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# Effect of probiotic supplementation in murines with induced osteoporosis: systematic review with meta-analysis

## Efeito da suplementação de probiótico em murinos com osteoporose induzida: revisão sistemática com meta-análise

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## ABSTRACT

Postmenopausal osteoporosis results in low bone mineral density, interference in bone microarchitecture, decreased bone strength, and an increased risk of fragility fractures. This systematic review aimed to increase the level of evidence on supplementation with bacteria of the genus Bifidobacterium in induced osteoporosis in murines, rats, and ovariectomized female mice (OVX). The search was initially conducted using the PubMed, Embase, ScienceDirect, Scopus, Web of Science, and Scielo databases in 2024, using the keywords “osteoporosis”, “murine”, “ovariectomized”, “OVX”, and “Bifidobacterium”. In total, 89 full articles, abstracts, or book chapters were found and after detailed screening, 5 studies met the inclusion criteria, using 66 animals, randomly divided between control and treatment groups. The results demonstrated that oral supplementation with bacteria of the genus Bifidobacterium significantly improves parameters indicative of bone health BMD, BMC, BV/TV%, and Tb.Sp. In conclusion, this meta-analysis provides evidence that bacteria of the genus Bifidobacterium are involved in anti-osteoporotic mechanisms in female OVX rats and mice, as a preclinical model, and suggests the need for further studies in postmenopausal women as a therapeutic alternative or complementary therapy to conventional osteoporosis treatments.

**Keywords:** ; Bone; Bifidobacterium; Ovariectomized; Rats; Mice.

## RESUMO

A osteoporose pós-menopausa resulta em baixa densidade mineral óssea, interferência na microarquitetura óssea, diminuição da resistência óssea e aumento do risco de fraturas por fragilidade. Esta revisão sistemática teve como objetivo aumentar o nível de evidência sobre a suplementação com bactérias do gênero Bifidobacterium na osteoporose induzida em murinos, ratos e camundongos fêmeas ovariectomizados (OVX). A busca foi realizada inicialmente nas bases de dados PubMed, Embase, ScienceDirect, Scopus, Web of Science e Scielo em 2024, utilizando as palavras-chave "osteoporosis", "murine", "ovariectomized", "OVX" e "Bifidobacterium". No total, foram encontrados 89 artigos completos, resumos ou capítulos de livros e após triagem detalhada, 5 estudos atenderam aos critérios de inclusão, utilizando 66 animais, divididos aleatoriamente entre grupos controle e tratamento. Os resultados demonstraram que a suplementação oral com bactérias do gênero Bifidobacterium melhora significativamente os parâmetros indicativos de saúde óssea DMO, CMO, BV/TV% e Tb.Sp. Em conclusão, esta meta-análise fornece evidências de que bactérias do gênero Bifidobacterium estão envolvidas em mecanismos anti-osteoporóticos em ratas e camundongos OVX fêmeas, como modelo pré-clínico, e sugere a necessidade de mais estudos em mulheres na pós-menopausa como alternativa terapêutica ou complementar terapêutica aos tratamentos convencionais da osteoporose.


**Palavras-chave:** Osso; Bifidobacterium; Ovariectomizado; Ratos; Camundongos.

## INTRODUCTION

Osteoporosis, from the Greek term "porous bone", is a common bone disease, affecting millions of people around the world. Although risk factors for osteoporosis vary depending on sex and age, osteoporotic fractures can result in substantial morbidity and mortality in both men and women (MITEK et al., 2019; RADOMINSKI et al., 2017). It is a chronic disease that affects the physical, emotional, and mental well-being of one in three women worldwide, and one in five men over the age of 50. According to the WHO (World Health Organization), this disease is defined as having a bone density of less than 2.5 SD (standard deviation) compared to an average healthy population of the same age and sex (DAMANI et al., 2022; KEEN; REDDIVARI, 2023; SALARI et al., 2021; TREVISAN et al., 2020).

The primary endocrine factors involved in the development of osteoporosis are the parathyroid hormone (PTH), vitamin D, calcitonin, and estrogen. PTH and vitamin D are directly connected: PTH can increase calcium absorption through the kidneys, bones, and intestine; promote the activity of osteoclasts; and activate vitamin D to form calcitriol, promoting intestinal calcium absorption. The roles of PTH and vitamin D are opposite to those of calcitonin, which binds to its receptor to reversibly block osteoclast function, thereby blocking bone resorption. Estrogen can also block bone resorption through interaction with tissue-specific receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ), to increase osteoclast apoptosis; a decrease in estrogen production in postmenopausal women is one reason why this population has a higher incidence of osteoporosis (ROONEY et al., 2023).

A growing body of preclinical and clinical literature shows that microorganisms, described as probiotics, are essential for improving intestinal absorption of calcium and other minerals and also for improving skeletal health (MCCABE; BRITTON; PARAMESWARAN, 2015; WEAVER, 2015). *Bifidobacteria* are probiotics commonly found throughout the colon of humans and animals, that are considered normal residents of the gastrointestinal tract (NIELSEN et al., 2003). These microbes are anaerobic, nonpathogenic, gram-positive, non-spore-forming, pleomorphic, and catalase-negative (ZACARÍAS et al., 2020). Different species and/or strains of bifidobacteria may have different beneficial effects on health, such as regulation of intestinal microbial homeostasis, repression of pro-carcinogenic enzymatic activities in the microbiota, production of vitamins, and bioconversion of various dietary compounds into bioactive

molecules (CHUGH; KAMAL-ELDIN, 2020). Most current evidence suggests the beneficial impact of oral probiotic supplementation during periods of rapid adolescent growth and also life stages characterized by greater bone mineral loss and osteoporotic fracture risk (DE SIRE et al., 2022; JIA et al., 2021; MCCABE; BRITTON; PARAMESWARAN, 2015).

In animal models, results demonstrated that oral probiotic supplementation is a safe and effective alternative to prevent bone loss and increase bone density in healthy or moderately inflamed individuals, as well as protecting against primary (estrogen deficiency) and secondary osteoporosis (COLLINS et al., 2017). However, due to different types of probiotic microorganisms, supplemented orally in isolation or in combination, these results need to be compiled and, if possible, secondary statistical analysis needs to be carried out, as preclinical models, to increase the level of evidence in the use of bacteria of the genus *Bifidobacterium* in the treatment of osteoporosis in ovariectomized murine females, and thus encourage the performance of randomized clinical studies (RCTs).

