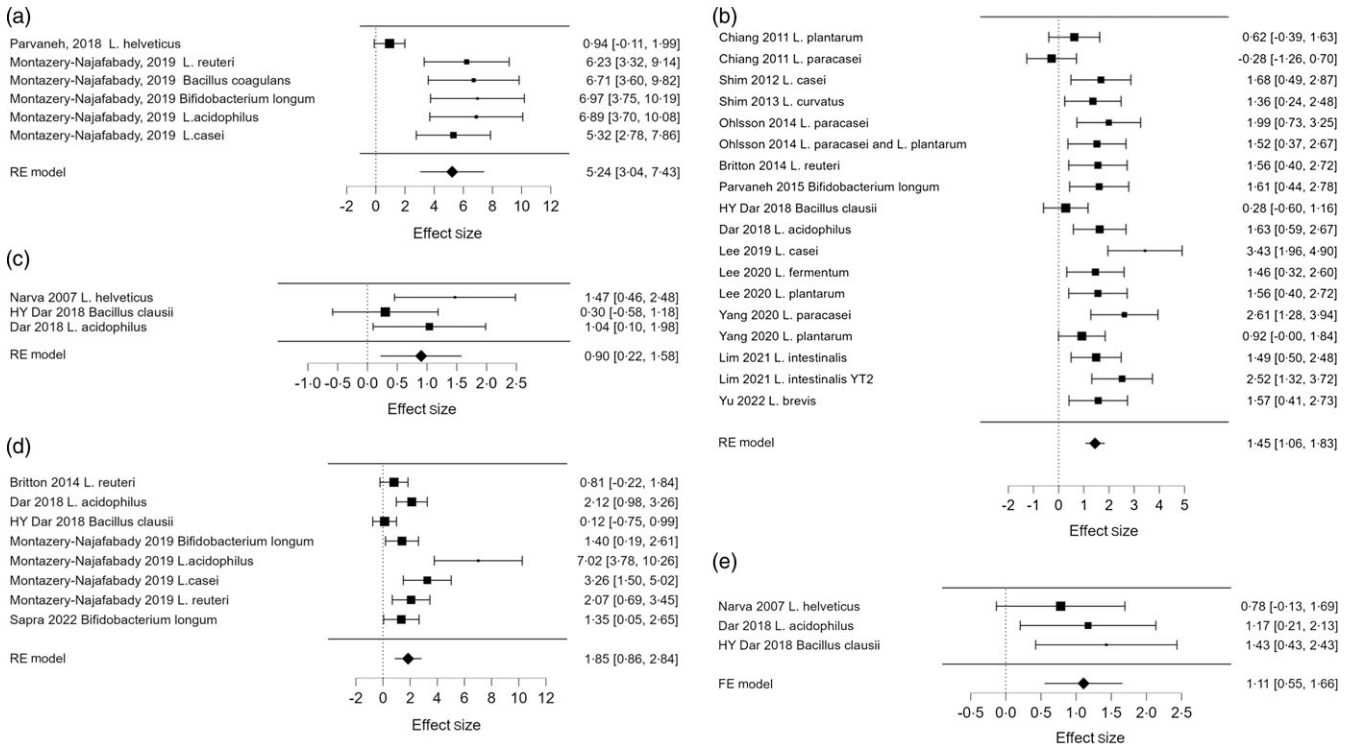

Fig. 2. Forest plot depicting pooled effect analysis on BMD of (a) femur total (b) femur trabecular (c) tibia trabecular (d) vertebra and (e) tibia cortical.

showed an increase while four showed a decrease in serum Ca upon treatment. In contrast, all three studies using *Bifidobacterium*[30,31,42] reported an increase in serum Ca after treatment. Pooled effect analysis was carried out on both of them using random effects model, and the results showed that both the probiotics had increased serum Ca over control (Fig. 6).

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Table 3. Summary of results of pooled analysis conducted on BMD and BV/TV data

Parameter	Model used	SMD	95 % CI and *P*	I^2 and *P*
1. BMD				
Femur total BMD	Random effects model	5·24	3·04, 7·43; $P<0·001$	79·03 %; $P<0·001$
Femur trabecular BMD	Random effects model	1·45	1·06, 1·83; $P<0·001$	52 %; $P<0·001$
Tibia trabecular BMD	Random effects model	0·9	0·22, 1·58; $P=0·009$	35 %; $P=0·2$
Tibia cortical BMD	Fixed effects model	1.11	0·55, 1·66; $P<0·001$	0 %; $P<0·001$
Vertebral BMD	Random effects model	1·85	0·86, 2·84; $P<0·001$	78·28 %; $P<0·001$
2. BV/TV				
Femur BV/TV	Random effects model	1·399	0·83, 1·96; $P<0·001$	75·98 %; $P<0·001$
Tibia BV/TV	Random effects model	2·76	0·43, 5·09; $P=0·02$	90·7 %; $P<0·001$
Vertebral BV/TV	Random effects model	2·26	0·75, 3·77; $P=0·003$	85·76 %; $P=0·001$

BMD, bone mineral density; BV/TV, bone volume fractions; SMD, standardised mean difference.

