Physiology International 113 (2026) 2, 183–208
DOI: 10.1556/2060.2026.00710

1828
AKADÉMIAI KIADÓ

Effects of exercise on bone health parameters in ovariectomized rats: A systematic review and meta-analysis

RINI MAYA PUSPITA[1,3]*, DENNY AGUSTININGSIH[2] and NUNUK PURWANTI[3]

[1] Doctoral Program in Medical and Health Sciences, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Special Region of Yogyakarta 55281, Indonesia

[2] Department of Physiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Special Region of Yogyakarta 55281, Indonesia

[3] Department of Dental Biomedical Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Special Region of Yogyakarta 55281, Indonesia

Received: July 22, 2025 • Revised manuscript received: February 7, 2026 • Accepted: March 9, 2026
Published online: April 15, 2026

ABSTRACT

Background: Osteoporosis is a major health concern in postmenopausal women, characterized by reduced bone mineral density (BMD) and deteriorated bone microarchitecture due to estrogen deficiency. While pharmacological therapies are widely used, exercise represents a promising non-pharmacological approach to enhancing bone health. *Purpose:* This systematic review and meta-analysis evaluated the effects of exercise interventions on BMD and bone-related biomarkers in ovariectomized (OVX) rats as a model of postmenopausal osteoporosis. *Methods:* A comprehensive search was conducted across Scopus, PubMed, and ScienceDirect databases for studies investigating exercise interventions in OVX rats. Random-effects meta-analyses were performed using Review Manager 5.4 to pool mean differences in BMD and bone-related biomarkers. Subgroup analyses were conducted according to skeletal site and exercise modality. Risk of bias was evaluated using the SYRCLE tool. *Results:* Twenty-nine studies were included, involving treadmill running (55%), swimming (14%), resistance training (7%), jumping exercises (17%), and downhill running (7%). Across 14 studies, OVX rats demonstrated significant reductions in BMD at the vertebrae, femur, and tibia (MD: 0.03 g cm^{-2}, $P < 0.0001$). Exercise partially reversed these deficits,

* Corresponding author. Doctoral Program in Medical and Health Sciences, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jl Farmako Sekip Utara Special Region Yogyakarta 55281, Indonesia. Tel.: +6281328751518. E-mail: rini_puspita@ugm.ac.id

yielding small but significant BMD at skeletal sites (MD: -0.01 g cm^{-2}, $P < 0.0001$). Moderate-intensity treadmill and jumping/plyometric exercise showed significant effects on BMD. Exercise decreases bone resorption biomarkers (TRACP/TRACP5b/TRAP, CTX-1/β-CTX/NTX) and balances bone formation biomarkers (OCN, ALP, P1NP), while regulating PPARγ, Wnt/β-catenin, sirtuins, RANKL, and oxidative stress. *Conclusion:* Exercise interventions significantly mitigate OVX-induced bone loss, improve BMD, and positively influence biomarkers of bone remodeling, supporting its role as a potential non-pharmacological strategy for managing postmenopausal osteoporosis.

KEYWORDS

exercise, bone health, ovariectomized rats, osteoporosis, bone mineral density, biomarkers

INTRODUCTION

Osteoporosis is a systemic skeletal disorder characterized by reduced bone mineral density (BMD) and deteriorated bone microarchitecture. This condition poses significant global health burden, particularly for postmenopausal women due to estrogen deficiency [1]. Over 200 million women are affected globally, with projections indicating a 240% increase in hip fractures among women by 2050 [2, 3]. In the U.S., 54 million adults over the age of 50 suffer from osteoporosis or low bone mass, with women accounting for 80% of these cases [4]. The economic impact is staggering, costing $17.9 billion annually in the U.S. and €37.5 billion in Europe. These costs are driven by fractures that lead to disability, reduced quality of life, and 20% mortality rate within one year of hip fractures [2, 3]. These figures underscore the urgency of addressing osteoporosis as a critical public health priority, particularly in aging populations in regions such as China and India, which are experiencing rapid rises in fracture rates [5, 6]. Current management strategies emphasize the use of antiresorptive drugs (such as bisphosphonates, denosumab) and anabolic agents (including teriparatide, romosozumab). These treatments can reduce fracture risk by 40–70%, yet they encounter several limitations, including rare side effects, high costs, and issues with patient adherence [7]. Despite their proven efficacy, fewer than 30% of high-risk patients receive these treatments, underscoring significant gaps in care models like Fracture Liaison Services (FLS) [8, 9]. Non-pharmacological approaches, particularly exercise, demonstrate promise in improving BMD, muscle strength, and balance through mechanical loading [10]. However, optimal exercise protocols remain undefined, with most guidelines recommending weight-bearing and resistance training without addressing hormonal interactions [11]. Emerging research emphasizes the necessity of integrating mechanical and hormonal pathways, as estrogen deficiency disrupts bone remodeling by elevating pro-osteoclastic cytokines such as Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α) and Receptor activator of NF-κB ligand (RANKL) expression [12].

The pathogenesis of osteoporosis is characterized by estrogen deficiency, which leads to increased bone resorption due to upregulated osteoclast activity and impaired osteoblast function [12]. Estrogen loss accelerates bone turnover by increasing sclerostin expression and oxidative stress, which suppresses Wnt/β-catenin signaling, a pathway critical for bone formation [12, 13]. Mechanistically, exercise counteracts these effects by stimulating bone formation through strain-induced osteocyte signaling and enhancing muscle-bone crosstalk [10, 14, 15].

Recent studies propose that postmenopausal bone loss creates a "window of opportunity" in which mechanical loading synergizes with residual hormonal pathways to optimize bone geometry [14, 15]. Ovariectomized (OVX) rats provide an excellent model for studying postmenopausal osteoporosis, as they closely mimic the hormonal changes and subsequent bone loss observed in humans [16]. This systematic review and meta-analysis aim to evaluate the effects of exercise interventions on BMD and bone-related biomarkers in OVX rats as a model of postmenopausal osteoporosis.

METHODS

Study design

This systematic review and meta-analysis were conducted in accordance with the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE), with the study protocol prospectively registered in PROSPERO (registration no. CRD42025645911). A comprehensive search strategy was implemented across multiple databases, including Scopus, PubMed, and ScienceDirect, on February 4, 2025. We utilized Boolean operators to combine keywords derived from free-text terms and Medical Subject Headings (MeSH) (see Table 1). Its reporting complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). To ensure literature saturation, we performed backward citation tracking to identify additional eligible studies.

Eligibility criteria

This systematic review will include in vivo studies utilizing OVX female rats as the primary model for osteoporosis, focusing on interventions involving any form of exercise, including weight-bearing, non-weight-bearing, or resistance training. Exclusion criteria comprise studies using non-OVX animal models, male subjects, exercise groups that combined with other interventions, ex vivo/in vitro experiments, human trials, in silico analyses, clinical trials, case reports, cross-over designs, observational studies, review articles, editorials, conference abstracts, and publications in languages other than English. Eligible studies must report outcomes related to bone health parameters, such as BMD, and/or bone-related biomarkers including osteocalcin (OCN), estrogen/estradiol (E2) levels, Alkaline Phosphatase (ALP), Tartrate-resistant Acid Phosphatase (TRAP), C-Terminal telopeptide of type I collagen (CTX-1), N-terminal telopeptide (NTX), Procollagen type I N-terminal propeptide (P1NP), RANKL, sclerostin, etc.

Table 1. The search strategy for eligible articles

Database	Search strategy
Scopus	(Exercise OR Training OR "Weight Bearing" OR "Non-Weight Bearing" OR Run OR Swim) AND (Bone OR Skeletal OR Musculoskeletal) AND ("Ovariectomized Rat")
PubMed	(Exercise OR Training OR "Weight Bearing" OR "Non-Weight Bearing" OR Run OR Swim) AND (Bone OR Skeletal OR Musculoskeletal) AND ("Ovariectomized Rat")
Science Direct	(Exercise OR Training OR Run OR Swim) AND (Bone OR Skeletal OR Musculoskeletal) AND ("Ovariectomized Rat") Filters: Research Article, English

Study selection

The search results were imported into the Rayyan.ai platform for systematic review management. After removing duplicate entries, two independent reviewers screened the remaining articles based on titles and abstracts. Full-text copies of studies that might be eligible were obtained and evaluated using predetermined inclusion and exclusion criteria. A third reviewer was consulted when the two primary reviewers couldn't agree on a study's eligibility.

Quality assessment

Critical judgment is conducted by two reviewers, with a third reviewer included in case of disagreements. The SYRCLE RoB assessment results are visualized as a traffic-light and a summary plot graph, which display outcomes as low, high, or unclear risk of bias. The overall certainty of evidence for each outcome was assessed using the GRADE approach adapted for preclinical animal studies [17]. Evidence from animal intervention studies was initially rated 'High' certainty and downgraded across five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Indirectness was considered inherent due to the translational gap between OVX rat models and postmenopausal women.

Data extraction and analysis

Three independent reviewers conducted data extraction using standardized forms that captured study metadata (author names, publication year), details of experimental design, animal model specifications, and both quantitative and qualitative outcomes assessing the effects of exercise on bone health. Extracted quantitative data for continuous outcomes (bone mineral density and bone-related biomarkers) were entered into Review Manager 5.4 and pooled using inverse-variance random-effects meta-analysis, generating mean differences (MD) with 95% confidence intervals (CI). Where sufficient data were available, subgroup meta-analyses were performed by skeletal site (vertebrae, femur, tibia) and by exercise modality (low- and moderate-intensity treadmill, weight-bearing treadmill, jumping/plyometrics) to explore differential effects and potential sources of heterogeneity. Statistical heterogeneity was quantified using the Chi-square test and I^2 statistic, and, when pooling was not appropriate due to limited or incomparable data, findings were synthesized narratively with results still categorized by exercise modality to facilitate comparison of efficacy patterns on bone health. Potential publication bias was evaluated visually using funnel plots for the principal meta-analysis outcomes.

