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Physical activity and fitness for the prevention and management of bone diseases

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Physical activity and fitness for the prevention and management of bone diseases

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Editorial: Physical activity and fitness for the prevention and management of bone diseases

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exercise, bone mineral density, non-communicable diseases, children, adults, mice, DXA, pQCT

Editorial on the Research Topic
Physical activity and fitness for the prevention and management of bone diseases

Osteoporosis, the most common bone disease, is a major non-communicable disease (NCD) and a public health problem. Approximately 50% of women and 20% of men will suffer at least one osteoporosis fracture above the age of 50 years (Holroyd et al., 2008). Physical inactivity and poor fitness are known as important factors behind the rise in NCDs and have been linked poor bone health and bone metabolism (Gil-Cosano et al., 2020; Ubago-Guisado et al., 2020; Jürimäe et al., 2021). There is a lack of proper exercise intervention studies specifically designed for improving bone outcomes at clinical sites at various life stages and under certain conditions, which is crucial to underline the importance of physical activity and fitness both on bone accrual and bone preservation. Research have predominantly used Dual-energy X-ray Absorptiometry (DXA) scans to quantify bone mass but data from 3-dimensional devices is scarce and may provide with additional cortical and trabecular bone parameters to better understand bone adaptations to exercise. This Research Topic of Frontiers in Physiology, "Physical activity and fitness for the prevention and management of bone diseases" contains 11 publications (9 original manuscripts and 2 systematic reviews and meta-analyses) investigating the contribution of exercise-induced mechanical stimulation on relevant bone outcomes in a variety of populations.

Two timely systematic reviews and meta-analyses were included in this Research Topic. Marmol-Perez et al. showed that the reduced number of exercise interventions performed to date (n = 8 studies) were inappropriate and therefore, ineffective to obtain any beneficial effect on bone health in children and adolescents with cancer during and after oncological treatment. Wu et al. assessed the effectiveness of Tai Chi on adult patients with rheumatoid arthritis (n = 9 studies) and observed that Tai Chi was a safe method in this population yet it did not improve pain or physical function outcomes.

Four original studies were published in young populations. Wang et al. performed a randomized controlled trial to investigate the effects of a 14-week cheerleading programme on areal bone mineral density (aBMD) outcomes (measured by DXA) and advanced

glycosylation end products in older adolescents (n = 46). Their findings support the use of cheerleading as a non-pharmacological intervention to improve aBMD (and physical fitness) by reducing advanced glycosylation end products. Kurgan et al. performed a randomized controlled trial to examine the influence of a 12-week (combined) exercise training and nutritional counselling (dairy intake) intervention on bone remodelling and metabolism in adolescent females with overweight/obesity (n = 30). In this study, the authors found that the intervention blunted the increase in sclerostin and augmented the increase in osteoprotegerin (OPG): receptor activator of nuclear factor kappa B Ligand (RANKL), following acute exercise. Interestingly, dairy product consumption did not further influence these responses. Mello et al. investigated the accuracy of different physical fitness tests for the screening of low aBMD in children aged 6–11 years (n = 160). In the same sample, Mello et al. also examined the associations between the performance in commonly used fitness tests in young populations with the aBMD obtained in different regions, observing site- and- test specific associations.

Three original studies were published in adult populations. Two of them, Fernandez et al. and Buchanan et al. used whole-body vibration training to stimulate bone. Fernandez et al. conducted a 12-month non-randomized clinical trial to evaluate the effects of high-frequency and combined amplitude stimuli whole-body vibration on bone parameters and bone metabolism markers in physically inactive postmenopausal women (n = 255) with 10-year major osteoporotic fracture risk. In their study, DXA was used to measure aBMD and high-resolution peripheral quantitative computed tomography (HR-pQCT) to measure bone microarchitecture. Despite the protocol was well tolerated, the study failed to detect a significantly change in any of the bone outcomes nor an amelioration was observed. Buchanan et al. performed a randomized crossover design study with 10 postmenopausal women to compare the responses of selected circulating microRNAs to a bout of resistance exercise and a bout of whole-body vibration and, its association with a bone resorption marker (tartrate-resistant acid phosphatase 5b, TRAP5b) The authors found that whole-body vibration altered circulating miR-21-5p expression and that increases in TRAP5b were associated with greater downregulation of this expression. Stunes et al. used a cross-over study design to evaluate the acute effects of two exercise sessions (resistance training and high-intensity interval training) on bone turnover markers in young adults of both sexes (n = 39) and elderly men (n = 14). The authors observed no differences in the response of most bone turnover markers between the sessions. However, the altered response of bone turnover markers was generally blunted after 24 h.

Two original studies were published in mice. Massing et al. in their randomized controlled trial (six experimental groups) compared the effects of various 14-week treadmill exercise programs on the loss of cortical and trabecular structures in postmenopausal ovariectomized C57BL/6J mice (n = 42 female mice). Micro-computed tomography (μCT) was used to measure bone structures and data on bone turnover markers was also obtained. Ovariectomy induced bone loss, however, none of the treadmill exercise programs was beneficial in preventing the loss of bone mass, challenging the idea that just treadmill training is suitable to stop ovariectomy-related bone loss. Finally, Huesa et al. investigated the effects of 3–7 weeks of moderate exercise (forced wheel walking) on early joint pathology in the mouse destabilization of the medial meniscus model (n = 42 male mice). Histology and μCT were used to assess the joints. Findings showed the protective effect of exercise against cartilage damage after 7 weeks of exercise, and a temporary protection against osteosclerosis after 3 weeks of exercise.

The present Research Topic provides a short summary of the progress on the topic of exercise and bone disease which will be useful from a clinical and public health perspective. It also highlights the current limitations and the necessity of more powerful study designs to further advance in the knowledge. Bone adaptations to exercise are greater during growth due to the bone cellular activity and therefore, preventative measures are highly recommended to build strong bones and reduce the odds of suffering bone diseases later in life. Researchers, when possible, must consider performing randomized controlled trials and avoid short interventions since the remodelling cycle may take at least 4 months for cortical bone and 7–8 months for trabecular bone (Agerbaek et al., 1991). In this sense, it is highly recommended implementing behavioural change techniques that increase motivation and therefore, adherence to the programmes. Whenever possible, the combination of DXA and QCT scans with bone turnover markers is also recommended to obtain a wider picture of bone adaptations.

Author contributions

All the authors have made a substantial contribution to the design of the article. EU-G and LG-M drafted the article. All the authors revised it critically and approved the version to be published.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Acute Effects of Strength and Endurance Training on Bone Turnover Markers in Young Adults and Elderly Men

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Context: Exercise is recognized as an important strategy to prevent bone loss, but its acute effects on bone turnover markers (BTMs) and related markers remain uncertain.

Objective: To assess the acute effects of two different exercise modes on BTMs and related markers in young adults of both sexes and elderly men.

Design, Setting, Participants: This was a three-group crossover within-subjects design study with a total of 53 participants—19 young women (aged 22–30), 20 young men (aged 21–30 years), and 14 elderly men (aged 63–74 years)—performing two different exercise sessions [strength training (ST) and high-intensity interval training (HIIT)] separated by 2 weeks, in a supervised laboratory setting.

Main Outcome Measures: Plasma volume-corrected serum measurements of the BTMs C-terminal telopeptide of type 1 collagen (CTX-I) and procollagen of type 1 N-terminal propeptide (P1NP), total osteocalcin (OC), sclerostin, and lipocalin-2 (LCN2) at baseline, immediately after, and 3 and 24 h after each of the two exercise modes were performed.

Results and Conclusion: Analyses revealed sex- and age-dependent differences in BTMs and related bone markers at baseline and time-, sex-, and age-dependent differences in response to exercise. No differences between exercise modes were observed for BTM response except for sclerostin in young men and LCN2 in elderly men. An acute, transient, and uniform increase in P1NP/CTX-1 ratio was found in young participants, demonstrating that beneficial skeletal effects on bone metabolism can be attained through both aerobic endurance and resistance exercise, although this effect seems to be attenuated with age. The acute effects of exercise on bone-related

biomarkers were generally blunted after 24 h, suggesting that persistent alterations following prolonged exercise interventions should be assessed at later time points.

Keywords: exercise, sclerostin, lipocalin-2, HIIT (high-intensity interval training), strength training, CTX-1, P1NP, osteocalcin

INTRODUCTION

Already in 1638, Galileo Galilei stated that "loading is required to preserve bone mass" (1). In the 1980s, the "mechanostat" theory, which describes the mechanism underlying the load-induced bone adaptation, was introduced by Harold Frost, who also identified the osteocyte as the "mechanostat" of bone (2, 3). Mechanical load causes dynamic fluid shifts in bone canalicular networks, which are sensed by bone-embedded osteocytes (4). The osteocytes convert the mechanical signal into biochemical signals transmitted to the effector cells, osteoclasts, and osteoblasts, impacting bone turnover (4).

Mechanical load can improve bone health and reduce the risk of osteoporosis; hence, physical activity and exercise are now regarded as beneficial interventions to prevent bone loss in the elderly (5) as well as in young subjects (6). In children, exercise/mechanical load is necessary to obtain an optimal peak bone mass to prevent future osteoporosis and fractures (7, 8). In the context of musculoskeletal health, strength training (ST) is widely advocated as a countermeasure for age- and lifestyle-related declines (9, 10). We have previously shown that maximal ST (MST) in hack squat provides a simple, low-volume training method to improve skeletal properties and neuromuscular function in young adults and postmenopausal women (11–13). Hack squat MST is a lower-extremity exercise that provides compressive load through the spine and the hip, which are sites particularly prone to osteoporotic fractures. Aerobic high-intensity interval training (HIIT) is a well-recognized intervention for cardiovascular and metabolic health that has become widely advocated in the last few years (14–16). HIIT is often performed as treadmill running, which has been shown to improve bone metabolism and bone mineral density (BMD), both acutely and long term (17, 18).

Most studies state a positive relationship between BMD and exercise in adults and the elderly and that exercise contributes to maintaining BMD and/or reducing bone loss (19). Conflicting results regarding the effects of training, as well as the load, intensity, and magnitude of necessary exercise, are present; however, high-impact activities (e.g., running, plyometrics, and jumping) are generally considered more osteogenic than lower-impact activities (e.g., cycling and swimming). In impact exercises, such as running/HIIT, ground reaction forces constitute peak force exerted on the skeleton, whereas in low-impact exercises, such as MST, the peak force is generated through muscle work (20, 21). Although HIIT and MST subsequently differ in skeletal loading characteristics, both training forms can be regarded as being osteogenic (12, 17, 18, 22).

Circulating bone turnover markers (BTMs) reflect dynamic changes and provide a metabolic image of bone metabolism (23). The recommended BTMs in clinical practice are serum levels of resorption marker C-terminal telopeptide of type 1 collagen (CTX-I), a product of the enzymatic degradation of collagen fibers in bone, and total procollagen of type 1 N-terminal propeptide (P1NP), a sensitive marker for processes in proliferating osteoblast (23–25). Osteocalcin (OC), which is synthesized during bone formation by mature osteoblasts, is often regarded as a bone formation marker (26) but might also be released during bone resorption in both its intact and fragmented forms and may therefore indicate general bone remodeling (27).

Bone resorption and formation are orchestrated by many substances, and measurements of these may yield insight into the mechanisms for alterations in serum levels of BTMs. Sclerostin is an osteocyte-specific secreted potent inhibitor of bone formation *via* inhibition of the Wnt/β-catenin pathway. Immobilization is associated with a rise in serum sclerostin and decreased BMD (28).

Lipocalin-2 (LCN2) has been proposed to act as an osteoblast mechano-responding gene, upregulated in osteoblasts during microgravity and correlated with poor osteoblast activity (29). Serum LCN2 was increased after 14 days in a bed rest study with male subjects, and *Lcn2* expression was upregulated in bones in different mouse models of unloading, which could be counteracted by physical activity (30). In contrast, serum LCN2 was found to increase after high-intensity acute exercise in young and middle-aged male runners (31).

A systematic review of BTMs in subjects with osteoporosis revealed possible exercise benefits in terms of improving bone formation and decreasing bone resorption biomarkers (32). Whereas some studies have reported beneficial acute effects of exercise on BTMs (17, 22, 33), a direct comparison of heavy resistance training and aerobic running exercise has not yet been performed under strictly controlled conditions. Hack squat ST and treadmill HIIT are two well-studied training interventions that are highly relevant in the context of musculoskeletal and cardiometabolic health (11–16). Also, the acute response of these interventions on other mechano-responsive biomarkers, such as sclerostin and LCN2, has been less investigated, along with possible sex and age differences.

In the current study, we aimed to characterize the respective responses of hack squat ST and treadmill HIIT across age and gender. Specifically, we sought to investigate the acute exercise-induced effects of these two interventions on serum BTMs (CTX-1 and P1NP), OC, sclerostin, and LCN2 in men and women 21 to 30 years of age and elderly men 63 to 74 years of age.

METHODS

Participants and Study Design

This was a three-group crossover within-subjects design study. Recruitment of participants took place from September 2018 to February 2019, through posters on local gyms and at the University of Science and Technology, Trondheim, Norway. The study was approved by the Regional Ethics Committee for Medical and Health Research (REK2018/926) and was performed in accordance with the Declaration of Helsinki.

A total of 53 men and women volunteered to participate in this study (19 young women (age 22–31 years), 20 young men (age 21–30 years), and 14 elderly men (age 63–74 years). Inclusion criteria were as follows: 1) did not suffer any significant illness relevant to the study, 2) free from chronic conditions and injuries that could influence blood samples or prevent the participants in performing the physical tests, and 3) not taking dietary supplements known to affect bone metabolism. All participants agreed to take part in the study by signing a consent form.

A flowchart of the study is presented in **Figure 1**. Each participant performed two training sessions, ST and HIIT, separated by 2 weeks. During both training sessions, a total of four blood samples were collected from each participant: at baseline (pre), immediately (0–5 min, post), and 3 and 24 h after training. The training sessions were performed in a supervised laboratory setting, and participants were asked to fast overnight, avoid dietary supplements, and exercise 48 h before the sessions. At the first visit (between 08:00 and 09:00), participants signed an informed consent form and answered a questionnaire regarding lifestyle, medical history, and use of oral contraceptives (young women). Overnight fasting baseline ($ST_{baseline}$) blood samples were drawn, and participants were tested for 1 repetition maximum (1RM) before the ST session, followed by post and 3 h after ST blood sampling. Between 08:00 and 10:00 the following day (second visit), blood samples were collected 24 h after ST, following an overnight fast. Body weight was obtained using an electronic scale, height was measured using a wall-mounted stadiometer, and body mass index (BMI; kg/m^2) was calculated. Further, after participants had a meal, their maximal oxygen uptake ($\dot{V}O_{2max}$) and maximal heart rate (HR) (HR_{max}) were measured. Two weeks later, between 08:00 and 09:00 (third visit), overnight fasting baseline ($HIIT_{baseline}$) blood samples were drawn, and participants took part in the HIIT session, followed by post and 3 h after HIIT blood sampling. Between 08:00 and 10:00 the following day (fourth visit), the blood samples after overnight fasting 24 h after HIIT were collected.

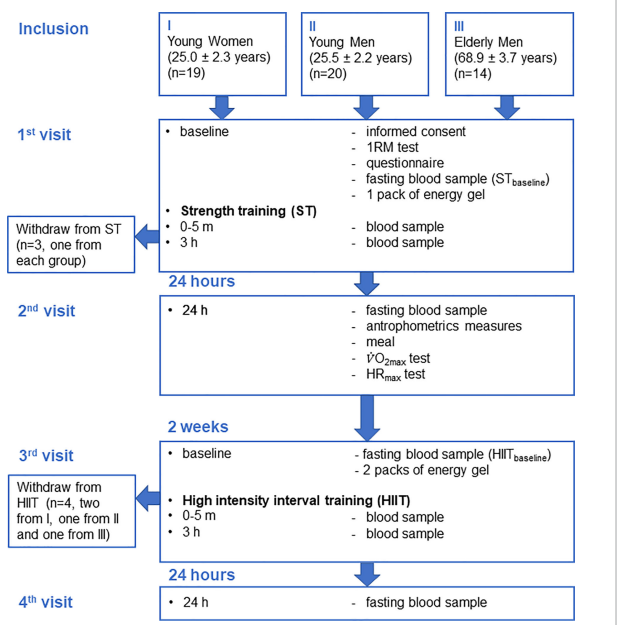

FIGURE 1 | Flowchart of the study.

One Repetition Maximum

1RM was tested in a seated leg press apparatus (TechnoGym, Cesena, Italy), according to a protocol by the American College of Sports Medicine (ACSM) (34). The protocol consisted of two warm-up sets and a maximum of five attempts to determine the maximum load of a single dynamic leg press movement. Participants were instructed to perform a leg press with a knee joint angle of 90°. The first warm-up set consisted of 5–10 repetitions at 40%–60% of assumed 1RM. The second warm-up set consisted of 3–5 repetitions at 60%–80% of assumed 1RM. After the second warm-up, the load was increased to the expected 1RM and the first attempt started. If the participant was able to complete one repetition with the given load, a new attempt with increased load was initiated after 3–5 min of rest, which was repeated until failure.

Maximal Oxygen Uptake and Maximal Heart Rate

Maximal oxygen uptake ($\dot{V}O_{2max}$) was measured on a treadmill (Woodway PPS Med, Weil am Rhein, Germany) with a Metamax II Portable device (Cortex, Leipzig, Germany). Participants warmed up for 10 min before the test. During the test, the minimum inclination on the treadmill was 5.3%, and the speed was increased every 2–3 min until the participant reached exhaustion. $\dot{V}O_{2max}$ was accepted when it leveled off despite further increases in workload and verified by lactate levels ≥8.0 mmol/L and respiratory exchange ratio ≥1.05. Lactate was measured with a biochemical analyzer (EKF Biosen C-line Diagnostics, Barleben, Germany), and HR was assessed with a Polar M200 monitor (Kempele, Finland). Maximal HR (HR_{max}) was set as the highest HR registered + 3 beats per minute.

Blood Sampling and Biochemical Analyses

Blood samples were collected by standard venipuncture. Blood was collected in vacuum tubes, let sit for 30 min at room temperature, and centrifuged (3,000 *g*/4°C/10 min) before serum was aliquoted and stored at −80°C until further analyses.

Serum albumin, creatinine, and total calcium (Ca) were analyzed with accredited analyses at the Department of Biochemistry, St. Olavs University Hospital, Trondheim, Norway. The bone turnover markers (BTMs) in serum resorption marker C-terminal telopeptide of type 1 collagen (CTX-I) and formation marker total procollagen of type 1 N-terminal propeptide (P1NP) were measured by accredited electrochemiluminescence immunoassays (Roche Cat# 11972308122, RRID:AB_2905599 and Roche Cat# 03141071190, RRID:AB_2782967, respectively), and total osteocalcin (OC) (intact (1-49) and large fragments (1-43)) by an accredited chemiluminescence assay (DiaSorin, Cat# 310950, RRID:AB_2917975;LIAISON® OSTEOCALCIN 1-49) at the Hormone Laboratory, Oslo University Hospital, Oslo, Norway. Serum sclerostin was analyzed by a human sclerostin ELISA kit (Biomedica Cat# BI-20492, RRID : AB_2894889), and serum LCN2 was analyzed by a human LCN2/NGAL DuoSet ELISA kit (R&D Systems Cat# DLCN20, RRID : AB_2894833). Analyses were done according to the manufacturers' instructions.

Strength Training

At the first visit, after the overnight fasting $baseline_{ST}$ blood samples were drawn, participants were offered one pack of energy gel, containing pure carbohydrates (60 ml, 87 kcal) (Go Isotonic Energy, Science in Sport, London, UK) before the ST session. The training was conducted in the same leg press apparatus as used for the 1RM testing procedure, consisting of 4 sets of 8–10 repetitions until failure, with 2–3-min rest between sets. The load was 80% of the participants measured 1RM. The training session was performed with a slow eccentric movement down to a 90° knee angle and maximal intended velocity in the concentric movement. Participants' mean values of 1RM are presented in **Table 1**.

High-Intensity Interval Training

At the third visit, 2 weeks after the ST session, the participants took part in a HIIT session on a treadmill, as previously described (35). The participants were offered two packs of energy gel before this session. Participants warmed up for 10 min before performing 4 × 4 min intervals, with 3 min of active rest, on a treadmill with a 5.3% incline. During the intervals, the treadmill speed was set to a target intensity of 90%–95% of each participant's HR_{max}, and during active rest periods, an intensity was set corresponding to 70% of HR_{max}. Participants' mean values of HR_{max} are presented in **Table 1**.

Corrections for Plasma Volume Changes

Plasma volume changes (ΔPV) can occur during and after exercise due to transient fluid shifts (hemodilution and hemoconcentration) (36). The ΔPV can affect the interpretation of biochemical measurements in blood, and changes in the concentrations of biomarkers in blood should therefore be adjusted for ΔPV in exercise studies (36, 37). The standard method for calculation of %ΔPV has been the Dill and Costill equation (37), requiring hematocrit (Htc) and hemoglobin (Hb) measurements. Changes in serum total calcium levels after exercise have been found to correlate well with %ΔPV and can therefore be used as a hemoconcentration biomarker in exercise studies (38). In the current study, all serum analyses at post-test, 3 h, and 24 h were corrected for %ΔPV using %Δ in [Ca] from corresponding baseline values, before comparisons. The following equations for corrections were used:

$$\mathrm{A})\ \%\Delta PV_{t1} = 100 * \left(\frac{[Ca]_{t1} - [Ca]_{baseline}}{[Ca]_{baseline}} \right)$$

TABLE 1 | Participants' baseline characteristics and measures.

	I, Young women (n = 19)		II, Young men (n = 20)		III, Elderly men (n = 14)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Age (years)	25.0 ± 2.3	22–30	25.5 ± 2.2	21–30	68.9 ± 3.7	63–74
Weight (kg)	65.6 ± 7.5	46.6–75.5	77.2 ± 10.3	62.8–103	79.2 ± 10.4	61.2–98.5
Height (cm)	169 ± 5.4	158–181	181 ± 6.2	168–193	180 ± 6.7	169–193
BMI ($kg \cdot m^{-2}$)	23.0 ± 2.9	16–28	23.5 ± 2.5	19–28	24.4 ± 2.5	20–28
$\dot{V}O_{2max}$						
($L \cdot min^{-1}$)	3.3 ± 0.5	2.26–4.05	4.8 ± 0.6	3.76–5.85	3.5 ± 0.5	2.64–4.81
($mL \cdot kg^{-1} \cdot min^{-1}$)	50.5 ± 6.3	35.7–60.3	62.6 ± 8.3	46.9–77.0	42.5 ± 5.4	34.0–51.3
HRmax (bmp)*	195 ± 9	179–210	196 ± 7	182–207	169 ± 17	143–197
1RM (kg)**	104 ± 25	64–140	134 ± 28	80–194	130 ± 17	98–164
sAlbumin (g/L)	44 ± 1.8	41–47	46 ± 1.8	42–49	43 ± 1.8	40–46
Reference***		36–48		36–48		36–45
sCreatinine (μmol/L)	73.8 ± 12.5	58–105	78.8 ± 7.8	63–90	84.3 ± 13.4	61–106
Reference***		45–90		60–105		60–105
sCalcium (nmol/L)	2.35 ± 0.04	2.26–2.46	2.40 ± 0.08	2.14–2.50	2.36 ± 0.06	2.22–2.44
Reference***		2.15–2.51		2.15–2.51		2.15–2.51

Data are in mean ± SD and range (minimum to maximum). Baseline serum levels are calculated mean values from $ST_{baseline}$ and $HIIT_{baseline}$.

BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen uptake; HR_{max}, maximal heart rate in beats per minute; 1RM, one repetition maximum; s, serum.

**Data of one young woman are missing.*

***Data of one young woman and one young man are missing.*

****Clinical reference ranges for validated analyses from Department of Biochemistry, St. Olavs University Hospital, Trondheim, Norway.*

$$B)\ [bone\quad marker\quad]corrected_{t1} = [bone\quad marker\quad]_{t1} * \left(1 - \left(\frac{\%\,\Delta PV_{t1}}{100}\right)\right)$$

where t_1 represents the time point for measurements (post-test, 3 h, or 24 h) after baseline in the applicable training mode. All serum analyses at post-tests and 3 and 24 h after the training intervention were performed with serum levels corrected for ΔPV as described (denoted as bone marker$_c$).

Statistical Analyses

Three missing values at individual time points (due to participant's absence) and one extreme outlier were replaced with the median value of the corresponding time point for analyses.

Data were assessed for normality using a Shapiro–Wilk test and checked for outliers by visual inspection by Q-Q plots. Skewness and kurtosis were analyzed by g1 and z-score.

A one-way ANOVA with a *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for each comparison was used to examine group differences in mean baseline values between the two young groups and the two male groups (calculated as mean values from $ST_{baseline}$ and $HIIT_{baseline}$) of BTMs, OC, sclerostin, and LCN2.

Two-way repeated-measures ANOVA (two-way-RM-ANOVA) with Geisser–Greenhouse correction to adjust for lack of sphericity was used to examine time and group main effects for CTX-1$_c$, P1NP$_c$, P1NP$_c$/CTX$_c$ ratio, OC$_c$, sclerostin$_c$, and LCN2$_c$ in each training mode. In case of a significant simple main effect of time, a within-group *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for each comparison was performed. Thereafter, a two-way-RM-ANOVA with Geisser–Greenhouse correction to adjust for lack of sphericity was used to examine the time and training mode main effects for each serum marker within each participant group. In case of significant training mode main effects, a Šidák multiple-comparison *post-hoc* test, with computed multiplicity adjusted p-values for each comparison, was performed for comparison of training mode at each time point.