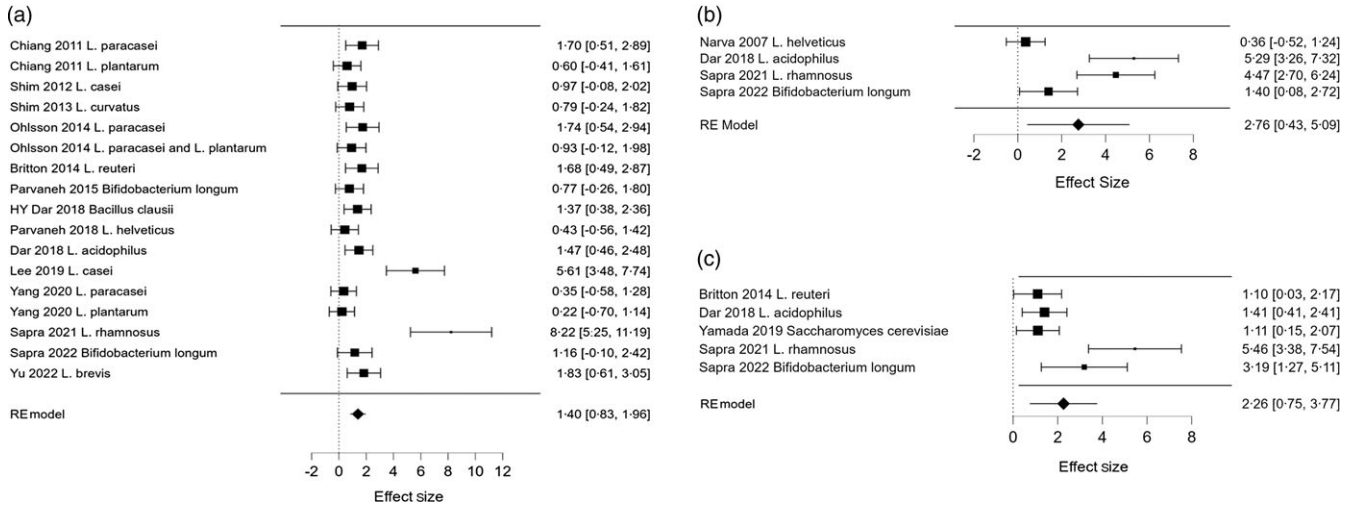

Fig. 3. Forest plot depicting pooled effect analysis on BV/TV of (a) femur (b) tibia and (c) vertebra.

However, the results were not statistically significant. A summary of the results is presented in Table 5.

Assessment of risk of bias

The twenty-two included studies were assessed for their risk of bias using the SYRCLE tool (online Supplementary Fig. 4). Briefly, none of the studies fulfilled all the ten criteria required for low risk of bias. Only one study accurately described the random sequence generation method used, and thus, the selection bias in the randomisation entry for all the other studies was judged as 'unclear risk'. The majority of the studies had similar baseline characteristics between control and experimental groups. The risk of bias was unclear for all the studies regarding allocation concealment, random housing and random outcome assessment. Blinding of outcome assessment was mentioned in only two studies and thus allocated a low-risk label. All the studies were allocated a low-risk label for attrition and reporting bias.

Publication bias

Publication bias was carried out qualitatively based on the funnel plot test and quantitatively based on Egger's test. We found significant publication bias for femur total BMD ($P<0·001$), femur trabecular BMD ($P<0·001$), vertebral BMD ($P<0·001$), BV/TV (femur, tibia and vertebral) ($P<0·001$), serum Ca ($P<0·001$) and Oc.S/B.S ($P=0·001$). We used Trim and Fill method to compute unbiased estimates for the above. However, the estimates for femur total BMD, vertebral BMD, femur BV/TV, vertebral BV/TV, serum Ca and Oc.S/B.S did not change after using Trim and Fill method. Adjusted estimates, using Trim and Fill method, were obtained for femur trabecular BMD (summary estimate: 1·14, 95 % CI 0·73, 1·55) and tibia BV/TV (summary estimate: 2·0, 95 % CI −0·37, 4·38). Publication bias was absent for tibia trabecular BMD, tibia cortical BMD, serum osteocalcin, serum CTX-1 and serum ALP. Funnel plots and *P*-value for Egger's test conducted are provided in the online Supplementary figures.

Discussion

Postmenopausal osteoporosis is a serious health issue in women, and it is a major public health concern worldwide. In recent years, the use of probiotics has gained popularity as a complementary therapy for various disorders. The regulation of bone homoeostasis by probiotics is proposed to be achieved through their immunomodulatory ability, which is mediated by