## METHODOLOGY

The systematic review to carry out the meta-analysis of this work was conducted in accordance with the recommendations of CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) and registered on the OSF platform (Open Science Framework) (https://osf.io/nrqja). For the report, the guidelines described in the Preferred Report Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed (MOHER et al., 2015).

### Search Strategy

The data and articles used in the systematic review were obtained during the month of January 2023, and the search strategy was repeated in April of the same year. The searches were carried out in the electronic scientific databases “Embase”, “PubMed”, “Scielo”, “ScienceDirect”, “Scopus”, and “Web of Science”, available on the Internet. For the searches, the keywords “osteoporosis”, “murine”, “ovariectomized”, “OVX”, and “Bifidobacterium” were used, which are accepted as Health Sciences Descriptors (DeCS) and Medical Subject Headings (MeSH). These terms were used together in English and Portuguese. To make the research more

comprehensive, at this stage, all results obtained were analyzed, without restrictions on dates, language of publication or category, including complete articles, abstracts, and book chapters.

Inclusion and Exclusion Criteria for Study Selection

The works that contained the keywords were selected and analyzed independently by two researchers from the group, and in case of disagreement, a third author was consulted. Duplicate records and works that were not related to the proposed objectives were manually excluded after analyzing the titles and/or abstracts. Complete works that studied the following P.I.C.O. were selected: (i) Population: ovariectomized rats (OVX); (ii) Intervention: supplementation with bacteria of the genus *Bifidobacterium*; (iii) Control: OVX female mice not supplemented in the diet with bacteria of the genus *Bifidobacterium*; (iv) Outcomes: evaluation of osteoporosis indicator parameters. Clinical trials in other species, pilot studies, review articles, and works that did not address the objective of this meta-analysis were excluded.

Data extraction

A spreadsheet was created with data from each study selected in the previous stage: author(s)/year of publication, *Bifidobacterium* used, duration of the experiment, species and number of animals used, and parameters analyzed (Table 1).

Parameters of Interest

We defined the trabecular fraction of the bone volume of the femur (BV/TV%), trabecular thickness of the femur (Tb.Th), trabecular spacing of the femur (Tb.Sp), bone mineral density of the trabecular portion of the femur (BMD), femoral bone mineral content (BMC), serum calcium (Ca) concentration, and serum phosphorus (P) concentration, as parameters of primary relevance for the objectives of this meta-analysis.

Quality of Studies and Risk of Bias

The methodological quality of individual studies was assessed by 2 authors, independently, based on the Collaborative Approach to Meta-analysis and Review of Animal Data from Experimental Studies checklists (MACLEOD et al., 2015) and the Risk of Bias Assessment Tools and Other Methodological Criteria for Published Animal

Studies (KRAUTH; WOODRUFF; BERO, 2013). These guidelines assess quality using the following criteria: (1) sample size calculation; (2) random allocation to treatment; (3) rearing conditions (e.g., breeding program, light/dark cycle, temperature, food type, access to water, and environmental enrichment); (4) blind evaluation of results; (5) compliance with animal welfare regulations; (6) disclosure of conflicts of interest; and (7) peer-reviewed publication. The quality scale varies from 0 to 7 points. A third reviewer resolved any differences between the 2 reviewers.

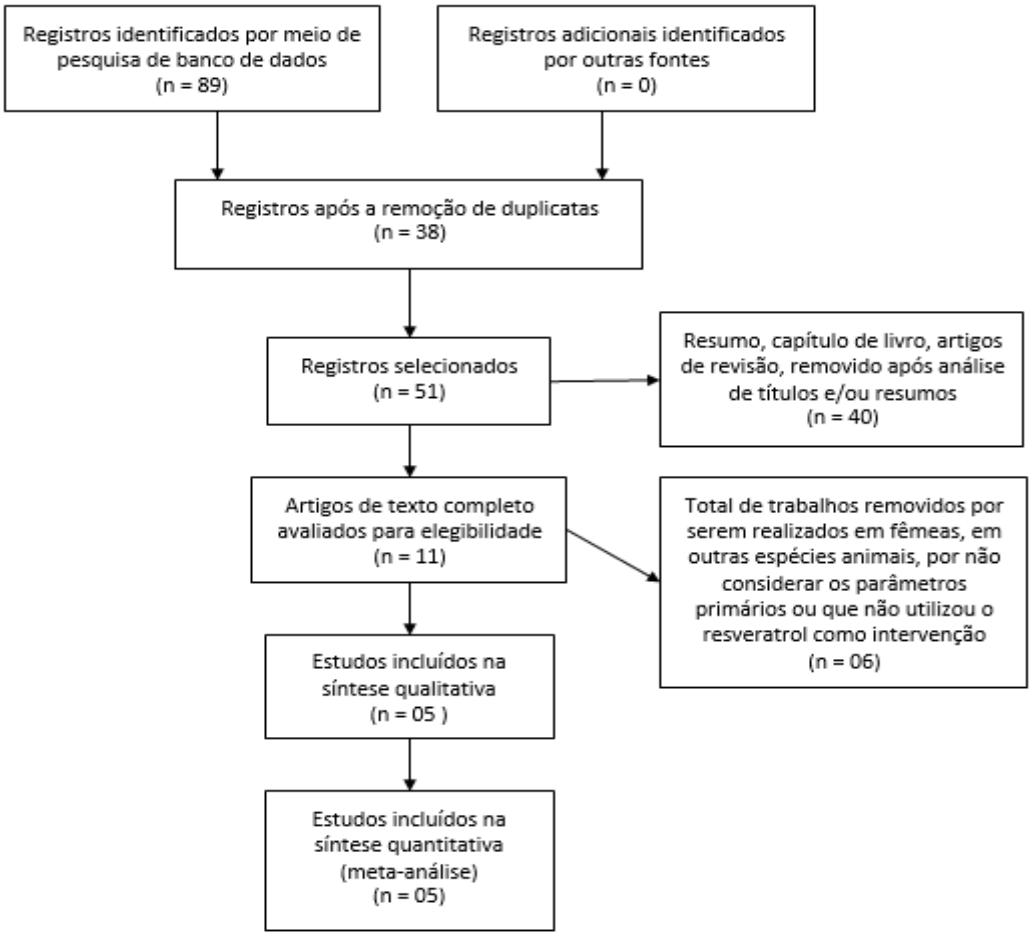
Statistical Analysis

We used RevMan 5.3 software to perform the meta-analysis calculations. For the analysis of the inverse variance, we used the means and standard deviations of the results of each study to compare the data found. Heterogeneity was calculated using the $Q^2$ and $I^2$ test, significance was defined at $p<0.10$ or $I^2>50\%$. Fixed effects of treatments were analyzed, however when we observed significant heterogeneity between studies, we analyzed random effects. BMD values were standardized using the Hedges adjustment method and implemented with RevMan 5.3. A value equal to or less than 5% was considered statistically significant.