Use of Artificial Intelligence tools

During the preparation of this work, Artificial Intelligence (AI) tools were used to improve the language and clarity of the manuscript. Specifically, Grammarly (Grammarly Inc.) was used for grammar correction and language refinement; ChatGPT (OpenAI) was used to assist with drafting and readability of selected text; QuillBot (QuillBot Inc.) for limited paraphrasing; and Perplexity AI (Perplexity AI Inc.) for preliminary literature exploration. In addition, Rayyan AI was used to facilitate systematic review screening, and RobVis was used to generate the risk-of-bias figures. Authors independently created and verified all scientific content, analyses, and conclusions. The authors subsequently reviewed and edited all AI-assisted content as needed and are fully responsible for the final version of this manuscript.

RESULTS

Study selection

Study selection process commenced with the identification of 1,264 records from three databases: Scopus (n = 217), PubMed (n = 52), and ScienceDirect (n = 995). After removing 60 duplicate records and 32 entries with incomplete metadata, 1,172 records were screened based on their titles and abstracts. Of these, 1,092 records were excluded for various reasons, including incorrect intervention (n = 404), irrelevant disease background (n = 226), inappropriate publication type (n = 197), unsuitable population (n = 154), flawed study design (n = 68), or unrelated outcomes (n = 43). From the remaining 80 reports sought for retrieval, 35 could not be obtained. A total of 45 reports were assessed for eligibility, of which 16 were excluded due to incompatible study designs (n = 9) or incomplete outcome data (n = 7). Ultimately, 29 studies were included for qualitative synthesis, and 14 studies provide sufficient quantitative data for meta-analysis (Fig. 1)

Risk of bias assessment

The risk-of-bias evaluation of 29 studies revealed a mixed pattern across the ten assessed domains (Figs 2 and 3). Baseline characteristics and complete outcome data demonstrated the strongest evidence of methodological rigor, with 29/29 studies (100%) rated at low risk. Random sequence generation showed predominantly low risk ratings, with 28/29 studies (96.6%). However, several critical methodological domains revealed substantial uncertainty or inadequate reporting. Randomization of outcome assessment and selective reporting emerged as a major weakness, with 29/29 studies (100%) classified as unclear. Similarly, allocation concealment, random housing, and blinding of housing exhibited limited explicit reporting (3/29; 1/29; and 1/29 classified as unclear, respectively). Blinding of outcome assessment data showed relatively favorable reporting, with 6/29 studies (20.6%) at low risk. In summary, while overt high-risk judgments were rare across all domains, the substantial proportion of unclear ratings, particularly in randomization of outcome assessment and selective reporting, substantially limits confidence in pooled estimates derived from this body of evidence.

Characteristics of studies

A total of 29 studies were summarized in Table 2, which investigates exercise interventions in OVX rat models of bone loss in postmenopausal osteoporosis. The studies include female Wistar rats (34%, n = 10), Sprague-Dawley rats (59%, n = 17), and other strains (7%, n = 2) with ages ranging from 5 weeks to 14 months, and sample sizes varying from 5 to 22 animals per group. The studies employed a variety of exercise protocols, including treadmill running (55%, n = 16), swimming (14%, n = 4), resistance training (7%, n = 2), jumping exercises (17%, n = 5), and downhill running (7%, n = 2) with intervention durations spanning from 4 to 36 weeks. The experimental designs typically compared sham-operated controls with OVX groups (with or without exercise), incorporating varying exercise intensities and different timings of exercise initiation post-surgery.

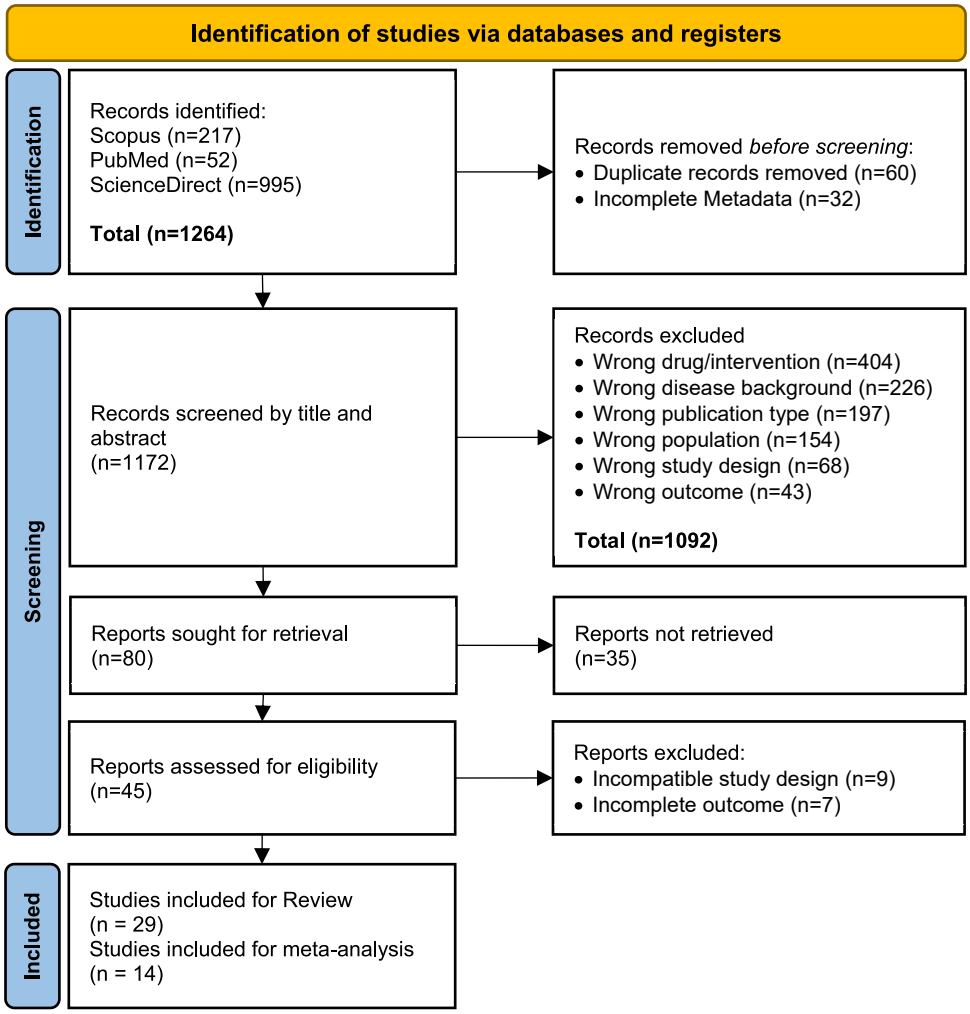

Fig. 1. PRISMA flowchart diagram

Exercise parameters exhibit substantial variation in intensity and progression. Treadmill exercise, gradually increase speed from 10 to 22 m min^{-1} or increase duration from 10 to 60 min. Some studies incorporated inclines of 0 to 15° or weighted backpacks of up to 20% of body weight. Resistance training involved ladder climbing with tail weights, with the weight gradually increasing from 50 to 100% of body weight. Jumping exercise involved jumping from an initial height of 25 cm, gradually increasing to 40 cm, while swimming protocols lasted up to 60 min per day. Most programs adhered to a 5-day-per-week regimen, with intervention durations spanning from 4 to 36 weeks either immediately or delayed for up to 16 weeks post-ovariectomy.

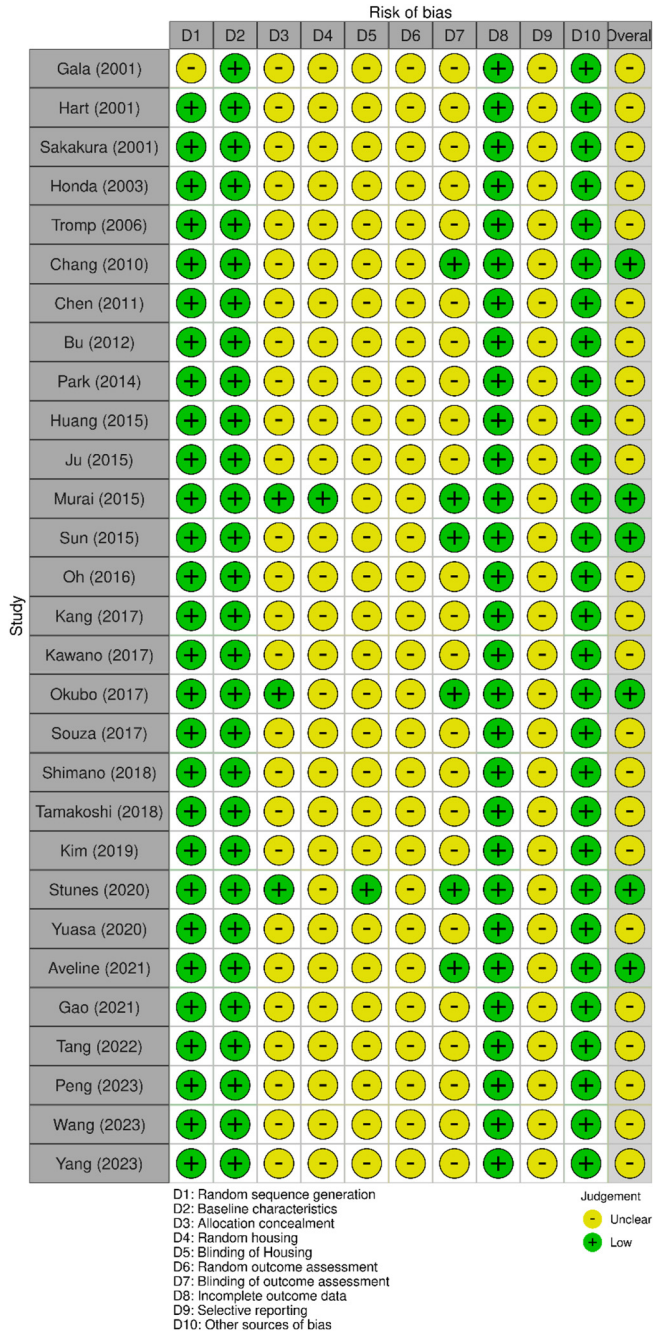

Fig. 2. Traffic-light plot of SYRCLE risk of bias results

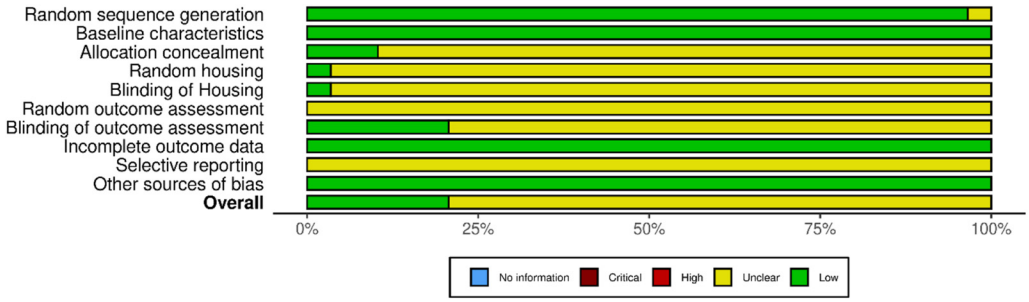

Fig. 3. Summary plot of SYRCLE risk of bias results

Meta-analysis results

Pairwise meta-analyses of data from 14 studies were conducted to evaluate the effects of exercise interventions on BMD in OVX rats, by skeletal site, and exercise modality-specific responses (Table 3).