For serum markers that exhibited a significant simple main effect of time, Pearson's correlation coefficients were used to determine the relationship between percentage change in plasma volume-corrected serum marker concentrations from baseline and the participants' 1RM and $\dot{V}O_{2max}$.

All statistical analyses were performed using SPSS (IBM SPSS, Inc., version 27, 2020) and GraphPad Prism (GraphPad Software, Inc., version 9.2.0, 2021). Figures were made in GraphPad Prism.

RESULTS

Participants and Baseline Measurements

A flowchart of the study design and participants is shown in **Figure 1**. Values for serum BTMs and related markers were normally distributed, with the expectation of one extreme outlier value for measurements of LCN2$_c$, which were replaced with the median value of the corresponding time point for further analyses. The participants' baseline characteristics, $\dot{V}O_{2max}$, HR_{max}, and 1RM are presented in **Table 1**. All three groups of participants were healthy, meaning an average BMI within the accepted normal range and fasting mean baseline serum levels of albumin, creatinine, and calcium within the clinical reference ranges (**Table 1**). A total of 396 blood samples (n = 50 and 4 time points in ST and n = 49 and 4 time points in HIIT) were included in the analyses.

Serum values for each group and exercise mode of calcium [Ca] and the %Δ[Ca] from the baseline used for correction of ΔPV (%ΔPV) are shown in **Table 2**.

The mean baseline levels of BTMs, OC, sclerostin, and LCN2 are presented in **Figure 2**. Young men had higher baseline CTX-1 levels than young women (mean difference (MD) = 0.17 μg/L, **Figure 2A**). Elderly men had lower baseline BTMs (CTX-1 and P1NP) and OC levels as compared to the young male group (CTX-1, MD = 0.35 μg/L; P1NP, MD = 28.6 μg/L; OC, MD = 1.4 nmol/L, **Figures 2A–C**). Elderly men had higher baseline sclerostin and LCN2 levels as compared to young men (sclerostin, MD = 23.2 pmol/L; LCN2, MD = 17.4 μm/L, **Figures 2D, E**).

Sex/Age and Time Effects

There were significant main effects of group (sex/age)-by-time on plasma volume-corrected levels of CTX-1$_c$, P1NP$_c$/CTX-1$_c$ ratio, and sclerostin$_c$ in ST ($F(6,141) = 5.48, 3.61$, and 2.18, respectively, $p < 0.05$ for all) and for CTX-1$_c$ and P1NP$_c$ in HIIT ($F(6,138) =$

TABLE 2 | Participants' serum total calcium [Ca] and %Δ[Ca] from baseline for correction of plasma volume changes.

Calcium (nmol/L)	I, Young women		II, Young men		III, Elderly men	
	ST (n = 18)	HIIT (n = 17)	ST (n = 19)	HIIT (n = 19)	ST (n = 13)	HIIT (n = 13)
Baseline (BL)	2.37 ± 0.07	2.34 ± 0.05	2.39 ± 0.08	2.42 ± 0.08	2.36 ± 0.05	2.35 ± 0.08
Post test	2.43 ± 0.09	2.49 ± 0.06	2.52 ± 0.08	2.47 ± 0.08	2.44 ± 0.05	2.40 ± 0.07
%Δ[Ca] from BL	6.62 ± 2.89	2.69 ± 2.40	5.25 ± 1.95	2.28 ± 1.97	3.61 ± 1.92	1.68 ± 1.93
3 h	2.40 ± 0.06	2.42 ± 0.05	2.45 ± 0.08	2.47 ± 0.09	2.38 ± 0.06	2.39 ± 0.08
%Δ[Ca] from BL	3.59 ± 2.90	1.71 ± 1.96	2.26 ± ′3.19	2.07 ± 2.7	0.92 ± 1.68	0.99 ± 1.90
24 h	2.38 ± 0.06	2.39 ± 0.07	2.41 ± 0.07	2.41 ± 0.07	2.34 ± 0.07	2.32 ± 0.06
%Δ[Ca] from BL	2.10 ± 2.60	0.47 ± 2.05	0.52 ± 3.10	−0.52 ± 3.09	−0.95 ± 1.72	−1.37 ± 2.00

Data are in mean ± SD.

ST, strength training; HIIT, high-intensity interval training; BL, baseline.

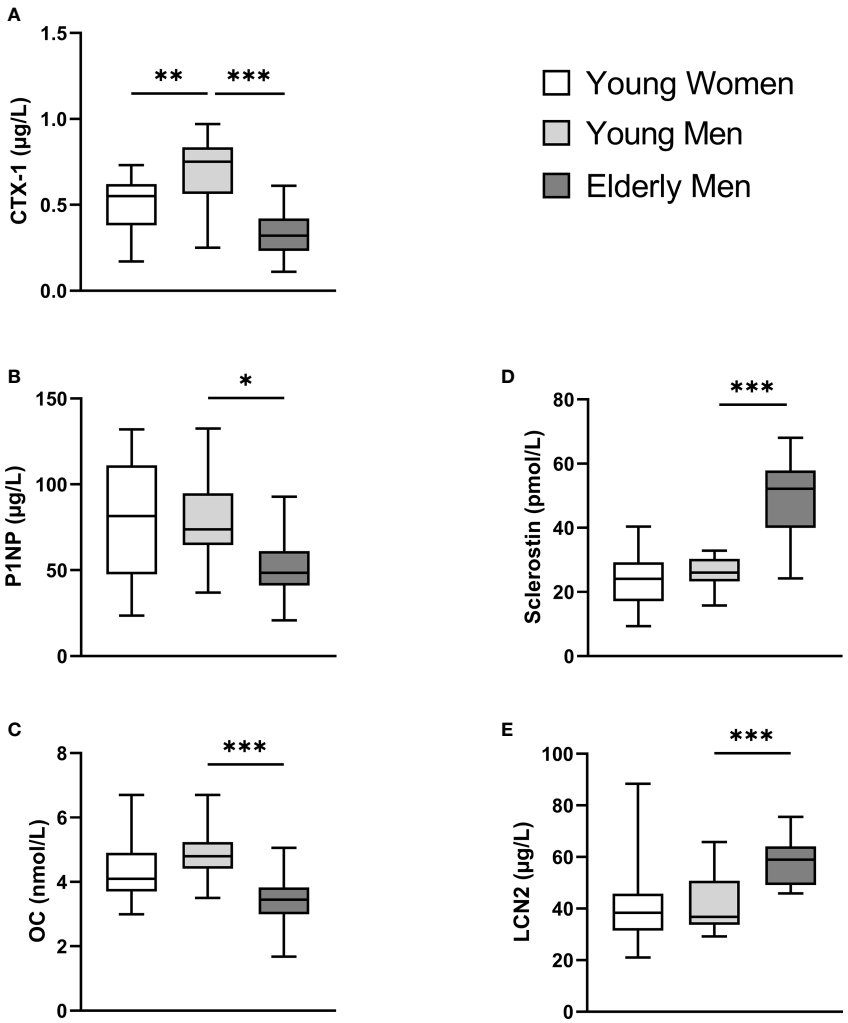

FIGURE 2 | Participants' baseline serum levels (calculated mean values from $ST_{baseline}$ and $HIIT_{baseline}$) of C-terminal telopeptide of type 1 collagen (CTX-I) **(A)**, total procollagen of type 1 N-terminal propeptide (P1NP) **(B)**, total osteocalcin (OC) **(C)**, sclerostin **(D)**, and lipocalin-2 (LCN2) **(E)**. A one-way ANOVA with a *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for comparisons was used to examine group differences between mean baseline values between young women versus young men and young versus elderly men. Data are presented as boxplot with range (minimum to maximum). *p < 0.05, **p < 0.01, ***p < 0.001.

7.81, p = 0.0001, and F(6,138) = 4.35, p = 0.0005, respectively). There was a significant simple main effect of group on all analyzed plasma volume-corrected serum markers (F(2,47) ranging from 3.40 to 39.9, p < 0.05 for all in ST and F(2, 44) ranging from 3.43 to 41.64, p < 0.05 for all in HIIT).

There was a significant simple main effect of time on most of the plasma volume-corrected serum markers in both exercise modes, and *post-hoc* Tukey multiple-comparison tests of the simple effect of time (from baseline) within-group and exercise mode showed the following.

Group I, Young Women

Serum $CTX\text{-}1_c$ decreased from baseline to post-test after ST training and after 3 h after both training modes, before returning to baseline levels after 24 h (**Figure 3A**). Serum $P1NP_c$ decreased from baseline to post-test and 3 h after ST training, increased from baseline to post-test after HIIT, and returned to baseline levels after 24 h for both exercise modes (**Figure 3B**). Serum $P1NP_c/CTX\text{-}1_c$ ratio increased from ST baseline to post-test after 3 h and from HIIT baseline to post-test and returned to baseline levels after 24 h for both exercise modes (**Figure 3C**). Serum OC_c decreased from baseline ST to post-test after 3 and 24 h. After HIIT, serum OC_c decreased from baseline to 3 h but returned to baseline values after 24 h (**Figure 3D**). There were no significant time effects on the serum levels of neither $sclerostin_c$ nor $LCN2_c$ after either exercise mode in the young female group (**Figures 3E, F**).

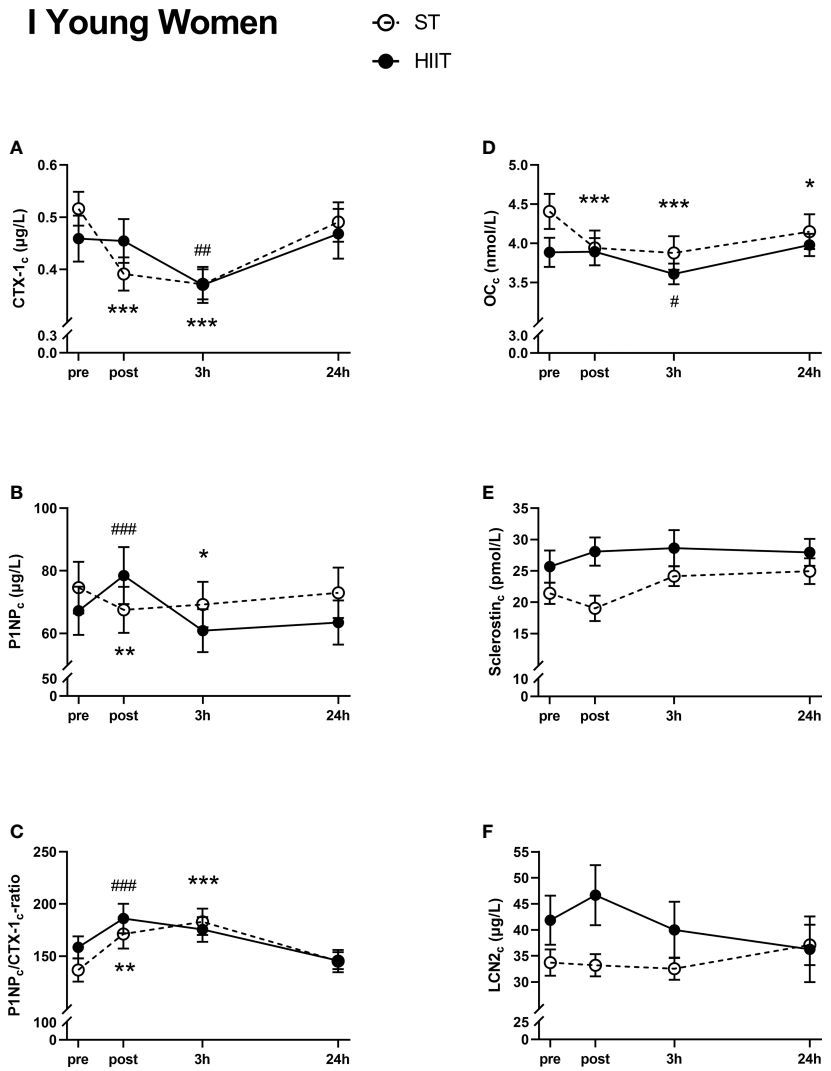

FIGURE 3 | Plasma volume-corrected serum levels of C-terminal telopeptide of type 1 collagen (CTX-I$_c$) **(A)**, total procollagen of type 1 N-terminal propeptide (P1NP$_c$) **(B)**, PINP$_c$/CTX-1$_c$ **(C)**, total osteocalcin (OC$_c$) **(D)**, sclerostin$_c$ **(E)**, and lipocalin-2 (LCN2$_c$) **(F)** in young women at baseline (pre), immediately after the strength training (ST) or high-intensity interval training (HIIT) sessions (post), and 3 and 24 h after exercise. Data are in mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus baseline after ST and $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 versus baseline after HIIT (two-way repeated-measures ANOVA and a within-group *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for each comparison).

Group II, Young Men

There was a significant decrease in serum CTX-1$_c$ from ST baseline to post-test and 3 h and from HIIT baseline to 3 h (**Figure 4A**). Serum CTX-1$_c$ returned to baseline values after 24 h for both exercise modes. Serum P1NP$_c$ decreased from ST baseline to post-test and increased from HIIT to post-test (**Figure 4B**). After 24 h, P1NP$_c$ returned to baseline levels. The serum P1NP$_c$/CTX-1$_c$ ratio was increased from baseline after 3 h in ST and from HIIT baseline to post-test and after 3 h and returned to baseline values for both after 24 h (**Figure 4C**). Serum OC$_c$ decreased from ST baseline to post-test and after 3 h and from HIIT baseline to 3 h before returning to baseline values after 24 h (**Figure 4D**). Serum sclerostin$_c$ increased from ST baseline to 24 h and increased from HIIT baseline to post-test and after 24 h (**Figure 4E**). There were no significant time effects on serum LCN2$_c$ after ST, but LCN2$_c$ increased from HIIT baseline to post-test and was thereafter decreased compared to baseline values after 24 h (**Figure 4F**).

Group III, Elderly Men

There were no significant time effects on serum CTX-1$_c$, except for a significant increase from baseline to 24 h after HIIT (**Figure 5A**). No time effects were seen in serum P1NP$_c$ levels after ST, while there was a significant increase from HIIT baseline to post-test followed by a decrease after 3 h, before returning to baseline values after 24 h (**Figure 5B**). There were no significant time effects on serum P1NP$_c$/CTX-1$_c$ ratio or serum OC$_c$ in neither of the two exercise modes (**Figures 5C, D**).

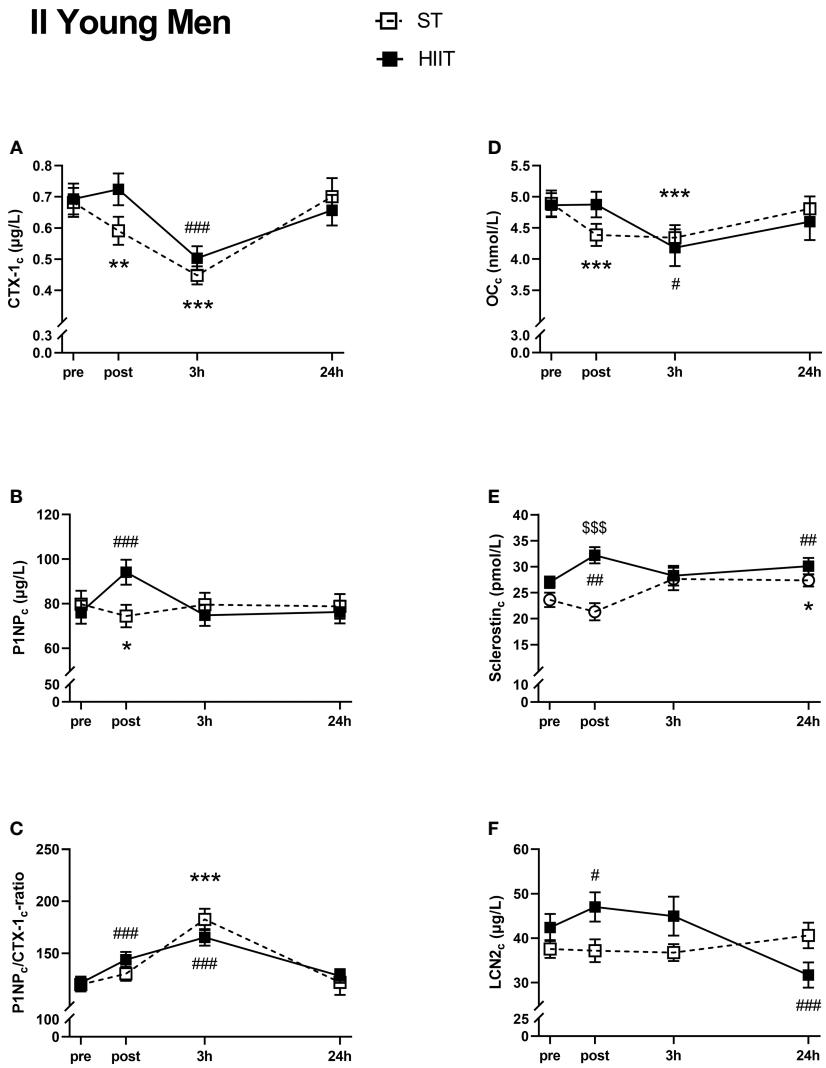

FIGURE 4 | Plasma volume-corrected serum levels of C-terminal telopeptide of type 1 collagen (CTX-I$_c$) **(A)**, total procollagen of type 1 N-terminal propeptide (P1NP$_c$) **(B)**, PINP$_c$/CTX-1$_c$ **(C)**, total osteocalcin (OC$_c$) **(D)**, sclerostin$_c$ **(E)**, and lipocalin-2 (LCN2$_c$) **(F)** in young men at baseline (pre), immediately after the strength training (ST) or high-intensity interval training (HIIT) sessions (post), and 3 and 24 h after exercise. Data are in mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus baseline after ST and $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 versus baseline after HIIT (two-way repeated-measures ANOVA and a within-group *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for each comparison). $^{\$\$\$}$p < 0.001 ST versus HIIT training modes (two-way repeated-measures ANOVA and a Šidák multiple-comparison *post-hoc* test for comparison of the two training modes at each time point).

Serum sclerostin$_c$ was not affected after ST at any measured time points, unlike after HIIT, where sclerostin$_c$ was significantly increased at post-test, after 3 and 24 h compared to baseline (**Figure 5E**). Serum LCN2$_c$ was not significantly affected by time after exercise, except for a small increase after 24 h compared to ST baseline (**Figure 5F**).

Exercise Mode and Time Effects

Since there was a significant main effect of the group on all plasma volume-corrected serum markers, the effect of exercise mode by time was analyzed separately in the groups I, II, and III for each serum analysis.

Group I, Young Women:

The two-way RM ANOVA showed no significant main effects of the exercise modes ST and HIIT for any of the plasma volume-corrected measured markers (CTX-1$_c$, $F(1,33) = 0.008$, $p = 0.93$; P1NP$_c$, $F(1,33) = 0.106$, $p = 0.75$; P1NP$_c$/CTX-1$_c$ ratio, $F(1,33) = 0.224$, $p = 0.64$; OC$_c$, $F(1,33) = 0.842$, $p = 0.37$; sclerostin, $F(1,33) = 3.52$, $p = 0.07$; LCN2$_c$, $F(1,33) = 1.94$, $p = 0.17$).

Group II, Young Men

There were no significant main effects of the exercise modes for any of the plasma volume-corrected serum markers, except for sclerostin$_c$ (CTX-1$_c$, $F(1,36) = 0.407$, $p = 0.53$; P1NP$_c$, $F(1,36) =$

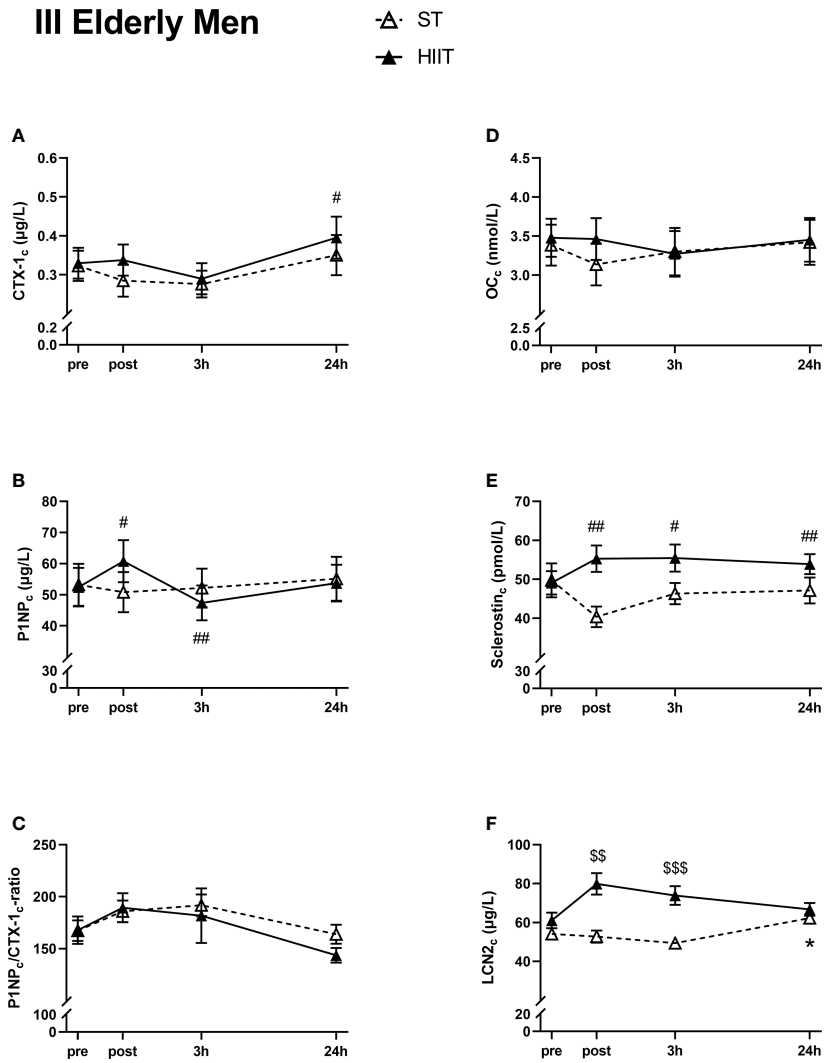

FIGURE 5 | Plasma volume-corrected serum levels of C-terminal telopeptide of type 1 collagen ($CTX\text{-}I_c$) **(A)**, total procollagen of type 1 N-terminal propeptide ($P1NP_c$) **(B)**, $PINP_c/CTX\text{-}1_c$ **(C)**, total osteocalcin (OC_c) **(D)**, $sclerostin_c$ **(E)**, and lipocalin-2 ($LCN2_c$) **(F)** in elderly men at baseline (pre), immediately after the strength training (ST) or high-intensity interval training (HIIT) sessions (post), and 3 and 24 h after exercise. Data are in mean ± SEM. *$p < 0.05$ versus baseline after ST and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus baseline after HIIT (two-way repeated-measures ANOVA and a within-group *post-hoc* Tukey's multiple-comparison test with computed multiplicity adjusted p-values for each comparison). $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ ST versus HIIT training modes (two-way repeated-measures ANOVA and a Šidák multiple-comparison *post-hoc* test for comparison of the two training modes at each time point).

0.085, p = 0.77; $P1NP_c/CTX\text{-}1_c$ ratio, F(1,36) = 0.009, p = 0.92; OC_c, F(1,36) = 0.008, p = 0.93; $sclerostin_c$, F(1,36) = 5.837, p = 0.021; $LCN2_c$, F(1,36) = 0.874, p = 0.36).

A Šidák multiple-comparison *post-hoc* test for comparison of exercise mode at each time point showed significantly higher $sclerostin_c$ in HIIT compared to ST at post-test immediately after exercise (MD 10.85 pmol/L) (**Figure 4E**).

Group III, Elderly Men

There was a significant main effect of exercise mode for $LCN2_c$, but not for $CTX\text{-}1_c$, $P1NP_c$, OC_c, and $sclerostin_c$ ($CTX\text{-}1_c$, F(1,24) = 0.261, p = 0.61; $P1NP_c$, F(1,24) = 0.007, p = 0.93; $P1NP_c/CTX\text{-}1_c$ ratio, F(1,24) = 0.295, p = 0.66; OC_c, F(1,24) = 0.081, p = 0.78; $sclerostin_c$, F(1,24) = 3.243, p = 0.08; $LCN2_c$, F(1,24) = 3.567, p < 0.001).

A Šidák multiple-comparison *post-hoc* test for comparison of exercise mode at each time point showed significantly higher levels of $LCN2_c$ in HIIT versus ST at post-test immediately after exercise (MD 27.21 µg/L), as well as 3 h after exercise (MD 24.56 µg/L) (**Figure 5F**).

Correlations

For plasma volume-corrected serum markers that exhibited a significant simple main effect of time, we analyzed the relationship between the percentage change and 1RM and $\dot{V}O_{2max}$. For young women, we found a negative correlation

between 1RM and CTX-1_c percentage changes from baseline to post-test and 3 h after ST ($r = -0.571$, $p = 0.013$, and $r = -0.471$, $p = 0.049$, respectively) and a positive correlation between 1RM and the percentage changes in $P1NP_c$ from baseline to post-test after HIIT ($r = 0.645$, $p = 0.013$). The change in OC_c was negatively correlated to 1RM at post-test after ST ($r = -0.664$, $p = 0.003$) and positively correlated to 1RM at post-test and 3 h after HIIT ($r = 0.596$, $p = 0.025$ and $r = 0.641$, $p = 0.014$, respectively), while changes in $sclerostin_c$ at post-test after HIIT were positively correlated to 1RM ($r = 0.645$, $p = 0.013$) in the young female group.

The plasma volume-corrected percentage changes in serum markers from baseline to post-test and 3 and 24 h after ST and HIIT, were not correlated to $\dot{V}O_{2max}$ in neither of the three groups nor with 1RM in the young and elderly male groups.