## RESULTS

Initially, 89 complete articles, abstracts, review articles, or book chapters were found that contained the keywords used in the bibliographic search. Of these, after independent screening of titles and abstracts by two researchers, 11 were potentially chosen for further evaluation and, following complete evaluation of the works, 05 experimental studies were selected, including one study that used female mice (SAPRA et al., 2022) and four others that used rats (JIA et al., 2021; MONTAZERI-NAJAFABADY et al., 2019, 2021; PARVANEH et al., 2015) and which met the inclusion criteria. The process of literature selection, study selection, and reasons for exclusion are shown in the PRISMA flow diagram (Figure 1).

**Figure 1** - PRISMA flow diagram showing the process of literature selection, study selection, and reasons for exclusion.




Source: authors

Characteristics of the studies included in the meta-analysis

The selected works studied the effects of treatment with different strains of *Bifidobacterium* on indicators of osteoporosis induced in OVX female mice. The doses used and the compositions are described in Table 1.

The duration of the studies included in the meta-analyses varied between 28 and 112 days and included a total of 54 ovariectomized female rats and 12 female mice, divided between control and treatment groups. The studies were carried out in the South and Southeast Asia and Middle East regions and published from 2015 to 2022.

The studies and the summary of the characteristics of the studies included in the meta-analysis are presented in Table 1. The results of the parameters chosen for analysis were expressed clearly, in such a way that they allowed the safe extraction of values to be meta-analyzed (Table 2).

**Table 1 -** Composition of *Bifidobacterium* and doses used in the studies

| Author/ Year | Probiotic | Dose | Composition |
|---|---|---|---|
| PARVANEH et al. (2015) | *Bifidobacterium longum* ATCC 15707 | 1 mL ($10^8$–$10^9$ CFU/mL/day) | *Bifidobacterium longum* activated in Broth (De Man Rogosa and Sharpe, Difco, Detroit, MI, USA) modified with 0.02% sodium carbonate, 0.01% calcium chloride dehydration, and 1% L-cysteine solution 0.05%. |
| MONTAZERI-NAJAFABADY et al. (2019) | *Bifidobacterium* | 1 mL ($10^9$ CFU/mL/day) | Total of 20 traditional fermented yogurt samples, produced on the northern coast of the Persian Gulf, were used to isolate *Bifidobacterium* spp from lactic acid. |
| JIA et al., (2021) | *Bifidobacterium longum BL986* | 1 mL ($10^7$ CFU/mL/day) | Commercial *Bifidobacterium longum BL986*. |
| MONTAZERI-NAJAFABADY et al. (2021) | *Bifidobacterium* | 1 mL ($10^9$ CFU/ml/day) | Total of 20 traditional fermented yogurt samples, produced on the northern coast of the Persian Gulf, were used to isolate *Bifidobacterium* spp from lactic acid |
| SAPRA et al. (2022) | *Bifidobacterium longum* UBBL-64 | 400 µL ($10^9$ CFU/day) | *Bifidobacterium longum* UBBL-64 (M1395) grown in Bifido broth containing 0.05% L-cysteine under anaerobic conditions. |

Source: authors.

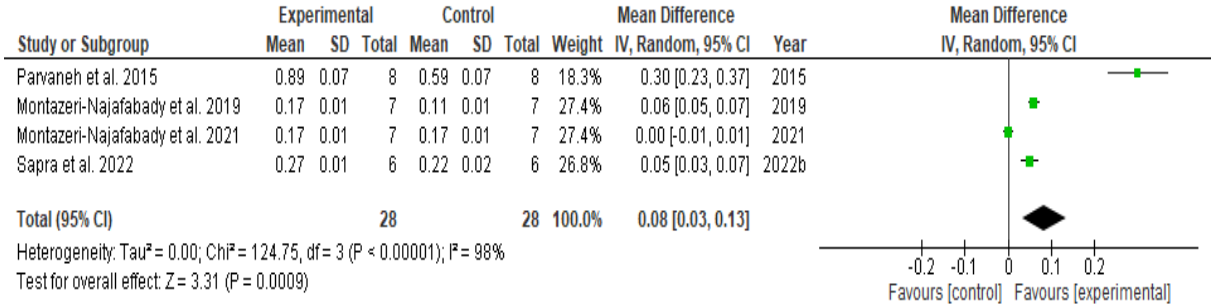
**Table 2 -** Characteristics of the studies included in the meta-analysis

| Authors/ Year | Experiment duration (days) | Species/sex | Animals per group | Meta-analyzed parameters |
|---|---|---|---|---|
| PARVANEH et al. (2015) | 112 | Sprague-Dawley rat/female | 8 | BMD, BV/TV%, Tb.Th, Tb.Sp, and Ca. |
| MONTAZERI-NAJAFABADY et al. (2019) | 28 | Sprague-Dawley rat/female | 7 | BMD, BMC, Ca, and P. |
| JIA et al., (2021) | 30 | Sprague-Dawley pathogen free rat (SPF)/ female | 5 | BV/TV%, Tb.Sp |
| MONTAZERI-NAJAFABADY et al. (2021) | 28 | Sprague-Dawley rat/female | 7 | BMD, BMC, Ca, P. |
| SAPRA et al. (2022) | 42 | C57BL/6 J pathogen free (SPF) mouse/ female | 6 | BMD, BV/TV%, Tb.Th, and Tb.Sp. |

Meta-analyzed parameters: Bone mineral density of the femoral portion (BMD); Femoral bone mineral content (BMC); Femoral bone volume fraction (BV/TV%); Trabecular thickness of the femur (Tb.Th); Trabecular spacing of the femur (Tb.Sp); Serum calcium concentration (Ca); and Serum phosphorus concentration (P). Source: authors

The meta-analysis of the results for trabecular bone mineral density, BMD, demonstrates that *Bifidobacterium longum* ATCC 15707, *Bifidobacterium longum* UBBL-64, and *Bifidobacterium* spp. are able to improve this parameter significantly (P=0.00009), when compared to the control groups (Figure 2).