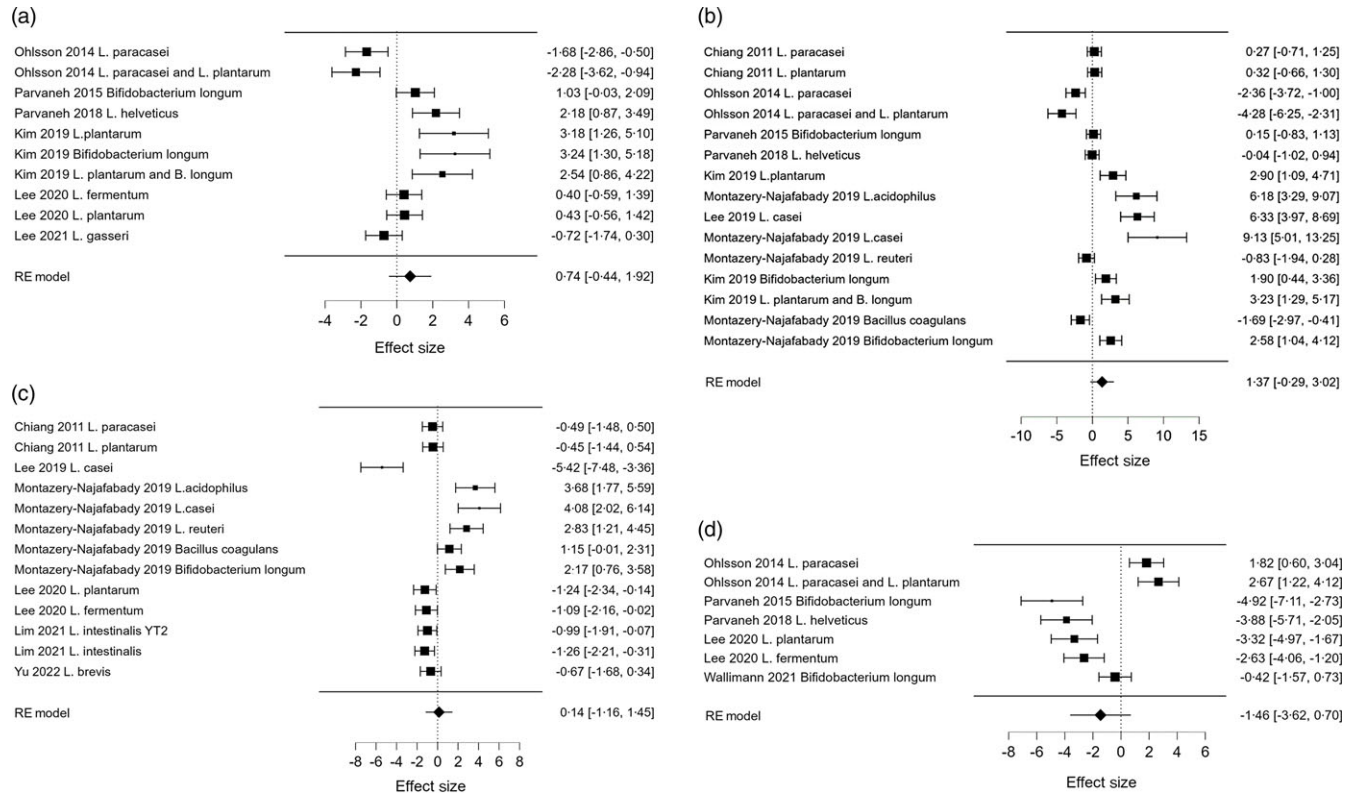

Fig. 4. Forest plot depicting pooled effect analysis on serum bone formation markers: (a) serum osteocalcin, (b) serum calcium, (c) serum ALP, and serum bone resorption marker: (d) serum CTX-1.

Table 4. Summary of the results of pooled analysis conducted on serum bone markers and histomorphometric parameters of bone turnover data

Parameter	Model used	SMD	95 % CI and *P*	I^2 and *P*
1. Serum bone markers				
Serum osteocalcin	Random effects	0·742	–0·44, 1·92; *P* = 0·218	88·68 %; *P* < 0·001
Serum Ca	Random effects	1·367	–0·29, 3·02; *P* = 0·106	95·24 %; *P* < 0·001
Serum ALP	Random effects	0·14	–1·16, 1·45; *P* = 0·832	93·45 %; *P* < 0·001
Serum CTX-1	Random effects	–1·46	–3·62, 0·70; *P* = 0·186	93·18 %; *P* < 0·001
2.Histomorphometric parameters				
BFR	Fixed effects	–0·66	–1·34, 0·02, *P* = 0·056	0 %; *P* = 0·852
Oc.S/B.S	Random effects	–1·0	–2·49, 0·49, *P* = 0·187	82·15 %, *P* = 0·006

SMD, standardised mean difference; ALP, alkaline phosphatase; CTX-1, C-terminal telopeptide; BFR, bone formation rate; Oc.S/B.S, osteoclast surface by bone surface.

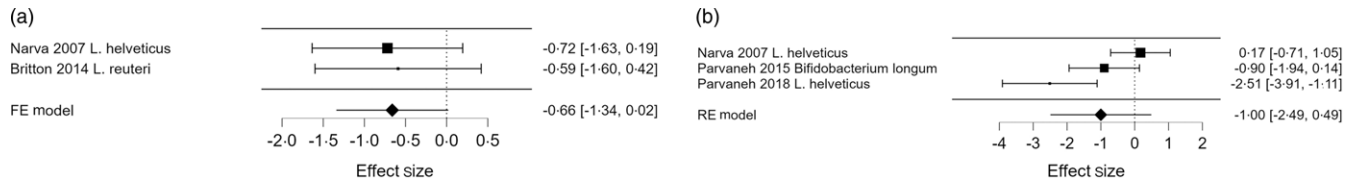

Fig. 5. Forest plot depicting pooled effect analysis on bone histomorphometric parameters: (a) BFR and (b) Oc.S/B.S.

the production of SCFA or the direct management of intestinal permeability[46]. SCFA have been shown to stimulate the expansion of regulatory T cells[47,48] that, along with CD8 + T cells, modulate the production of Wnt10b[49]. Wnt10b acts on stromal cells and osteoblasts to promote bone formation[50]. In addition to their role in regulating regulatory T cells, probiotics also help maintain the integrity of intestinal epithelial cells[51], which is critical for separating commensal bacteria from mucosal immune cells. In osteoporosis, the integrity of intestinal epithelial cells is often compromised[52], leading to the expansion of Th17 cells that produce osteoclastogenic inflammatory cytokines[53]. These cytokines promote the formation of bone-resorbing osteoclasts, leading to bone loss. Probiotics have been shown to improve epithelial barrier function and restrict the expansion