DISCUSSION

In the current study, we investigated the acute effects of two well-recognized training interventions for cardiometabolic and musculoskeletal health, namely, treadmill HIIT and hack squat ST. The respective responses to bone turnover and mechano-responsive biomarkers in young men and women, and in elderly men, were explored.

The elderly men displayed significantly lower baseline values of the BTMs CTX-1 and P1NP, as well as OC, whereas LCN2 and sclerostin levels were higher, the latter by twofold, compared to those in the young male participants. Both training modes induced an acute decrease in plasma volume-corrected CTX-1_c in the young groups, whereas $PINP_c$ levels were divergently affected, with HIIT promoting a transient increase and ST a transient decrease. Both training modes evoked a rise in the ratio of $P1NP_c$/CTX-1_c at post-test and/or after 3 h in the young groups, but not in elderly men. A significant increase in $sclerostin_c$ was seen in young and elderly men after HIIT, which lasted for 24 h. Moreover, $LCN2_c$ levels were elevated immediately after HIIT in young men but decreased after 24 h as compared to baseline. Correlations between simple time effects changes in BTMs and related bone markers and the participants' muscle strength and cardiovascular fitness were solely seen in the young female group.

Age and Sex Differences in Baseline Levels of Bone Turnover Markers, Osteocalcin, Sclerostin, and Lipocalin-2

In line with previous studies, we observed significantly lower baseline levels of BTMs and OC in elderly men compared to young men, reflecting an age-induced decline in bone turnover (39). This could partly be due to the high level of baseline sclerostin in this group, as sclerostin acts as a strong inhibitor of bone formation. The role of LCN2 in bone is not fully settled, but it has been shown to inhibit osteoclast generation (40) and is associated with reduced osteoblast activity (29), which could reduce bone turnover. Baseline levels of sclerostin and LCN2 were higher in the elderly men compared to the young male participants. Moreover, young men displayed higher levels of CTX-1 than young women, confirming previous studies (41, 42), while baseline levels of P1NP, OC, sclerostin, and LCN2 did not differ between the young groups.

Age and Sex Differences in Training-Induced Changes in Serum Markers

The alterations in BTMs after training were most pronounced in the young participants. This indicates that the acute effects of training on bone turnover decrease by age, as also suggested in a recent systematic review by Smith et al. (19). In both groups of young participants, we observed an immediate decrease in the serum bone resorption marker CTX-1_c after both training modes, which returned to baseline level after 24 h. A decline in $P1NP_c$ levels was also seen in these groups after ST. Notably, $P1NP_c$ levels increased transiently in all groups after HIIT. This was paralleled by a rise in sclerostin in the two male groups and by an increase in $LCN2_c$ in the young male group. When calculating the ratio of $P1NP_c$/CTX-1_c, we observed an increase in both sexes, after both training modes, at post-test and/or after 3 h, suggesting a favorable acute effect of training on bone formation. In line with this, Whipple et al. found that resistance training with moderate intensity acutely reduced the serum bone resorption marker type I collagen N-telopeptide (NTX) for at least 8 h post-exercise in untrained young men (43). In contrast, Mieszkowski et al. reported that CTX-1 levels increased acutely after exercise in physically active young men, with no change in P1NP levels (44).

In contrast to the young participants, we observed no alterations after ST in elderly men, whereas HIIT induced altered CTX-1_c and $P1NP_c$ levels. Moreover, no significant time effects were seen on $P1NP_c$/CTX-1_c ratio after either exercise mode. These results are partly in line with a study by Maïmoun et al., who found no significant change in CTX-1 in elderly participants after a maximal incremental exercise test (45); however, both elderly men and women were included, and P1NP was not measured in this study. In continuation of this, a more recent study reported beneficial effects of plyometric exercise on the P1NP/CTX-1 ratio in premenopausal women, an effect that was blunted in postmenopausal women (33). The latter study reported the effect of BTMs as a formation/resorption ratio, using P1NP and CTX-1 (33). In the current study, we present P1NP/CTX-1 ratio, as part of a strategy to overcome the existing discrepancy between studies, as also suggested and used in several other reports (46–48).

We observed an acute decrease in OC_c at post-test and/or 3 h after both exercise modes in young participants, but not in the elderly men. Other studies show conflicting results regarding the effect of exercise on serum OC levels, as reviewed by Mohammad Rahimi et al. (49), making comparisons difficult. The interpretation of serum OC levels is further complicated by the fact that it exists in the circulation in two forms, namely, a carboxylated (cOC) form reflecting bone mineralization and an undercarboxylated (ucOC) form acting as a hormone, i.e., energy metabolism (50).

Few Differences in Exercise Mode Effects on Serum Markers

The hack squat ST and treadmill HIIT induced similar effects on BTMs in young women and men. Potential differences between these exercise modes in the mechanisms accounting for the BTM response are still unclear. Treadmill HIIT yields a substantially higher metabolic load than hack squat ST. In the context of bone strain rate, however, running exercise (as in HIIT) is commonly regarded as an impact exercise, where ground reaction forces constitute the peak forces exerted on the skeleton, whereas ST provides peak forces through muscle work, being referred to as low-impact exercise (20, 21). A substantial amount of research has demonstrated that distinct changes in BTMs can occur following both high- and low-impact exercise, despite differences in metabolic load (20–22, 51). The same has also been reported with BMD adaptations from long-term training interventions (18). Previous studies of acute exercise have reported similar BTM responses as the results of our study, following resistance, plyometric, and endurance exercise, when investigated separately (17, 22, 51). Collectively, these findings suggest that BTMs can be acutely secreted from bone cells, reflecting changes in bone homeostasis induced by both high- and low-impact exercise despite differences in the initiating stimulus.

The BTM response was essentially similar after ST and HIIT among the young participants, whereas the immediate response in serum sclerostin$_c$ differed, with an increase in level after HIIT and a decrease after ST in young men. Kouvelitoti et al. investigated the acute response in BTMs after interval treadmill running versus interval cycling (low mechanical load) in young women and found no differences in CTX-1 but an acute increase in sclerostin immediately after both exercise modes, which contrasts with the findings in young women in the current study (52). Acute physical activity is postulated to inhibit sclerostin (53), and plyometric exercise has been found to cause an immediate and transient increase in serum sclerostin levels in men, but not in prepubertal boys and girls (54). We speculate that the high load and rapid execution (i.e., high rate of force development) in our ST model could be the decisive factor regarding the sclerostin response, as this type of execution is more similar to plyometric exercise. Still, sclerostin's response to exercise is not yet clear and requires further research, as conflicting reports exist regarding circulating sclerostin alterations, from elevation, no effect, and suppression (55).

In the elderly men, we observed a significant difference in LCN2$_c$ levels between the two exercise modes, as it was increased immediately and 3 h after HIIT before returning to baseline values but was increased at 24 h only after ST. Few studies have explored the effects of exercise on circulating LCN2 in humans, and since it is regarded as a mechanosensor (29), we find it interesting that the acute effects of the two exercise modes differ in the current study, even though in elderly men only. The increase in LCN2$_c$ after HIIT in the elderly men corresponds to the results from Ponzetti et al. describing increased serum LCN2 immediately after acute high-intensity exercise in young and middle-aged male runners (31), suggesting that HIIT is a potent stimulus for LCN2 response. In the young male participants, we also found an acute simple time effect on LCN2$_c$, which increased immediately after HIIT and then decreased after 24 h, but these effects were not significantly different from ST.

Effect of Participants' Fitness Level and Acute Responses of Exercise

The participants included in this study all exhibited higher aerobic capacity, measured by $\dot{V}O_{2max}$ (between 8% and 18% above the average) for their sex and age compared to reference data from healthy Norwegian men and women aged 20–90 (56). The participants' fitness level might modulate BTMs and related bone marker responses to acute training. Mieszkowski et al. described that serum CTX-1 increased after a Wingate high-intensity anaerobic test in physically active men, but not in professional gymnastics athletes (44), while Scott et al. found no significant differences in β-CTX response between endurance-trained and recreationally active men after exhaustive running (57). Interestingly, we found no association between 1RM or $\dot{V}O_{2max}$ and changes in plasma volume-corrected BTMs and related markers among the male participants, indicating that in the current setting, neither muscle strength nor cardiovascular fitness affected the training-induced changes in BTMs and related bone markers, in young and elderly men. In young women, muscle strength (1RM) was correlated with the significant main effect of time changes in all measured BTMs and related markers, but no correlations were detected with $\dot{V}O_{2max}$. The baseline levels of BTMs and related bone markers in the young women showed some variations between the two exercise modes' baseline levels, which could not be detected among the male participants. Circulating levels of CTX-1 might vary during the menstrual cycle (58), and the use of oral contraceptives is known to decrease bone turnover (59), which partly can explain the observed variations in baseline values in the female group. We did not collect data on the menstrual cycle in the current study, but ten of the 19 women included reported the use of oral contraceptives. When analyzing the baseline levels of BTMs between users/non-users of oral contraceptives, we found no significant differences, possibly due to the relatively small sample size.

Implications for Long-Term Training Adaptations

We do not know how acute and transient changes to individual BTMs and related markers translate in the long-term regarding changes in BMD and microarchitecture and, ultimately, to bone strength and future fracture risk. A recent systematic review of the effects of acute exercise on BTMs in middle-aged and older adults concluded that acute exercise is an effective tool to modify BTMs, but the response appears to be dependent on exercise modality, intensity, age, and sex (19). The current study indicates that both high-intensity

running exercise and lower-extremity ST can provide favorable effects on bone metabolism, at least in younger individuals, which agrees with our previous findings of improved skeletal properties following 12 weeks of hack squat MST (11–13). Further research is needed to unravel whether this also applies to treadmill HIIT. The observed acute effects in BTMs and related biomarkers returned to baseline levels after 24 h, indicating that persistent training-induced changes from an intervention should be measured at later time points.

CONCLUSION

Despite being inherently different in skeletal loading characteristics, HIIT and ST induced similar effects on bone metabolism markers in young adults of both sexes. The two exercise modes did not diverge in BTM and OC_c responses but did for $sclerostin_c$ in young men and $LCN2_c$ in elderly men. Baseline fitness measures were not correlated to changes in BTMs and related bone markers in the male groups, while muscle strength measured by 1RM was associated with changes in BTMs and related markers in the young female group.

Collectively, these findings demonstrate that beneficial skeletal effects on bone metabolism can be attained through both aerobic endurance and resistance exercise, although this effect seems to be attenuated with age. Mostly, these effects were blunted after 24 h, suggesting that persistent alterations following prolonged exercise interventions should be assessed at later time points.

STUDY STRENGTHS AND LIMITATIONS

Our study has several strengths. We included participants across sex and age. All baseline and 24 h after exercise blood samples were taken in the morning, after overnight fasting, to rule out diurnal variations. The energy intake was controlled and similar for all participants during exercise sessions, and sessions were supervised in a laboratory setting with close individual monitoring by qualified staff. All analyses of serum markers were performed with concentrations corrected for potential ΔPV, using changes in total calcium as a hemoconcentration biomarker. All participants were thoroughly tested regarding their muscular strength and cardiovascular fitness. The current study also has some limitations. We did not measure BMD in our participants, so we cannot rule out that the bone status by means of BMD could affect our results.

The baseline and 24-h blood samples were drawn in the morning after overnight fasting. Participants received equal amounts of carbohydrate energy gel before performing the exercise, and it cannot be ruled out that the serum BTMs measured at post-test and 3 h after exercise samples might have been affected by nutrition intake. Studies have demonstrated that intake of carbohydrates causes a decrease in serum BTMs, especially CTX-1, for up to 2 h after intake (60–62). However, Sale et al. suggested that even though carbohydrate feeding during exercise reduces overall bone turnover in the hours following exercise, the balance between resorption and formation markers is maintained (63). A recent review stated circadian variation for BTMs CTX-1 and OC, with nighttime or early morning peak, but with less amplitude for P1NP and none for sclerostin (64). We tried to minimize the effects of circadian variation for BTMs by drawing blood at the same time of the day for all participants.

We measured serum tOC and were therefore unable to reveal a possible shift between the two forms of circulating OC (bone mineralization related carbonylated (cOC) and undercarboxylated (ucOC)). The optimal time for blood sampling for assessment of BTMs and related bone markers after training is not established. Ideally, blood tests should be taken every 30 min post-exercise for an extended period. For the feasibility of the study, we chose four time points for blood sampling (baseline, immediately after, 3, and 24 h after the exercise sessions). We cannot, however, rule out that we have "missed" the participants' individual peak responses in BTMs, OC, sclerostin, and LCN2 after exercise. Also, for the feasibility of the study and to minimize the number of visits for the participants, the order of the exercise sessions was not randomized. Preferably, both sexes should have been evaluated also in the elderly group, but we chose to prioritize men in an attempt to undermine the estrogen component related to aging. In the context of skeletal health, it could be argued that elderly women would be more relevant, although previous studies suggest that comparable results as in the present study can be expected (33, 65).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Norwegian Regional Ethics Committee for Medical and Health Research (REK2018/926). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: MM, US, and EW. Data collection and curation: CB, NA, MB, MM, and EW. Analysis: MS and AS. Formal analyses, statistics, and visualization: AS. Funding acquisition: MM, US, and AS. Project administration: MM. Supervision: MM and EW. Validation: MM and AS. Writing—original draft: AS. Writing—review and editing: all authors.

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Effects of cheerleading practice on advanced glycation end products, areal bone mineral density, and physical fitness in female adolescents

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Background: Exercise has been widely reported to promote bone health, but it is unknown whether is associated with a reduction in advanced glycosylation end products (AGEs). This study aimed to investigate the effects of 14 weeks of cheerleading exercise on areal bone mineral density (aBMD) and AGEs.

Methods: In this study, 46 female teenagers (age, 19.52 ± 1.21 years; body mass index, 20.15 ± 2.47 kg/m^2) were randomly divided into a cheerleading group (CHE, n = 21) and a control group (CON, n = 25). The CHE group was subjected to cheerleading practice twice a week for 14 weeks; the CON group maintained their daily routine. Dual-energy X-ray absorptiometry was used to measure aBMD, and autofluorescence (AF) values were used to reflect AGEs. Physical fitness testing all-in-one machines are used to test body composition, cardiorespiratory fitness, muscle fitness and flexibility. A mixed ANOVA model was used to examine the effect of the intervention on each outcome. A multiple mediation model with covariates for physical activity and eating behaviors was performed to explore the mediators between cheerleading exercise and aBMD.

Results: After 14 weeks of cheerleading practice, 1) aBMD increased significantly in both groups with significantly higher increases in the CHE group ($p < 0.05$). 2) AGEs significantly decreased in the CHE group (–2.7%), but not in the CON group ($p > 0.05$). 3) Vertical jumps and sit-ups significantly increased in the CHE group ($p < 0.05$), but not in the CON group ($p > 0.05$). 4) ΔAF values was significantly negatively correlated with Δ aBMD ($r = -0.302$, $p < 0.05$). 5) ΔAF values mediated the effect of exercise on the aBMD (indirect effect: 0.0032, 95% CI 0.0002–0.0079).

Conclusion: Cheerleading practice improved aBMD and physical fitness and reduced AGEs accumulation in female adolescents. The effect of exercise on aBMD was partially mediated by AGEs.

KEYWORDS

cheerleading practice, female adolescents, areal bone mineral density, advanced glycosylation end products, physical fitness

Introduction

Adolescent bone health is an important public health issue. In recent years, adolescent bone health has been challenged by a range of lifestyle changes, such as reduced physical activity, obesity, High-fat and sucrose diet, and eating disorders (Hallal et al., 2006; Lorincz et al., 2010; Hills et al., 2011). Low bone mineral density is due to a failure to increase peak bone mass in adolescence and a decrease in bone mass in young adulthood. Studies have shown that sedentary behavior, obesity, and eating disorders can contribute to low bone mineral density in adolescents and are important risk factors for adolescent bone health (Pelegrini et al., 2020; Zuckerman-Levin et al., 2014; Pollock, 2015). Female adolescents may be at greater risk for bone health as most adolescent girls do not meet physical activity guidelines and are more likely to have an eating disorder (Treuth et al., 2007; Bland et al., 2020; Thornton and Gordon, 2017). Therefore, it is important to take care of adolescent bone health issues, especially for female adolescents.

The harmful effects of Advanced glycosylation end products (AGEs) on bone health have been one of the greatest concerns of researchers in recent years. (Sanguineti et al., 2014; Lamb et al., 2018). AGEs are a complex and heterogeneous group of compounds (Semba et al., 2010; Vistoli et al., 2013). AGEs have a significant impact on human health (Yamamoto and Sugimoto, 2016). Researchers investigated 9,203 patients, whose bone mineral density (BMD) was assessed by quantitative ultrasound techniques, and their AGEs were assessed by measuring skin autofluorescence (AF) values, suggesting that the accumulation of AGEs was a detrimental factor for bone health (Tabara et al., 2019). AGEs could affect bones throughout a person's entire life span (Vashishth et al., 2001; Thomas et al., 2018). Studies have found that the accumulation of AGEs might be a risk factor for osteoporosis and fractures in the older population (Asadipooya and Uy, 2019). Baxter et al. (Baxter-Jones et al., 2011) showed that childhood and adolescence are prime periods for bone mineral accumulation, with stable peak bone mass levels being reached during the first 2 decades. The pentosidine, a type of AGEs, was negatively correlated with the bone mineral content in adolescents suggesting that the accumulation of AGEs may affect peak bone mass in young people (Kindler et al., 2019). AGEs might affect bone via the following mechanisms. AGEs physically affect the properties of bone material and bone mass through their accumulation in collagen fibers (Yamamoto and Sugimoto, 2016). In addition, AGEs adversely affect human interstitial bone marrow stem cells and play a harmful role in the pathogenesis of skeletal disorders (Kume et al., 2005).

Exercise can be a non-medical intervention to promote bone health. The frequency and intensity of exercise is important in influencing the bone health of adolescents (Ishikawa et al., 2013; Rowlands et al., 2020). Appropriately designed aerobic exercise and resistance training can improve bone health in young people (Greene et al., 2006; Lloyd et al., 2014). Cheerleading is an aerobic dance sport that includes many aerobic and self-weight training elements and has been found to benefit the development of cardiorespiratory endurance and strength in adolescents (Krivoruchko, 2018). Studies have shown that aerobic dance are beneficial for improving women's bone health (Na and Kritpet, 2015; Yang et al., 2022). The beneficial effects of exercise on bone may be related to appropriate mechanical stress stimulation (Li et al., 2019) and the secretion of muscle-derived cytokines (Colaianni et al., 2019).

Exercise may also be an effective way to reduce AGEs. Karine et al. (Rodrigues et al., 2018) found that exercise reduced AGEs levels in inactive patients with acquired immunodeficiency virus (HIV), suggesting that short-term moderate-intensity aerobic training could reduce AGEs levels in the body. Reduced accumulation of AGEs in the body by exercise may be associated with decreased hyperglycemia (Reddy et al., 2019) and increased clear efficiency (Shen et al., 2020). However, it is unclear whether cheerleading, one of the more popular sports among female adolescents, can reduce the accumulation of AGEs and promote bone health in female adolescents. It is also unclear whether the improvement in bone health from exercise is associated with a reduction in the accumulation of AGEs in the body. Few studies explore the effects of exercise on bones and AGEs simultaneously.

In general, most of the research on the damage to bone health caused by AGE accumulation has been epidemiological (Li et al., 2012; Rodrigues et al., 2018; Tabara et al., 2019) and animal studies (van Dongen et al., 2021), but there has been a lack of research on the effects of exercise on AGE accumulation and bone health in humans. Therefore, this study explores the effects of cheerleading on AGEs, aBMD, and physical fitness in female adolescents, and provides a practical basis for intervention strategies to improve the overall health of female adolescents.

Materials and methods

Participants

From March 2021 to July 2021, A total of 50 female adolescents were recruited as participants, and they were randomly divided into a cheerleading group and a control group (CON, n = 25). Four participants dropped out of cheerleading practice, three because of inability to persist and one because of relocation (CHE, n = 21) (Figure 1). All participants had never been trained in cheerleading before. Participants were informed of the whole process of the experiment and the experimental procedure is in accordance with the Declaration of Helsinki. The study was

TABLE 1 A typical cheerleading practice schedule.

Intervention Content	Density	Intensity	Equipment	Time (min)
Preparation exercise	Preparation exercises 1 set, 2 reps a movement	50–60% HR_{max}	Playground	5
1) Dynamic preparation activities (neck, shoulder, spine, pelvis, rump, knee, ankle)				
2) Arm control exercises: Lateral arm raises, small up and down arm vibrations, small back and forth arm vibrations, lateral arm raises in clockwise circles, lateral arm raises in counterclockwise circles, etc. kicking exercises: front kick, side kick, beside kick, Sucking leg jump, back stomp run, trot pony jump.	Arm control and kicking exercises 2 sets of 4 reps of one movement	55–65% HR_{max}	flower ball	15
3) Learn the 8 hand positions: (upper M, lower M, W-movement, high V, inverted V, T-movement, diagonal movement, short T-movement) learn the basic leg steps:(stand with legs together, stand with legs apart, lunge standing)	Divide the new movements into stages, gradually learning 8 new hand positions and basic leg steps, practicing 4 sets of 8 reps of each movement	55–65% HR_{max}	flower ball	40
4) Practice the basic hand positions and leg movements learned in Part 3	Dance the basic hand positions and leg movements learned in part 3 for 4 consecutive 8 beats	65–75% HR_{max}	flower ball	30
5) Relaxation exercises: stretching the large arms, small arms and wrists. Stretching the front and back of the legs and the outside of the legs	Relaxation exercises in 2 sets of 4 reps for each movement	50–60% HR_{max}	Playground	10

approved by the Ethics Committee with approval number 202216003.

Study design and procedures

The CHE group conducted the cheerleading practice for 14 weeks in two different ways. A: the intervention took place every Monday in the gym; B: every Saturday they trained independently at home via the internet, practicing the cheerleading motions they had learned in class. The training recordings were transmitted through photos and videos to chat software, which was monitored and checked by the researcher. The duration of each training session was 100 min. The control group lived in the same environment as the CHE group, maintained their daily routine and received a health education before the start of the experiment. The volume, intensity and density of a typical training session have been shown in Table 1. All practice schedules can be found in the supplementary material. Height, weight, body mass index (BMI), body composition, aBMD, and AGEs levels were measured before and after the intervention. All tests were carried out by trained professional researchers.

Physical activity, eating behavior, exercise enjoyment, mental health measurement

A short version of the International Physical Activity Questionnaire (IPAQ) (Lee et al., 2011) was used to assess the daily energy expenditure of participants. This self-administered questionnaire consists of 7 items. Six of the items were about the time spent on vigorous exercise, moderate exercise, and walking. The last question was about the amount of time spent sitting each day.

Eating behavior was measured using the Three-Factor Eating Questionnaire (TFEQ) (de Lauzon et al., 2004), which measures participants' eating habits in three dimensions: cognitive restriction, uncontrolled eating, and emotional eating. The Eating Behavior Scale which consists of 21 items is a 4-point response scale (absolutely correct/basically correct/basically incorrect/absolutely incorrect). The total score was obtained by summing the three dimensions' scores.

Sports enjoyment was measured using the Physical Activity Enjoyment Scale (PACES) (Gnambs and Staufenbiel, 2018; Teques et al., 2020), which consists of 16 items.

Anthropometric measurements

Researchers first used the Physical Fitness Assessment System TA106 (IDONG; Shenzhen Taishan Sports Technology Co., Ltd.,). to measure height and weight. During the test, the participant stood naturally with their hands down at their sides and their eyes looking straight ahead, and the results were automatically saved and recorded. Body composition was measured using a bioelectrical impedance method (Inbody 230, Biospace Inc., Seoul, Korea). During the test, participants were asked to stand naturally, keeping the spine in a neutral position. Participants held electrode rods in their hands and took off

their shoes and socks, placing their bare feet on metal electrodes to ensure direct contact between the skin and the metal electrodes. The percentage of body muscle and fat will be automatically saved and recorded.

Bone parameters measurement

Areal bone mineral density at the distal end of the radius was measured using dual-energy x-ray absorptiometry Bone mineral Densitometer DXA-IMAX (Kang Rong Xin Intelligent Medical System Co., Ltd., Xi'an, China), which is examined and calibrated before each test to ensure accuracy. Calibration was performed using a phantom with the same shape as the real bone until the measured aBMD reading was the same as the actual labeled value of the phantom, then the calibration was successful. The coefficient of variation of aBMD measured by repeated measurements on the same day and multiple days using a 4 mm phantom was ≤0.01. The dominant hand of all participants is the right hand. The participant sits in a specific position and uniformly places her right hand into the aBMD testing device, placing the end of the wrist at the infra-red crossing marked on the device as required, with the eyes looking straight ahead. The test result indicators aBMD, Bone mineral content (BMC) and will be automatically saved and recorded.

AGEs measurement

Autofluorescence (AF) value, an indicator of the accumulation of AGEs in the body, was measured by the Advanced Glycation End Product Reader for AGEs READER (Diagnoptics Technologies B.V Co., Ltd., Netherlands). This method is so simple as to be widely used (Meerwaldt et al., 2004). Participants were asked to not use skin care products such as sunscreen on the arm area before measurement. Calibration is carried out before each test using the manufacturer's matching kit until the calibration is successful with an autofluorescence value of 2.65. Participants place the forearm of the right hand on the device and the average of three consecutive measurements is taken to determine the AF level. The test was conducted with the participant seated in a designated position, with the palm side of the lower arm approximately 10 cm in front of the elbow joint pressed against the excitation point of the device, and the AGEs READER excitation light source illuminating approximately 1 cm 2 of skin on the palm side of the lower arm to measure AF at a peak wavelength of 370 nm. The measurement should be taken on normal skin, avoiding visible blood vessels, scars, lesions, or other skin abnormalities. The measured data is automatically stored and recorded by the skin fluorescence detector.