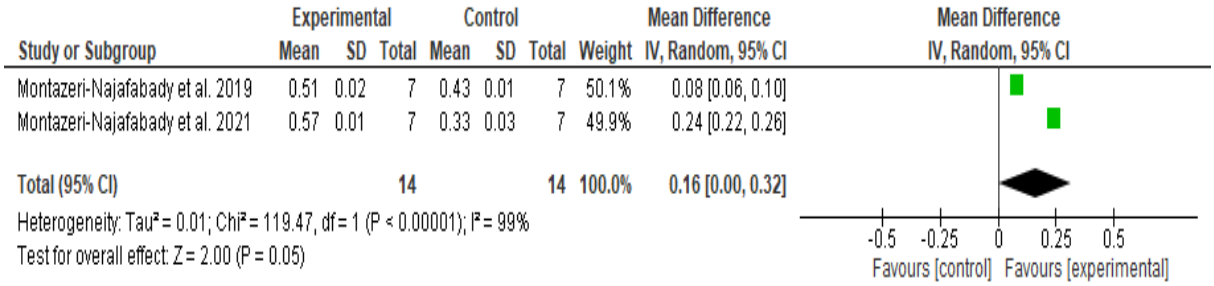
**Figure 2 -** Forest plot for the effect of consumption of bacteria of the genus *Bifidobacterium* on bone mineral density of the trabecular portion of the femur (BMD), expressed as mean differences between the intervention and control diets.




Source: authors

The meta-analysis of the results for femoral bone mineral content, BMC, demonstrates that *Bifidobacterium* spp. is able to improve this parameter significantly (P≤0.05), when compared to the control groups (Figure 3).

**Figure 3 -** Forest plot for the effect of consumption of bacteria of the genus Bifidobacterium on (a) levels of femoral bone mineral content, BMC, expressed as mean differences between the intervention and control diets.

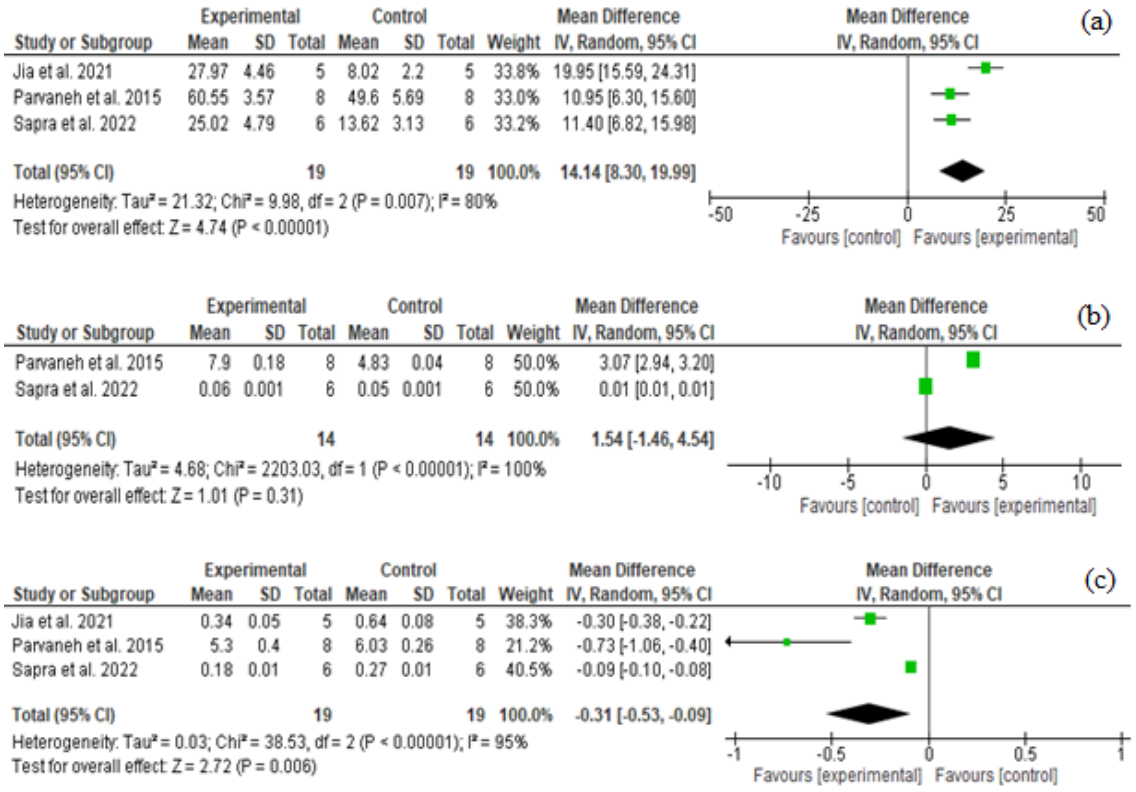



Source: authors

The meta-analysis of results for BV/TV% demonstrates that the probiotics *Bifidobacterium longum* ATCC 15707 and *Bifidobacterium longum* UBBL-64 are able to improve this parameter significantly (P<0.00001), when compared to the control groups (Figure 4a). The meta-analysis of the results for Tb.Th demonstrates that neither

*Bifidobacterium longum* ATCC 15707 nor *Bifidobacterium longum* UBBL-64 prebiotics improved this parameter (P=0.31) (Figure 4b). The statistical results that evaluated trabecular spacing in the femur (Tb.Sp) after interventions with *Bifidobacterium longum* ATCC 15707 and *Bifidobacterium longum* UBBL-64 revealed a significant difference (P=0.06) when compared to the control group (Figure 4c).