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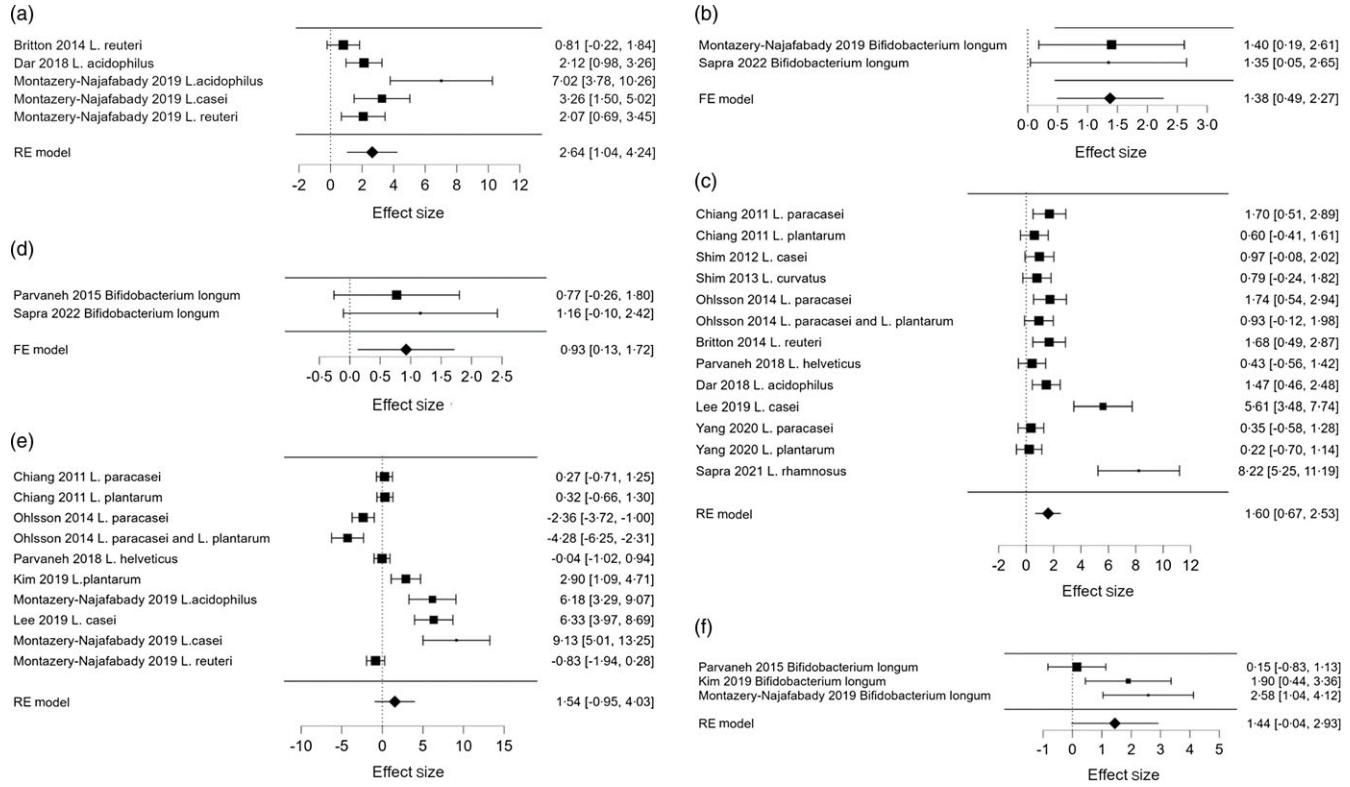

Fig. 6. Forest plot depicting comparative analysis of (a) vertebral BMD- using *Lactobacillus* (b) vertebral BMD- using *Bifidobacterium* (c) femur BV/TV- using *Lactobacillus* (d) femur BV/TV- using *Bifidobacterium* (e) serum calcium- using *Lactobacillus* and (f) serum calcium- using *Bifidobacterium.*

Table 5. Comparative analysis of *Lactobacillus* and *Bifidobacterium*

Parameter		*Lactobacillus*	*Bifidobacterium*
Vertebral BMD	Model used	Random effects	Fixed effects
	SMD	2·643	1·38
	95 % CI and *P*	1·04, 4·24; $P = 0·001$	0·49, 2·27; $P = 0·002$
	I^2 and *P*	82·85 %; $P = 0·003$	0 %; $P = 0·95$
Femur BV/TV	Model used	Random effects	Fixed effects
	SMD	1·604	0·925
	95 % CI and *P*	0·674, 2·535; $P < 0·001$	0·127, 1·724; $P = 0·023$
	I^2 and *P*	51·83 %; $P < 0·001$	0 %; $P = 0·639$
Serum Ca	Model used	Random effects	Random effects
	SMD	1·542	1·445
	95 % CI and *P*	–0·946, 4·03; $P = 0·224$	–0·041, 2·931; $P = 0·057$
	I^2 and *P*	96·7 %; $P < 0·001$	73·83 %; $P = 0·016$

BMD, bone mineral density; SMD, standardised mean difference; BV/TV, bone volume fractions.

of Th17 cells[54], thereby preventing the development of osteoclastogenic inflammation and bone loss.