The skeletal site analysis revealed OVX effects across anatomical regions, with vertebrae demonstrating the most affected (MD: 0.03 g cm^{-2}, 95% CI: 0.01 to 0.04, I^2 = 94%, P = 0.0006), followed by femur (MD: 0.04 g cm^{-2}, 95% CI: 0.03 to 0.04, I^2 = 67%, P < 0.0001) and tibia (MD: 0.04 g cm^{-2}, 95% CI: 0.02 to 0.06, P = 0.0001). The heterogeneity was high (I^2 = 94%), which may reduce the reliability of the result. To test the robustness of the results and identify the source of this high variance, we performed a sensitivity analysis by sequentially excluding each study to evaluate its impact on the overall result. Leave-one-out sensitivity analysis identified Tromp et al. (2006) [22] as the primary source of heterogeneity in the vertebrae subgroup; exclusion of this study reduced the I^2 from 94% to 0% (MD: 0.03 g cm^{-2}, 95% CI: 0.03 to 0.04) and the overall I^2 from 93% to 45% (MD: 0.03 g cm^{-2}, 95% CI: 0.03 to 0.04), while the pooled effect estimates remained consistent in direction and significance (Fig. 4).

When stratified by exercise intervention versus OVX controls, vertebrae showed attenuated benefit (MD: −0.01 g cm^{-2}, 95% CI: −0.02 to 0.00, I^2 = 87%, P = 0.01), whereas femur maintained significant positive effects (MD: −0.02 g cm^{-2}, 95% CI: −0.02 to −0.01, I^2 = 78%, P = 0.002), and tibia exhibited marginal improvement (MD: −0.02 g cm^{-2}, 95% CI: −0.03 to 0.00, I^2 = N/A, P = 0.06). Exercise modality-specific subgroup analyses demonstrated that moderate-intensity treadmill exercise produced the most pronounced osteogenic stimulus in both vertebrae (MD: −0.02 g cm^{-2}, 95% CI: −0.03 to −0.00, P = 0.02) and femur (MD: −0.03 g cm^{-2}, 95% CI: −0.04 to −0.01, P = 0.0004), while jumping/plyometric protocols yielded consistent skeletal benefits across vertebrae (MD: −0.02 g cm^{-2}, 95% CI: −0.04 to 0.00, I^2 = 83%, P = 0.05) and femur (MD: −0.02 g cm^{-2}, 95% CI: −0.02 to −0.01, I^2 = 0%, P < 0.0001), suggesting that high-impact loading stimuli effectively counteract OVX-induced bone loss. These findings collectively support the implementation of structured, moderate-to-high-intensity mechanical loading regimens as therapeutic strategies to mitigate postmenopausal bone loss, while highlighting the need for exercise prescription tailored to specific skeletal sites and biomechanical characteristics.

Table 2. Data extraction for study characteristics

Author (year)	Animal strain	Age (weight)	Groups (*n* per group)	Exercise model
Gala et al. [18]	Female Wistar rats	15 weeks old (252 g)	SHAM (22), OVX (22), OVX + EX (22)	Treadmill running: 1,080 m day^{-1} at 8 m min^{-1}, 5 days $week^{-1}$, for 12 weeks
Hart et al. [19]	Female Sprague-Dawley rats	12–14 months old (327–335 g)	CON (9), EX-SWI (8)	Swimming: 60 min day^{-1}, 5 days $week^{-1}$, for 12 weeks
Sakakura et al. [20]	Female Wistar rats	24 weeks old (386 g)	SHAM (5), SHAM + EX-TM (6), OVX (6), OVX + EX-TM (6)	Treadmill running: Gradual increase to 25 m min^{-1} with a 5° incline for 30 min day^{-1}, for 8 weeks
Honda et al. [21]	Female Wistar rats	9 months old (308–309 g)	SHAM (9), SHAM + EX-JMP (12), OVX (10), OVX + EX-JMP (11)	Jumping: 10 jumps/day at a height of 40 cm, 5 days $week^{-1}$, for 8 weeks
Tromp et al. [22]	Female Wistar rats	12 weeks old (193–236 g)	SHAM (10), OVX (10), EX–TM (10), OVX + EX–TM (10)	Treadmill running with backpack (20% body weight): 15 min day^{-1}, 5 days $week^{-1}$, for 19 weeks (started 5 weeks post-OVX). Velocity gradually increased to 16.8 m min^{-1}, average velocity 12.7 m min^{-1}
Chang et al. [23]	Female Sprague-Dawley rats	8 weeks old (229 ± 9 g)	OVX (15), OVX + EX–TM (15), EX–TM (15), CON (15)	Treadmill running: 12 m min^{-1}, 60 min day^{-1}, 5 days $week^{-1}$, for 36 weeks (started 16 weeks after surgery)
Chen et al. [24]	Female Sprague-Dawley rats	3 months old (235 ± 8 g)	SHAM (10), OVX (10), OVX + EX–TM (10)	Treadmill running: 18 m min^{-1}, at 0% grade, 40 min day^{-1}, 4 days $week^{-1}$, for 14 weeks
Bu et al. [25]	Female Sprague-Dawley rats	3 months old (235 ±8 g)	SHAM (10), OVX (10), OVX + EX–TM (10)	Treadmill running: 18 m min^{-1}, at 0% grade for 40 min, 4 days $week^{-1}$ for 14 weeks
Park et al. [26]	Female Sprague-Dawley rats	6 weeks old (N/R)	CON (7), EX-SWI (7), EX-RT (Climb)	Swimming: 1 h day^{-1}, 6 times/week. Climbing to reach a water bottle, which is set at 20 cm high and gradually elevated to 2 m over 1 week. For 8 weeks

(continued)

Table 2. Continued

Author (year)	Animal strain	Age (weight)	Groups (*n* per group)	Exercise model
Huang et al. [27]	Female Sprague-Dawley rats	36 weeks old (370–412 g)	SHAM (9), OVX (8), OVX + EX–TM (8)	Treadmill running: progressive increase to 16 m min^{-1} for 60 min, 5 days $week^{-1}$, for 12 weeks
Ju et al. [28]	Female Fisher 344 rats	18 weeks old (165–167 g)	SHAM (9), SHAM + EX–SWI (9), OVX (9), OVX + EX–SWI (9)	Swimming: 60 min day^{-1}, 5 days $week^{-1}$, for 12 weeks
Murai et al. [29]	Female Wistar rats	15 weeks old (250–300 g)	SHAM (8), OVX + EX-DR (6)	Downhill running: 20 m min^{-1} for 30 min per session, 5 days a week for 12 weeks
Sun et al. [30]	Female Sprague-Dawley rats	3-month-old (230 ± 10 g)	OVX (15), SHAM (15), OVX + EX–TM (15)	Treadmill exercise: gradually increased from 10 to 20 m min^{-1}, at 5% incline, 10–60 min/session for 1 week; 22–24 m min^{-1} for 60 min/session for the remaining 53 days
Oh et al. [31]	Female Sprague-Dawley rats	6 weeks old (130–160 g)	SHAM (6), SHAM + EX–SWI (6), OVX (10), OVX + EX–SWI (11)	High-intensity intermittent swimming training (HIST): 14 repetitions of 20-s bouts with 10-s pauses. Weight equivalent to 14–16% of body weight, for 8 weeks
Kang et al. [32]	Female Sprague-Dawley rats	8 weeks old (N/R)	SHAM (8), OVX (8), OVX + EX–DR (8), OVX + EX–UR (8)	Downhill training (16 m min^{-1}, −15°) and Uphill (16 m min^{-1}, +15°) and for 60 min day^{-1}, 5 days $week^{-1}$, for 8 weeks
Kawano et al. [33]	Female Wistar rats	6 weeks old (N/R)	OVX (5), OVX + EX–TM (5)	Treadmill running: 10 m min^{-1} for the first 5 min and 20 m min^{-1} for the next 25 min; the inclination was set to 0°. Each rat ran 30 min day^{-1}, 5 days $week^{-1}$, for 3 months
Okubo et al. [34]	Female Wistar rats	10 weeks old (199.75 ± 7.45 g)	SHAM (10), OVX (10), SHAM + EX–JMP (10), OVX + EX–JMP (10)	Jumping: 20 times/day, 5 days $week^{-1}$, to a height of 40 cm for 12 weeks (started 60 days after surgery)

(continued)