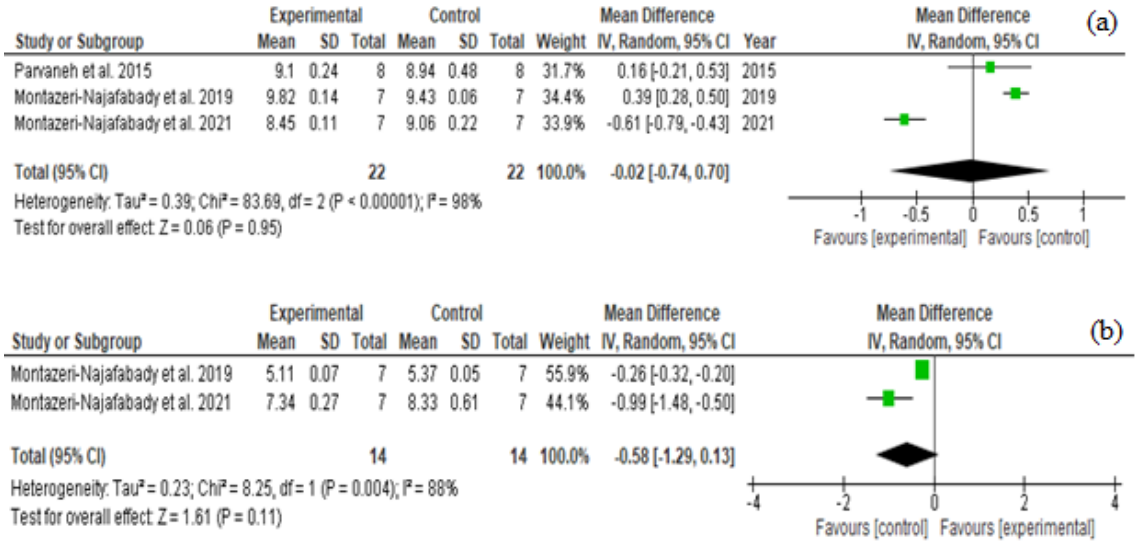
**Figure 4 -** Forest plots for the effects of consumption of bacteria of the genus *Bifidobacterium* on: (a) trabecular femur bone volume fraction (BV/TV%); (b) trabecular femoral thickness (Tb.Th); and (c) femoral trabecular spacing (Tb.Sp), expressed as mean differences between the intervention and control diets.




Source: authors

In the meta-analysis of the results related to Ca, it was observed that the rats in the groups supplemented with *Bifidobacterium* spp. did not demonstrate significant improvement (P=0.95) compared to the control groups during the experimental period (Figure 5a). Rats supplemented with *Bifidobacterium* spp. also did not demonstrate significant improvement in serum phosphorus concentration (P=0.11) compared to the control groups during the experimental period (Figure 5b).

**Figure 5 -** Forest plots for the effects of consumption of bacteria of the genus *Bifidobacterium* on (a) serum calcium levels and (b) serum phosphorus levels, expressed as mean differences between the intervention and control diets.




Source: authors

Study and Risk of Bias

The results of our risk of bias assessment of the five studies included in this systematic review are presented in Table 3. None of the studies described performing a sample size calculation and blind assessment of results. Only one study did not describe having carried out random allocation to treatment (JIA et al., 2021). All studies described having maintained adequate breeding conditions (e.g., breeding program, light/dark cycle, temperature, type of food, access to water, and environmental enrichment), and compliance with animal welfare regulations, and cited having disclosed possible conflicts of interest and peer-reviewed publication. The studies presented low risk of bias in most methodological criteria and achieved the desired quality.

## DISCUSSION

Osteoporosis is a systemic bone disease characterized by reduced bone mass and deterioration of bone microarchitecture, leading to bone fragility and an increased risk of fractures (SŁUPSKI; JAWIEŃ; NOWAK, 2021). In women, bones are smaller and thinner and during menopause there is a drop in estrogen levels, which triggers rapid acceleration of bone loss, beginning at least one year before menopause and continuing

for another three years, before slowing down slightly, and which can last for up to eight subsequent years. Even in the postmenopausal phase, this condition of bone loss may persist, as demonstrated through monitoring of bone loss rates and bone resorption markers (NOVRIADI et al., 2023).

**Table 3.** Evaluation criteria for study selection.

| Study | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| PARVANEH et al. (2015) | N | Y | Y | N | Y | Y | Y |
| MONTAZERI-NAJAFABADY et al. (2019) | N | Y | Y | N | Y | Y | Y |
| JIA et al., (2021) | N | N | Y | N | Y | Y | Y |
| MONTAZERI-NAJAFABADY et al. (2021) | N | Y | Y | N | Y | Y | Y |
| SAPRA et al. (2022) | N | Y | Y | N | Y | Y | Y |

Risk of bias: #, YES; ∗, NO. Risk of bias assessment questions: (1) sample size calculation; (2) random allocation to treatment; (3) rearing conditions (e.g., breeding program, light/dark cycle, temperature, food type, access to water, and environmental enrichment); (4) blind evaluation of results; (5) compliance with animal welfare regulations; (6) disclosure of conflicts of interest; and (7) peer-reviewed publication. Source: authors

In this sense, a range of studies have been performed with preclinical models to better understand the pathophysiology of osteoporosis, enabling analysis of clinical conditions and observation of opportunistic reuse of existing compounds, which have contributed to the development of medicines. However, the available treatments do not reduce the occurrence of fractures, which may also be related to the incorrect prescription and use of medications by patients, to the detriment of possible side effects and a long period of treatment (KHOSLA; HOFBAUER, 2017).

Despite advances, a concerning gap in patient care remains (COMPSTON; MCCLUNG; LESLIE, 2019), as conventional treatments with risedronate or bisphosphonates reserved for people over 60 years of age have demonstrated side effects that compromise therapeutic adherence, such as gastrointestinal lesions in the upper portion of the tract and esophageal irritation and dyspepsia (ARANTES; SILVA;

LAZARETTI-CASTRO, 2010; NOVRIADI et al., 2023; YAMAMOTO et al., 2019). After treatment with the monoclonal antibody, denosumab, patients presented hypocalcemia, resulting from the decrease in the absorption potential generated by medication with this mineral. Furthermore, patients may develop angioedema, erythema multiforme, dermatitis, rash, and urticaria and an important barrier to this treatment is discontinuation, which should not be abrupt due to the potential to generate bone injuries (COMPSTON; MCCLUNG; LESLIE, 2019; TSVETOV et al., 2020). In addition, recommended treatments based on hormone replacement have been subject to the occurrence of adverse effects, mainly cardiovascular complications and cancer induction (higher incidences of breast and uterus) (AIBAR-ALMAZÁN et al., 2022; GROUP ON HORMONAL FACTORS IN BREAST CANCER, 2019). With the same aim, diets with high doses of calcium have been shown to increase the supply of this mineral, however, they have important limitations, such as an increased risk of heart and kidney disease (HAMILTON; TERENTYEV, 2019; MADISON; KIECOLT-GLASER, 2019).