In this meta-analysis, we aimed to investigate the effect of probiotics on postmenopausal bone health by analysing the available data from both animal and human studies. However, most of the available research are carried out on animal models, and the human studies, although conducted, are inadequate for meta-analysis. Therefore, we performed our meta-analysis with the studies in the preclinical models of postmenopausal osteoporosis. Ovariectomised rodents are a well-established and preferred experimental model for investigating postmenopausal osteoporosis, as it closely mimics the disease's characteristics. This preclinical model exhibits significantly reduced BMD and BV/TV in the femur, tibia and vertebra, as compared with control animals (sham-operated). Furthermore, ovariectomised animals demonstrate increased bone turnover, which can be evaluated by analysing bone cell activity through histology or by measuring serum biochemical markers of bone formation and resorption[55].

BMD is a measure of the amount of minerals contained in a certain volume of bone. About 50 % of trabecular and 30 % of cortical bone is lost by women during the course of their lifetime, about half of which is lost during the first 10 years after menopause[56,57]. Our meta-analysis found that probiotics

significantly increase BMD in ovariectomised animals at all sites except tibia cortical. The highest effect was observed in femur total BMD.

To address concerns about the potential for errors in BMD measurements due to high variability[58], we also examined the more reliable micro-CT data performed in animal models, specifically, BV/TV. BV/TV is defined as the volume of mineralised bone per unit volume of the sample and is a preferred measurement of bone quality, as it provides a more accurate representation of the actual bone volume. The pooled effect analysis using a random effects model showed that probiotic treatment significantly increases bone volume compared with the control in all the studied skeletal sites. The most significant effects were observed in the tibia and vertebra.

The currently available treatments for postmenopausal osteoporosis can be broadly classified into two categories: antiresorptives and anabolics. While antiresorptives inhibit bone resorption, anabolics increase bone formation, thereby enhancing bone mass[59]. To gain further insight into the mechanism of action of probiotics, we investigated their impact on the hallmarks of bone formation and resorption. To analyse the effect of probiotic treatment on bone formation, we examined data that reported serum bone formation marker levels like serum osteocalcin, Ca and ALP. Osteocalcin is a protein produced by osteoblasts during bone formation[60,61], Ca is an essential component of bone mineralisation[62] and ALP is an enzyme that plays a vital role in bone mineralisation[63], about 50 % of the total ALP activity in serum of normal healthy adults arises from bone[64]. A pooled effect analysis using a random effects model showed that probiotic treatment tended to increase all three serum bone formation markers, but the effects were not statistically significant.

Similarly, data were analysed to evaluate the effect of probiotics on bone resorption by analysing serum CTX-1 levels. CTX of fibrillar collagens such as collagen type I and type II are peptide fragments that are produced during bone resorption, and hence serum CTX-1 represents an important marker of the same[65]. The findings indicated that, while not statistically significant, probiotics treatment had a suppressing effect on bone resorption.

We further analysed data to evaluate the effect of probiotics on BFR, which is the amount of mineralised bone formed per unit time per unit volume of bone surface. BFR slows down in osteoporosis, and decreased BFR can lead to bone fragility and increased fractures[66–68]. Pooled effect analysis on the BFR data reported in two studies using fixed effects model revealed a decrease in BFR after probiotic treatment which was not statistically significant. However, accuracy of the result obtained might be affected due to involvement of only two studies.

Increased bone resorption by osteoclasts and inability of osteoblasts to make up for this bone loss leads to weakening of bone in osteoporosis[69–71]. Finally, we investigated whether probiotic treatment could decrease the osteoclast surface and identified three studies that reported Oc.S/B.S data. Pooled effect analysis showed a decrease in osteoclast surface over bone surface upon probiotic treatment. This was again statistically not significant.

Together, these results revealed that probiotic treatment has a positive effect on bone mass in postmenopausal animal models of osteoporosis, evidenced by increases in BMD and BV/TV in the femur, tibia and vertebrae. Additionally, probiotic treatment displayed a tendency towards increased serum bone formation markers and decreased bone resorption markers. It should be noted, however, that the limited number of studies reporting serum bone turnover markers and the high heterogeneity among the studies mean that these results should be interpreted with caution. Further research is necessary to validate the effects of probiotics on serum bone turnover markers.

Although the primary aim of this meta-analytical study was to assess the overall effect of probiotic supplementation on osteoporotic bone, we also checked if a particular probiotic genus produced a better overall effect over the other. The majority of studies selected for this meta-analysis used either *Lactobacillus* sp. or *Bifidobacterium* sp., which are the major genera of gut microbiota[72]. The unavailability of sufficient data limited our analysis to only BMD, BV/TV and serum Ca. The results of the analysis showed that both *Lactobacillus* and *Bifidobacterium* probiotics were effective in improving bone health, as seen in the increase in BMD and BV/TV over control. However, the effect size was larger for *Lactobacillus* compared with *Bifidobacterium*, indicating that *Lactobacillus* may be more effective in improving bone health. It is important to note that the number of studies reporting on *Bifidobacterium* is limited, and further studies are needed to confirm these findings.

With respect to serum Ca, both probiotics showed an increase, although not statistically significant. Notably, in studies using *Bifidobacterium*, all three reported an increase, while only six out of ten studies using *Lactobacillus* reported an increase. This suggests *Bifidobacterium* may be more effective in raising serum Ca levels. However, due to significant study heterogeneity, further research is required for confirmation.