Physical fitness measurements

Physical fitness (PF) tests were performed using Health Fitness Instrument TA106 (IDONG; Shenzhen Taishan Sports Technology Co., Ltd.). The main tests include vertical jump, sit-ups, balance, sit and reach test, systolic and diastolic blood pressure. Throughout the test, participants followed the instructions of the staff and completed each step correctly. In the vertical jump test, participants were asked to jump vertically upwards with their hands naturally down and knees slightly bent, jumping twice to take their highest value. In the sit-up test, participants were asked to start in a flat position, with their hands by their ears, and finish once with their elbows touching their knees. In the balance test, participants close their eyes at the instruction of the machine, naturally lift either foot and naturally lower their arms until they cannot maintain their balance, the machine automatically records the time taken to maintain standing and the maximum result is recorded after two tests. In sit and reach tests, participants were asked to sit with knees straight, upper limbs extended and arms straight, with the participant sitting on the machine with the entire upper body bent forward so that the fingers reached the furthest scale on the device scale to obtain the best result. The electronic spirometer was used to measure the vital capacity of the participants. The vital capacity index was obtained using vital capacity divided by body weight. The step test was used to measure the participants' cardiorespiratory fitness. Participants followed the beats of the metronome and went up and down the steps at a rate of 30 steps/min for a total of 3 min and then rested for 4 min. Heart rate during rest was recorded using photoplethography and the step index was calculated using the following formula.

$$\text{Step index} = 180 \times 100/2\,(\text{P1} + \text{P2} + \text{P3})$$

P1, P2 and P3 are the heart rates for the three recovery periods of 1–1 min 30 s, 2–2 min 30 s and 3 min–3 min 30 s after exercise.

Statistical analysis

The sample size required for this study was calculated by G Power version 3.1.9.7 (Kiel University, Germany). It was calculated that a sample size of at least 44 participants was required to achieve 95% statistical power and this study met the minimum sample requirement. All data were tested for normal distribution by Kolmogorov-Smirnov statistic and descriptive statistics were used as mean ± standard deviation. Independent samples *t*-test was used to test for differences in baseline indicators between the two groups. A mixed ANOVA model (within-subjects factor: 2 time × between-subjects factor: 2 groups) was used to explore the effects of exercise on AGEs, bone, and PF, and post hoc analysis was performed with Bonferroni. The effect sizes were expressed using η_p^2. Spearman correlation analysis was

TABLE 2 Basic participant characteristics (mean ± SD).

	CHE Group	CON Group	Total
Age (year)	18.48 ± 0.75	20.40 ± 0.71	19.52 ± 1.21
Height (cm)	164.01 ± 5.22	161.67 ± 5.97	162.73 ± 5.71
Weight (kg)	55.21 ± 7.79	52.00 ± 8.07	53.47 ± 8.02
BMI (kg/m^2)	20.51 ± 2.68	19.84 ± 2.30	20.15 ± 2.47
Systolic pressure (mmHg)	112.80 ± 10.80	111.20 ± 9.35	111.93 ± 9.95
Diastolic pressure (mmHg)	71.50 ± 8.45	73.28 ± 7.44	72.47 ± 7.78
TFEQ-21	49.26 ± 6.67	50.86 ± 8.55	50.07 ± 7.65
UE	22.81 ± 3.80	23.57 ± 4.73	23.20 ± 4.28
CR	13.70 ± 3.56	23.57 ± 4.73	23.20 ± 4.28
EE	12.74 ± 9.65	13.07 ± 1.98	12.91 ± 1.82
PACES	37.96 ± 9.65	35.50 ± 10.01	36.71 ± 9.82
IPAQ (met/min·week)	2,204.27 ± 1703.06	1985.84 ± 1364.10	2093.07 ± 1529.39
Low PA level	1 (4.8%)	1 (4.0%)	2 (100.0%)
Moderate PA level	16 (76.2%)	20 (80.0%)	36 (100.0%)
Vigorous PA level	4 (19.0%)	4 (16.0%)	8 (100.0%)

Abbreviations: CHE, cheerleading group; CON, control group; BMI, body mass index; TFEQ: three-factor eating questionnaire; UE: uncontrolled eating; CR: cognitive restraint; EE: emotional eating; PACES: physical activity enjoyment scale; IPAQ: international physical activity questionnaire; PA: physical activity.

TABLE 3 AF, aBMD, and physical fitness in the pre-and post-intervention.

	CHE Group				CON Group				Interaction	
	Pre	Post	*P*	ES	Pre	Post	*P*	ES	*P*	η_p^2
aBMD (g/cm^2)	0.37 ± 0.02	0.38 ± 0.01	0.000	0.396	0.37 ± 0.01	0.37 ± 0.01[a]	0.001	0.228	0.134	0.050
BMC (g)	4.57 ± 0.62	4.68 ± 0.62	0.123	0.053	4.48 ± 0.44	4.51 ± 0.50	0.552	0.008	0.454	0.013
AF	1.77 ± 0.06	1.72 ± 0.06	0.004	0.175	1.74 ± 0.08	1.77 ± 0.09[a]	0.096	0.062	0.001	0.209
Muscle mass (kg)	37.76 ± 2.89	38.57 ± 2.58	0.002	0.211	36.89 ± 3.54	36.71 ± 3.45	0.356	0.020	0.003	0.187
%BF	25.60 ± 6.23	23.99 ± 6.13	0.000	0.291	23.36 ± 6.23	22.05 ± 6.13	0.001	0.249	0.584	0.007
VJ (cm)	23.73 ± 4.66	26.04 ± 4.12	0.004	0.178	21.94 ± 3.67	22.50 ± 4.30[a]	0.417	0.015	0.084	0.066
SRT (cm)	15.83 ± 6.22	14.74 ± 6.18	0.173	0.043	13.66 ± 5.52	12.98 ± 5.21	0.334	0.022	0.705	0.003
Sit-ups (reps/min)	32.40 ± 7.41	34.70 ± 7.82	0.062	0.077	28.28 ± 7.42	28.48 ± 6.90[a]	0.856	0.001	0.203	0.037
Balance (s)	35.80 ± 34.71	53.47 ± 37.79	0.019	0.118	36.64 ± 36.26	53.23 ± 37.10	0.017	0.123	0.914	0.000
VCI (ml/kg)	55.98 ± 10.12	57.04 ± 8.57	0.456	0.013	56.08 ± 8.73	57.07 ± 9.50	0.446	0.013	0.942	0.000
Step test index	55.21 ± 13.01	49.14 ± 10.42	0.000	0.281	60.09 ± 11.36	52.96 ± 9.04	0.000	0.391	0.596	0.006

Abbreviations: CHE, cheerleading group; CON, control group; aBMD, areal bone mineral density; BMC, bone mineral content; AF, autofluorescence; %BF, body fat percentage; VJ, vertical jump; SRT, sit and reach test; VCI, vital capacity index; Interaction: interactive effects; ES, effect size.
[a]Indicates a significant difference compared to the post-exercise CHE, group.
Autofluorescence (AF) value, an indicator of the accumulation of advanced glycosylation end products in the body.

used to examine the association between the aBMD and the change in AF values.

A parallel multi-mediator model was performed using the PROCESS macro in SPSS to examine whether the effect of exercise on aBMD was directly and/or indirectly carried through changes in body composition (muscle mass and body fat percentage), AF values, and physical fitness. When the independent variable (exercise) entered the model, it was set as a dummy variable (no exercise = 0; exercise = 1). The mediating variables were selected as those with significant differences between groups after exercise. Physical activity and eating behaviour at baseline were added to the mediation model as covariates. Since previous studies have shown that these two covariates are closely associated with the status of

TABLE 4 Correlation analysis between ΔAF and BMD-related indicators.

	ΔAF	*P*
ΔaBMD	−0.302	0.042
aBMD (Post-exercise)	−0.228	0.128

Abbreviations: aBMD, areal bone mineral density; AF, autofluorescence; ΔAF, change of AF, value; ΔaBMD, change of aBMD.

aBMD and AF values (Waqas et al., 2020; Del et al., 2021). The bootstrap confidence interval (CI) method was used to infer direct, indirect, and overall effects in the mediated model, with confidence intervals not containing 0 considered significant, and the bootstrap sample was 5,000.

A p-value < 0.05 was considered statistically significant. SPSS version 23.0 (Provided by SPSS Inc., Chicago, Illinois, United States) software was used for statistical analysis.

Results

Participants

A total of 46 female adolescents were eventually included in this study. Participants had a mean age of 19.52 ± 1.21 years, a mean height of 162.73 ± 5.71 cm, and a mean BMI of 20.15 ± 2.47 kg/m^2. Table 2 shows the baseline characteristics of the participants, with no significant differences between the two groups in height, weight, BMI, physical activity, eating behavior, exercise enjoyment (p > 0.05). Table 3 shows the pre-intervention data for aBMD, BMC, AGEs, and PF, none of which were significantly different between the two groups before the intervention (p > 0.05).

Bone parameters

For aBMD there was no significant time × group interaction effect (p = 0.134, η_p^2 = 0.050) (Figure 2A). Simple effect for time showed that aBMD increased significantly in both the CHE group (p = 0.000, η_p^2 = 0.396) and the CON group (p = 0.001, p = 0.228). Simple effects for groups showed the aBMD of the CHE group was significantly higher than that of the CON group (p = 0.006, η_p^2 = 0.158) after the cheerleading practice (Figure 2B).

Autofluorescence

For AF values, a significant time × group interaction was found (p = 0.001, η_p^2 = 0.209) (Figure 2C). Simple effects analysis for time showed a significant decrease of 2.7% in the CHE group (p = 0.004, η_p^2 = 0.175), while there was no significant change in the CON group (p = 0.096, η_p^2 = 0.062). Simple effects analysis for groups showed AF values was significantly higher in the CON group than in the CHE group (p = 0.048, η_p^2 = 0.086) after the cheerleading practice (Figure 2D).

Physical fitness

For vertical jump, there was no significant time × group interaction effect (p = 0.084, η_p^2 = 0.066) (Figure 2E). Simple

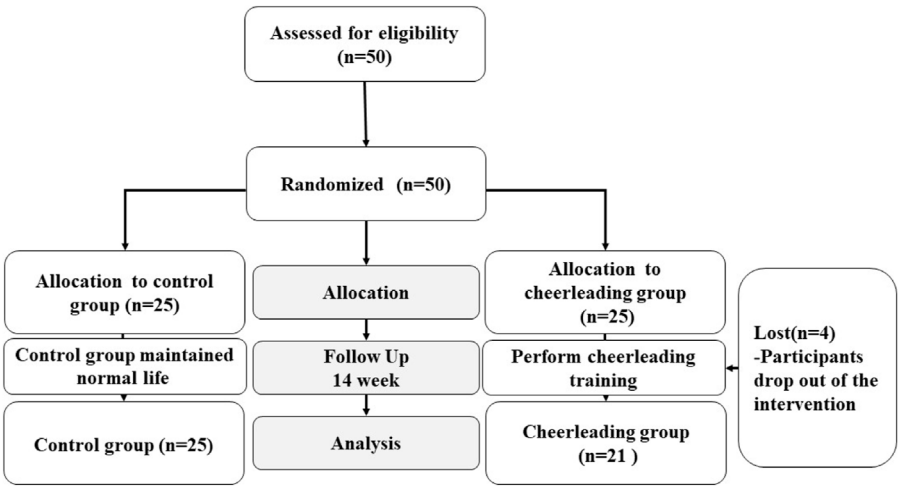

FIGURE 1
Participants' recruitment and intervention flow diagram.

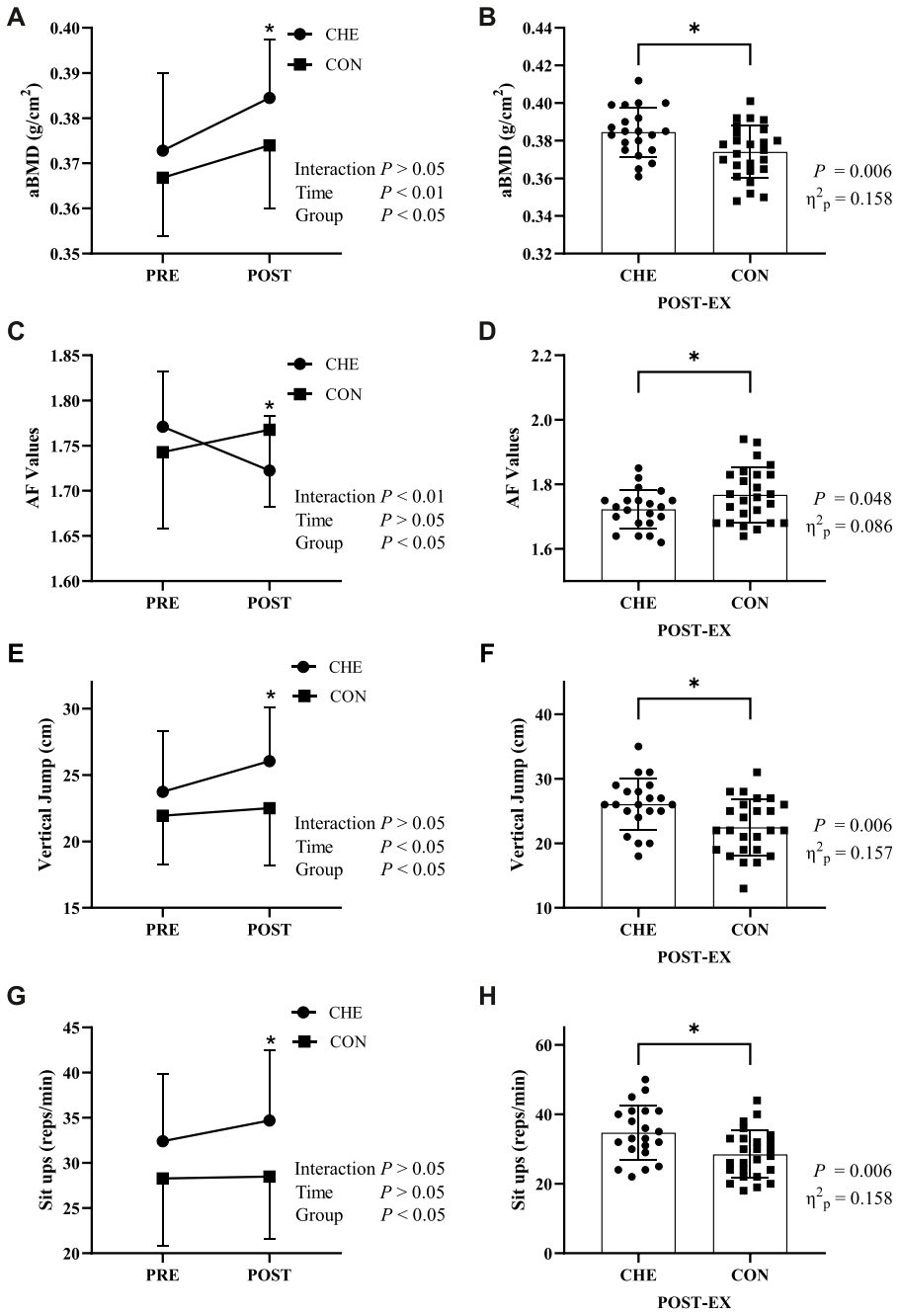

FIGURE 2
Changes in indicators before (PRE) and after (POST) the intervention in the cheerleading group (CHE) and the control group (CON). **(A,B)** areal bone mineral density (aBMD); **(C,D)** auto-fluorescence (AF) Values; **(E,F)** vertical jump; **(G,H)** sit-ups. * indicates a significant difference between the CHE and CON groups. Interaction: interactive effects; Time: time main effect; Group: group main effect.

effects analysis for time showed a significant increase of 12.7% in the CHE group (p = 0.003, η_p^2 = 0.185), while there was no significant change in the CON group (p = 0.411, η_p^2 = 0.015). Simple effects analysis for groups showed vertical jump was significantly higher in the CHE group than in the CON group (p = 0.006, η_p^2 = 0.157) after the cheerleading practice (Figure 2F).

For sit-ups, there was no significant time × group interaction effect (p = 0.203, η_p^2 = 0.037) (Figure 2G). Simple effects analysis

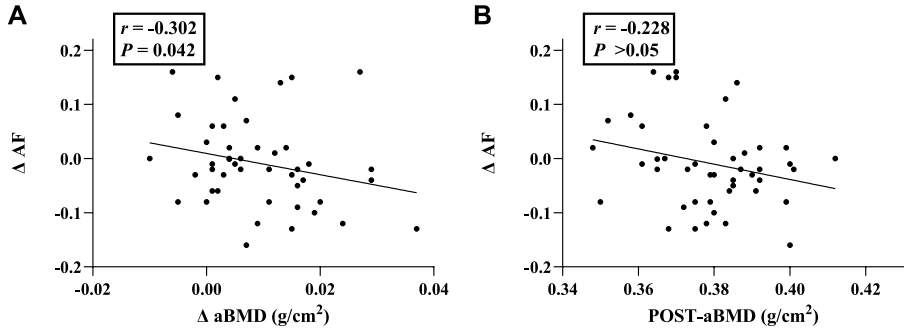

FIGURE 3
Correlation analysis between ΔAF and aBMD-related indicators. **(A)** Correlation of ΔAF with Δ aBMD; **(B)** Correlation of ΔAF with aBMD (Post-exercise). Abbreviations: aBMD, areal bone mineral density; AF, autofluorescence; ΔAF, change of AF value; ΔaBMD, change of aBMD.

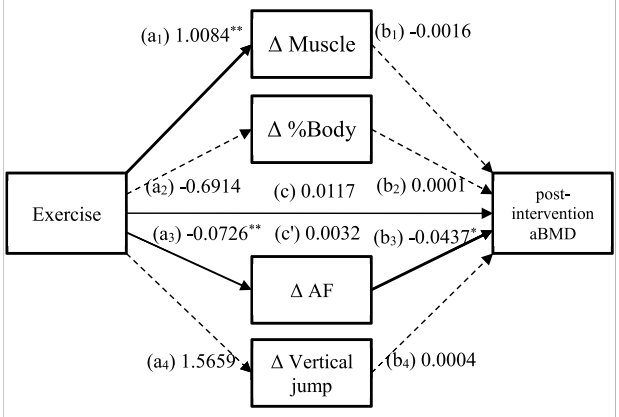

FIGURE 4
Mediated model of the relationship between exercise and post-intervention aBMD. a_1, a_2, a_3, a_4, b_1, b_2, b_3, b_4: path coefficients from the bootstrap procedure; c: the total effect of exercise on the post-intervention aBMD; c′: the indirect effect of exercise on the post-intervention aBMD. The solid paths are significant ($p < 0.05$) and the dashed paths are not significant ($p > 0.05$). The covariates (physical activity, eating behaviour) are not shown on the graph for overall clarity. Abbreviations: aBMD, areal bone mineral density; AF, autofluorescence; ΔAF, change of AF value (Figure 4).

for time showed no significant increase in both the CHE group and the CON group. Simple effects analysis for groups showed sit-ups was significantly higher in the CHE group than in the CON group ($p = 0.006$, $\eta_p^2 = 0.158$) (Figure 2H).

Correlation analysis

As shown in Table 4, ΔAF values were significantly negatively correlated with Δ aBMD ($r = -0.302$, $p = 0.042$) (Figure 3).

Mediation analysis

Table 5 summarises the indirect effects of exercise on post-intervention aBMD through changes in body composition, AF values, and physical fitness. Of the four mediators, the indirect effect of ΔAF values (a3b3 = 0.0032, 95% CI 0.0002–0.0079) was significant only because the CI did not contain 0. It had a total effect of 0.0117 and a direct effect of 0.0085, with the indirect effect accounting for 27.4% of the total effect. This suggests that 27.4% of the effect of exercise on post-intervention aBMD was mediated through ΔAF values (Figure 4).

Discussion

Our primary finding was a significant increase in aBMD and a significant decrease in AGEs after 14 weeks of cheerleading practice. We observed an increase in vertical jumps and sit-ups in the cheerleading group. Moreover, the change in aBMD was found to be negatively correlated with the change in AF values, and changes in AF values mediated part of the effect of exercise on aBMD. Our results suggest that exercise could improve bone health in female adolescents and that this could be due to the reduction in AGEs levels in the body.

In this study, we found significant improvements in aBMD following cheerleading practice, which is similar to the results of other studies on using exercise to improve bone health (Zhao et al., 2014; Al et al., 2019; Perkins et al., 2019). Cheerleading evolved from competitive gymnastics and includes many jumping movements, and Zhao et al. (Zhao et al., 2014) found that using jumping movements as the main form of exercise significantly increased BMD in specific areas, such as the femoral neck and greater trochanter. In addition to this, cheerleading is also a typical aerobic exercise. Al et al. (2019) conducted aerobic exercise training with 65 female participants

TABLE 5 Mediated analysis of the relationship between exercise and post-intervention aBMD.

IV	Mediators	DV	IDEs	95%	CI
Exercise	Δ Muscle mass	post-intervention aBMD	−0.0016	−0.0067	0.0029
	Δ %body fat		0.0000	−0.0012	0.0016
	Δ AF		0.0032	0.0002	0.0078
	Δ Vertical jump		0.0006	−0.0013	0.0029

Physical activity and eating behavior at baseline were added as covariates in all models. Abbreviations: IV, independent variables; DV, dependent variable; IDE, indirect effect; aBMD, areal bone mineral density; AF, autofluorescence; ΔAF, change of AF, value.

for a total of 60 min, three times a week for 12 weeks, and found that the aerobic exercise practice significantly increased the participants' BMD levels. Exercise probably increases bone mineral density in several ways: 1. Cheerleading has a physiological impact benefit on human bone in the upper and lower extremities, where bone tissue is stretched to activate the Wnt/β-catenin signaling pathway through cell-localized mechanosensors, leading to bone formation. 2. Irisin increases. Irisin is a hormone secreted by the skeletal muscles during exercise. Studies have shown that in healthy populations, irisin levels are positively associated with bone mineral status and circulating osteocalcin (Colaianni et al., 2019). Bone mineral status and circulating osteocalcin play an important role in bone formation, suggesting that irisin may increase BMD by increasing bone mineral status and osteocalcin. In addition, irisin can induce MC3T3-E1 osteoblast growth at the junction of the skeletal muscle and the bone (Zhang et al., 2017). Therefore, cheerleading would provide a non-medical means to improve peak bone mass in adolescents.

This study observed a significant decrease in the AF values of the cheerleading group after 14 weeks of intervention with cheerleading. The AF value is an indicator used to evaluate the level of accumulation of AGEs in the body. At baseline, although the difference was not significant, the cheerleading group also had a slightly higher mean level of AGEs than the control group, while after the exercise, the cheerleading group had a significantly lower level of AGEs than the control group. This suggests that cheerleading exercises were effective in reversing the accumulation of AGEs levels in the body. The same results were found in a similarly designed study by Karine et al. (Rodrigues et al., 2018), who found that after 3 months of aerobic exercise practice, the higher levels of AGEs in previously physically inactive human immunodeficiency virus-infected individuals decreased to the same level as those in normal individuals. All this evidence suggests that lack of physical activity may lead to higher levels of AGEs accumulation, and that exercise may be effective in reducing AGEs accumulation, returning them to normal levels, and preventing the risk of chronic diseases.

There are three main ways in which AGEs are derived in humans: 1) exogenous intake into the body (e.g., the ingestion of foods containing high levels of AGEs); 2) endogenous formation and retention in the body (e.g., the carbonyl group of reducing sugars or aldehydes combined with lysine and arginine amino acid residues); 3) unhealthy lifestyles such as sedentary behavior, lack of exercise (Strizich et al., 2018), smoking, and long-term alcohol intake can lead to the production of AGEs (Hayashi et al., 2013; Leung et al., 2016; Lopez-Moreno et al., 2017). Researchers have suggested that younger people are vulnerable to the effects of AGEs, with Putte et al. (Van Putte et al., 2016) finding that AGEs begin to accumulate in people as young as 20 years old, and then seemingly increase steadily. Exercise probably reduces the accumulation of AGEs in the body in several ways. 1. Exercise helps alleviate hyperglycemia. Exercise has been found to alleviate hyperglycemia associated with obesity by improving insulin resistance (Reddy et al., 2019), and hyperglycemia itself might be a direct source of AGEs production and accumulation. 2. Exercise enhances the ability of the kidneys to clear AGEs. The kidneys' proximal tubular cells play an important role in the disposal of plasma AGEs (Shen et al., 2020), and aerobic and resistance exercises significantly enhance the kidneys' filtration ability, and improve kidney function (Wu et al., 2020). Thus, the kidneys could clear AGEs more efficiently. 3. Anti-inflammatory effects of exercise. The body's inflammatory response will activate relevant immune cells, including macrophages and dendritic cells, and induce a switch in the metabolism of these inflammatory cells towards glycolysis. During glycolysis, the precursor substances of AGEs, methyglyoxal, and glyoxal, are produced. Exercise has an anti-inflammatory effect (Sohail et al., 2019), and thus helps to reduce the accumulation of AGEs associated with inflammation.