Preclinical models that reproduce the pathophysiological effects of postmenopausal osteoporosis in humans adopt the use of ovariectomized mice as a validated experimental model (KALU, 1991), because after surgical intervention, the results demonstrated a reduction in diaphyseal bone mineral density and impairment in biomechanical properties, when compared to control animals (ARTONI DE CARVALHO et al., 2023). Thus, the use of animals, female OVX rats and mice, is a widely used experimental model for evaluating the effects of supplementation with functional foods, probiotics and prebiotics, as an alternative therapy to those traditionally used for maintaining bone health, in studies that analyzed parameters indicative of osteoporosis (ARTONI DE CARVALHO et al., 2023; JIA et al., 2021; MONTAZERI-NAJAFABADY et al., 2019, 2021; PARVANEH et al., 2015; SAPRA et al., 2022).

The beneficial results observed in studies using probiotics are due to the ability of this dietary supplement to reduce the occurrence of senescence-induced osteoporosis, prevent bone loss induced by OVX, inhibit the differentiation of osteoclast precursor cells *in vitro*, and accelerate the remodeling of the callosal cartilage of the fracture (LEGETTE et al., 2012; LYU et al., 2023).

In this meta-analysis, the results increased the level of evidence that oral supplementation with bacteria of the genus *Bifidobacterium* is able to significantly

improve the BV/TV%, BMD, and BMC parameters and that these are related to the composition, structure, organization, and mechanical resistance of the bone tissue of the femur and are determining factors in the occurrence of fractures (HELGASON et al., 2008; MATHEY et al., 2007). These positive effects on BMD and BMC reiterate the pro-regenerative relationship, mainly in preserving the barrier and intestinal microbiota (ROBERTS et al., 2020, 2023). This genus of bacteria in the intestinal microbiota stimulates the production of short-chain fatty acids (SCFA), which reduce luminal pH and increase calcium solubility and transcellular absorption, mainly in the distal colon (DAVIS et al., 2020; MONTAZERI-NAJAFABADY et al., 2021). In addition, it has the ability to positively regulate the secreted protein acidic and rich in cysteine (SPARC), a glycoprotein that encodes a non-collagenous protein necessary in the bone extracellular matrix for the calcification of collagen in bone, and a Ca-binding receptor. Thus, this genus is associated with remodeling, repair, tissue development, cell renewal, and bone mineralization, resulting in stronger bones (PARVANEH et al., 2015; RIBEIRO et al., 2014) and bone morphogenetic protein 2 (BMP2) and playing an important role in regulating the microenvironment of the bone marrow matrix and promoting the osteogenic differentiation of mesenchymal stem cells that are involved in signaling pathways associated with the organization of the extracellular matrix, differentiation of osteoblasts, ossification, bone development, chondrocyte differentiation, and bone morphogenesis (CAI et al., 2020).

A growing body of data demonstrates that estrogen deficiency, during menopause in women and induced in an animal model by ovariectomy, leads to alterations in the intestinal microbiota, which may contribute to the development of osteoporosis resulting from increased bone remodeling and decreased bone formation in each remodeling unit, resulting in a loss of bone mass (IBÁÑEZ et al., 2019; LI et al., 2016, 2020; PARVANEH et al., 2015; XU et al., 2017). The administration of probiotics can have favorable effects, delaying the process (BEHERA et al., 2020; LI et al., 2016).

Excessive recovery is a risk factor for fracture, which may be important in small bones (HEANEY, 2006; MADISON; KIECOLT-GLASER, 2019) and supplementation of probiotics from the genus Bifidobacterium were shown to inhibit bone remodeling, through the ability to modulate osteoclastogenesis in terms of the differentiation of Tregs, Bregs, and Th17 cells, a fundamental role of the “Breg – Treg – Th17” cellular axis in postmenopausal osteoporotic mouse models, however, the metabolites involved

in the “immunoporotic” potential need to be elucidated (SAPRA et al., 2022; SRIVASTAVA et al., 2022).

Osteoporosis is accompanied by changes in the microstructure of bone tissue, such as reductions in BV/TV% and Tb.Th and an increase in Tb.Sp, as well as total porosity and risk of bone fracture. Meta-analyses highlighted these characteristics of osteoporosis in the OVX and non-probiotic supplemented groups, as well as in others not included in this systematic review (ABDUL-MAJEED; MOHAMED; SOELAIMAN, 2015; JIA et al., 2021; PARVANEH et al., 2015; SAPRA et al., 2022). *B. longum* supplementation demonstrated a beneficial effect on trabecular microstructure and BV/TV, the main determinants of bone fracture and bone strength, and that have a positive relationship with bone loss (LEGRAND et al., 2000). Furthermore, supplementation decreased Tb.Sp and total porosity of the femur and, in parallel, increased BV/TV and led to an improvement in femoral BMD. Thus, Tb.Sp as well as BV/TV are some of the risk predictors of bone fracture and osteoporosis, which were beneficially modulated by *B. longum* supplementation. Similar results regarding changes in trabecular microstructure due to probiotic consumption have also been reported by other studies (SHIM et al., 2013).

Meta-analyses of serum calcium and phosphorus values revealed no difference between the treated and control groups and unlimited oral intake of these minerals met the animals' basal nutritional needs. Although the ingestion of probiotic microorganisms has a beneficial effect, due to the concentrations having a wide range of serum values, the results did not show a significant effect, which was also observed in humans (KEMI; KÄRKKÄINEN; LAMBERG-ALLARDT, 2006).

Assessment of bias

In this systematic review with meta-analysis, after a rigorous selection process, studies were included because they presented rigorous methodology and were well described. Randomization was considered an important methodological requirement, however, studies that did not mention the sequence of randomization and allocation concealment were included when we understood that the animals used in the experiments presented homogeneity in weight, sex, stabilization time after OVX, and route of administration of the supplement. The consequence of this consideration was the possibility of including conflicting results, which in our opinion increases the level of evidence of the result. Heterogeneous results between studies may be due to several

factors, including differences in the duration of treatment, method of administration of *Bifidobacterium*, and age of the animals at the beginning of the experimental period (LEGETTE et al., 2012).