Table 2. Continued

Author (year)	Animal strain	Age (weight)	Groups (*n* per group)	Exercise model
Souza et al. [35]	Female Holstman rats	N/R (240–250 g before grouping)	SHAM (11), OVX (11), SHAM + EX–RT (11), OVX + EX–RT (11)	Resistance training: Climbing a vertical ladder (1.1 m × 0.18 m × 2 cm, 80° incline) while wearing weights on their tails, every 3 days for 12 weeks
Shimano et al. [36]	Female Wistar rats	8 weeks old (approx. 200 g)	OVX (8), OVX + EX–JMP (8), SHAM (8), SHAM + EX–JMP (8)	Jump exercise: 20 jumps/day, 5 days $week^{-1}$ at an initial height of 25 cm, gradually increased to 40 cm, for 12 weeks
Tamakoshi et al. [37]	Female Wistar rats	7-week-old (191–202 g)	SHAM (6), OVX (8), OVX + EX–TM (6), OVX + EX–UR (6), OVX + EX-DR (6)	Treadmill running: 20 m min^{-1}, 30 min, 5 days $week^{-1}$, for 8 weeks starting at 1 week after surgery; Uphill: +10%, Normal Treadmill: 0%, Downhill: −10% slope
Kim et al. [38]	Female Sprague-Dawley rats	8 weeks old (N/R)	SHAM (8), OVX (8), OVX + EX–TM CR (8), OVX + EX–TM IR (8)	Continuous running (CR): 16 m min^{-1}, 0° slope, 60 min day^{-1}, 5 days $week^{-1}$ for 6 weeks. Interval running (IR): 30 min day^{-1}, 5 days $week^{-1}$ for 6 weeks, alternating between 3 min high intensity (23 m min^{-1}, +15° slope) and 2 min incomplete rest, repeated five times. Velocity and slope gradually increased every 2 weeks
Stunes et al. [39]	Female Sprague Dawley rats	12 weeks of age (231 ± 19 g)	SHAM (12), OVX + EX–JMP (12)	Plyometric jumping exercise: 3 days a week for 8 weeks, 3 sets of 6 jumps with progressive jumping height
Yuasa et al. [40]	Female Sprague-Dawley rats	16-week-old (375–394 g)	SHAM (20), OVX + EX–TM (20)	Low-intensity treadmill exercise: 12–15 m min^{-1}, 5% incline, for 60 min day^{-1}, 5 days $week^{-1}$, for 4 or 8 weeks, starting 8 weeks after ovariectomy

(continued)

Table 2. Continued

Author (year)	Animal strain	Age (weight)	Groups (*n* per group)	Exercise model
Aveline et al. [41]	Female Wistar rats	5 weeks old (N/R)	SHAM (12), OVX (12), OVX + EX–JMP (12)	Jumping: 10 drops per day from 45 cm height, 5 days a week for 8 weeks
Gao et al. [42]	Female Sprague-Dawley rats	3-month-old (272 ± 14 g)	SHAM (20), OVX (40), OVX + EX–TM (20)	Treadmill exercise: 20 m min^{-1} for 60 min, 5 days per week for 17 weeks
Tang et al. [43]	Female Sprague-Dawley rats	3-month-old (210 ± 8 g)	SHAM (10), OVX (10), OVX + EX–TM (10)	Treadmill running: 20 m min^{-1} while bearing 35% of their body weight, 20 min, 6 days $week^{-1}$ for 10 weeks
Peng et al. [44]	Female Sprague-Dawley rats	3-month-old (approx. 270 g)	CON (8), OVX (8), OVX + EX–TM (8)	Treadmill exercise: 20 m min^{-1}, 40 min day^{-1}, 5 days $week^{-1}$, 5° slope, for 12 weeks (starting 14 days post-OVX)
Wang et al. [45]	Female Sprague-Dawley rats	9-week-old (180–200 g)	SHAM (6), OVX (6), OVX + EX–RT (6)	Resistance exercise (ladder climbing): 2 weeks of adaptive training (Started at 50% body weight, increased to 75%, then 100%.), then 12 weeks of formal training. 5 days $week^{-1}$, 4 sets of 3 repetitions, 1 min rest at top, 2 min rest between sets
Yang et al. [46]	Female Sprague-Dawley rats	3-month-old (272 + 14 g)	SHAM (20), OVX (20), OVX + EX–TM (20)	Moderate intensity treadmill exercise program: 20 m min^{-1} for 60 min in the morning, 5 days per week for 17 weeks

OVX, ovariectomized; SHAM, sham-operated; EX, exercise; OVX + EX, ovariectomized with exercise intervention; SHAM + EX, sham-operated with exercise intervention; CON, non-ovariectomized control; TM, treadmill running; SWI, swimming; JMP, jumping or plyometric jumping exercise; RT, resistance training; CR, continuous running; IR, interval running; UR, Uphill running; DR, Downhill running.

Bone-related biomarker result

The analysis of bone-related biomarkers revealed consistent patterns of ovariectomy-induced alterations and exercise-mediated amelioration across multiple biological pathways (Table 4).

Ovariectomy significantly elevated bone resorption biomarkers, including TRACP/TRACP5b/TRAP, CTX-1/β-CTX/NTX, compared with sham-operated controls. Exercise

Table 3. Summary of pair-wise meta-analysis results

Outcomes	Studies (*n*)	Animals (*n*)	MD (95% CI)	I^2	*P*-value
Sham vs OVX					
BMD Skeletal Site Sub-Group	**9**	**367**	**MD: 0.03 g cm^{-2} (0.02 to 0.04)**	**93%**	**<0.0001***
- Vertebrae	6	152	MD: 0.03 g cm^{-2} (0.01 to 0.04)	94%	**0.0006***
- Femur	7	175	MD: 0.04 g cm^{-2} (0.03 to 0.04)	67%	**<0.0001***
- Tibia	1	40	MD: 0.04 g cm^{-2} (0.02 to 0.06)	N/A	0.0001*
OVX vs OVX-Exercise					
BMD Skeletal Site Sub-Group	**9**	**385**	**MD: −0.01 g cm^{-2} (−0.02 to −0.01)**	**82%**	**<0.0001***
- Vertebrae	7	169	MD: −0.01 g cm^{-2} (−0.02 to −0.00)	87%	**0.01***
- Femur	7	176	MD: −0.02 g cm^{-2} (−0.02 to −0.01)	78%	**0.002***
- Tibia	1	40	MD: −0.02 g cm^{-2} (−0.03 to 0.00)	N/A	0.06
BMD Exercise Sub-Group	**9**	**345**	**MD: −0.01 g cm^{-2} (−0.02 to −0.01)**	**83%**	**<0.0001***
• **Treadmill**	**6**	**245**	**MD: −0.01 g cm^{-2} (−0.02 to 0.00)**	**77%**	**0.002***
- Low-intensity Treadmill - Vertebrae	3	88	MD: −0.01 g cm^{-2} (−0.02 to 0.00)	70%	0.20
- Low-intensity Treadmill - Femur	3	98	MD: −0.01 g cm^{-2} (−0.03 to 0.00)	86%	0.17
- Low-intensity Treadmill - Tibia	1	40	MD: −0.02 g cm^{-2} (−0.03 to 0.00)	N/A	0.06
- Moderate-intensity Treadmill - Vertebrae	1	20	MD: −0.02 g cm^{-2} (−0.03 to 0.00)	N/A	0.02*
- Moderate-intensity Treadmill - Femur	1	20	MD: −0.03 g cm^{-2} (−0.04 to −0.01)	N/A	0.0004*
- Weight-Bearing Treadmill - Vertebrae	1	19	MD: 0.00 g cm^{-2} (−0.00 to 0.00)	N/A	0.6
• **Jumping/Pyelometrics**	**3**	**100**	**MD: −0.02 g cm^{-2} (−0.03 to −0.01)**	**51%**	**<0.0001***
- Jumping - Vertebrae	2	42	MD: −0.02 g cm^{-2} (−0.04 to 0.00)	83%	0.05
- Jumping - Femur	3	58	MD: −0.02 g cm^{-2} (−0.02 to −0.01)	0%	**<0.0001***

BMD, bone mineral density; MD, mean difference; CI, confidence interval; I^2, Higgins I-squared heterogeneity statistic; OVX, ovariectomized; SHAM, sham-operated control; P, statistical significance level; *, statistically significant at $P < 0.05$; N/A, not applicable (single study, heterogeneity not calculated).

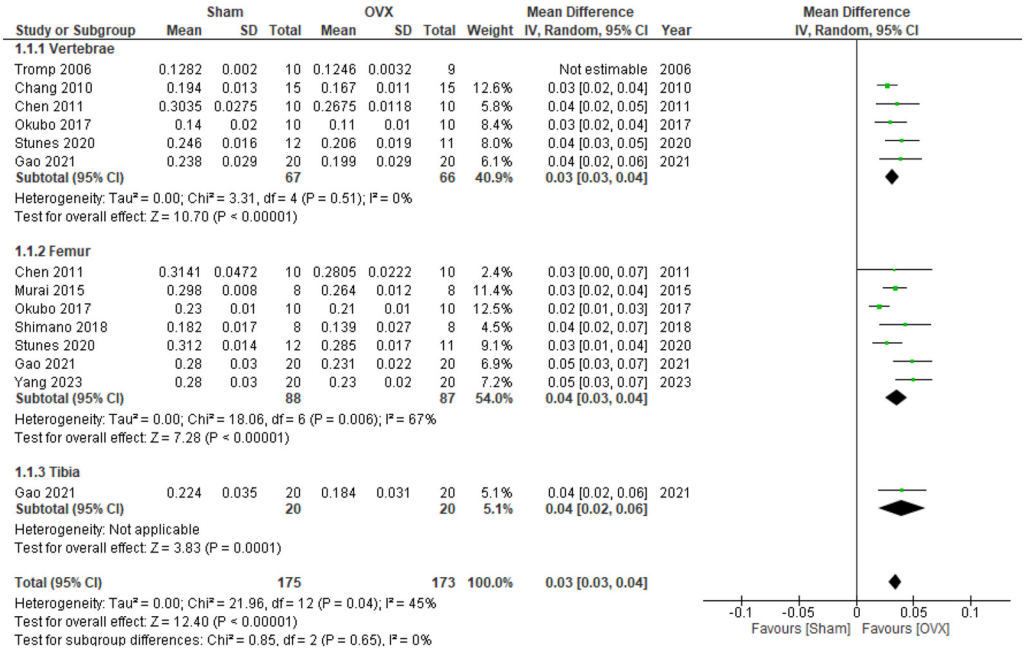

Fig. 4. Forest plot of BMD in Sham vs OVX groups after Leave-One-Out Sensitivity Analysis

interventions in OVX rats consistently reduced these elevated resorption biomarkers toward sham levels, and balanced the formation biomarkers OCN, ALP, and P1NP. Specifically, for CTX-1, Huang et al. (2015) [27] showed that treadmill training reduced CTX-1 levels from 15.2 ± 0.6 to 12.3 ± 1.5 (approximately 19% reduction), and Stunes et al. (2020) [39] reported that jumping exercise decreased CTX-1 from 12.2 ± 3.4 to 9.9 ± 1.9 (approximately 19% reduction). The studies above show that exercise reduces collagen degradation across two different exercise modalities. Hormonal analyses demonstrated that ovariectomy markedly reduced E2 levels, whereas exercise elevated them.