This study found that changes in aBMD were negatively correlated with changes in AF values and that changes in AF values mediated part of the effect of exercise on of aBMD. This suggests that the elevation in aBMD resulting from exercise may be associated with a decrease in AGEs. Previous studies have suggested that exercise could improve BMD by increasing beneficial mechanical stress on the skeleton and promoting irisin, which improves bone health (Saito and Marumo, 2010). Our study provides evidence on the possible mechanisms by which exercise improves bone health by reducing AGEs levels. AGEs have harmful effects on bone health. Studies have shown that AGEs reduce bone strength and density in humans due to increased non-enzymatic cross-linking (Yamamoto and

Sugimoto, 2016) and the induction of apoptosis in osteoblasts via the ages/rage/caspase-3 signaling pathway (Liu et al., 2016). However, exercise can significantly reduce the high levels of AGEs accumulated in the body, and bring them back to the normal range, while at the same time, an increase in aBMD has been observed in this period. Therefore, the evidence from this study suggests that exercise could increase bone mineral density by reducing the accumulation of AGEs in the body, and that a reduction in AGEs is one of the mechanisms by which exercise improves bone health.

This study showed that after 14 weeks of cheerleading practice, the cheerleading group had a significantly higher vertical jump and sit-ups than the control group. Previous studies have found that exercises such as cheerleading, which involves a lot of running and jumping, can significantly improve lower limb and core muscle strength (Wisloff et al., 2004). The reasons for this might be the following. Firstly, cheerleading involves many movements such as kicks, squats, and big jumps, and is beneficial for the growth of muscles (Wisloff et al., 2004). Secondly, the large number of movement changes during the limited duration of cheerleading also helps to increase the neural control of muscles (Soendenbroe et al., 2022). Thirdly, the complex movements involved in cheerleading also improve the ability of the participants to synergistically contract the various muscle groups.

The strength of this study is that, to our knowledge, it is the first to examine both the effects of exercise on bone health and the levels of AGEs. Secondly, we have used an intelligent all-in-one machine to conduct the physical fitness test, with intelligent human voice instructions and prompts, thus avoiding the bias of manual measurements. At the same time, there were several limitations to this study. Firstly, the number of outdoor exercise practices was less per week. However, the longer durations of each practice and the online practices compensate for this to some extent. Secondly, this study only measured aBMD in participants' carpal bones, and future studies are encouraged to test participants for whole-body aBMD. Thirdly, although the participants were theoretically informed about dietary intake during the outdoor practice, we did not systematically control and track participants' dietary intakes during the study. Therefore, it is suggested that future studies conduct a comprehensive assessment of participants' diets.

Conclusion

In conclusion, cheerleading practice increased aBMD and physical fitness in female adolescents, and also decreased the levels of AGEs. Further analysis revealed that changes in aBMD were negatively correlated with changes in AGEs, and the effect of exercise on aBMD is partly mediated by AGEs. These findings suggest that cheerleading may be an effective non-pharmacological intervention to increase aBMD by reducing the accumulation of AGEs in female adolescents. Future studies should investigate the effects of exercise on aBMD and AGEs when combined with more stringent dietary control.

Data availability statement

Data are available by contacting the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the School of Physical Education, Shaanxi Normal University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LW and HZ designed the project. HZ and YL conducted experiments. HZ analysed the data. HZ wrote the manuscript. HZ, TX, and YQ collected the data. JZ and HZ revised the manuscript. HZ and LW contributed equally to the manuscript. All authors read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effects of combined amplitude and high-frequency vibration on physically inactive osteopenic postmenopausal women

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Purpose: To evaluate whole-body vibration (WBV) osteogenic potential in physically inactive postmenopausal women using high-frequency and combined amplitude stimuli.

Methods: Two-hundred fifty-five physically inactive postmenopausal women (55–75 years) with 10-year major osteoporotic fracture risk (3%–35%) participated in this 18-month study. For the first 12 months, the vibration group experienced progressive 20-min WBV sessions (up to 3 sessions/week) with rest periods (30–60 s) between exercises. Frequencies (30–50 Hz), with low (0.2–0.4 mm) and high (0.6–0.8 mm) amplitude stimuli were delivered *via* PowerPlate Pro5 platforms producing accelerations of (0.75–7.04 g). The last 6 months for the treatment group were a follow-up period similar to control. Serum bone remodelling markers [C-terminal crosslinked telopeptide of type-1 collagen (CTX), procollagen type-1 N-terminal propeptide (P1NP), bone alkaline phosphatase (BAP) and sclerostin] were measured at fasting. CTX and P1NP were determined by automated chemiluminescence immunoassay, bone alkaline phosphatase (BAP) by automated spectrophotometric immunoassay, and sclerostin by an enzyme-immunoassay. Bone mineral density (BMD) of the whole-body, proximal femur and lumbar vertebrae was measured by dual-energy X-ray absorptiometry (DXA). Bone microarchitecture of the distal non-dominant radius and tibia was measured by high-resolution peripheral quantitative computed tomography (HR-pQCT).

Results: Femoral neck (p = 0.520) and spine BMD (p = 0.444) failed to improve after 12 months of WBV. Bone macro and microstructural parameters were not impacted by WBV, as well as estimated failure load at the distal radius (p = 0.354) and tibia (p = 0.813). As expected, most DXA and HR-pQCT parameters displayed age-related degradation in this postmenopausal population. BAP

and CTX increased over time in both groups, with CTX more marginally elevated in the vibration group when comparing baseline changes to month-12 (480.80 pmol/L; p = 0.039) and month-18 (492.78 pmol/L; p = 0.075). However, no differences were found when comparing group concentrations only at month-12 (506.35 pmol/L; p = 0.415) and month-18 (518.33 pmol/L; p = 0.480), indicating differences below the threshold of clinical significance. Overall, HR-pQCT, DXA bone parameters and bone turnover markers remained unaffected.

Conclusion: Combined amplitude and high-frequency training for one year had no ameliorating effect on DXA and HR-pQCT bone parameters in physically inactive postmenopausal women. Serum analysis did not display any significant improvement in formation and resorption markers and also failed to alter sclerostin concentrations between groups.

KEYWORDS

whole-body vibration, age-related bone loss, fracture risk, dose-response, postmenopausal women

Introduction

Osteoporosis affects skeletal integrity, whereby bone mineral density (BMD) is decreased in addition to microarchitectural deterioration (Kanis et al., 2019). This results in an increased risk of fractures impacting mobility (Kanis et al., 2019) and quality of life (Rhodes et al., 2000), with repercussions at an individual and societal level worldwide (Rashki Kemmak et al., 2020; Salari et al., 2021). In Europe, fracture rates and treatment costs are projected to surge by 25% (Willers et al., 2022). Recent European statistics have also demonstrated a stark contrast in the numbers of osteoporotic cases between males and females, with females affected nearly four times as much (Willers et al., 2022). This increased risk in women is largely attributed to the effect of menopause (The ESHRE Capri Workshop Group, 2010), and despite advances in prevention, screening and management of this condition, osteoporosis continues to remain a significant challenge worldwide (Salari et al., 2021; Willers et al., 2022). For this reason, females are the primary focus of this study.

Several pharmacological treatments and exercise interventions are relied upon to manage osteoporosis (Beck, 2022; Palacios, 2022). However, the difficulty with medication is finding the appropriate balance between evidence-based medicine and proven treatment strategies (Ragucci and Shrader, 2011; Gregson et al., 2022). This is further complicated when integrating suitable exercise interventions for the management of this condition. Previous studies have highlighted the effectiveness of exercise in the management of osteoporosis (Gupta and March, 2016; Koshy et al., 2022). However, significant consideration is required since exercise can substantially increase fall risk and needs to be adapted to the individual (Gupta and March, 2016; Benedetti et al., 2018). Furthermore, prevention with physical activity interventions has not always yielded positive effects (Rhodes et al., 2000; Borer, 2005; Ma et al., 2013). As a result, modalities such as whole-body vibration (WBV) were proposed to harness the potential of physical activity by combining simplicity, ease of administration and encouraging adherence. Since WBV can be easily adapted into an everyday routine, it has garnered a lot of attention over the years as a potential treatment modality for osteoporosis prevention, particularly in postmenopausal women (Marin-Puyalto et al., 2018). WBV involves the transmission of mechanical stimuli delivered *via* different vibration platforms (vertical, rotational, or lateral planes) that transfer forces to skeletal segments like other forms of exercise (Marin-Puyalto et al., 2018). The effects of WBV have been extensively investigated (Verschuren et al., 2004; Gusi et al., 2006; Von Stengel et al., 2011a; Von Stengel et al., 2011b; Slatkovska et al., 2011; Verschueren et al., 2011; Lai et al., 2013; Stolzenberg et al., 2013; Slatkovska et al., 2014; Liphardt et al., 2015; de Oliveira et al., 2019) resulting in disparities between studies.

Following seminal studies by Rubin et al. (2004) to evidence the impact of WBV on bone, abundant studies ranging from short to long-duration exposure to WBV have been tested (Gómez-Cabello et al., 2014; Kiel et al., 2015). In the 11-week study published by Gomez-Cabello et al. (2014), bone parameters in a relatively small population were examined using high-amplitude and high-frequency vibration stimuli. Particular focus was on dual-energy X-ray absorptiometry (DXA) derived parameters at the femoral neck and lumbar spine along with added peripheral quantitative computer tomography (pQCT) parameters examining the cortical and trabecular BMD of the radius and tibia. Despite this combination, no conclusive effects on bone were identified. Similarly, studies 6-month in length showed parallels in signal characteristics observed in the 11-week study; however, muscle strength and hip density were also incorporated into the protocol (Verschueren et al., 2004; Verschueren et al., 2011).

Interestingly, two studies of the same 6-month duration explored effects on bone turnover markers (cross-linked C-telopeptide of type I collagen and osteocalcin) alongside DXA outcomes using similar high-frequency and high-amplitude stimuli. Both reached similar conclusions with varying bone and serum results despite utilising similar vibration platforms (Verschuren et al., 2004; Sen et al., 2020). Some studies of much longer duration (12 months or greater) have shifted the focus more towards incorporating advancements in 3D imaging modalities, moving away from the reliance on traditional 2D DXA parameters to draw more in-depth conclusions associated with longitudinal changes in bone (Slatkovska et al., 2011). Studies have experimented with a range of frequencies (Slatkovska et al., 2011) and exposure intervals (Liphardt et al., 2015), using High Resolution Peripheral Computer Tomography (HR-pQCT) (Slatkovska et al., 2011; Liphardt et al., 2015) or novel Magnetic Resonance Imagining (MRI) techniques (Rajapakse et al., 2021) to examine the effects of WBV on bone geometry and microstructural parameters. However, the extensive focus on 3D imaging modalities has exacerbated differences of opinion regarding the efficacy of WBV on bone.

These studies alone highlight several gaps in the literature. Since the osteogenic response to physical activity reduces with age (Rubin et al., 1992), alterations in the length and frequency of sessions could be necessary (Gómez-Cabello et al., 2014). Weekly sessions have varied between one and three, with some varying the training intensity by modifying the signal characteristics and rest periods (Beck and Norling, 2010; Slatkovska et al., 2011; Sen et al., 2020). Despite modifications to signal attributes seen in studies to date, the role of amplitude in WBV bone response is still in question. Physical activity is known to produce a range of amplitudes depending on the type of exercise performed (Deere et al., 2016). Since WBV is a substitute for physical activity, it is essential to replicate this aspect as closely as possible. Frequency variations (high/low), varying amplitudes (<1 g/> 1 g), as well as exposure intervals to the vibration stimuli, have significantly differed between studies and are critical elements of any vibration signal (Prisby et al., 2008). The lack of studies investigating combined amplitude creates a new research opportunity. Physical activity levels prior to intervention were also alluded to as a possible explanation for the lack of observable effect (Rajapakse et al., 2021), which has not been a consideration in studies thus far. Accounting for this might reveal a relationship between WBV and osteogenic potential. Finally, several studies have already explored the response of formation and resorption of bone markers but have not investigated sclerostin, which is known to increase with age and fluctuate based on activity levels (Robling et al., 2008; Amrein et al., 2012). The inclusion of this could capture the subtle responses to combined amplitude stimuli.

In light of the differences outlined above, this study aimed to investigate the role of high-frequency and combined amplitude stimuli over 12 months on physically inactive postmenopausal women. The primary research objective was to investigate changes specific to the femoral neck, while the secondary objective was to measure bone geometry and microarchitectural changes when exposed to such stimuli. In addition, a 6-month follow-up after vibration training was added to observe any sustained osteogenic benefits. It was hypothesised that bone parameters would be improved, with positive osteogenic effects sustained following WBV therapy.

Materials and methods

Ethical approval

Written informed consent for all experiment protocols was obtained from each participant and conformed to the standards set by the latest revision of the Declaration of Helsinki and the committee of human rights protection, southeast, France (N°0908095). ClinicalTrials.gov Identifier (NCT01982214).

Participant information and study design

255 sedentary (< 2 h of physical activity/week at recruitment) postmenopausal women (55–75 years) with absent menses > 1 year, participated in this 18-month non-randomised clinical trial (Supplementary Material S1; Figures 1, 2). Physical activity levels were screened using a questionnaire and further estimated by a computerized self-administered self-assessment questionnaire (QUANTAP, Version 2.0, Université Poincaré de Nancy, France) to obtain an overview of the participants' activity levels at recruitment (Supplemental Material S1). The first 12 months consisted of the vibration protocol (vibration group) and regular visits (control group). The 6 months thereafter was a follow-up period for both groups. Since difficulties with randomisation were anticipated, participants were given the choice to select their preferred group allocation for the duration of the study. Potential bias resulting from this was effectively addressed since participants were matched according to their FRAX scores for major osteoporotic fracture (MOF) 10 year absolute risk ranging from (3%–35%) to ensure group comparability.

Training program

Only the vibration group carried out this protocol. All participants familiarised themselves with the vibration protocol and equipment beforehand. Twenty minute sessions of light squatting and stretching exercises (max 3 sessions/week, up to 130 sessions total) during the 12-month vibration protocol were performed and recorded in the participants' logbook. These

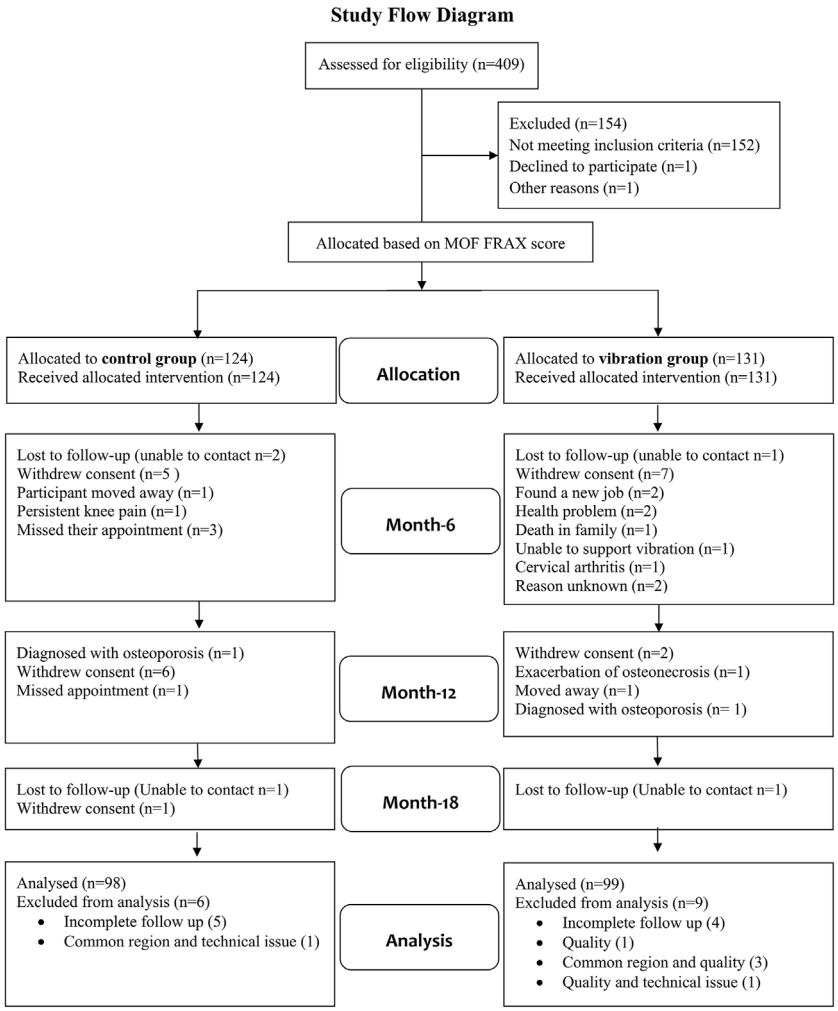

FIGURE 1
Study flow chart.

exercises were for the sole purpose of maintaining the participants' motivation given the duration of the vibration training sessions. The first session was shorter and interspersed with rest periods, and a fitness instructor constantly ensured the participants' safety.

The vibration characteristics were: frequency (30–50 Hz), with a combination of low-amplitude (0.2–0.4 mm) and high-amplitude (0.6–0.8 mm) stimuli were delivered using PowerPlate Pro5 airdaptive system (Performance Health Systems, LLC, NorthBrook, IL, United States) to generate an acceleration profile of (0.75–7.04 g). This system delivered vibration stimuli tri-axially (X,Y,Z axis) and possessed a self-adjusting air cushion system that distributed participants' weight across the platform to mitigate against signal dampening. The chosen vibration amplitudes and frequencies were applicable to elderly people to obtain an acceleration close to 3 g, reproduced by fast walking or osteogenic sports (Vainionpää et al., 2006). Initially, all participants started with low-amplitude stimuli; however, exercise repetition, vibration frequency and amplitude progressively changed throughout the study (Supplementary Material S2). All participants were requested not to modify their regular activity levels, avoiding impact activities/sports throughout the entire study.

Biochemistry

All samples were obtained in the morning following an overnight fast. Serum concentrations of C-terminal crosslinked telopeptide of type-1 collagen (CTX, pmol/L) and procollagen type-1 N-terminal propeptide (P1NP, μg/L) were determined by automated chemiluminescence immunoassay

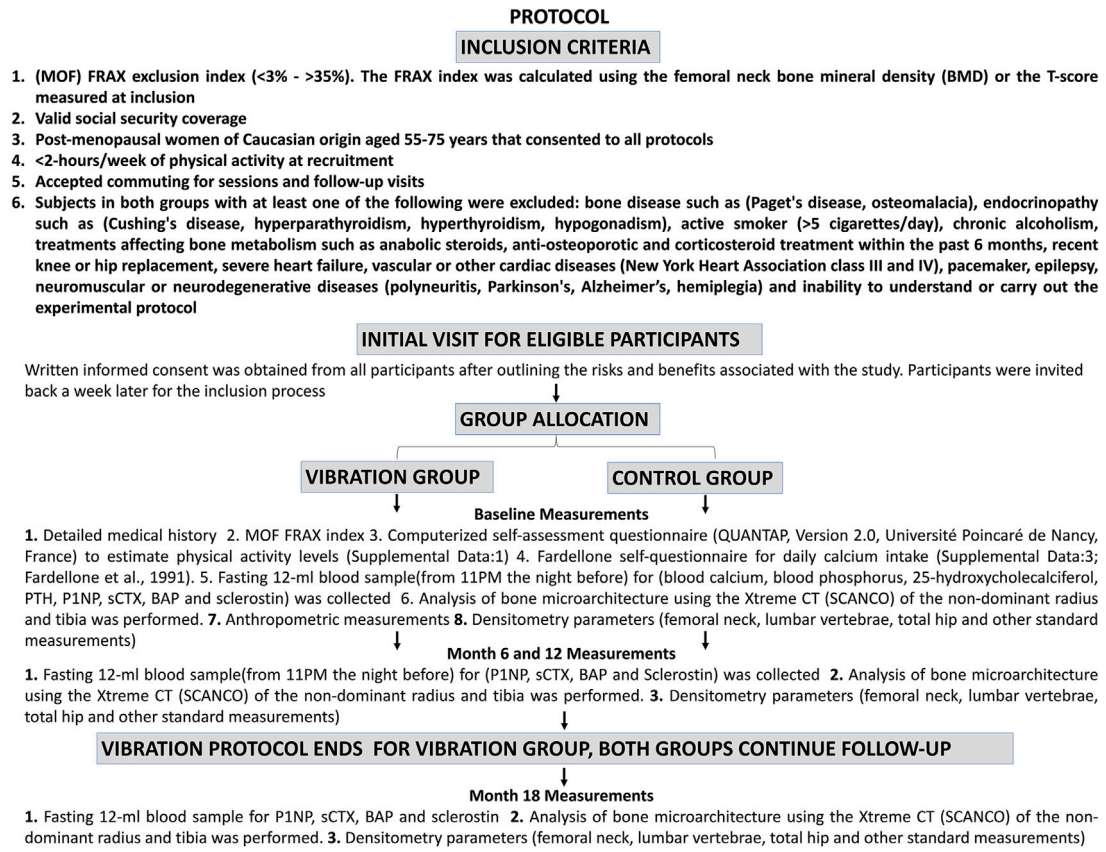

FIGURE 2
Protocol flow chart.

(IDS-iSYS automated analyser, Boldon, United Kingdom), while bone alkaline phosphatase (BAP, µg/L) was measured using an automated spectrophotometric immunoassay (IDS-iSYS automated analyser, Boldon, United Kingdom). Intra-assay CV were < 4.9%, < 3.0%, and < 2%, while inter-assay CV were < 8.8%, < 5.3, and < 9% for CTX, P1NP and BAP respectively. An enzyme-immunoassay (EIA) kit was used to quantify sclerostin levels (SOST, ng/ml) (Quidel Corporation San Diego, CA, United States) with precision CV's of 3.7%–4.2% and 4.3%–4.8% for within-run and between run respectively.

Dual-energy X-ray absorptiometry

Fat, lean body and total body mass in grams, along with standard BMD measurements at the femoral neck (g/cm^2), total hip (g/cm^2), and lumbar spine (L1-L4) (g/cm^2), were measured using DXA (GE, Lunar iDXA, Milwaukee, WI, United States). Additional femoral neck analyses were performed using 3D-Shaper (Galgo Medical, Barcelona, Spain) to obtain cortical vBMD (mg/cm^3) and trabecular vBMD (mg/cm^3) of the femur and femoral neck. Lumbar spine trabecular bone score (g/cm^2) was attained using TBS iNsight (Medimaps, Geneva, Switzerland). All measurements were performed by the same qualified technician according to manufacturer's guidelines.

High-resolution peripheral quantitative computed tomography measurements

All microarchitectural bone measurements were obtained using HR-pQCT (Scanco Medical, Bassersdorf, Switzerland) of the non-dominant radius and tibia unless a fracture was reported in the region of interest. Scans were performed by the same qualified operator by positioning the reference line at the endplate of the radius and tibia on an anteroposterior scout view. Using this line, the standard scan region of interest was found automatically with the first slice 9.5 mm and 22.5 mm down to the reference line for the distal radius and tibia respectively. The following settings were used: peak energy, 60 kVp; X-ray tube current, 900 mA; matrix size, 1,536 × 1,536.A slack of 110 CT slices were acquired over a 9 mm

length consisting of 110 CT slices with an isotropic voxel size of 82 μm, with an effective dose of 3 μSv in approximately 3 min. Image quality was scored ranging from grade 1 (highest quality) to grade 5 (unacceptable), using manufacturers' recommendations (Scanco Medical). A preliminary quality grading was made prior to image acquisition, and repeated measurements were made for all scans with insufficient quality (grade 4 or 5). Thus, only scans with quality grade 1–3 (none, minor or moderate motion artefacts) were used for subsequent image analysis. After manual correction of the periosteal and endosteal contours when needed, total vBMD was calculated as the total amount of mineral divided by the total bone volume within the periosteal contour. This volume was then separated into cortical and trabecular compartments, each compartment being analysed separately. Images were analysed using the advanced cortical evaluation protocol provided by the manufacturer (Burghardt et al., 2010).

The following parameters were measured for the radius and tibia and analysed using Image Processing Language (IPL; version-6, Scanco Medical): total BMD (mg HA/cm^3), trabecular bone volume fraction (BV/TV, %), trabecular inhomogeneity (Tb.1/N.SD; mm), trabecular vBMD (mg HA/cm^3), cortical vBMD (mg HA/cm^3), cortical area (Ct.Ar; mm), cortical thickness (Ct.Th; mm), cortical porosity (Ct.Po; %) and cortical perimeter (Ct.Pm; mm). Finite element analysis using the advanced evaluation script (version 1.0, Scanco Medical) evaluated failure load (FL; kN), compartment load distribution ($Load_{trab.prox,}$ $Load_{trab.dist;}$ %) and average Von Mises stress ($Stress_{VM}$; Mpa) of the cortical and trabecular compartments. The coefficient of variation has been established elsewhere (Vico et al., 2008). The reproducibility of HR-pQCT density measurements ranged from 0.5% to 1.1%. In comparison, reproducibility of the structural parameters (number of trabeculae, trabecular thickness, and cortical thickness) was slightly lower, with coefficients ranging from 0.7% to 4.5%. The reproducibility of the measurement was similar at the distal radius and the distal tibia. Finally, total volume (mm^3) and muscle volume fraction (MV/TV, %) of the distal tibia was performed using soft tissue analysis script provided by Scanco Medical (Erlandson et al., 2017).

Statistical analysis

To estimate the effect size, the DXA BMD at the femoral neck was used. An analysis of four cohort studies (Arlot et al., 1997; Pedrazzoni et al., 2003; Gjesdal et al., 2004; Emaus et al., 2006) evaluating average bone loss in postmenopausal women aged between 55 and 75 years estimated the change in BMD over 1 year and found that a loss of 1% per year was observed. Another study evaluating the effects of walking on BMD in postmenopausal women (Martyn-St James and Carroll, 2008) reported an effect of $^+$2% compared to controls. Additionally, another study comparing the effects of walking with those of WBV (Gusi et al., 2006) observed a $^+$2% difference in WBV from walking on BMD. Thus, we wished to demonstrate an effect of $^+$4% with the WBV compared to the control group (a Delta of 0.04). Considering this desired delta, with a standard deviation of 0.10, a power of 80% and an alpha of 5%, the number of subjects required would consist of 100 participants in each group. A total of 240 women was expected to be included, 120 women per group, taking into account those who would be lost at follow-up.