## CONCLUSION

The results of this study allow us to conclude that oral supplementation of bacteria of the genus *Bifidobacterium* improves bone mineral density and bone mineral content of the femur in ovariectomized rats, in a preclinical model. Furthermore, the results revealed significant beneficial effects on the bone volume fraction of the trabecular portion of the femur and trabecular spacing of the femur and a beneficial effect on the trabecular spacing of the femur. However, due to the low evidence found, the current meta-analysis suggests that further studies are necessary, using probiotic bacteria from this genus of bacteria, in order to increase the level of evidence, enabling these probiotics to be used as a complementary therapy to conventional treatments, or even as a new therapeutic approach for postmenopausal osteoporosis in women.

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## REFERENCES

ABDUL-MAJEED, S.; MOHAMED, N.; SOELAIMAN, I.-N. The use of delta-tocotrienol and lovastatin for anti-osteoporotic therapy. **Life Sciences**, v. 125, p. 42–48, 15 mar. 2015.

AIBAR-ALMAZÁN, A. et al. Current Status of the Diagnosis and Management of Osteoporosis. **International Journal of Molecular Sciences**, v. 23, n. 16, p. 9465, 21 ago. 2022.

ARANTES, H. P.; SILVA, A. G. DA; LAZARETTI-CASTRO, M. Bisphosphonates in the treatment of metabolic bone diseases. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 54, n. 2, p. 206–212, mar. 2010.

ARTONI DE CARVALHO, J. A. et al. Prebiotics improve osteoporosis indicators in a

preclinical model: systematic review with meta-analysis. **Nutrition Reviews**, v. 81, n. 8, p. 891–903, 10 jul. 2023.

BEHERA, J. et al. The role of gut microbiota in bone homeostasis. **Bone**, v. 135, p. 115317, 1 jun. 2020.

CAI, H. et al. BMP2 induces hMSC osteogenesis and matrix remodeling. **Molecular Medicine Reports**, v. 23, n. 2, p. 125, 8 dez. 2020.

CHUGH, B.; KAMAL-ELDIN, A. Bioactive compounds produced by probiotics in food products. **Current Opinion in Food Science**, v. 32, p. 76–82, 1 abr. 2020.

COLLINS, F. L. et al. The Potential of Probiotics as a Therapy for Osteoporosis. In: BRITTON, R. A.; CANI, P. D. (Eds.). . **Bugs as Drugs**. [s.l.] NIH Public Access, 2017. v. 5p. 213–233.

COMPSTON, J. E.; MCCLUNG, M. R.; LESLIE, W. D. Osteoporosis. **The Lancet**, v. 393, n. 10169, p. 364–376, jan. 2019.

DAMANI, J. J. et al. The Role of Prunes in Modulating Inflammatory Pathways to Improve Bone Health in Postmenopausal Women. **Advances in Nutrition**, v. 13, n. 5, p. 1476–1492, 2 set. 2022.

DAVIS, E. C. et al. Microbiome Composition in Pediatric Populations from Birth to Adolescence: Impact of Diet and Prebiotic and Probiotic Interventions. **Digestive Diseases and Sciences**, v. 65, n. 3, p. 706–722, 31 mar. 2020.

DE SIRE, A. et al. Role of Dietary Supplements and Probiotics in Modulating Microbiota and Bone Health: The Gut-Bone Axis. **Cells**, v. 11, n. 4, p. 743, 21 fev. 2022.

GROUP ON HORMONAL FACTORS IN BREAST CANCER, C. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. **The Lancet**, v. 394, n. 10204, p. 1159–1168, 28 set. 2019.

HAMILTON, S.; TERENTYEV, D. Altered Intracellular Calcium Homeostasis and Arrhythmogenesis in the Aged Heart. **International Journal of Molecular Sciences**, v. 20, n. 10, p. 2386, 14 maio 2019.

HEANEY, R. P. Calcium intake and disease prevention. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 50, n. 4, p. 685–693, ago. 2006.

HELGASON, B. et al. Mathematical relationships between bone density and mechanical properties: A literature review. **Clinical Biomechanics**, v. 23, n. 2, p. 135–146, fev. 2008.

IBÁÑEZ, L. et al. Gut microbiome and bone. **Joint Bone Spine**, v. 86, n. 1, p. 43–47, 1 jan. 2019.

JIA, L. et al. Probiotics ameliorate alveolar bone loss by regulating gut microbiota. **Cell Proliferation**, v. 54, n. 7, 7 jul. 2021.

KALU, D. N. The ovariectomized rat model of postmenopausal bone loss. **Bone and mineral**, v. 15, n. 3, p. 175–91, dez. 1991.

KEEN, M. U.; REDDIVARI, A. K. R. **Osteoporosis In Females**. [s.l.] StatPearls Publishing, 2023.

KEMI, V. E.; KÄRKKÄINEN, M. U. M.; LAMBERG-ALLARDT, C. J. E. High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. **British Journal of Nutrition**, v. 96, n. 3, p. 545–552, 17 set. 2006.

KHOSLA, S.; HOFBAUER, L. C. Osteoporosis treatment: recent developments and ongoing challenges. **The Lancet Diabetes & Endocrinology**, v. 5, n. 11, p. 898–907, nov. 2017.

KRAUTH, D.; WOODRUFF, T. J.; BERO, L. Instruments for Assessing Risk of Bias and Other Methodological Criteria of Published Animal Studies: A Systematic Review. **Environmental Health Perspectives**, v. 121, n. 9, p. 985–992, set. 2013.

LEGETTE, L. L. et al. Prebiotics Enhance Magnesium Absorption and Inulin-based Fibers Exert Chronic Effects on Calcium Utilization in a Postmenopausal Rodent Model. **Journal of Food Science**, v. 77, n. 4, p. 88–94, abr. 2012.

LEGRAND, E. et al. Trabecular Bone Microarchitecture, Bone Mineral Density, and Vertebral Fractures in Male Osteoporosis. **Journal of Bone and Mineral Research**, v. 15, n. 1, p. 13–19, 1 jan. 2000.

LI, J.-Y. J.-Y. et al. Sex steroid deficiency–associated bone loss is microbiota dependent and prevented by probiotics. **Journal of Clinical Investigation**, v. 126, n. 6, p. 2049–2063, 25 abr. 2016.

LI, S. et al. Gut microbiome and osteoporosis. **Bone & Joint Research**, v. 9, n. 8, p. 524–530, 1 ago. 2020.

LYU, Z. et al. Modulation of bone remodeling by the gut microbiota: a new therapy for osteoporosis. **Bone Research**, v. 11, n. 1, p. 31, 9 jun. 2023.