Beyond traditional bone turnover biomarkers, exercise modulated key molecular signaling involved in bone metabolism and oxidative stress. Ovariectomy upregulated peroxisome proliferator-activated receptor gamma (PPARγ), increased triglycerides, and altered runt-related transcription factor 2 (Runx2) expression, while exercise downregulated PPARγ and normalized triglyceride content in OVX rats. Exercise also restored Wnt/β-catenin signaling by increasing *β*-catenin and phosphorylated glycogen synthase kinase-3 beta (P-GSK-3β) expression, which were suppressed by ovariectomy. Additionally, exercise enhanced bone morphogenetic proteins (BMPs), sirtuin 1 and 6 (SIRT1, SIRT6), and reduced RANKL-induced osteoclastogenesis, while reducing osteopontin, sclerostin, and leptin levels. Resistance training specifically increased matrix metalloproteinase-2 (MMP-2) activity and improved calcium and phosphorus levels in both sham and OVX rats. Exercise also attenuated oxidative stress by increasing superoxide dismutase (SOD) activity in OVX rats to levels approaching or exceeding sham controls, and partially normalized ALP and estrogen receptor alpha (ERα) mRNA expression.

Table 4. The results of bone-related biomarkers

Markers	Results	Reference(s)
ALP	OVX decreases ALP compared with SHAM; exercise in OVX partially normalizes ALP toward SHAM levels	[18, 20, 32, 38, 41, 43]
OCN/Gla-OCN/Glu-OCN	OVX elevates circulating osteocalcin (or its fractions) compared with SHAM, whereas exercise in OVX tends to reduce or normalize osteocalcin levels compared with OVX alone	[21, 27, 30, 31, 32, 34, 36, 38, 41, 44, 46]
CTX-1/β-CTX/NTX	OVX enhances CTX-1, β-CTX, and NTX relative to SHAM; exercise in OVX consistently lowers these markers, often approaching SHAM levels	[27, 30, 38, 39, 41, 44, 45]
P1NP	OVX modestly elevates formation marker P1NP; exercise in OVX shows a slight reduction versus OVX	[39, 44]
TRACP/TRACP 5 b/TRAP	OVX increases TRACP/TRACP 5 b/TRAP activity compared with SHAM; exercise in OVX reduces these activities toward control levels	[18, 20, 43, 45, 46]
Estradiol/Estrogen (E2)	OVX markedly reduces E2 relative to SHAM; exercise in OVX produces small, inconsistent increases in E2 levels	[20, 24, 25, 32, 38, 43]
PPARγ, Runx2, TG	OVX upregulates PPARγ and increases triglycerides with altered Runx2; exercise in OVX downregulates PPARγ and normalizes triglyceride content	[24, 25, 27]
β-catenin, P-GSK-3β	OVX suppresses β-catenin and P-GSK-3β; exercise in OVX restores or increases these signaling proteins toward SHAM	[25]
MMP-2, Calcium, Phosphorus	OVX reduces MMP-2 activity and mineral levels; resistance training in both SHAM and OVX increases MMP-2 and improves calcium/phosphorus, particularly in exercised OVX rats	[35]
Osteopontin	OVX stimulates osteopontin relative to SHAM; exercise in OVX markedly lowers osteopontin	[36]

(continued)

Table 4. Continued

Markers	Results	Reference(s)
BMPs, SIRT1, SIRT6,	OVX with interval running (OVX-IR) enhances BMPs and sirtuins versus OVX control	[38]
Sclerostin, Leptin	OVX boosts sclerostin and leptin; exercise in OVX reduces sclerostin and leptin	[27, 39, 44]
SOD (oxidative stress marker)	OVX decreases SOD activity compared with SHAM; exercise in OVX increases SOD toward/above SHAM	[44]
ALP mRNA, ERα mRNA	OVX increases ALP and ERα mRNA expression vs SHAM and is reduced partially in exercise-OVX towards SHAM	[33]
RANKL expression	OVX upregulated RANKL expression, while exercise downregulated RANKL expression	[39, 44]

ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; TRACP, tartrate-resistant acid phosphatase; TRACP 5b, tartrate-resistant acid phosphatase 5b isoform; OVX, ovariectomized; SHAM, sham-operated control; OCN, osteocalcin; Gla-OCN, gamma-carboxylated osteocalcin; Glu-OCN, undercarboxylated osteocalcin; CTX-1, C-terminal telopeptide of type I collagen; β-CTX, beta C-terminal telopeptide of type I collagen; NTX, N-terminal telopeptide of type I collagen; P1NP, procollagen type I N-terminal propeptide; E2, estradiol/estrogen; PPARγ, peroxisome proliferator-activated receptor gamma; Runx2, runt-related transcription factor 2; TG, triglycerides; β-catenin, beta-catenin; P-GSK-3β, phosphorylated glycogen synthase kinase-3 beta; MMP-2, matrix metalloproteinase-2; BMPs, bone morphogenetic proteins; SIRT1, sirtuin 1; SIRT6, sirtuin 6; RANKL, receptor activator of nuclear factor kappa-B ligand; SOD, superoxide dismutase; ALP mRNA, alkaline phosphatase messenger RNA; ERα mRNA, estrogen receptor alpha messenger RNA.

Publication bias

The funnel plot comparing BMD between Sham and OVX groups (Fig. 5A) shows a relatively symmetric distribution of studies around the pooled mean difference line across vertebrae, femur, and proximal tibia, suggesting low risk of small-study or publication bias for this contrast. In contrast, the funnel plot for BMD in OVX versus OVX-Exercise groups (Fig. 5B) demonstrates a slightly asymmetric pattern driven mainly by one outlying vertebral study on the left side, indicating a potential small-study effect that warrants cautious interpretation of the exercise-related benefit.

Overall certainty of evidence

GRADE assessment indicated that the overall certainty of evidence was Very Low across all outcomes (Table 5). For both the Sham vs. OVX and OVX vs. OVX-Exercise comparisons, all BMD outcomes at the vertebrae, femur, and tibia were downgraded due to serious risk of bias (in SYRCLE domains, 100% of studies rated 'unclear' for randomization of outcome assessment

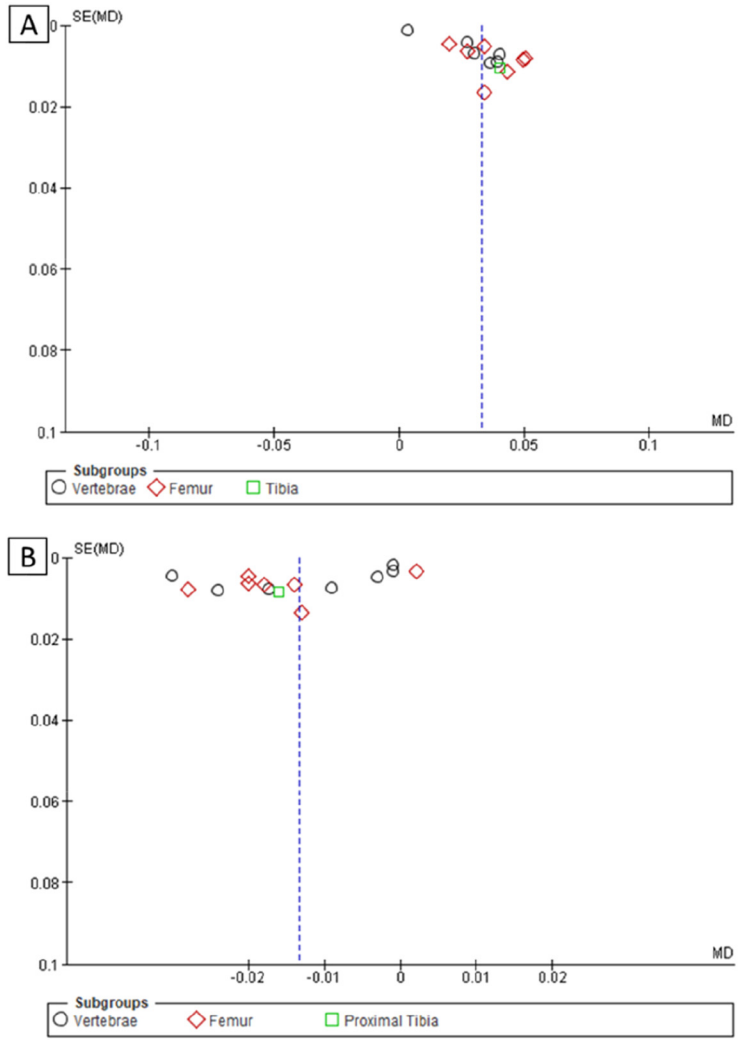

Fig. 5. Funnel plot of BMD in (A) Sham vs OVX groups and (B) OVX vs OVX-Exercise groups

and selective reporting; >90% unclear for allocation concealment, random housing, and blinding of housing). Serious indirectness (OVX rat model as a surrogate for human postmenopausal osteoporosis) and serious inconsistency (I^2 ranging from 67 to 94% for multi-study outcomes) suggest variability across experimental protocols, animal characteristics, and intervention parameters, with additional downgrading for imprecision in tibia outcomes (single study, small sample) and suspected publication bias in the OVX vs. OVX-Exercise vertebral comparison (funnel plot asymmetry). The certainty for biomarker outcomes (CTX-1, osteocalcin, TRAP, ALP) was also rated Very Low due to the same risk of bias and indirectness concerns,

Table 5. GRADE summary of findings for the certainty of evidence

Outcome	Studies (Animals)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Certainty
Sham vs. OVX							
BMD – Vertebrae	5 (133)*	⊕ Serious[a]	Not serious[b]	⊕ Serious[c]	Not serious	Not serious[d]	⊕⊕◯◯ Low
BMD – Femur	7 (175)	⊕ Serious[a]	⊕ Serious[e]	⊕ Serious[c]	Not serious	Not serious[d]	⊕◯◯◯ **Very Low**
BMD – Tibia	1 (40)	⊕ Serious[a]	N/A[f]	⊕ Serious[c]	⊕ Serious[g]	Not serious	⊕◯◯◯ **Very Low**
OVX vs. OVX-Exercise							
BMD – Vertebrae	7 (169)	⊕ Serious[a]	⊕ Serious[h]	⊕ Serious[c]	Not serious	⊕ Serious[i]	⊕◯◯◯ **Very Low**
BMD – Femur	7 (176)	⊕ Serious[a]	⊕ Serious[j]	⊕ Serious[c]	Not serious	Not serious	⊕◯◯◯ **Very Low**
BMD – Tibia	1 (40)	⊕ Serious[a]	N/A[f]	⊕ Serious[c]	⊕ Serious[k]	Not serious	⊕◯◯◯ **Very Low**
Bone biomarkers	2–9 (narrative)	⊕ Serious[a]	N/A[l]	⊕ Serious[c]	⊕ Serious[g]	Not assessed	⊕◯◯◯ **Very Low**

Notes: * Post-sensitivity analysis: Tromp et al. (2006) [22] excluded as the identified source of heterogeneity.