An independent samples *t*-test was used to assess differences in baseline characteristics and for the selection of appropriate covariates. All quantities were analysed in STATA-17 (StataCorp LP, College Station, Texas, United States) using a linear mixed model with a random intercept and robust standard errors. This model was selected given the multiple timepoints associated with each participant and the ability to handle missing values. Fixed factors were group (2 levels: control, vibration) and month (4 levels: 0,6,12,18), while the random factor was participants (n = 197). Age was treated as a continuous factor. Overall model fit and normality was assessed using quartile-quartile plots of the residuals. All dependant variables during the vibration phase were analysed in the following way: (Baseline vs. month-6 and Baseline vs. month-12). Baseline compared with follow-up used: (Baseline vs. month-18). Finally, to analyse the prolonged effect of vibration (Month-12 vs. Month-18) was compared. Bonferroni correction was applied for all comparisons with results presented as contrasts between groups, and the significance level was set to $p < 0.05$.

Results

This non-randomised clinical trial matched participants according to their FRAX scores for major osteoporotic fracture (MOF) 10 years-absolute risk ranging from (3%–35%) to ensure group comparability. Statistical analysis was performed using 197 participants (Figure 1) having four timepoints (baseline, 6, 12 and 18 months). For all 197 participants, HR-pQCT image quality and common region were (≤ 3) and (≥ 70%) respectively (Table 1; Figure 1). Participants' baseline characteristics can be found in (Tables 2, 3). Vibration protocol adherence was good with 110.02 ± 14.25 (mean ± SD) vibration sessions from a maximum of 130 completed by the vibration group. All within groups differences are presented in (Tables 4–7) and significant between group differences are presented in (Table 8).

Dual-energy X-ray absorptiometry and 3D-Shaper

All DXA and 3D-Shaper parameters are presented in (Table 4). Other than a similar and continued decrease over

TABLE 1 Breakdown of the total number of participants analysed for HR-pQCT parameters, based on having 4-time points, image quality (≤ 3) and common region (≥ 70%). Total number of participants (n = 197): Control (n = 98), Vibration (n = 99). Common region for tibia (mean ± SD): Control (89.05 ± 7.27) Vibration (88.05 ± 9.06). Common region for radius (mean ± SD): Control (82.00 ± 10.64) Vibration (82.70 ± 12.41).

Total number of participants analysed for HR-pQCT parameters					
Group					
Control			Vibration		
Radius only	Tibia only	Radius and Tibia	Radius only	Tibia only	Radius and Tibia
10	33	55	9	26	64

time in all participants, no significant improvement in BMD of the femoral neck, total hip, total cortical and trabecular vBMD of the femur, cortical and trabecular vBMD at the femoral neck, total BMD, bone mass, and BMD and trabecular bone score of the spine (L1-L4) were observed.

A group by month interaction was observed for fat, lean and total body mass (Table 8). Fat mass for the vibration group was significantly lower compared to control, towards the end of the vibration period (−776.06 g, p = 0.043). For lean body mass, a reduction at month-12 compared to baseline produced a difference of (−519.11 g, p = 0.009) between the vibration group compared to control. Total body mass decreased from baseline to month-12 with group differences of (−1,285.72 g, p = 0.003) compared to control. Although not statistically significant, differences from month-18 to baseline demonstrated that these effects were not long lasting.

Tibia high-resolution peripheral quantitative computed tomography

All tibia HR-pQCT parameters are presented in (Table 5). No significant improvement in total BMD compared to control was observed throughout the vibration phase (p = 1.00). No apparent differences could be observed for trabecular volume resulting from vibration exposure. In contrast, BV/TV exhibited a group by month interaction in the vibration group when comparing baseline to month-12 (p = 0.023). However, despite this significant difference between groups, the overall change reflected in BV/TV was negligible (0.0009%, p = 0.050). Trabecular inhomogeneity remained unchanged. No improvement in cortical area or thickness was observed. Apart from a gradual decrease over time no differences in cortical vBMD and perimeter were noted between groups. Finally, cortical porosity continued to increase over time in both the control and training group. Total muscle volume continued to decrease over time and MV/TV showed no overall differences between groups.

Radius high-resolution peripheral quantitative computed tomography

All radius HR-pQCT parameters are presented in (Table 6). No improvement in total bone mineral density was evident. Trabecular volume, BV/TV as well as trabecular inhomogeneity remained unaltered. No significant interactions were observed for any cortical parameters. For cortical area, thickness, perimeter and cortical vBMD no improvement resulting from vibration could be detected. Finally, both groups experienced an increase in cortical porosity levels over time.

Finite element analysis

All finite element parameters for the radius and tibia are presented in (Tables 5, 6). WBV failed to improve finite element parameters in the vibration group. Failure load continued to worsen over time in both the tibia and radius. The ratio of the load supported by the trabecular bone and the load by the whole bone at the distal tibial site continued to decrease (p = 0.016) throughout. In contrast, these changes were not significantly different at the proximal end (p = 0.185). For the radius, both the distal and proximal sites showed reductions over time with values of (p < 0.001) and (p = 0.008), respectively. Cortical Von Mises stress for the tibia and radius moderately decreased over time, however these changes were not statistically significant. Trabecular Von Mises stress on the other hand demonstrated no significant changes over time for the tibia, however, significant decreases in stress loads over time were seen for the radius (p = 0.009).

Serum analysis

All serum markers are presented in (Table 7). Interaction effects between month and group were observed for serum CTX (p = 0.015) (Table 8; Figure 3). Although both groups

TABLE 2 Participants' characteristics and DXA parameters at baseline (See Supplementary Material S3) and (Fardellone et al., 1991) for Fardellone self-questionnaire. (See Supplementary Material S1) for lifetime activity questionnaire (QUANTAP). QUANTAP total lifetime activity and current level of physical activity at recruitment were reported in Kilojoules/Kilograms (Kj/Kg).

	Group				
	Control (n = 98)		Vibration (n = 99)		p-value
	Mean	SD	Mean	SD	
Participants' baseline characteristics					
Age	66.1	5.39	63.27	4.94	< 0.001
(MOF) Frax score	6.04	2.63	5.41	2.09	0.062
(Fardelonne) daily calcium intake (mg)	762.24	383.92	677.9	323.02	0.099
(QUANTAP) total lifetime activity at recruitment (Kj/kg)	108,8338.88	625,610.81	112,2443.84	579,877.59	0.698
(QUANTAP) current physical activity levels at recruitment (Kj/kg)	22,965.06	16,743.94	24,198.09	15,973.68	0.607
Calcium (mmol/L)	2.29	0.25	2.28	0.08	0.739
Phosphate (mmol/L)	1.21	0.19	1.21	0.18	0.983
25-OH Vitamin D (µg)	27.04	10.91	29.73	9.82	0.07
Parathyroid hormone (ng/L)	29.94	11.35	29.77	10.44	0.911
Height (cm)	159.33	5.82	158.12	5.43	0.133
Body mass (kg)	66.8	13.15	63.5	10.71	0.058
BMI (kg/m^2)	26.21	4.84	25.11	4.14	0.089
DXA and 3D-Shaper					
Total BMD (g/cm^2)	1.02	0.09	1.01	0.1	0.371
Total T-score (SD)	0.05	0.93	−0.08	0.98	0.371
Fat mass (g)	26,845.52	9,543.16	24,673.83	7,314.66	0.074
Lean mass (g)	37,858.96	4,363.89	36,812.57	4,169.27	0.087
Body mass (g)	66,806	13,157.84	63,545.19	10,713.35	0.058
BMD spine L1-L4 (g/cm^2)	1.06	0.16	1.04	0.15	0.398
T-score spine L1-L4 (SD)	−0.86	1.35	−1.01	1.21	0.398
Trabecular bone score L1-L4 (g/cm^2)	1.26	0.09	1.28	0.1	0.162
BMD femoral neck (g/cm^2)	0.85	0.09	0.84	0.09	0.253
T-score femoral neck (SD)	−1.07	0.73	−1.2	0.79	0.253
BMD Total Hip (g/cm^2)	0.89	0.1	0.87	0.11	0.071
T-score total hip (SD)	−0.88	0.85	−1.11	0.88	0.071
3D-Shaper total cortical vBMD (mg/cm^3)	803.9	84.05	788.19	84.62	0.193
3D-Shaper total trabecular vBMD (mg/cm^3)	140.42	33.09	135.05	35.21	0.272
3D-Shaper cortical vBMD of the neck (mg/cm^3)	803.7	74.97	786.53	74.77	0.109
3D-Shaper trabecular vBMD of the neck (mg/cm^3)	187.91	37.06	185.99	37.51	0.719

experienced an increase in CTX concentrations, a more marginal elevation was noted in the vibration group. Compared to baseline, the vibration group at month 12 produced greater differences in CTX concentrations compared to control (480.80 pmol/L; p = 0.039). However, no differences between groups were observed when only comparing groups at month-12 (506.35 pmol/L; p = 0.415) and month-18 (518.33 pmol/L; p = 0.480). No significant differences in sclerostin concentration could be observed. Apart from significant increases over time (p < 0.001), no other differences were observed for BAP. Where P1NP was concerned, no significant alterations were observed.

Discussion

This non-randomised clinical trial matched participants according to their FRAX scores for major osteoporotic fracture (MOF) 10 years-absolute risk ranging from (3%–35%) to ensure group comparability investigated the effects of WBV on 197 postmenopausal women over 12 months, with an additional 6-month follow-up to observe post-vibratory outcomes. Treatment responses were measured using DXA, HR-pQCT, and serum analysis. Fat, lean body, and total body mass assessed by DXA very marginally decreased in the vibration group during the vibration phase. No significant

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TABLE 3 HR-pQCT (Radius and Tibia) and serum parameters at baseline.

	Group				
	Control (n = 98)		Vibration (n = 99)		p-value
	Mean	SD	Mean	SD	
HR-pQCT (Tibia)					
Total BMD (mgHA/cm^3)	257.5	49.15	253.4	48.44	0.576
Trabecular vBMD (mgHA/cm^3)	154.53	34.96	152.4	35.93	0.688
BV/TV (%)	0.13	0.03	0.13	0.03	0.686
Trabecular inhomogeneity (Tb.1/N.SD; mm)	0.29	0.16	0.35	0.38	0.157
Cortical area (mm^2)	96.6	14.51	92.39	15.72	0.065
Cortical vBMD (mgHA/cm^3)	795.6	60.3	806.31	64.9	0.256
Cortical perimeter (mm)	99.49	7.38	99.86	6.7	0.728
Cortical thickness (mm)	1.07	0.18	1.02	0.18	0.091
Cortical porosity (%)	0.08	0.03	0.07	0.03	0.051
Ultimate load (N)	−8028.69	1,265.94	−8020.71	1,320.72	0.967
Trabecular load vs. whole bone load (distal)	0.52	0.09	0.53	0.1	0.397
Trabecular load vs. whole bone load (proximal)	0.32	0.08	0.33	0.09	0.32
Trabecular Von Mises stress (MPa)	52.76	7.11	54.06	7.18	0.225
Cortical Von Mises stress (MPa)	86.01	2.21	86.19	2.41	0.612
Total Muscle Volume (mm^3)	19,625.05	4,851.78	18,770.96	3,965.69	0.201
MV/TV	0.55	0.15	0.57	0.13	0.453
HR-pQCT (Radius)					
Total BMD (mgHA/cm^3)	295.95	67.08	295.05	59.35	0.934
Trabecular vBMD (mgHA/cm^3)	149.62	39.62	142.75	41.17	0.321
BV/TV (%)	0.12	0.03	0.12	0.03	0.319
Trabecular inhomogeneity (Tb.1/N.SD; mm)	0.29	0.21	0.33	0.22	0.368
Cortical area (mm^2)	48.88	8.26	48.73	7.64	0.915
Cortical vBMD (mgHA/cm^3)	847	56.1	863.29	59.37	0.101
Cortical perimeter (mm)	68.8	10.67	66.76	5.04	0.145
Cortical thickness (mm)	0.81	0.16	0.82	0.15	0.748
Cortical porosity (%)	0.025	0.013	0.02	0.009	0.011
Ultimate load (N)	−2,859.75	551.01	−2901.77	541.36	0.654
Trabecular load vs. whole bone load (distal)	0.44	0.07	0.44	0.1	0.952
Trabecular load vs. whole bone load (proximal)	0.16	0.06	0.16	0.06	0.656
Trabecular Von Mises stress (MPa)	42.42	6.29	43.61	7.24	0.31
Cortical Von Mises stress (MPa)	78.92	3.81	79.99	3.55	0.092
Serum					
P1NP (μg/L)	55.77	23.87	56.17	18.39	0.897
CTX (pmol/L)	4,141.05	2,379.15	4,332.82	1,836.07	0.527
BAP (μg/L)	12.25	4.12	11.71	3.6	0.334
Sclerostin (ng/ml)	0.64	0.15	0.6	0.15	0.062

differences at the femoral neck or for all other DXA and serum parameters were observed except for an 11% increase in CTX levels in the vibration group, a non-clinically relevant variation which remained present at month-18. Furthermore, no demonstrable benefits from vibration were detected for all bone geometry and microarchitectural parameters measured by HR-pQCT. Finite element analysis demonstrated a continuous decrease in failure load, and disproportionate stress loads were observed for the cortical and trabecular compartments in both groups. Finally, no changes in muscle

TABLE 4 Summary of all DXA and 3D-Shaper parameters.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Total BMD (g/cm²)											$p = 0.0980$	$p<0.001$
Month 6 vs. Baseline	−0.005	−0.51	0.00	−0.008	−0.002	−0.004	−0.36	0.01	−0.007	−0.001		
Month 12 vs. Baseline	−0.011	−1.07	0.00	−0.014	−0.008	−0.007	−0.72	0.00	−0.011	−0.004		
Month 18 vs. Baseline	−0.011	−1.08	0.00	−0.016	−0.006	−0.010	−1.02	0.00	−0.014	−0.007		
Fat mass (g)											$p = 0.0107$	$p = 0.262$
Month 6 vs. Baseline	368.804	1.37	0.32	−135.817	873.425	56.752	0.23	1.00	−333.840	447.344		
Month 12 vs. Baseline	947.120	3.53	0.00	253.847	1640.393	170.848	0.69	1.00	-298.648	640.345		
Month 18 vs. Baseline	616.845	2.30	0.11	−70.477	1304.166	554.351	2.25	0.03	25.956	1082.746		
Lean mass (g)											$p = 0.0268$	$p < 0.001$
Month 6 vs. Baseline	522.864	1.38	0.00	185.875	859.852	168.943	0.46	0.98	−150.549	488.434		
Month 12 vs. Baseline	878.791	2.32	0.00	520.487	1237.095	359.627	0.98	0.01	71.778	647.476		
Month 18 vs. Baseline	759.616	2.01	0.00	409.126	1110.106	476.753	1.30	0.00	132.296	821.210		
Body mass (g)											$p = 0.0015$	$p = 0.005$
Month 6 vs. Baseline	885.310	1.33	0.00	211.027	1559.593	222.018	0.35	1.00	−310.917	754.954		
Month 12 vs. Baseline	1808.351	2.71	0.00	952.759	2663.943	522.380	0.82	0.10	−57.210	1101.970		
Month 18 vs. Baseline	1350.420	2.02	0.00	521.401	2179.439	1014.991	1.60	0.00	398.553	1631.429		
BMD spine (g/cm²)											$p = 0.4447$	$p < 0.001$
Month 6 vs. Baseline	−0.007	−0.64	0.21	−0.015	0.002	−0.007	−0.68	0.04	−0.014	0.000		
Month 12 vs. Baseline	−0.011	−1.04	0.00	−0.020	−0.002	−0.012	−1.18	0.00	−0.020	−0.005		
Month 18 vs. Baseline	−0.007	−0.62	0.35	−0.016	0.003	−0.014	−1.33	0.00	−0.022	−0.005		
Trabecular bone score L1-L4 (g/cm²)											$p = 0.5035$	$p < 0.001$
Month 6 vs. Baseline	0.005	0.41	1.00	−0.008	0.019	0.010	0.75	0.41	−0.004	0.023		
Month 12 vs. Baseline	−0.004	−0.33	1.00	−0.017	0.009	−0.005	−0.37	1.00	−0.017	0.007		
Month 18 vs. Baseline	−0.005	−0.37	1.00	−0.019	0.009	−0.014	−1.13	0.02	−0.028	−0.001		
BMD femoral neck (g/cm²)											$p = 0.5201$	$p = 0.180$
Month 6 vs. Baseline	0.002	0.24	1.00	-0.004	0.008	−0.004	−0.43	0.46	−0.009	0.002		
Month 12 vs. Baseline	−0.001	−0.13	1.00	−0.006	0.004	−0.004	−0.51	0.19	−0.010	0.001		
Month 18 vs. Baseline	−0.003	−0.33	1.00	−0.010	0.004	−0.008	−0.98	0.01	−0.015	−0.002		
BMD Total Hip (g/cm²)											$p = 0.4923$	$p < 0.001$
Month 6 vs. Baseline	−0.003	−0.39	0.17	−0.008	0.001	−0.002	−0.19	1.00	−0.005	0.002		

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TABLE 4 (*Continued*) Summary of all DXA and 3D-Shaper parameters.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Month 12 vs. Baseline	−0.006	−0.72	0.00	−0.011	−0.002	−0.003	−0.38	0.16	−0.007	0.001		
Month 18 vs. Baseline	−0.008	−0.89	0.00	−0.013	−0.003	−0.008	−0.90	0.00	−0.012	−0.003		
3D-Shaper total cortical vBMD (mg/cm^3)											$p = 0.3041$	$p = 0.001$
Month 6 vs. Baseline	−3.247	−0.40	1.00	−9.769	3.274	1.349	−0.17	1.00	−7.985	5.287		
Month 12 vs. Baseline	−3.129	−0.39	1.00	−9.308	3.051	1.149	0.15	1.00	−5.052	7.351		
Month 18 vs. Baseline	−5.710	−0.71	0.15	−12.409	0.990	−6.931	−0.88	0.08	14.370	0.509		
3D-Shaper total trabecular vBMD (mg/cm^3)											$p = 0.0807$	$p < 0.001$
Month 6 vs. Baseline	−1.142	−0.81	0.35	−2.732	0.447	0.825	0.61	1.00	−1.209	2.859		
Month 12 vs. Baseline	−2.707	−1.93	0.00	−4.621	−0.794	−0.470	−0.35	1.00	−2.387	1.448		
Month 18 vs. Baseline	−3.669	−2.61	0.00	−5.986	−1.353	−2.925	−2.17	0.05	−5.813	−0.038		
3D-Shaper cortical vBMD of the neck (mg/cm^3)											$p = 0.9032$	$p = 0.005$
Month 6 vs. Baseline	−1.555	−0.19	1.00	−7.752	4.641	−1.425	−0.18	1.00	−8.257	5.407		
Month 12 vs. Baseline	−0.416	−0.05	1.00	−6.388	5.556	1.138	0.14	1.00	−4.901	7.176		
Month 18 vs. Baseline	−4.495	−0.56	0.27	−10.396	1.407	−5.740	−0.73	0.24	−13.094	1.614		
3D-Shaper trabecular vBMD of the neck (mg/cm^3)											$p = 0.3418$	$p = 0.030$
Month 6 vs. Baseline	−0.731	−0.39	1.00	−2.915	1.452	0.328	0.18	1.00	−2.218	2.875		
Month 12 vs. Baseline	−2.875	−1.53	0.18	−6.384	0.635	−0.951	−0.51	1.00	−3.218	1.316		
Month 18 vs. Baseline	−2.969	−1.58	0.11	−6.271	0.334	−3.791	−2.04	0.02	−7.260	−0.323		

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TABLE 5 Summary of all HR-pQCT parameters at the distal tibia.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Total BMD (mgHA/cm^3)											$p = 0.0063$	$p = 0.149$
Month 6 vs. Baseline	0.108	0.04	1.000	−1.107	1.323	−1.009	−0.40	0.154	−2.201	0.184		
Month 12 vs. Baseline	−1.841	−0.71	0.023	−3.517	−0.164	−1.346	−0.53	0.014	−2.509	−0.183		
Month 18 vs. Baseline	−2.186	−0.85	0.007	−3.970	−0.402	−3.187	−1.26	0.000	−4.565	−1.809		
Trabecular vBMD (mgHA/cm^3)											$p = 0.0159$	$p = 0.076$
Month 6 vs. Baseline	0.257	0.17	1.000	−0.508	1.021	0.082	0.05	1.000	−0.675	0.839		
Month 12 vs. Baseline	−0.828	−0.54	0.165	−1.819	0.163	0.290	0.19	1.000	−0.456	1.036		
Month 18 vs. Baseline	−0.769	−0.50	0.576	−1.989	0.450	−0.198	−0.13	1.000	−1.116	0.721		
BV/TV (%)											$p = 0.0231$	$p = 0.049$
Month 6 vs. Baseline	0.000	0.17	1.000	0.000	0.001	0.000	0.10	1.000	−0.001	0.001		
Month 12 vs. Baseline	−0.001	−0.52	0.213	−0.002	0.000	0.000	0.23	1.000	0.000	0.001		
Month 18 vs. Baseline	−0.001	−0.52	0.506	−0.002	0.000	0.000	−0.11	1.000	−0.001	0.001		
Trabecular inhomogeneity (Tb.1/N.SD; mm)											$p = 0.3615$	$p = 0.149$
Month 6 vs. Baseline	0.002	0.57	1.000	−0.006	0.009	0.005	1.47	1.000	−0.009	0.019		
Month 12 vs. Baseline	0.003	1.17	1.000	−0.011	0.018	0.017	4.91	0.159	−0.003	0.038		
Month 18 vs. Baseline	0.005	1.69	1.000	−0.007	0.017	0.010	2.90	0.962	−0.009	0.029		
Cortical area (mm^2)											$p = 0.1317$	$p = 0.822$
Month 6 vs. Baseline	0.359	0.37	1.000	−1.247	1.966	0.127	0.14	1.000	−0.857	1.110		
Month 12 vs. Baseline	−0.306	−0.32	1.000	−1.837	1.226	0.267	0.29	1.000	−0.798	1.332		
Month 18 vs. Baseline	0.470	0.49	1.000	−0.932	1.873	−0.208	−0.22	1.000	−1.326	0.911		
Cortical vBMD (mgHA/cm^3)											$p = 0.9484$	$p = 0.049$
Month 6 vs. Baseline	−3.907	−0.49	0.034	−7.637	−0.176	−6.048	−0.75	0.000	−9.305	−2.790		
Month 12 vs. Baseline	−6.078	0.76	0.002	−10.503	−1.654	−9.481	−1.18	0.000	−13.111	−5.851		
Month 18 vs. Baseline	−9.814	−1.23	0.000	−14.344	−5.284	−13.523	−1.68	0.000	−17.747	−9.299		
Cortical perimeter (mm)											$p = 0.3602$	$p = 0.065$
Month 6 vs. Baseline	−0.155	−0.16	0.216	−0.349	0.040	−0.147	−0.15	0.280	−0.341	0.048		
Month 12 vs. Baseline	0.790	0.79	1.000	−1.792	3.371	−0.413	−0.41	0.001	−0.691	−0.136		
Month 18 vs. Baseline	−0.258	−0.26	0.082	−0.534	0.018	−0.408	−0.41	0.002	−0.709	−0.107		
Cortical thickness (mm)											$p = 0.3772$	$p = 0.972$
Month 6 vs. Baseline	−0.001	−0.05	1.000	−0.014	0.013	−0.001	−0.09	1.000	−0.012	0.011		

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TABLE 5 (*Continued*) Summary of all HR-pQCT parameters at the distal tibia.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Month 12 vs. Baseline	−0.001	−0.13	1.000	−0.015	0.012	0.002	0.22	1.000	−0.009	0.014		
Month 18 vs. Baseline	0.000	0.01	1.000	−0.012	0.012	0.000	−0.03	1.000	−0.013	0.013		
Cortical porosity (%)											$p = 0.6070$	$p = 0.559$
Month 6 vs. Baseline	0.001	1.52	1.000	−0.002	0.005	0.002	2.81	0.094	0.000	0.004		
Month 12 vs. Baseline	0.003	3.79	0.147	−0.001	0.007	0.005	6.50	0.000	0.002	0.007		
Month 18 vs. Baseline	0.005	6.23	0.003	0.001	0.009	0.006	7.82	0.000	0.003	0.008		
Ultimate load (N)											$p = 0.8138$	$p = 0.145$
Month 6 vs. Baseline	30.062	−0.37	1.000	−65.523	125.647	70.369	−0.88	0.299	−24.280	165.017		
Month 12 vs. Baseline	74.798	−0.93	0.484	−38.158	187.754	86.170	−1.07	0.312	−30.849	203.190		
Month 18 vs. Baseline	61.717	−0.77	0.883	−50.621	174.056	131.971	−1.65	0.019	14.108	249.835		
Trabecular load vs. whole bone load (distal)											$p = 0.4204$	$p = 0.016$
Month 6 vs. Baseline	−0.006	−1.13	0.278	−0.014	0.002	−0.005	−0.90	0.566	−0.012	0.003		
Month 12 vs. Baseline	−0.002	−0.46	1.000	−0.011	0.006	−0.011	−2.07	1.000	−0.033	0.011		
Month 18 vs. Baseline	−0.006	−1.18	0.363	−0.015	0.002	−0.001	−0.27	1.000	−0.010	0.007		
Trabecular load vs. whole bone load (proximal)											$p = 0.4699$	$p = 0.185$
Month 6 vs. Baseline	−0.004	−1.30	0.872	−0.012	0.003	−0.002	−0.66	1.000	−0.009	0.005		
Month 12 vs. Baseline	0.000	−0.03	1.000	−0.008	0.008	−0.009	−2.63	0.925	−0.025	0.007		
Month 18 vs. Baseline	−0.003	−0.85	1.000	−0.012	0.007	−0.001	−0.34	1.000	−0.009	0.007		
Trabecular Von Mises stress (MPa)											$p = 0.8662$	$p = 0.156$
Month 6 vs. Baseline	−0.473	−0.90	1.000	−1.566	0.619	−0.573	−1.06	1.000	−1.762	0.616		
Month 12 vs. Baseline	−0.379	−0.72	1.000	−1.595	0.837	−1.270	−2.35	0.516	−3.222	0.682		
Month 18 vc Baseline	−0.487	−0.92	1.000	−1.793	0.819	−1.022	−1.89	0.349	−2.445	0.401		
Cortical Von Mises stress (MPa)											$p = 0.0927$	$p = 0.477$
Month 6 vs. Baseline	−0.135	−0.16	1.000	−0.431	0.162	−0.179	−0.21	0.334	−0.425	0.068		
Month 12 vs. Baseline	−0.232	−0.27	0.194	−0.518	0.054	0.011	0.01	1.000	−0.295	0.318		
Month 18 vs. Baseline	−0.146	−0.17	1.000	4.446	0.155	−0.226	−0.26	0.588	−0.586	0.134		
Total Muscle Volume (mm^3)											$p = 0.5400$	$p = 0.008$
Month 6 vs. Baseline	37.341	0.19	1.000	454252	528.935	−115.230	−0.61	1.000	450.794	220.333		
Month 12 vs. Baseline	166.399	0.85	1.000	−356.981	689.779	−166.633	−0.89	1.000	−536.316	203.050		
Month 18 vs. Baseline	−117.924	−0.60	1.000	−471.104	235.255	−256.935	−1.37	0.224	−582.550	68.679		

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TABLE 5 (*Continued*) Summary of all HR-pQCT parameters at the distal tibia.