MACLEOD, M. R. et al. Risk of Bias in Reports of In Vivo Research: A Focus for Improvement. **PLOS Biology**, v. 13, n. 10, p. e1002273, 13 out. 2015.

MADISON, A.; KIECOLT-GLASER, J. K. Stress, depression, diet, and the gut microbiota: human–bacteria interactions at the core of psychoneuroimmunology and nutrition. **Current Opinion in Behavioral Sciences**, v. 28, n. 2, p. 105–110, ago. 2019.

MATHEY, J. et al. Modulation of soy isoflavones bioavailability and subsequent effects on bone health in ovariectomized rats: the case for equol. **Osteoporosis International**, v. 18, n. 5, p. 671–679, 29 mar. 2007.

MCCABE, L.; BRITTON, R. A.; PARAMESWARAN, N. Prebiotic and Probiotic Regulation of Bone Health: Role of the Intestine and its Microbiome. **Current Osteoporosis Reports**, v. 13, n. 6, p. 363–371, 30 dez. 2015.

MITEK, T. et al. Genetic Predisposition for Osteoporosis and Fractures in Postmenopausal Women. In: **Advances in experimental medicine and biology**. [s.l.] Adv Exp Med Biol, 2019. v. 1211p. 17–24.

MOHER, D. et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. **Systematic Reviews**, v. 4, n. 1, p. 1, 1 dez. 2015.

MONTAZERI-NAJAFABADY, N. et al. Supportive Role of Probiotic Strains in Protecting Rats from Ovariectomy-Induced Cortical Bone Loss. **Probiotics and Antimicrobial Proteins**, v. 11, n. 4, p. 1145–1154, 17 dez. 2019.

MONTAZERI-NAJAFABADY, N. et al. Exploring the bone sparing effects of postbiotics in the post-menopausal rat model. **BMC Complementary Medicine and Therapies**, v. 21, n. 1, p. 155, 28 dez. 2021.

NIELSEN, D. S. et al. Case Study of the Distribution of Mucosa-Associated Bifidobacterium Species, Lactobacillus Species, andOther Lactic Acid Bacteria in the HumanColon. **Applied and Environmental Microbiology**, v. 69, n. 12, p. 7545–7548, dez. 2003.

NOVRIADI, R. et al. Well-defined multispecies probiotic and enzyme combination outperforms traditional fermented probiotic applications in an intensive Pacific white shrimp, Litopenaeus vannamei (Boone, 1931), culture system. **Journal of the World Aquaculture Society**, v. 54, n. 1, p. 156–166, 24 fev. 2023.

PARVANEH, K. et al. Probiotics ( Bifidobacterium longum ) Increase Bone Mass Density and Upregulate Sparc and Bmp-2 Genes in Rats with Bone Loss Resulting from Ovariectomy. **BioMed Research International**, v. 2015, p. 1–10, 2015.

RADOMINSKI, S. C. et al. Diretrizes brasileiras para o diagnóstico e tratamento da osteoporose em mulheres na pós-menopausa. **Revista Brasileira de Reumatologia**, v. 57, p. 452–466, 2017.

RIBEIRO, N. et al. Role of SPARC in Bone Remodeling and Cancer-Related Bone Metastasis. **Journal of Cellular Biochemistry**, v. 115, n. 1, p. 17–26, 14 jan. 2014.

ROBERTS, J. L. et al. Bifidobacterium adolescentis supplementation attenuates fracture-induced systemic sequelae. **Biomedicine & Pharmacotherapy**, v. 132, p. 110831, dez. 2020.

ROBERTS, J. L. et al. Bifidobacterium longum supplementation improves age-related delays in fracture repair. **Aging Cell**, v. 22, n. 4, p. 1–17, 27 abr. 2023.

ROONEY, A. M. et al. PTH Treatment Increases Cortical Bone Mass More in Response

to Compression than Tension in Mice. **Journal of Bone and Mineral Research**, v. 38, n. 1, p. 59–69, 12 jan. 2023.

SALARI, N. et al. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. **Journal of Orthopaedic Surgery and Research**, v. 16, n. 1, p. 609, 17 out. 2021.

SAPRA, L. et al. Bifidobacterium longum Ameliorates Ovariectomy-Induced Bone Loss via Enhancing Anti-Osteoclastogenic and Immunomodulatory Potential of Regulatory B Cells (Bregs). **Frontiers in Immunology**, v. 13, 25 maio 2022.

SHIM, K.-S. et al. Lactobacillus fermentation enhances the inhibitory effect of Hwangryun-haedok-tang in an ovariectomy-induced bone loss. **BMC Complementary and Alternative Medicine**, v. 13, n. 1, p. 106, 16 dez. 2013.

SŁUPSKI, W.; JAWIEŃ, P.; NOWAK, B. Botanicals in Postmenopausal Osteoporosis. **Nutrients**, v. 13, n. 5, p. 1609, 11 maio 2021.

SRIVASTAVA, R. K. et al. World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (WCO-IOF-ESCEO 2022). **Aging Clinical and Experimental Research**, v. 34, n. S1, p. 35–474, 26 ago. 2022.

TREVISAN, C. et al. The Impact of Smoking on Bone Metabolism, Bone Mineral Density and Vertebral Fractures in Postmenopausal Women. **Journal of Clinical Densitometry**, v. 23, n. 3, p. 381–389, 1 jul. 2020.

TSVETOV, G. et al. Denosumab-induced hypocalcemia in patients with osteoporosis: can you know who will get low? **Osteoporosis International**, v. 31, n. 4, p. 655–665, 14 abr. 2020.

WEAVER, C. M. Parallels Between Nutrition and Physical Activity: Research Questions in Development of Peak Bone Mass. **Research Quarterly for Exercise and Sport**, v. 86, n. 2, p. 103–106, 3 abr. 2015.

XU, X. et al. Intestinal microbiota: a potential target for the treatment of postmenopausal osteoporosis. **Bone Research**, v. 5, n. 1, p. 17046, 4 out. 2017.

YAMAMOTO, K. et al. Symptoms and Upper Gastrointestinal Mucosal Injury Associated with Bisphosphonate Therapy. **Internal Medicine**, v. 58, n. 8, p. 1049–1056, 15 abr. 2019.

ZACARÍAS, M. F. et al. Effects of conventional and nonconventional drying on the stability of Bifidobacterium animalis subsp. lactis INL1. **International Journal of Dairy Technology**, v. 73, n. 3, p. 625–633, 9 ago. 2020.