Overall, our study has several strengths, including the exclusive focus on preclinical models of postmenopausal osteoporosis, which allowed us to obtain a comprehensive understanding of the effect of probiotics on postmenopausal bone loss. Moreover, we investigated multiple outcomes related to bone health, including BMD, BV/TV, BFR and serum bone turnover markers to improve our understanding of the effect of probiotics on osteoporotic bone. However, we acknowledge several limitations that should be considered. Firstly, most of the studies included in our analysis were conducted on animal models, which may limit the generalisability of our findings to humans. Secondly, the duration of treatment varied widely among the studies, which could have influenced our results. Thirdly, the heterogeneity of the studies included in our analysis was high, which could have affected the accuracy of our results. Finally, the number of studies reporting serum bone formation/resorption markers was limited, and our results should be interpreted with caution.

Future studies should focus on conducting randomised controlled trials on humans to further investigate the effect of probiotics on postmenopausal bone health. These studies should also aim to standardise the duration and dosage of probiotic treatment to obtain more accurate and reliable results.

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Additionally, future studies should aim to identify the specific strains of probiotics that are most effective in improving bone health and the mechanisms by which they exert their effects. This information would be useful in the development of probiotic-based therapies for the prevention and treatment of osteoporosis.

In conclusion, our meta-analysis suggests that probiotics have the potential to improve postmenopausal bone health in the preclinical models of the disease. Probiotic supplementation could be a simple and safe strategy for preventing or delaying the onset of osteoporosis in women after menopause. However, more studies are needed to confirm these findings and to identify the specific strains of probiotics and mechanisms involved in the observed effects.

Acknowledgements

This study was supported by funding from the Science and Engineering Research Board (SERB), Government of India (KS). Research fellowship from the Indian Council of Medical Research-Junior Research Fellowship (SB) is also acknowledged.

S. B.: Investigation, Data curation and Writing – original draft preparation. K. S.: Writing – review and editing, Supervision, and Study design.

Shibani Bose and Kunal Sharan declare that they have no conflict of interest.

The data that support the findings of this study are available from the corresponding author (KS) upon reasonable request.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114523002362

References

1. Brandi ML (2009) Microarchitecture, the key to bone quality. Rheumatology 48, iv3–8.
2. Kalpakcioglu BB, Morshed S, Engelke K, *et al.* (2008) Advanced imaging of bone macrostructure and microstructure in bone fragility and fracture repair. *J Bone Joint Surg Am* **90**, 68–78.
3. Neer RM, Arnaud CD, Zanchetta JR, *et al.* (2001) Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* **344**, 1434–1441.
4. Miller PD, Hattersley G, Riis BJ, *et al.* (2016) Effect of abaloparatide *v.* placebo on new vertebral fractures in postmenopausal women with osteoporosis: a randomized clinical trial. *JAMA* **316**, 722–733.
5. Behera J, Ison J, Tyagi SC, *et al.* (2020) The role of gut microbiota in bone homeostasis. *Bone* **135**, 115317.
6. Ding K, Hua F & Ding W (2020) Gut microbiome and osteoporosis. *Aging Dis* **11**, 438–447.
7. Rettedal EA, Ilesanmi-Oyelere BL, Roy NC, *et al.* (2021) The gut microbiome is altered in postmenopausal women with osteoporosis and osteopenia. *JBMR Plus* **5**, e10452.
8. Wang H, Liu J, Wu Z, *et al.* (2023) Gut microbiota signatures and fecal metabolites in postmenopausal women with osteoporosis. *Gut Pathog* **15**, 33.
9. Ling CW, Miao Z, Xiao ML, *et al.* (2021) The association of gut microbiota with osteoporosis is mediated by amino acid metabolism: multiomics in a large cohort. *J Clin Endocrinol Metab* **106**, e3852–e3864.
10. Ilesanmi-Oyelere BL, Roy NC & Kruger MC (2021) Modulation of bone and joint biomarkers, gut microbiota, and inflammation status by synbiotic supplementation and weight-bearing exercise: human study protocol for a randomized controlled trial. *JMIR Res Protoc* **10**, e30131.
11. Rodrigues FC, Castro AS, Rodrigues VC, *et al.* (2012) Yacon flour and bifidobacterium longum modulate bone health in rats. *J Med Food* **15**, 664–670.
12. Howarth GS & Wang H (2013) Role of endogenous microbiota, probiotics and their biological products in human health. *Nutrients* **5**, 58–81.
13. Clarke G, Stilling RM, Kennedy PJ, *et al.* (2014) Minireview: gut microbiota: the neglected endocrine organ. *Mol Endocrinol* **28**, 1221–1238.
14. Villa JKD, Diaz MAN, Pizziolo VR, *et al.* (2017) Effect of vitamin K in bone metabolism and vascular calcification: a review of mechanisms of action and evidences. *Crit Rev Food Sci Nutr* **57**, 3959–3970.
15. van Wijngaarden JP, Doets EL, Szczecińska A, *et al.* (2013) Vitamin B_{12}, folate, homocysteine, and bone health in adults and elderly people: a systematic review with meta-analyses. *J Nutr Metab* **2013**, 486186.
16. Ohlsson C, Engdahl C, Fåk F, *et al.* (2014) Probiotics protect mice from ovariectomy-induced cortical bone loss. *PLoS One* **9**, e92368.
17. McCabe LR, Irwin R, Schaefer L, *et al.* (2013) Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. *J Cell Physiol* **228**, 1793–1798.
18. Collins FL, Rios-Arce ND, Schepper JD, *et al.* (2019) Beneficial effects of Lactobacillus reuteri 6475 on bone density in male mice is dependent on lymphocytes. *Sci Rep* **9**, 14708.
19. Behera J, Ison J, Voor MJ, *et al.* (2021) Probiotics stimulate bone formation in obese mice via histone methylations. *Theranostics* **11**, 8605–8623.
20. Higgins JPT, Chandler J, Cumpston M, *et al.* (2019) *Cochrane Handbook for Systematic Reviews of Interventions*. Chichester, England: John Wiley & Sons.
21. Cochran WG (1954) The combination of estimates from different experiments. *Biom* **10**, 101–129.
22. Vesterinen HM, Sena ES, Egan KJ, *et al.* (2014) Meta-analysis of data from animal studies: a practical guide. *J Neurosci Methods* **221**, 92–102.
23. Hooijmans CR, Rovers MM, de Vries RB, *et al.* (2014) SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* **14**, 43.
24. Narva M, Rissanen J, Halleen J, *et al.* (2007) Effects of bioactive peptide, valyl-prolyl-proline (VPP), and lactobacillus helveticus fermented milk containing VPP on bone loss in ovariectomized rats. *Ann Nutr Metab* **51**, 65–74.
25. Parvaneh M, Karimi G, Jamaluddin R, *et al.* (2018) Lactobacillus helveticus (ATCC 27558) upregulates Runx2 and Bmp2 and modulates bone mineral density in ovariectomy-induced bone loss rats. *Clin Interv Aging* **13**, 1555–1564.
26. Shim KS, Kim T, Ha H, *et al.* (2012) Hwangryun-haedok-tang fermented with lactobacillus casei suppresses ovariectomy-induced bone loss. *Evid Based Complement Alternat Med* **2012**, 325791.