[a] Risk of bias: Downgraded −1 (serious). Randomization of outcome assessment unclear in 29/29 studies (100%); selective reporting unclear in 29/29 (100%); allocation concealment unclear in 26/29 (90%); random housing unclear in 28/29 (97%); blinding of personnel unclear in 27/29 (93%).

[b] Not downgraded. After exclusion of Tromp et al. (2006) [22] via leave-one-out sensitivity analysis, I^2 reduced from 94% to 0% ($P = 0.51$); pooled effect remained consistent in direction and significance.

[c] Downgraded −1 (serious). OVX rat model used as surrogate for human postmenopausal osteoporosis; inherent translational gap in species, bone physiology, and remodeling rate.

[d] Not serious. Funnel plot showed relatively symmetric distribution.

[e] Downgraded −1 (serious). $I^2 = 67\%$, $P = 0.006$.

[f] Not applicable — single study; heterogeneity cannot be assessed.

[g] Downgraded −1 (serious). Single study or limited studies with small sample size.

[h] Downgraded −1 (serious). $I^2 = 87\%$.

[i] Downgraded −1 (serious). Funnel plot asymmetry driven by one outlying vertebral study.

[j] Downgraded −1 (serious). $I^2 = 78\%$.

[k] Downgraded −1 (serious). Single study; 95% CI includes null ($P = 0.06$).

[l] Not assessed – synthesized narratively due to limited and incomparable quantitative data.

compounded by limited study numbers that precluded meta-analytic pooling. Therefore, while the findings provide valuable preclinical insight, the overall certainty of evidence remains limited.

DISCUSSION

This systematic review and meta-analysis synthesized 29 studies qualitatively and 14 quantitatively to examine how different exercise modalities affect bone health in OVX rat models. Ovariectomy caused marked BMD loss at the vertebrae, femur, and tibia, while exercise partially reversed these deficits, producing significant BMD gains at vertebral and femoral sites but not at the tibia. Treadmill running and jumping/plyometric were shown to have osteogenic responses, and exercise broadly shifted bone biomarker profiles from a resorptive toward a more balanced or mildly anabolic state, attenuating increases in TRACP/TRACP5b/TRAP, CTX-1/β-CTX/NTX, and normalizing formation markers such as OCN, ALP, and P1NP while favorably regulating PPARγ, Wnt/β-catenin, sirtuins, RANKL and oxidative stress.

Similar to the results of our study in OVX rat models, human studies have demonstrated that weight-bearing and resistance exercises significantly enhance BMD at critical sites such as the lumbar spine, femoral neck, and total hip. For instance, a systematic review involving postmenopausal women reported standardized mean differences (SMDs) of 0.29 for lumbar spine, 0.27 for femoral neck, and 0.41 for total hip BMD after exercise interventions, underscoring the osteogenic potential of physical activity in mitigating bone loss caused by estrogen deficiency [47, 48]. Additionally, studies have shown that exercise reduces markers of bone resorption and increases markers of bone formation, consistent with the biomolecular findings in OVX rats, where exercise shifts the balance towards bone formation [49]. These parallels underscore the relevance of OVX rat models in studying postmenopausal osteoporosis and the translational value of exercise interventions.

However, some divergences exist between animal and human studies. While OVX rat models often focus on specific molecular pathways (e.g., Wnt/β-catenin signaling) to elucidate exercise-induced changes in bone remodeling, human studies tend to emphasize clinical outcomes such as fracture risk reduction or long-term BMD improvements. For example, prolonged exercise protocols in humans (e.g., 12 months or more) have been found to produce more substantial BMD gains compared to shorter interventions [50, 51]. Furthermore, aquatic exercises, which are less impactful in OVX rats due to reduced mechanical loading, have shown benefits in human populations by improving balance and preventing falls [52]. These differences highlight the importance of considering biomechanical and physiological variations when translating findings from animal models to clinical settings.

Our findings align with the current understanding of osteoporosis pathophysiology, particularly the role of estrogen deficiency in disrupting bone remodeling processes. Estrogen plays a critical role in maintaining skeletal homeostasis by promoting osteoprotegerin (OPG) expression and suppressing RANKL expression, thereby inhibiting osteoclast activity and promoting osteoblast function. Estrogen also activates Wnt/β-catenin signaling pathway to enhance osteoblast differentiation. In its absence, as seen in postmenopausal osteoporosis or OVX models, there is an upregulation of RANKL expression and pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-α), leading to increased osteoclastogenesis and bone resorption [53–55].

This imbalance results in elevated bone turnover, with bone resorption exceeding formation, ultimately causing bone loss. The studies in this review demonstrate that exercise counteracts these effects by modulating these molecular pathways. For instance, interventions like treadmill running and resistance training have been shown to reduce RANKL expression, normalize OPG levels [39], and suppress inflammatory cytokines, thereby restoring balance to bone remodeling processes [56].

When estrogen levels in tissues decrease, extragonadal tissues synthesize estrogen through the aromatization of testosterone and androstenedione by enzyme CYP19 aromatase, the sole member of family 19 of the cytochrome P450 enzymes. Exercise has been shown to enhance CYP19 aromatase expression in osteoblasts and osteocytes, ensuring sufficient estrogen in bone to maintain bone homeostasis [57–59]. During exercise, osteoblast cell proliferation is enhanced, intracellular signaling pathways that support bone formation are boosted, and Estrogen Receptor alpha (ERα) signaling is activated. The presence of estrogen further promotes ERα transcription in bone and other estrogen-responsive tissues [60, 61].

Moreover, exercise exerts anabolic effects by enhancing mechanical loading on bones, which stimulates osteocyte signaling and promotes osteoblast activity. Osteocytes are the primary bone cells responsible for perceiving mechanical signals, transforming them into biological messengers, and transmitting these signals intercellularly through the highly specialized Lacunocanalicular Network (LCN). Interstitial fluid flows through LCN in the bone matrix because of mechanical strain [15, 62]. These mechanical signals are transferred from osteocytes' processes to surrounding cells via gap junctions and ion channels, controlling the activities of osteoblasts, osteoclasts, and the extracellular matrix (ECM) of the bone microenvironment, leading to upregulation of bone turnover and subsequent bone deposition [62, 63].

Mechanical stimulation in exercise activates Wnt/β-catenin signaling and reduces oxidative stress markers such as reactive oxygen species (ROS), both of which are disrupted during estrogen deficiency [54, 56]. Mesenchymal stem cells proliferate and differentiate when the Wnt/β-catenin signaling pathway is activated [64], increases the population of osteoblast progenitor cells, reduces the apoptosis of mature osteoblasts [65], and inhibits osteoclast differentiation, resulting in enhanced bone formation [66].

Tissue crosstalk may also be modulated by molecular signals originating in muscle tissue that act on bone tissue, particularly during mechanical stimulation. Research has shown that during exercise, skeletal muscle releases myokines such as myostatin and irisin, which promote osteoblast differentiation [62, 67, 68]. Mechanical loading reduces sclerostin expression in osteocytes, thereby activating Wnt/β-catenin signaling, which promotes osteoblast differentiation and bone formation [65, 66]. Additionally, exercise enhances the production of cytokines that prevent bone resorption, such as IL-2, IL-10, IL-12, IL-13, IL-18, and IFN, while decreasing the secretion of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α [67].

Exercise improves biomarkers of bone formation (e.g., OCN) while reducing markers of resorption (e.g., CTX-1), as evidenced in OVX rat studies included in this review. These findings highlight exercise's dual role in mitigating the catabolic effects of estrogen deficiency and enhancing anabolic processes, making it a valuable non-pharmacological strategy for managing osteoporosis. By addressing both structural and molecular deficits caused by estrogen loss, exercise provides a holistic approach to preserving bone health in postmenopausal conditions.

LIMITATION OF THE STUDY

A thorough search across multiple databases, strict research selection criteria, and comprehensive extraction of various exercise parameters and bone health outcomes are the strengths of this systematic review. The use of SYRCLE for quality assessment enhances the robustness of bias assessment. However, the review also has limitations. Primarily, it focuses on animal studies (OVX rats), which, while informative, may not fully translate to human conditions due to physiological differences. Publication bias may also exist because studies with favorable findings are more likely to be published. Moreover, although overt high-risk judgments were rare, the predominance of unclear ratings in critical methodological domains, particularly randomization of outcome assessment and selective reporting, raises substantial concerns about possible selection, detection, and reporting bias. The limited explicit reporting of allocation concealment, random housing procedures, and blinding of housing (collectively unclear in the vast majority of studies) further constrains confidence in the observed effect estimates. These substantial gaps in methodological transparency warrant cautious interpretation of the pooled findings and highlight the need for future studies to adhere to rigorous reporting standards. When assessed using the GRADE framework adapted for preclinical animal studies, these methodological gaps (together with high statistical heterogeneity and inherent indirectness of the OVX rat model) resulted in Low-to-Very Low certainty of evidence for the primary outcomes. Additionally, most included studies used female Sprague-Dawley or Wistar rats, with limited representation of other strains, ages, or disease comorbidities (e.g., obesity, metabolic dysfunction) relevant to diverse human populations. Further research should focus on longitudinal studies to assess the long-term effects of exercise on bone health in OVX rats. Additionally, studies comparing different exercise modalities directly, using standardized methods for measuring BMD and biomarkers, and exploring the molecular mechanisms by which exercise affects bone remodeling, could provide valuable insights into developing effective exercise interventions for postmenopausal women.