	Control					Vibration					*p*-value group*month	*p*-value month
	Contrast	% change	*p*-value	95% lower bound	95% upper bound	Contrast	% change	*p*-value	95% lower bound	95% upper bound		
MV/TV											$p = 0.1708$	$p = 0.914$
Month 6 vs. Baseline	−0.002	−0.41	1.000	−0.011	0.007	0.000	−0.04	1.000	−0.007	0.006		
Month 12 vs. Baseline	−0.009	−1.62	0.062	−0.018	0.000	0.001	0.13	1.000	−0.007	0.008		
Month 18 vs. Baseline	−0.006	−1.11	0.776	−0.017	0.005	−0.003	−0.51	1.000	−0.011	0.005		

parameters evaluated by HR-pQCT at the distal tibia were detected.

The primary objective was to assess the effect of WBV on the femoral neck BMD, given that this parameter is a strong predictor of hip and spine fractures in this type of population (Crandall et al., 2021). Some studies have found neutral effects of WBV at the hip and femoral neck (Lau et al., 2011; Ma et al., 2016; Oliveira et al., 2016; Jepsen et al., 2017; Luo et al., 2017) while one demonstrated positive effects (Sitjà-Rabert et al., 2012). Although the objective of previous studies has been on frequency variation and exposure length, none have incorporated combined amplitude in the experimental protocol. Therefore, unique to this study was the incorporation of high and low-amplitude stimuli with an incremental increase throughout the vibration protocol. By further tailoring the signal characteristics that have generally been sufficient to stimulate bone formation (Santos et al., 2017), this protocol was expected to better replicate the role of mechanical stimulation, in particular the ground reaction forces produced during regular jumping and physical activity and to demonstrate its tolerance in this population. However, despite this combination of amplitude and frequency, no discernible benefits were observed at the femoral neck, spine, or HR-pQCT parameters. The continued reduction in failure load with unequal load distributions between cortical and trabecular compartments is consistent with elevated fracture risk (Boutroy et al., 2008) in this population, not significantly negated by vibration therapy.

The results from this study are difficult to compare, given the unique signal characteristics. However, considering studies 12 months in duration, Slatkovska et al. (2011) share several similarities (population size, age range and 20-min training sessions with predominantly European participants) with the current study. Although Slatkovska et al. (2011) mainly focused on frequency variation (90 Hz and 30 Hz), the frequency range in the present study (30 Hz–50 Hz) fell between the two training groups. This is a particular strength as it helps to further expand upon the range of frequencies previously tested while incorporating the application of combined amplitude. However, both Slatkovska et al. (2011) and the present study failed to see any benefits on bone after 12 months of WBV exposure. Another one-year longitudinal study (Liphardt et al., 2015), which incorporated a follow-up period to observe sustained osteogenic benefits of WBV, also failed to detect changes in the bone and observed no improvement in load distribution between groups, which indicates no improvement in fracture risk. Though these studies employed different platforms and signal attributes, the consistency in HR-pQCT results of bone geometry and microarchitecture remains a crucial finding.

The current results can be further contrasted with other studies using the PowerPlate device. Sen et al. (2020) and Verschueren et al. (2004) utilised high-frequency stimuli for 6 months to measure BMD changes at one or more sites (hip,

TABLE 6 Summary of all HR-pQCT parameters at the distal radius.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Total BMD (mgHA/cm^3)											$p = 0.4353$	$p = 0.092$
Month 6 vs. Baseline	0.51	0.17	1.00	−1.87	2.89	−0.95	−0.32	1.00	−3.25	1.36		
Month 12 vs. Baseline	−1.93	−0.65	0.25	−4.43	0.58	−1.73	−0.59	0.58	−4.47	1.01		
Month 18 vs. Baseline	−3.71	−1.25	0.08	−7.66	0.25	−4.09	−1.39	0.00	−6.92	−1.26		
Trabecular vBMD (mgHA/cm^3)											$p = 0.5609$	$p = 0.010$
Month 6 vs. Baseline	0.04	0.03	1.00	−0.98	1.06	0.10	0.07	1.00	−0.95	1.15		
Month 12 vs. Baseline	−0.75	−0.50	0.48	−1.89	0.38	0.25	0.17	1.00	−1.00	1.50		
Month 18 vs. Baseline	−2.28	−1.52	0.12	−4.85	0.29	−0.78	−0.54	0.83	−2.16	0.61		
BV/TV (%)											$p = 0.0112$	$p = 0.011$
Month 6 vs. Baseline	0.00	0.05	1.00	0.00	0.00	0.00	0.09	1.00	0.00	0.00		
Month 12 vs. Baseline	0.00	−0.54	0.39	0.00	0.00	0.00	0.22	1.00	0.00	0.00		
Month 18 vs. Baseline	0.00	−1.52	0.11	0.00	0.00	0.00	−0.53	0.88	0.00	0.00		
Trabecular inhomogeneity (Tb.1/ N.SD; mm)											$p = 0.1465$	$p = 0.037$
Month 6 vs. Baseline	0.01	2.45	0.27	0.00	0.02	0.01	4.13	0.02	0.00	0.03		
Month 12 vs. Baseline	0.00	1.49	1.00	−0.01	0.02	0.01	3.30	1.00	−0.01	0.03		
Month 18 vs. Baseline	0.01	2.52	0.27	0.00	0.02	0.01	4.13	0.26	0.00	0.03		
Cortical area (mm^2)											$p = 0.3697$	$p = 0.260$
Month 6 vs. Baseline	0.37	0.75	0.88	−0.30	1.04	−0.19	−0.39	1.00	−0.73	0.36		
Month 12 vs. Baseline	−0.02	−0.03	1.00	−0.80	0.77	−0.14	−0.28	1.00	−0.69	0.42		
Month 18 vs. Baseline	1.51	3.10	1.00	−2.81	5.84	−0.55	−1.14	0.18	−1.23	0.12		
Cortical vBMD (mgHA/cm^3)											$p = 0.4968$	$p = 0.582$
Month 6 vs. Baseline	−0.92	−0.11	1.00	−5.36	3.51	−3.55	−0.41	0.22	−8.05	0.94		
Month 12 vs. Baseline	−3.86	−0.46	0.40	−9.43	1.70	−6.90	−0.80	0.00	−11.65	−2.15		
Month 18 vs. Baseline	−11.69	−1.38	0.52	−29.67	6.28	−7.65	−0.89	0.00	−12.45	−2.85		
											$p = 0.1963$	$p = 0.056$

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TABLE 6 (*Continued*) Summary of all HR-pQCT parameters at the distal radius.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Cortical perimeter (mm)												
Month 6 vs. Baseline	−1.37	−1.99	1.00	−4.18	1.45	−0.08	−0.11	1.00	−0.31	0.16		
Month 12 vs. Baseline	−1.49	−2.17	0.97	−4.30	1.32	−0.23	−0.35	0.04	−0.45	−0.01		
Month 18 vs. Baseline	−1.54	−2.24	0.89	−4.35	1.27	−0.36	−0.55	0.00	−0.61	−0.12		
Cortical thickness (mm)											$p = 0.1879$	$p = 0.616$
Month 6 vs. Baseline	0.00	0.55	1.00	−0.01	0.02	−0.01	−0.71	0.30	−0.01	0.00		
Month 12 vs. Baseline	0.00	−0.10	1.00	−0.01	0.01	0.00	−0.30	1.00	−0.01	0.01		
Month 18 vs. Baseline	0.01	1.49	1.00	−0.03	0.06	−0.01	−0.71	0.86	−0.02	0.00		
Cortical porosity (%)											$p = 0.7435$	$p = 0.059$
Month 6 vs. Baseline	0.00	7.22	0.35	0.00	0.00	0.00	8.05	0.05	0.00	0.00		
Month 12 vs. Baseline	0.00	8.08	0.21	0.00	0.00	0.00	8.74	0.06	0.00	0.00		
Month 18 vs. Baseline	0.01	56.38	0.98	−0.01	0.04	0.00	14.13	0.00	0.00	0.00		
Ultimate load (N)											$p = 0.3537$	$p = 0.005$
Month 6 vs. Baseline	18.16	−0.64	1.00	−55.29	91.61	52.41	−1.81	0.94	−45.08	149.90		
Month 12 vs. Baseline	80.07	−2.80	0.00	16.85	143.28	64.08	−2.21	0.01	11.54	116.62		
Month 18 vs. Baseline	39.49	−1.38	0.93	−33.88	112.87	86.96	−3.00	0.00	25.80	148.13		
Trabecular load vs. whole bone load (distal)											$p = 0.4963$	$p<0.001$
Month 6 vs. Baseline	0.00	−0.39	1.00	−0.02	0.01	0.00	0.81	1.00	−0.01	0.02		
Month 12 vs. Baseline	−0.01	−2.84	0.16	−0.03	0.00	−0.01	−2.49	0.39	−0.03	0.00		
Month 18 vs. Baseline	0.01	1.41	1.00	−0.01	0.02	0.00	−0.10	1.00	−0.02	0.02		
Trabecular load vs. whole bone load (proximal)											$p = 0.3569$	$p = 0.008$
Month 6 vs. Baseline	0.00	−3.03	0.66	−0.01	0.00	0.00	0.69	1.00	−0.01	0.01		
Month 12 vs. Baseline	−0.01	−3.52	0.49	−0.01	0.00	0.00	−2.17	1.00	−0.01	0.00		
Month 18 vs. Baseline	0.00	0.40	1.00	−0.01	0.01	0.00	0.43	1.00	−0.01	0.01		
											$p = 0.8640$	$p = 0.009$

(Continued on following page)

TABLE 6 (*Continued*) Summary of all HR-pQCT parameters at the distal radius.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
Trabecular Von Mises stress (MPa)												
Month 6 vs. Baseline	−0.14	−0.34	1.00	−1.66	1.37	−0.27	−0.62	1.00	−1.99	1.45		
Month 12 vs. Baseline	−1.44	−3.38	0.11	−3.05	0.17	−1.08	−2.48	0.33	−2.56	0.40		
Month 18 vs. Baseline	0.02	0.06	1.00	−1.64	1.68	−0.62	−1.43	1.00	−2.24	0.99		
Cortical Von Mises stress (MPa)											$p = 0.4670$	$p = 0.175$
Month 6 vs. Baseline	−0.34	−0.43	1.00	−1.25	0.57	−1.15	−1.44	1.00	−3.38	1.08		
Month 12 vs. Baseline	−0.70	−0.89	0.27	−1.63	0.22	−0.41	−0.51	0.94	−1.16	0.35		
Month 18 vs. Baseline	−0.92	−1.16	0.40	−2.23	0.40	−0.71	−0.88	0.06	−1.43	0.02		

femoral neck, and lumbar region) along with serum bone turnover markers (CTX and osteocalcin). In both studies, the vibration groups demonstrated an improvement in BMD; however, bone turnover marker results were inconsistent. In the present study, no significant benefits were observed at the crucial DXA sites. However, from baseline to the end of the training period, CTX was elevated in both groups with marginally higher concentrations in the vibration group. Nevertheless, when comparing months 12 and 18 alone these differences were not statistically different.

Several explanations could account for the marginal increase in CTX concentrations. Firstly, the two previous studies (Verschueren et al., 2004; Sen et al., 2020) were of much shorter duration, hence CTX would not have changed as it did in this study. Secondly, when taken together, the increased BAP activity with the rising CTX concentrations is indicative of the stimulated bone remodeling process in postmenopausal women (Eastell et al., 2016). Finally, the marginal elevation in CTX concentrations in the vibration group could also be linked with the small reduction in body mass resulting from WBV exposure. The impact of weight loss attributed to dieting and/or exercise on bone turnover markers (CTX) has been previously reported (Eastell and Szulc, 2017).

Sen et al. (2020) and Verschueren et al. (2004) examined the response of WBV on osteocalcin, as a bone formation marker. No group differences were reported by Verschueren et al. (2004) while a decrease was reported by Sen et al. (2020). In the present study, the effects on bone formation were studied using P1NP which was not altered due to vibration training. Elevation in P1NP was observed by Corrie et al. (2015) in a study using a PowerPlate device. However, the age of the participants was considerably higher (79–82 years) than the current study, the training duration was only twelve weeks and focused on both men and women in a small population. It is also important to note that no BMD measurements were taken during this study.

WBV response on bone turnover markers has not been extensively studied, however, the few that have investigated its effects (Russo et al., 2003; Verschueren et al., 2004; Corrie et al., 2015; Kiel et al., 2015) focused mainly on markers of formation and resorption. Even fewer have explored the role of sclerostin in the context of WBV. Sclerostin has emerged over the years as a marker essential to skeletal physiology and homeostasis and is found to be mostly expressed by osteocytes (Poole et al., 2005). It interacts with the Wnt signalling pathway (Baron and Kneissel, 2013) leading to the formation or resorption of bone. Moreover, sclerostin concentrations have been shown to increase or decrease based on the response to mechanical stimuli (Robling et al., 2008) and are also known to increase with age (Amrein et al., 2012). The goal of measuring sclerostin was to evaluate if the signal characteristics in this study could be detected. Since no differences between groups were observed, this further highlights that the effects of combined amplitude stimuli were undetected. However, it does pose the question of whether combined amplitude exposure of longer duration could produce significant group differences.

TABLE 7 Summary of all serum parameters.

	Control					Vibration						
	Contrast	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	**Contrast**	**% change**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**	***p*-value month**
PINP (ug/L)											$p = 0.3254$	$p = 0.509$
Month 6 vs. Baseline	0.23	0.41	1.00	−2.34	2.79	−0.76	−1.36	1.00	−3.80	2.27		
Month 12 vs. Baseline	−0.43	−0.76	1.00	−4.07	3.22	1.05	1.88	1.00	−2.64	4.74		
Month 18 vs. Baseline	−0.61	−1.10	1.00	−5.11	3.88	−2.03	−3.61	1.00	−6.27	2.21		
CTX (pmol/L)											$p = 0.0155$	$p < 0.001$
Month 6 vs. Baseline	223.34	5.39	0.39	−95.26	541.94	169.85	3.92	1.00	−161.59	501.28		
Month 12 vs. Baseline	25.42	0.61	1.00	−326.68	377.52	506.29	11.68	0.00	136.70	875.88		
Month 18 vs. Baseline	304.45	7.35	021	−77.31	686.22	797.43	18.40	0.00	361.07	1233.79		
BAP (ug/L)											$p = 0.3818$	$p < 0.001$
Month 6 vs. Baseline	1.29	10.57	0.00	0.70	1.89	1.21	10.31	0.00	0.53	1.88		
Month 12 vs. Baseline	2.37	19.33	0.00	1.56	3.17	2.51	21.41	0.00	1.71	3.31		
Month 18 vs. Baseline	3.48	28.45	0.00	2.47	4.50	2.87	24.47	0.00	1.95	3.78		
Sclerostin (ng/mL)											$p = 0.0881$	$p = 0.772$
Month 6 vs. Baseline	0.01	1.96	0.89	−0.01	0.04	−0.01	−1.62	1.00	−0.03	0.02		
Month 12 vs. Baseline	0.00	−0.36	1.00	−0.03	0.02	0.00	0.65	1.00	−0.02	0.03		
Month 18 vs. Baseline	0.01	1.11	1.00	−0.02	0.03	0.00	0.73	1.00	−0.02	0.03		

Previous research has also explored age-related responses to WBV (Slatkovska et al., 2011; Marín-Cascales et al., 2018); however, the relevance within the context of high-frequency and combined amplitude training has not been tested. Moreover, since fracture risk increases substantially beyond age 64 (Arlot et al., 1997; Emaus et al., 2006), this breakpoint provides an opportunity to ascertain dose and bone response associated with age, resulting from WBV exposure. These age-related differences were explored using retrospective statistical analysis by incorporating age groups (≤ 64 and > 64) into the fixed effects of the aforementioned statistical model to further examine whether any effects of WBV could be detected (Supplementary Material S8–S11). This was done to elucidate any preventative effects given that WBV is relied upon more as a preventive treatment modality. Despite such attempts, no significant differences between age groups were noted. Both bone and serum parameters demonstrated no significant improvement, and more importantly, parameters including P1NP, which is a marker sensitive to bone formation (Gillett et al., 2021), exhibited no significant improvement resulting from vibration therapy. As a result,

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TABLE 8 Interaction effects table.

	Control		Vibration						
	Contrast	**% change**	**Contrast**	**% change**	**Difference compared to vibration group**	***p*-value**	**95% lower bound**	**95% upper bound**	***p*-value group*month**
DXA Fat mass (g)									$p = 0.0107$
Month 6 vs. Baseline	268.74	1.01	−47.37	−0.19	−316.11	0.573	−894.894	262.665	
Month 12 vs. Baseline	752.91	2.82	−23.16	−0.09	−776.06	0.043	−1535.777	−16.35	
Month 18 vs. Baseline	326.41	1.22	267.62	1.07	−58.79	1.000	−846.76	729.182	
Lean mass (g)									$p = 0.0268$
Month 6 vs. Baseline	501.40	1.33	146.61	0.40	−354.79	0.132	−776.399	66.814	
Month 12 vs. Baseline	837.14	2.21	318.02	0.86	−519.12	0.009	−936.298	−101.941	
Month 18 vs. Baseline	697.33	1.84	415.26	1.13	−282.07	0.390	−728.222	164.085	
Body mass (g)									$p = 0.0015$
Month 6 vs. Baseline	766.60	1.15	98.48	0.15	−668.11	0.121	−1448.131	111.907	
Month 12 vs. Baseline	1577.93	2.37	292.20	0.46	−1285.72	0.003	−2223.718	−347.732	
Month 18 vs. Baseline	1005.83	1.51	674.80	1.05	−331.03	1.000	−1270.346	608.277	
Tibia HR-pQCT Total BMD (mgHA/cm^3)									$p = 0.0063$
Month 6 vs. Baseline	0.64	0.25	−0.46	−0.18	−1.09151	0.272	−2.637	0.454	
Month 12 vs. Baseline	−0.81	−0.31	−0.32	−0.13	0.49332	1.000	−1353	2.34	
Month 18 vs. Baseline	−0.65	−0.25	−1.67	−0.66	−1.02255	0.686	−3.056	1.011	
Trabecular vBMD (mgHA/cm^3)									$p = 0.0159$
Month 6 vs. Baseline	−0.02	−0.01	−0.21	−0.13	−0.18782	1.000	−1.167	0.791	
Month 12 vs. Baseline	−1.37	−0.89	−0.25	−0.16	1.11947	0.052	−0.008	2.247	
Month 18 vs. Baseline	−1.57	−1.02	−0.99	−0.64	0.58315	0.941	−0.803	1.969	
BVTV (%)									$p = 0.0231$
Month 6 vs. Baseline	0.00	−0.02	−0.00011	−0.09	−0.00009	1.000	−0.001	0.001	
Month 12 vs. Baseline	0.00	−0.88	−0.00017	−0.13	0.00096	0.050	0.000	0.002	
Month 18 vs. Baseline	0.00	−1.06	−0.00082	−0.64	0.00054	0.825	−0.001	0.002	
Serum CTX (pmol/L)									$p = 0.0155$
Month 6 vs. Baseline	255.47	6.12	203.73	4.85	−51.74	1.000	−468.701	365.225	
Month 12 vs. Baseline	87.79	2.10	568.60	13.53	480.8	0.039	17.735	943.873	
Month 18 vs. Baseline	397.14	9.51	889.92	21.18	492.78	0.075	−33.247	1018.812	

this further substantiates that WBV has neither osteogenic benefits nor negates fracture risk in this type of physically inactive population regardless of the signal characteristics.

The minimal reductions in fat, lean body and total body mass observed towards the end of the vibration protocol could be linked with a few different possibilities. Firstly, the vibration group was aware that they were undergoing treatment, and the possible impact of this awareness cannot be discounted as an influencing factor. As a result, participants could have been influenced in their dietary habits. Nevertheless, aside from this possibility, the results indicate an interesting effect between groups in that the control group saw more significant weight increases over time than the vibration group. The present study is not the only one to report this effect since the impact of vibration on weight has previously been reported by many (Cristi-Montero, 2013). Several hypotheses exist and involve the role of the sympathetic nervous system (Alavinia et al., 2021), osteocalcin and sclerostin (Wang et al., 2021). A recent pilot study also suggested the role of SMP30 on fat mass,

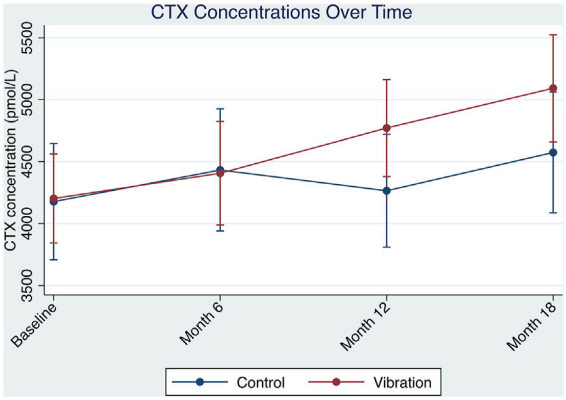

FIGURE 3
Group by month interaction for serum CTX comparing control (n = 98) and vibration (n = 99) groups. Values depicted are estimated marginal means derived from the linear mixed model plotted with 95% CI's. Vibration period (Baseline to Month-12) in the vibration group and regular visits for the control group (Baseline to Month-12). Follow-up visits for both groups (Month-12–Month-18).

given its role in lipid regulation (Pérez-Gómez et al., 2020). Further studies are needed to fully understand the mechanism as it remains largely unknown and poorly understood.

Limitations

To the best of our knowledge, this study was the first to comprehensively investigate the effects of WBV by including an additional 6-month follow-up period to examine bone geometry and microarchitecture, muscle (HR-pQCT distal tibia), serum and DXA with a particular focus on the femoral neck, using high-frequency and combined amplitude training. Despite this, there are several limitations:

1) This study investigated the effects of WBV in a group of Caucasian European postmenopausal women; however, future research will have to explore the effect of menopause on WBV using broader clinical criteria by accounting for the age, stage of menopause, level of exercise and diverse ethnicities.
2) This study was primarily designed to observe the overall effects of WBV on a large physically inactive group and was not specifically tailored to extract age-related responses. However, given the large population, age range, and adherence to the study protocol, a retrospective statistical analysis, showed that 12 months of WBV treatment did not produce any preventative effects specific to the bone.
3) Finally, the exercise profile of all participants at recruitment relied on several questionnaires to account for their physical activity levels. While this provided a fair estimate of both their general and physical activity levels at recruitment, future studies will need to select parameters involving a combination of physiological and questionnaire-based measures to attain a more accurate and comprehensive assessment.
4) In future studies, closer nutritional monitoring between groups along with the use of Magnetic Resonance Imagining (MRI) could help to assess the effects of WBV on weight.

Conclusion

In conclusion, this 18-month non-randomised clinical trial matched participants according to their (MOF) FRAX scores for major osteoporotic fracture and used WBV for 12 months with combined amplitude and high-frequency vibration to improve bone geometry and bone microarchitecture. This study failed to significantly detect an improvement in bone outcomes of physically inactive osteopenic postmenopausal women. Although the 12-month training protocol was well tolerated, DXA BMD measurements of the lumbar spine, femoral neck and hip were not significantly improved compared to control. Moreover HR-pQCT analysis of cortical and trabecular compartments, including finite element analysis did not demonstrate significant improvement resulting from WBV training. Serum markers (P1NP, BAP and sclerostin) also showed no response to mechanical stimuli. A marginal increase in CTX concentrations was observed; however, there was no indication that this could have resulted from vibration exposure. Nevertheless, future studies could expand upon these results by accounting for stratification in age, and stage of menopause to draw further conclusions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by committee of human rights protection, southeast, France (N°0908095). The patients/participants provided their written informed consent to participate in this study.