27. Lee YM, Kim IS & Lim BO (2019) Black rice (Oryza sativa L.) fermented with lactobacillus casei attenuates osteoclastogenesis and ovariectomy-induced osteoporosis. *Biomed Res Int* **2019**, 5073085.
28. Shim KS, Kim T, Ha H, *et al.* (2013) Lactobacillus fermentation enhances the inhibitory effect of Hwangryun-haedok-tang in an ovariectomy-induced bone loss. *BMC Complement Altern Med* **13**, 106.
29. Lim EY, Song EJ, Kim JG, *et al.* (2021) Lactobacillus intestinalis YT2 restores the gut microbiota and improves menopausal symptoms in ovariectomized rats. *Benef Microbes* **12**, 503–516.
30. Montazeri-Najafabady N, Ghasemi Y, Dabbaghmanesh MH, *et al.* (2019) Supportive role of probiotic strains in protecting rats from ovariectomy-induced cortical bone loss. *Probiotics Antimicrob Proteins* **11**, 1145–1154.
31. Parvaneh K, Ebrahimi M, Sabran MR, *et al.* (2015) Probiotics (Bifidobacterium longum) increase bone mass density and upregulate Sparc and Bmp-2 genes in rats with bone loss resulting from ovariectomy. *Biomed Res Int* **2015**, 897639.
32. Dar HY, Pal S, Shukla P, *et al.* (2018) Bacillus clausii inhibits bone loss by skewing Treg-Th17 cell equilibrium in postmenopausal osteoporotic mice model. *Nutrition* **54**, 118–128.
33. Lee S, Jung DH, Park M, *et al.* (2021) The effect of lactobacillus gasseri BNR17 on postmenopausal symptoms in ovariectomized rats. *J Microbiol Biotechnol* **31**, 1281–1287.
34. Lee CS, Kim JY, Kim BK, *et al.* (2021) Lactobacillus-fermented milk products attenuate bone loss in an experimental rat model of ovariectomy-induced post-menopausal primary osteoporosis. *J Appl Microbiol* **130**, 2041–2062.
35. Britton RA, Irwin R, Quach D, *et al.* (2014) Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell Physiol* **229**, 1822–1830.
36. Sapra L, Dar HY, Bhardwaj A, *et al.* (2021) Lactobacillus rhamnosus attenuates bone loss and maintains bone health by skewing Treg-Th17 cell balance in Ovx mice. *Sci Rep* **11**, 1807.
37. Dar HY, Shukla P, Mishra PK, *et al.* (2018) Lactobacillus acidophilus inhibits bone loss and increases bone heterogeneity in osteoporotic mice via modulating Treg-Th17 cell balance. *Bone Rep* **8**, 46–56.
38. Wallimann A, Hildebrand M, Groeger D, *et al.* (2021) An exopolysaccharide produced by bifidobacterium longum 35624® inhibits osteoclast formation via a TLR2-dependent mechanism. *Calcif Tissue Int* **108**, 654–666.
39. Yang LC, Lin SW, Li IC, *et al.* (2020) Lactobacillus plantarum GKM3 and Lactobacillus paracasei GKS6 supplementation ameliorates bone loss in ovariectomized mice by promoting osteoblast differentiation and inhibiting osteoclast formation. *Nutrients* **12**, 1914.
40. Yu J, Hang Y, Sun W, *et al.* (2022) Anti-osteoporotic effect of lactobacillus brevis AR281 in an ovariectomized mouse model mediated by inhibition of osteoclast differentiation. *Biol* **11**, 359.
41. Chiang SS & Pan TM (2011) Antiosteoporotic effects of Lactobacillus -fermented soy skim milk on bone mineral density and the microstructure of femoral bone in ovariectomized mice. *J Agric Food Chem* **59**, 7734–7742.
42. Kim DE, Kim JK, Han SK, *et al.* (2019) Lactobacillus plantarum NK3 and bifidobacterium longum NK49 alleviate bacterial vaginosis and osteoporosis in mice by suppressing NF-κB-linked TNF-α expression. *J Med Food* **22**, 1022–1031.
43. Sapra L, Shokeen N, Porwal K, *et al.* (2022) Bifidobacterium longum ameliorates ovariectomy-induced bone loss via enhancing anti-osteoclastogenic and immunomodulatory potential of regulatory B cells (Bregs). *Front Immunol* **13**, 875788.
44. Yamada T, Park G, Node J, *et al.* (2019) Daily intake of polyamine-rich Saccharomyces cerevisiae S631 prevents osteoclastic activation and bone loss in ovariectomized mice. *Food Sci Biotechnol* **28**, 1241–1245.
45. Ryan R (2016) Cochrane Consumers and Communication Group: Meta-Analysis. Cochrane Consumers and Communication Review Group. http://cccrg.cochrane.org (accessed July 2023).
46. Li C, Pi G & Li F (2021) The role of intestinal flora in the regulation of bone homeostasis. *Front Cell Infect Microbiol* **11**, 579323.
47. Davie JR (2003) Inhibition of histone deacetylase activity by butyrate. *J Nutr* **133**, 2485s–2493s.
48. Sanford JA, Zhang LJ, Williams MR, *et al.* (2016) Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks immune tolerance of the epidermis to TLR ligands. *Sci Immunol* **1**, eaah4609.
49. Tyagi AM, Yu M, Darby TM, *et al.* (2018) The microbial metabolite butyrate stimulates bone formation via T Regulatory cell-mediated regulation of WNT10B expression. Immunity 49, 1116–1131.e1117.
50. Bodine PV, Zhao W, Kharode YP, *et al.* (2004) The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. *Mol Endocrinol* **18**, 1222–1237.
51. Ohland CL & Macnaughton WK (2010) Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* **298**, G807–819.
52. Guan Z, Luo L, Liu S, *et al.* (2022) The role of depletion of gut microbiota in osteoporosis and osteoarthritis: a narrative review. *Front Endocrinol (Lausanne)* **13**, 847401.
53. Li L, Rao S, Cheng Y, *et al.* (2019) Microbial osteoporosis: the interplay between the gut microbiota and bones via host metabolism and immunity. *Microbiologyopen* **8**, e00810.
54. Tanabe S (2013) The effect of probiotics and gut microbiota on Th17 cells. *Int Rev Immunol* **32**, 511–525.
55. Turner AS (2001) Animal models of osteoporosis–necessity and limitations. *Eur Cell Mater* **1**, 66–81.
56. Finkelstein JS, Brockwell SE, Mehta V, *et al.* (2008) Bone mineral density changes during the menopause transition in a multiethnic cohort of women. *J Clin Endocrinol Metab* **93**, 861–868.
57. Riggs BL & Melton LJ 3rd (1992) The prevention and treatment of osteoporosis. *N Engl J Med* **327**, 620–627.
58. Nguyen TV, Sambrook PN & Eisman JA (1997) Sources of variability in bone mineral density measurements: implications for study design and analysis of bone loss. *J Bone Miner Res* **12**, 124–135.
59. Tu KN, Lie JD, Wan CKV, *et al.* (2018) Osteoporosis: a review of treatment options. *Pharm Therapeut* **43**, 92–104.
60. Singer FR & Eyre DR (2008) Using biochemical markers of bone turnover in clinical practice. *Cleve Clin J Med* **75**, 739–750.
61. Ivaska KK, Hentunen TA, Vääräniemi J, *et al.* (2004) Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption *in vitro*. *J Biol Chem* **279**, 18361–18369.
62. Walker HK, Hall WD & Hurst JW (1990) *Clinical Methods: The History, Physical, and Laboratory Examinations*. Boston: Butterworths. Copyright © 1990, Butterworth Publishers, a division of Reed Publishing.
63. Harris H (1990) The human alkaline phosphatases: what we know and what we don't know. *Clin Chim Acta* **186**, 133–150.
64. Green S, Anstiss CL & Fishman WH (1971) Automated differential isoenzyme analysis. II. The fractionation of serum alkaline phosphatases into 'liver', 'intestinal' and 'other' components. *Enzymologia* **41**, 9–26.