CONCLUSIONS

Exercise emerges as a promising non-pharmacological strategy for mitigating postmenopausal bone loss through integrated mechanical, biomolecular, hormonal, and inflammatory mechanisms. Despite the Low-to-Very Low certainty of evidence as assessed by GRADE, the consistent direction of BMD improvements and favorable modulation of bone turnover markers across exercise types and skeletal sites provide valuable preclinical insights supporting the rationale for exercise in clinical osteoporosis management, particularly as an adjunctive strategy to optimize pharmacological therapies or for patients intolerant of or refusing medication. However, the high proportion of unclear risk-of-bias ratings and the inherent translational limitations of the OVX rat model necessitate cautious interpretation of these findings. Future animal studies should employ standardized outcome assessments, longer follow-up periods, and direct comparative protocols to identify exercise prescriptions that maximize bone mechanical properties and fracture resistance, which attributes are more predictive of clinical fracture risk than BMD alone. Well-designed clinical trials are needed to confirm these preclinical findings in postmenopausal women.

Author contributions: Rini Maya Puspita: conceptualization, writing original draft and editing, methodology, formal analysis, data curation. Denny Agustiningsih: writing review and editing, methodology, supervision. Nunuk Purwanti: writing review and editing, analysis, supervision.

Statement for conflicts of interest: The authors declare no competing interests.

Consent to publish: The final manuscript has been reviewed, approved, and published with approval from all authors.

Funding: No specific grant from a public, private, or nonprofit funding organization was obtained for this study.

ACKNOWLEDGMENTS

We would like to express our gratitude to the reviewers for their time and expertise during the peer review of this systematic review and meta-analysis.

REFERENCES

1. Aibar-Almazan A, Voltes-Martinez A, Castellote-Caballero Y, Afanador-Restrepo DF, Carcelen-Fraile MC, Lopez-Ruiz E. Current status of the diagnosis and management of osteoporosis. Int J Mol Sci 2022; 23: 9465. https://doi.org/10.3390/ijms23169465
2. Cauley JA. Public health impact of osteoporosis. J Gerontol A Biol Sci Med Sci 2013; 68(10): 1243–51. https://doi.org/10.1093/gerona/glt093
3. Harvey N, Dennison E, Cooper C. Osteoporosis: impact on health and economics. Nat Rev Rheumatol 2010; 6(2): 99–105. https://doi.org/10.1038/nrrheum.2009.260
4. Sarafrazi N, Wambogo EA, Shepherd JA. Osteoporosis or low bone mass in older adults: United States, 2017–2018. NCHS Data Brief 2021; 405: 1–8. https://doi.org/10.15620/cdc:103477
5. Lin X, Xiong D, Peng YQ, Sheng ZF, Wu XY, Wu XP, et al. Epidemiology and management of osteoporosis in the people's Republic of China: current perspectives. Clin Interv Aging 2015; 10: 1017–33. https://doi.org/10.2147/cia.s54613
6. Dong Y, Kang H, Peng R, Song K, Guo Q, Guan H, et al. Global, regional, and national burden of low bone mineral density from 1990 to 2019: results from the global burden of disease study 2019. Front Endocrinol (Lausanne) 2022; 13: 870905. https://doi.org/10.3389/fendo.2022.870905
7. Lorentzon M. Treating osteoporosis to prevent fractures: current concepts and future developments. J Intern Med 2019; 285(4): 381–94. https://doi.org/10.1111/joim.12873
8. Adami G, Fassio A, Gatti D, Viapiana O, Benini C, Danila MI, et al. Osteoporosis in 10 years time: a glimpse into the future of osteoporosis. Ther Adv Musculoskelet Dis 2022; 14: 1759720X221083541. https://doi.org/10.1177/1759720x221083541
9. Clynes MA, Harvey NC, Curtis EM, Fuggle NR, Dennison EM, Cooper C. The epidemiology of osteoporosis. Br Med Bull 2020; 133(1): 105–17. https://doi.org/10.1093/bmb/ldaa005

10. Santos L, Elliott-Sale KJ, Sale C. Exercise and bone health across the lifespan. Biogerontology 2017; 18(6): 931–46. https://doi.org/10.1007/s10522-017-9732-6
11. Harding AT, Beck BR. Exercise, osteoporosis, and bone geometry. Sports (Basel) 2017; 5(2): 29. https://doi.org/10.3390/sports5020029
12. Streicher C, Heyny A, Andrukhova O, Haigl B, Slavic S, Schüler C, et al. Estrogen regulates bone turnover by targeting RANKL expression in bone lining cells. Sci Rep 2017; 7(1): 6460. https://doi.org/10.1038/s41598-017-06614-0
13. Föger-Samwald U, Dovjak P, Azizi-Semrad U, Kerschan-Schindl K, Pietschmann P. Osteoporosis: pathophysiology and therapeutic options. EXCLI J 2020; 19: 1017–37. https://doi.org/10.17179/excli2020-2591
14. Hughes JM, Guerriere KI, Popp KL, Castellani CM, Pasiakos SM. Exercise for optimizing bone health after hormone-induced increases in bone stiffness. Front Endocrinol (Lausanne) 2023; 14: 1219454. https://doi.org/10.3389/fendo.2023.1219454
15. Yuan Y, Chen X, Zhang L, Wu J, Guo J, Zou D, et al. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. Prog Biophys Mol Biol 2016; 122(2): 122–30. https://doi.org/10.1016/j.pbiomolbio.2015.11.005
16. Johnston BD, Ward WE. The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women. Biomed Res Int 2015; 2015: 635023. https://doi.org/10.1155/2015/635023
17. Hooijmans CR, de Vries RBM, Ritskes-Hoitinga M, Rovers MM, Leeflang MM, IntHout J, et al. Facilitating healthcare decisions by assessing the certainty in the evidence from preclinical animal studies. PLoS One 2018; 13(1): e0187271. https://doi.org/10.1371/journal.pone.0187271
18. Gala J, Díaz-curiel M, de la Piedra C, Calero J. Short- and long-term effects of calcium and exercise on bone mineral density in ovariectomized rats. Br J Nutr 2001; 86(4): 521–7. https://doi.org/10.1079/bjn2001428
19. Hart KJ, Shaw JM, Vajda E, Hegsted M, Miller SC. Swim-trained rats have greater bone mass, density, strength, and dynamics. J Appl Physiol (1985) 2001; 91(4): 1663–8. https://doi.org/10.1152/jappl.2001.91.4.1663
20. Sakakura Y, Shide N, Tsuruga E, Irie K, Yajima T. Effects of running exercise on the mandible and tibia of ovariectomized rats. J Bone Miner Metab 2001; 19(3): 159–67. https://doi.org/10.1007/s007740170036
21. Honda A, Sogo N, Nagasawa S, Shimizu T, Umemura Y. High-impact exercise strengthens bone in osteopenic ovariectomized rats with the same outcome as sham rats. J Appl Physiol (1985) 2003; 95(3): 1032–7. https://doi.org/10.1152/japplphysiol.00781.2002
22. Tromp AM, Bravenboer N, Tanck E, Oostlander A, Holzmann PJ, Kostense PJ, et al. Additional weight bearing during exercise and estrogen in the rat: the effect on bone mass, turnover, and structure. Calcif Tissue Int 2006; 79(6): 404–15. https://doi.org/10.1007/s00223-006-0045-z
23. Chang TK, Huang CH, Huang CH, Chen HC, Cheng CK. The influence of long-term treadmill exercise on bone mass and articular cartilage in ovariectomized rats. BMC Musculoskelet Disord 2010; 11: 185. https://doi.org/10.1186/1471-2474-11-185
24. Chen Y, Wang S, Bu S, Wang Y, Duan Y, Yang S. Treadmill training prevents bone loss by inhibition of PPARγ expression but not promoting of Runx2 expression in ovariectomized rats. Eur J Appl Physiol 2011; 111: 1759–67. https://doi.org/10.1007/s00421-010-1820-0
25. Bu S, Chen Y, Wang S, Zhang F, Ji G. Treadmill training regulates β-catenin signaling through phosphorylation of GSK-3β in lumbar vertebrae of ovariectomized rats. Eur J Appl Physiol 2012; 112(9): 3295–304. https://doi.org/10.1007/s00421-011-2306-4
26. Park J, Omi N. The effects of different exercise modes for preventing endothelial dysfunction of arteries and bone loss in ovariectomized rats. J Exerc Nutr Biochem 2014; 18(2): 133–9. https://doi.org/10.5717/jenb.2014.18.2.133

27. Huang TH, Su IH, Lewis JL, Chang MS, Hsu AT, Perrone CE, et al. Effects of methionine restriction and endurance exercise on bones of ovariectomized rats: a study of histomorphometry, densitometry, and biomechanical properties. J Appl Physiol (1985) 2015; 119(5): 517–26. https://doi.org/10.1152/japplphysiol.00395.2015
28. Ju YI, Sone T, Ohnaru K, Tanaka K, Fukunaga M. Effect of swimming exercise on three-dimensional trabecular bone microarchitecture in ovariectomized rats. J Appl Physiol (1985) 2015; 119(9): 990–7. https://doi.org/10.1152/japplphysiol.00147.2015
29. Murai IH, Roschel H, Pabis LVS, Takayama L, de Oliveira RB, dos Santos Pereira RT, et al. Exercise training, creatine supplementation, and bone health in ovariectomized rats. Osteoporos Int 2015; 26(4): 1395–404. https://doi.org/10.1007/s00198-014-3017-6
30. Sun X, Li F, Ma X, Ma J, Zhao B, Zhang Y, et al. The effects of combined treatment with Naringin and treadmill exercise on osteoporosis in ovariectomized rats. Sci Rep 2015; 5: 13009. https://doi.org/10.1038/srep13009
31. Oh T, Tanaka S, Naka T, Igawa S. Effects of high-intensity swimming training on the bones of ovariectomized rats. J Exerc Nutr Biochem 2016; 20(3): 39–45. https://doi.org/10.20463/jenb.2016.09.20.3.6
32. Kang YS, Kim CH, Kim JS. The effects of downhill and uphill exercise training on osteogenesis-related factors in ovariectomy-induced bone loss. J Exerc Nutr Biochem 2017; 21(3): 1–10. https://doi.org/10.20463/jenb.2017.0010
33. Kawano M, Itomine I, Monma M, Asakawa K, Toyoda A, Miyaguchi Y. Effects of combined treatment with fermented soybean (natto) intake and exercise on bone metabolism in ovariectomized rats. SAGE Open Med 2017; 5: 2050312117725643. https://doi.org/10.1177/2050312117725643
34. Okubo R, Sanada LS, Castania VA, Louzada MJQ, de Paula FJA, Maffulli N, et al. Jumping exercise preserves bone mineral density and mechanical properties in osteopenic ovariectomized rats even following established osteopenia. Osteoporos Int 2017; 28(4): 1461–71. https://doi.org/10.1007/s00198-017-3905-7
35. Souza MVC, Lino ADS, Ruffoni LGD, Domingos MM, Barbosa MR, Rodrigues MFC, et al. Resistance training and hormone replacement increase MMP-2 activity, quality and quantity of bone in ovariectomized rats. Motriz: Revista De Educação Física 2017; 23(4): 1–8. https://doi.org/10.1590/s1980-6574201700040003
36. Shimano RC, Yanagihara GR, Macedo AP, Yamanaka JS, Shimano AC, Tavares JMRS, et al. Effects of high-impact exercise on the physical properties of bones of ovariectomized rats fed to a high-protein diet. Scand J Med Sci Sports 2018; 28(5): 1523–31. https://doi.org/10.1111/sms.13058
37. Tamakoshi K, Nishii Y, Minematsu A. Upward running is more beneficial than level surface or downslope running in reverting tibia bone degeneration in ovariectomized rats. J Musculoskelet Neuronal Interact 2018; 18(4): 493–500.
38. Kim JS, Jeon J, An JJ, Yi HK. Interval running training improves age-related skeletal muscle wasting and bone loss: experiments with ovariectomized rats. Exp Physiol 2019; 104(5): 691–703. https://doi.org/10.1113/ep087458
39. Stunes AK, Erben RG, Schüler C, Eriksen EF, Tice M, Vashishth D, et al. Skeletal effects of plyometric exercise and metformin in ovariectomized rats. Bone 2020; 132: 115193. https://doi.org/10.1016/j.bone.2019.115193
40. Yuasa Y, Miyakoshi N, Kasukawa Y, Nagahata I, Akagawa M, Ono Y, et al. Effects of bazedoxifene and low-intensity aerobic exercise on bone and fat parameters in ovariectomized rats. J Bone Miner Metab 2020; 38(2): 179–87. https://doi.org/10.1007/s00774-019-01045-5
41. Aveline P, Cesaro A, Mazor M, Best TM, Lespessailles E, Toumi H. Cumulative effects of strontium ranelate and impact exercise on bone mass in ovariectomized rats. Int J Mol Sci 2021; 22(6): 3040. https://doi.org/10.3390/ijms22063040

42. Gao L, Li Y, Yang YJ, Zhang DY. The effect of moderate-intensity treadmill exercise on bone mass and the transcription of peripheral blood mononuclear cells in ovariectomized rats. Front Physiol 2021; 12: 729910. https://doi.org/10.3389/fphys.2021.729910
43. Tang L, Zhao T, Kang Y, An S, Fan X, Sun L. MSTN is an important myokine for weight-bearing training to attenuate bone loss in ovariectomized rats. J Physiol Biochem 2022; 78(1): 61–72. https://doi.org/10.1007/s13105-021-00838-5
44. Peng X, Gao B, Wang X, Qin X, Peng M, Zeng X. Hyperbaric oxygen and treadmill exercise partially prevented bone loss and bone microarchitecture deterioration in ovariectomized rats. Diving Hyperb Med 2023; 53(2): 111–9. https://doi.org/10.28920/dhm53.2.111-119
45. Wang Q, Weng H, Xu Y, Ye H, Liang Y, Wang L, et al. Anti-osteoporosis mechanism of resistance exercise in ovariectomized rats based on transcriptome analysis: a pilot study. Front Endocrinol (Lausanne) 2023; 14: 1162415. https://doi.org/10.3389/fendo.2023.1162415
46. Yang YJ, Li Y, Gao L. Postmenopausal osteoporosis: effect of moderate-intensity treadmill exercise on bone proteomics in ovariectomized rats. Front Surg 2023; 9: 1000464. https://doi.org/10.3389/fsurg.2022.1000464
47. Mohebbi R, Shojaa M, Kohl M, von Stengel S, Jakob F, Kerschan-Schindl K, et al. Exercise training and bone mineral density in postmenopausal women: an updated systematic review and meta-analysis of intervention studies with emphasis on potential moderators. Osteoporos Int 2023; 34(7): 1145–78. https://doi.org/10.1007/s00198-023-06682-1
48. Benedetti MG, Furlini G, Zati A, Mauro GL. The effectiveness of physical exercise on bone density in osteoporotic patients. Biomed Res Int 2018; 2018: 4840531. https://doi.org/10.1155/2018/4840531
49. Marini S, Barone G, Masini A, Dallolio L, Bragonzoni L, Longobucco Y, et al. The effect of physical activity on bone biomarkers in people with osteoporosis: a systematic review. Front Endocrinol (Lausanne) 2020; 11: 585689. https://doi.org/10.3389/fendo.2020.585689
50. Oliveira TP, Espada MC, Massini DA, Robalo RAM, Almeida TAF, Hernández-Beltrán V, et al. Effects of exercise and sports intervention and the involvement level on the mineral health of different bone sites in the leg, hip, and spine: a systematic review and meta-analysis. Int J Environ Res Public Health 2023; 20(15): 6537. https://doi.org/10.3390/ijerph20156537
51. Hejazi K, Askari R, Hofmeister M. Effects of physical exercise on bone mineral density in older postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. Arch Osteoporos 2022; 17(1): 102. https://doi.org/10.1007/s11657-022-01140-7
52. Moreira LDF, de Oliveira ML, Lirani-Galvão AP, Marin-Mio RV, dos Santos RN, Lazaretti-Castro M. Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. Arq Bras Endocrinol Metabol 2014; 58(5): 514–22. https://doi.org/10.1590/0004-2730000003374
53. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005; 115(12): 3318–25. https://doi.org/10.1172/jci27071
54. Cheng CH, Chen LR, Chen KH. Osteoporosis due to hormone imbalance: an overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. Int J Mol Sci 2022; 23(3): 1376. https://doi.org/10.3390/ijms23031376
55. Khosla S, Pacifici R. Estrogen deficiency and the pathogenesis of osteoporosis. In: Dempster DW, Bouxsein ML, Cauley JA, Cosman F, editors. Marcus and Feldman's osteoporosis. Massachusetts, USA: Academic Press; 2021. p. 773–97.
56. Li L, Chen X, Lv S, Dong M, Zhang L, Tu J, et al. Influence of exercise on bone remodeling-related hormones and cytokines in ovariectomized rats: a model of postmenopausal osteoporosis. PLoS One 2014; 9(11): e112845. https://doi.org/10.1371/journal.pone.0112845

57. Agustiningsih D, Soejono SK, Soesatyo MHNE. Exercise induces the synthesis of estrogen in ovariectomized sprague-Dawley rats ventricular myocardium through increase expression of CYP19 aromatase. Sport Sci Health 2015; 11(3): 337–43. https://doi.org/10.1007/s11332-015-0245-x
58. Blakemore J, Naftolin F. Aromatase: contribution to physiology and disease in women and men. Physiology (Bethesda) 2016; 31(4): 258–69. https://doi.org/10.1152/physiol.00054.2015
59. Rachner TD, Gobel A, Jaschke NP, Hofbauer LC. Challenges in preventing bone loss induced by aromatase inhibitors. J Clin Endocrinol Metab 2020; 105(10): dgaa463. https://doi.org/10.1210/clinem/dgaa463
60. Devlin MJ. Estrogen, exercise, and the skeleton. Evol Anthropol 2011; 20(2): 54–61. https://doi.org/10.1002/evan.20299
61. Shi V, Morgan EF. Estrogen and estrogen receptors mediate the mechanobiology of bone disease and repair. Bone 2024; 188: 117220. https://doi.org/10.1016/j.bone.2024.117220
62. Chang X, Xu S, Zhang H. Regulation of bone health through physical exercise: mechanisms and types. Front Endocrinol (Lausanne) 2022; 13: 1029475. https://doi.org/10.3389/fendo.2022.1029475
63. Li MCM, Chow SKH, Wong RMY, Qin L, Cheung WH. The role of osteocyte-specific molecular mechanism in regulation of mechanotransduction – a systematic review. J Orthop Translat 2021; 29: 1–9. https://doi.org/10.1016/j.jot.2021.04.005
64. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, et al. Wnt/β-catenin signaling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther 2022; 7: 3. https://doi.org/10.1038/s41392-021-00762-6
65. Hong AR, Kim SW. Effects of resistance exercise on bone health. Endocrinol Metab (Seoul) 2018; 33(4): 435–44. https://doi.org/10.3803/enm.2018.33.4.435
66. Hu L, Chen W, Qian A, Li Y. Wnt/β-catenin signaling components and mechanisms in bone formation, homeostasis and disease. Bone Res 2024; 12(1): 39. https://doi.org/10.1038/s41413-024-00342-8
67. Zhang L, Zheng YL, Wang R, Wang XQ, Zhang H. Exercise for osteoporosis: a literature review of pathology and mechanism. Front Immunol 2022; 13: 1005665. https://doi.org/10.3389/fimmu.2022.1005665
68. Kumar S, Smith C, Clifton-Bligh RJ, Beck BR, Girgis CM. Exercise for postmenopausal bone health - can we raise the bar? Curr Osteoporos Rep 2025; 23(1): 20. https://doi.org/10.1007/s11914-025-00912-7