65. Risteli L & Risteli J (1993) Biochemical markers of bone metabolism. *Ann Med* **25**, 385–393.
66. Malluche HH, Davenport DL, Lima F, *et al.* (2022) Prevalence of low bone formation in untreated patients with osteoporosis. *PLoS One* **17**, e0271555.
67. Whyte MP, Bergfeld MA, Murphy WA, *et al.* (1982) Postmenopausal osteoporosis. A heterogeneous disorder as assessed by histomorphometric analysis of Iliac crest bone from untreated patients. *Am J Med* **72**, 193–202.
68. Arlot ME, Delmas PD, Chappard D, *et al.* (1990) Trabecular and endocortical bone remodeling in postmenopausal osteoporosis: comparison with normal postmenopausal women. *Osteoporos Int* **1**, 41–49.
69. Udagawa N, Takahashi N, Jimi E, *et al.* (1999) Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. *Bone* **25**, 517–523.
70. Fujiwara Y, Piemontese M, Liu Y, *et al.* (2016) RANKL (Receptor activator of NFκB ligand) produced by osteocytes is required for the increase in B cells and bone loss caused by estrogen deficiency in mice. *J Biol Chem* **291**, 24838–24850.
71. Xiong J, Piemontese M, Onal M, *et al.* (2015) Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. *PLoS One* **10**, e0138189.
72. Tap J, Mondot S, Levenez F, *et al.* (2009) Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* **11**, 2574–2584.

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