Author contributions

PF: data collection and analysis, manuscript writing; MP: experiment setup, data collection and organisation; HL: experiment setup, data collection and organisation; MN: statistical review; CB: processing of biological samples; M-HL: participant recruitment and follow-up; HM: participant recruitment and follow-up; TT: conceptualisation of the experiment, experimental setup, participant recruitment and follow-up; LV: conceptualisation of the experiment, experimental setup, manuscript review and editing. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.952140/full#supplementary-material

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Circulating microRNA responses to acute whole-body vibration and resistance exercise in postmenopausal women

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Evaluating alterations in circulating microRNA (c-miRNA) expression may provide deeper insight into the role of exercise in the attenuation of the negative effects of aging on musculoskeletal health. Currently, there are sparse data on c-miRNA responses to acute exercise in postmenopausal women. The purpose of this study was to characterize the effects of acute bouts of resistance exercise and whole-body vibration on expression of selected c-miRNAs in postmenopausal women aged 65-76 years (n=10). We also examined relationships between c-miRNAs and muscle strength and bone characteristics. This randomized crossover design study compared c-miRNA responses to a bout of resistance exercise (RE) (3 sets 10 reps 70% 1 repetition maximum (1RM), 5 exercises) and a bout of whole-body vibration (WBV) (5 sets 1 min bouts 20Hz 3.38mm peak to peak displacement, Vibraflex vibration platform). DXA was used to measure body composition and areal bone mineral density (aBMD) of the total body, AP lumbar spine, and dual proximal femur. pQCT was used to measure tibia bone characteristics (4%, 38%, 66% sites). Blood samples were collected before exercise (Pre), immediately-post (IP), 60 minutes post (60P), 24 hours (24H), and 48 hours (48H) after exercise to measure serum miR-21-5p, -23a-3p, -133a-3p, -148a-3p (qPCR) and TRAP5b (ELISA). There was a significant modality × time interaction for c-miR-21-5p expression ($p=0.019$), which decreased from 60P to 24H after WBV only. TRAP5b serum concentrations significantly increased IP then decreased below Pre at 24H for both WBV and RE ($p<0.01$). Absolute changes in TRAP5b were negatively correlated with c-miR-21-5p fold changes ($r= -0.642$ to -0.724, $p<0.05$) for both exercise modalities. There were significant negative correlations between baseline c-miRNAs and bone status variables ($r= -0.639$ to -0.877, $p<0.05$). Our findings suggest that whole-body vibration is a sufficient mechanical stimulus for altering c-miR-21-5p expression, whereas a high intensity resistance exercise protocol did not elicit any c-miRNA responses in postmenopausal women. Increases in the bone resorption

marker, TRAP5b, were associated with greater downregulation of c-miR-21-5p expression.

KEYWORDS

microRNA, bone markers, aging, muscle mass, bone density

Introduction

Life expectancy worldwide has increased by five years between 2000 and 2016, the fastest increase since the 1960s. Unfortunately, our ability to live longer also has increased the incidence of noncommunicable diseases such as osteoporosis from low bone mass and sarcopenia due to low muscle mass. This increase in disease in the elderly has increased healthcare costs numbering in the billions of dollars (1). The current gold standard for diagnosis of osteoporosis is areal bone mineral density (aBMD) measured by dual energy x-ray absorptiometry (DXA). While DXA has high validity and precision, tracking of disease progression is limited due to the long periods of time between scans necessary to detect changes. To assess the relationship between osteoblast and osteoclast activity, it is possible to measure serum levels of bone turnover markers, though they are not currently accepted for use in diagnosing osteoporosis (2). Recently, blood assessments of microRNAs (miRNA, miR) have been suggested to be valuable markers of bone-related diseases, including osteoporosis (3).

MiRNAs are short (18-25nt), noncoding strands of RNA that influence genes post-transcriptionally. They are typically negative regulators of genetic expression by interfering with or destroying their associated messenger RNA (mRNA) target (4). Multiple RNAs are targetable by a single miRNA, and greater than 60% of human protein-coding genes are affected by them (5). Typically, only a few miRNAs are expressed for a given tissue (6) so the presence of c-miRNAs gives a basis for use as biomarkers for various tissue and physiological systems. There is evidence that deregulated miRNAs affect bone metabolism and have potential as biomarkers for osteoporosis, including miR-21-5p, -23a-3p, -124, -125b-3p, -133a-3p, -148a-3p (7) (Supplementary Table S1). MiR-21-5p promotes bone formation by inhibiting Small Mothers Against Decapentaplegic 7 (SMAD 7), a protein that inhibits osteoblast differentiation *via* the bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β) pathways. However, it also inhibits programmed cell death protein 4 (PDCD4) in osteoclasts, leading to increased osteoclast differentiation. MiR-23a-3p has a negative impact on bone by decreasing osteoblast differentiation. MiR-133a-3p promotes bone resorption by inhibiting the inhibitors of osteoclastogenesis. MiR-148a-3p has doubly negative impacts on bone status by increasing resorption through osteoclastogenesis and decreasing formation by inhibiting osteoblasts (7).

Aging is also associated with gradual loss of muscle mass from loss of muscle fibers and reduction in the cross-sectional area of remaining fibers and reductions in functional strength (8). The loss of muscle mass has implications, especially in the lower limbs, for increasing fall risk. Currently, there are multiple approaches for diagnosing sarcopenia with varying degrees of success in predicting falls (9). Diagnostic tests involve assessment of appendicular lean mass expressed relative to height squared by DXA, typically utilized in conjunction with a functional performance test like gait speed or muscle strength (10). Potential serum markers investigated for sarcopenia include inflammatory cytokines, anabolic hormones, and antioxidants, but these are not muscle-specific and may not reflect the physiology of skeletal muscle (11). MiR-21-5p, -23a-3p, -133a-3p, -148a-3p also have RNA targets in skeletal muscle (Supplementary Table S1). Targeting PDCD4 in fibers, miR-21-5p prevents myoblasts from undergoing apoptosis (12). MiR-23a-3p positively affects muscle by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) which reduces myostatin, a myokine that inhibits cellular growth (13). MiR-133a-3p has binding targets for serum response factor (SRF), which is responsible for muscle proliferation and differentiation (14). MiR-148a-3p positively affects muscle by inhibiting rho associated coiled-coil containing protein kinase 1 (ROCK1), a negative regulator of myoblast differentiation (15).

Though aging is associated with decreased muscle mass and strength (8), and increased bone loss (16), physical activity can attenuate or reverse these negative effects. *In vivo* animal models and cell culture studies have documented that mechanical loading increased bone formation rates (17) and suppressed osteoclast formation (18), and that WBV treatment inhibited osteoclast formation (19, 20). In middle-aged and older humans, chronic resistance exercise increases muscle strength (21) and improves aBMD or attenuates its loss (22, 23). Whole-body vibration (WBV) training also improves lower body muscle strength (24) and attenuates or reverses bone loss (25). Interventions combining resistance exercise and WBV have additive effects on muscle strength gains in postmenopausal women (26).

Future endeavors to reduce deterioration with aging will require a better understanding of human genes, their expression

and regulation (27), and how expression can be favorably manipulated with interventions. From a genetic perspective, characterizing circulating miRNA (c-miRNA) responses to exercise is still in its infancy (28), with few published studies in older populations and none in postmenopausal women or with WBV. Exercise influences physiological processes within various systems and expression changes in c-miRNA may potentially reflect the adaptations occurring within target tissues, though there is currently limited evidence (29). Aging research specifically may benefit from this line of research, as serum sample collection is relatively non-invasive in a population where bone and muscle tissue collection may be difficult (29). With the ability of c-miRNA to be taken up by tissues (30), expression in serum may also be indicative of a functional role in adaptations to exercise. However, the paucity and inconsistent results in recent literature characterizing c-miRNA and exercise responses make their use as exercise biomarkers limited at present (31). More exercise studies, conducted in a variety of populations using various modalities, are needed to fully characterize c-miRNA expression responses to exercise before they can be reliably used as biomarkers (31). Therefore, the primary purposes of this study were to: (1) characterize the effects of acute bouts of resistance exercise (RE) and whole-body vibration (WBV) on expression of selected c-miRNAs in postmenopausal women aged 65-76 years; and (2) determine whether exercise responses of c-miRNA and the bone resorption marker, tartrate-resistant acid phosphatase 5b (TRAP5b) are correlated. Based on their biological effects on bone and muscle tissue, it was hypothesized that c-miRNA (c-miR-21-5p, -23a-3p, -133a-3p, -148a-3p) expression would increase immediately post exercise for both protocols as a result of plasma volume shifts, followed by downregulation at 24 hours and 48 hours post exercise. TRAP5b absolute changes would be positively correlated with c-miR-133a-3p and -148a-3p fold changes and negatively correlated with c-miR-21-5p and -23a-3p fold changes.

Methods

Participants

Fourteen participants were initially enrolled in the study; 4 participants were excluded prior to testing due to voluntary termination (n=2), injury outside of the study (n=1), and an inability to give blood draws (n=1). In total, 10 participants, 65-76 years of age were used in the final analysis. Inclusion criteria were: women 60–85 years of age; independent living; greater than 5 years postmenopausal. Exclusion Criteria were: current smokers; individuals with metabolic disease (e.g., diabetes), cancer, or uncontrolled hypertension; individuals taking medications, other than bisphosphonates, known to affect bone metabolism, such as hormone replacement therapy, antidepressants, or glucocorticoids; individuals who had sustained a fracture within the previous 12 months; and individuals with metal implants or joint replacement at the hip or spine. Participants provided written informed consent and this study was approved by the University of Oklahoma Health Sciences Center (OUHSC) Institutional Review Board (IRB#9569)

Research design

This within-subjects randomized crossover study design for RE and WBV conditions required 9 visits: consenting, blood pressure, and questionnaires (visit 1); bone scans, familiarization of resistance exercise equipment and whole body vibration, and functional performance measures of handgrip strength and jump power (visit 2); 1 repetition maximum (1 RM) testing of leg press, shoulder press, latissimus (lat) pulldown, leg extension, and hip adduction (visit 3); resistance exercise testing (visit 4); whole body vibration testing (visit 7); Visits 4 and 7 were randomized with a minimum of a 10-day washout period between exercise visits. Visits 5, 6, 8, and 9 consisted of blood draws that occurred between 8:00-9:00 a.m. 24 and 48 hours after each exercise visit. Medical clearance was obtained before Visit 2. Study visits are illustrated in Supplementary Figure S1.

An *a priori* power analysis was performed with G*Power 3.1.9.2 software based on the study by Daniels et al. (32) with power set at 0.8, alpha at 0.05 for detecting changes in blood miRNA expression over 5 timepoints. Calculated effect sizes were 0.34 and 0.41, requiring sample sizes of 10 to 12 for 80% power.

Questionnaires

Questionnaires were completed by participants to determine inclusion/exclusion criteria and collect information to reduce the potential confounding influence of physical activity, diet, and menstrual history. An in-house health status questionnaire was used to determine if participants meet study criteria and if they had any preexisting conditions that warranted exclusion and used to record medications taken by the participants. An in-house menstrual history questionnaire provided information about menstrual history and any hormone replacement therapy. Physical activity status was determined by the International Physical Activity Questionnaire (IPAQ) designed to designate low (<600 MET min/week), moderate (≥600 to<3,000 MET min/week), or high (≥3,000 MET min/week) physical activity levels per week (33), and the Bone-Specific Physical Activity Questionnaire (BPAQ) that quantified exposure to bone loading physical activity throughout the lifespan (34). Daily calcium Intake was estimated from a food frequency questionnaire that included supplements (35).

Anthropometric measurements and blood pressure

Height was measured to the nearest 0.5 cm using a wall stadiometer (PAT #290237, Novel Products, Rockton, IL). Weight was measured to the nearest 0.1 kg with a digital electronic scale (BWB-800, Tanita Corporation of America, Inc., Arlington Heights, IL). Participants' resting blood pressure was measured with an automatic blood pressure monitor (Omron, Japan) on the left arm. Participants with values above 140 mmHg systolic or 90 mmHg diastolic pressure were excluded from further study participation.

Dual energy X-ray absorptiometry

The Lunar Prodigy DXA (GE Healthcare, enCORE software, version 16, Madison, MI) was used to measure areal bone mineral density (aBMD) and body composition. The four scan sites were the total body, AP lumbar spine (L1-L4), and dual proximal femur (femoral neck, trochanter, and total hip). Total body scans provided body composition of the whole body and appendicular skeletal muscle mass (ASMM) for potential sarcopenia classification. Participants were instructed to remove jewelry, shoes, and lie supine and centered on the scanning table with the top of their head approximately 2-3 cm below the horizontal white line for the total body scan. Hips and shoulders were adjusted, as necessary, to position the participant evenly in the middle of the scanning field. Straps were used below the knee and at the ankles to maintain leg positioning. For the spine scan, the legs were raised, and a foam block placed underneath so the knees were bent at a 45-to-60-degree angle. The dual femur scan required the foam block and straps to be removed. A brace was placed between the ankles and strapped in place. The left femur was positioned directly parallel with the table, then the right femur. Scans were analyzed with encore software, v16 (GE Healthcare, Madison, WI). Quality Assurance (QA) tests were performed and documented before each scanning day for calibration of the device. *In vivo* precision (RMS CV%) for aBMD for our lab are 1.27% for total body, 1.8% for AP spine, and 1.0 – 1.79% for hip variables. For soft tissue variables, RMS CV% are 1.21% for bone-free lean body mass, 1.74% fat mass, and 2.08% for appendicular skeletal muscle mass. Hydration status was determined with a urine refractometer (VEE GEE CLX-1, Rose Scientific Ltd., Alberta, Canada). Acceptable hydration for body composition determination is a urine specific gravity between 1.004-1.029 (36).

Peripheral quantitative computed tomography

An XCT-3000 bone scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany) was used for the epiphyseal and diaphyseal bone measurements of the non-dominant tibia at the 4%, 38%, and 66% sites. Integrated software v6.00 (Stratec Medizintechnik GmbH, Pforzheim, Germany) was used for analysis. Prior to scanning, non-dominant tibia length (mm) was assessed using a tape measure. Participants were instructed to sit in a chair and cross their non-dominant leg over their dominant knee. Scans were obtained with a 0.4 mm voxel size, 2.2 mm slice, and a 20 mm/s scan speed. At the distal tibia (4%), cont mode 3 at 169 mg/cm3 and peel mode 4 at 650 mg/cm3 with a 10% peel were used to determine total vBMD (mg/cm3), total bone area (mm2), trabecular vBMD (mg/cm3), trabecular area (mm2), and bone strength index (BSI) (mg/mm4). For 38% and 66% tibia, cont mode 2 at 710 mg/cm3 was used to define total vBMD (mg/cm3), total bone area (mm2), cortical vBMD (mg/cm3), cortical area (mm2), cortical thickness (mm), while cont mode 2 at 480 mg/cm3 was used to obtain resistance to torsional deformation polar moment of inertia (Ipolar) (mm4) and torsional polar strength for strength-strain index (pSSI) (mm3). Muscle cross-sectional area (MCSA) (mm2) was also calculated for the 66% tibia site. RMS CV% for all pQCT variables for our lab range from 0.68 – 3.07% for the 4% site, 0.29 – 0.61% for the 38% site, and 0.49 – 1.85% for the 66% site.

Strength testing

Leg press, shoulder press, lat pulldown, leg extension, and hip adduction isotonic machines (Cybex, Medway, MA) were used for this study. Trained personnel were present to instruct participants on appropriate lifting technique. Participants warmed up for 5 min at a self-selected comfortable pace and resistance on a stationary bicycle (828E, Monark, Vansbro, Sweden). The 1RM protocol for each piece of equipment was: (1) proper positioning based on manufacturer recommendations; (2) complete a warmup set of 5-10 repetitions at ~50% of estimated maximal strength; (3) after 1 min rest, another set of 3-5 repetitions at ~75% of estimated maximal strength; (4) After 2 min rest, the load was increased for 1 repetition, with this step repeating, until a maximum was achieved. Maximum strength was achieved within 5 attempts.

Acute exercise protocols

Participants performed 2 upper body and 3 lower body resistance exercises in the following order: leg press, shoulder press, lat pulldown, leg extension, and hip adduction. There were 3 sets of 10 repetitions per exercise at 70-75% of 1RM with 2-3 min of rest between sets and exercises. Higher intensities were chosen based on the safety considerations and effectiveness of improving muscle and bone strength in an elderly population (37). Each repetition consisted of ~1 second each during the

eccentric and concentric phases, with minimal time spent isometrically. One participant failed at 8/10 repetitions on the third set of shoulder press and the weight was reduced by 2.5kg to complete the last two repetitions. All other participants completed every repetition for all sets and exercises at the prescribed load.

The WBV protocol required participants to stand barefoot with knees bent at 30° and their second toe in line with the dot located between positions 1 and 2 on the Vibraflex Vibration Platform (Orthometrix, Inc., Naples, FL). Each of the 5 bouts were performed for one min at a 20 Hz frequency with a 3.38 mm peak-to-peak displacement and a peak acceleration for each vibration bout of approximately 2.7g and 1 min of rest between bouts to restore mechanosensitivity to bone cells (38). Peak acceleration was calculated from the following formula for side-alternating vibration platforms: G-Force= $(A(2\pi f)2)/9.81$ (39). The knee-bent positioning with high amplitude and low frequency on an oscillating platform has been shown through meta-analysis to be the most effective protocol for stimulating bone formation (25). Four participants reported minor side effects from the WBV exposures, including itchiness (n=2), dizziness (n=1), and low back tightness (n=1).

Blood sampling

Blood samples (8.5 ml) were collected *via* venipuncture by a registered phlebotomist. Sampling times are illustrated in Supplementary Figure S2. Baseline samples (Pre) on exercise days were collected at 8:00 am after an 8h overnight fast to measure c-miRNA and TRAP5b, with further sampling immediately (IP), 60 minutes (60P), 24 hours (24H), and 48 hours (48H) post-exercise. Blood draws at 24H and 48H occurred between 8-9:00 am. One participant did not go to the clinic for her 24-hour blood draw, thus n=9 for that timepoint. For the exercise day blood draws, two hematocrit tubes were filled from the serum separator tubes for measurement of hematocrit (HCT) and plasma volume shifts. Percent change in plasma volume (%ΔPV) was determined with the following equation:

$$\%\Delta PV = 100/(100 - HCT\ Pre) * 100((HCT\ Pre - HCT\ Post)/HCT\ Post)$$

(40) and applied to TRAP5b concentrations with the following equation:

$$Corrected\ Concentration = Uncorrected\ value * ((100 + \%\Delta PV)/100$$

Blood lactate was analyzed with the Lactate Plus lactate analyzer (Sport Resource Group Inc., Minneapolis, MN) pre and immediately post-exercise. Blood samples then were allowed to clot for 30 min and centrifuged at 2,000 g for 15 min to obtain serum, which was were equally aliquoted into 8 microtubes and frozen at -84°C until analysis.

TRAP5b assays

MicroVue™ Commercial EIA kits were used to measure TRAP5b (Quidel, Athens, OH) in duplicate. All assays were performed following step by step instructions included with each kit. Intra-assay CV% ranged from 1.3-7.9% and the inter-assay CV% was 7.2%.

MicroRNA quantification

Target miRNAs (miR-21-5p, -23a-3p, -133a-3p, -148a-3p) were selected based on their regulatory functions on bone metabolism and muscle tissue (7, 41) (Supplementary Table S1). MiRNA analyses were performed by TAmiRNA GmbH miRNA quantification service (Vienna, Austria). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) following procedures included in the kit. Standardized methods and quality controls were performed according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiment Guidelines (MIQE) (42) to ensure quality assurance for the miRNA workflow. The reactions were run in duplicate. Serum samples were thawed on ice and centrifuged at 12,000g for 5 min for cellular debris removal. 200µl of serum were used for sample lysis by mixing with 1,000µl QIAzol Lysis Reagent and 1µl of synthetic spike-in, Uni-Spike-In 4 (UniSp4) (Exiqon, Vedbaek, Denmark), to control for variance of cDNA synthesis and qPCR. After incubation at room temperature for 10 min, RNA extraction was performed using 200µl chloroform and phase separation achieved by centrifugation at 12,000g for 15 min at 4°C. 650µl of the upper aqueous phase was transferred to a new collection tube and mixed with 7µl glycogen. Samples were transferred to an miRNeasy mini column, and RNA was precipitated with 750µL of ethanol followed by washing with RPE and RWT buffer. RNA was eluted in 30µL of nuclease-free water and stored at −80°C until further analysis. Detection of hemolysis was performed using the Nanodrop OD414 measurement. Spike-in controls were used for quality control: 1. Uni-Spike-In 4 (UniSp4) to monitor RNA extraction efficiency and assess the overall variability of the assay; and 2. Cel-miR-39-3p was added after RNA isolation to monitor the presence of inhibitors and variability during cDNA synthesis and qPCR. The CV% were 1.62% for the UniSp4 RNA spike-in and 1.32% for the cel-miR-39-3p cDNA spike-in.

cDNA synthesis

The extracted RNA was transcribed to cDNA using the Universal cDNA Synthesis Kit II (Exiqon). The protocol was

modified in that 2μL of total RNA was used per 10 μL reverse transcription (RT) reaction. Polymerase chain reaction amplification was performed in a 96-well plate format using custom Pick-&-Mix plates (Exiqon) in a Roche LC480 II instrument (Roche, Mannheim, Germany) and EXiLENT SYBR Green Master Mix (Exiqon) with the following settings: 95°C for 10 min, 45 cycles of 95°C for 10 seconds and 60°C for 60 seconds, followed by melting curve analysis.

Quantification of miRNA expression

Quantification of expression was determined by assessing the quantification cycle (Cq) of selected c-miRNA utilizing the 2nd derivative method (43). Quality control data for RNA spike-ins are provided in Supplementary Figure S3. Data was normalized to the RNA spike-in control (UniSP4) that was added in before RNA extraction with the equation Cq = Cq (UniSP4) – Cq(miRNA). The 2-ΔΔCt was used to calculated fold changes from pre values (44).

The following formula was used to adjust miRNA expression responses for plasma volume changes with an alteration to the formula to account for exponential expression changes in quantification cycle (Cq) values for miRNA expression with the formula %ΔPV = (log(100)/log(100) – log(HCT Pre)) * log (100)* ((log(HCT Pre) – log(HCT Post))/log(HCT Post)). The correction factor was subtracted from the Cq values for each miRNA from each exercise sample.

Data analyses

Statistical analyses were performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL), version 24. Relative expressions of c-miRNA are reported as mean ± standard error (SE), with other descriptive data reported as mean ± standard deviation (SD). Normality of dependent variables was assessed *via* Shapiro-Wilk tests. For participant characteristics, bone and muscle variables, normality was only violated for current BPAQ scores, IPAQ, and 66% total volumetric bone mineral density. For miRNAs, all pre-exercise log2-tranformed values were normally distributed. The 24H timepoint on the RE day for miR-21-5p was non-normally distributed, as well as WBV miR-133a-3p 48H and RE miR-133a-3p IP; however, the data for each timepoint followed normal straight-line Q-Q plots. Separate Kruskal-Wallis tests were run for separate modalities to see differences between timepoints and Mann-Whitney U tests to compare timepoints to pre-exercise expression. Paired t-tests were performed between pre-exercise WBV and RE for c-miRNAs.

Two-way mixed-model repeated measures ANOVA [modality × time] were used to assess changes across time for c-miRNAs, lactate, and TRAP5b between the two exercise modalities. ANOVAs included modality × time for all five timepoints (Pre, IP, 60P, 24H, 48H), and separate ANOVAs for the 3-exercise day timepoints (pre, IP, 60P) and recovery days (Pre, 24H, 48H) for the c-miRNA. For significant modality × time interactions, one-way ANOVAs across time within each modality with Bonferroni corrections and paired t-tests between modalities at each timepoint were used for *post-hoc* pairwise comparisons. Pre-exercise data was set as the control to calculate fold change of c-miRNA expression for subsequent timepoints (IP, 60P, 24H, 48H). Pearson's correlation coefficients were utilized for normally distributed variables and Spearman's rank rho for the four non-normally distributed variables to determine associations between microRNAs and muscle strength, bone variables, and TRAP5b. MiRNA fold change (FC) effect sizes for differential expression were ≥ 2 for upregulation and ≤ 0.5 for down regulation. Effect sizes for the two-way ANOVA results are reported as partial eta squared (ηp2) with small, medium, and large effect sizes indicated by ηp2 values of 0.0099, 0.0588, and 0.1379, respectively (45). The level of significance was set at $p \leq 0.05$.

Results

Participant characteristics

Participant characteristics and physical performance measures are found in Table 1 and Supplementary Table S2. Calcium intake ranged from 270-1,690 mg/day, with the mean above the recommended 1,000mg/day (46). Additionally, 8 participants were considered highly active according to the IPAQ questionnaire with 2 classified as moderately active (33). According to the European Working Group on Sarcopenia in Older People (EWGSOP) 2018 criteria (ASMM)< 15kg or ASMM/height^2< 5.5kg/m2) (47), 2 participants were sarcopenic based on ASMM alone, but none had ASMM/height^2 values below 5.5kg/m2. Supplementary Table S3 shows the DXA variables for the total body, spine, and dual femur scans. Assessment of bone status by T-scores (48), determined 1 participant was osteoporotic, 7 were osteopenic, and 2 had normal bone status. Supplementary Table S4 depicts pQCT variables for the 4%, 38%, and 66% non-dominant tibia sites, respectively.

Lactate and plasma volume

Table 2 displays exercise responses for plasma volume changes and lactate concentrations for the pre, immediate post (IP), and 60 min post-exercise (60P) timepoints. For lactate there was a significant modality × time interaction ($p<0.001$) and significant main effects for modality ($p=0.01$) and time ($p<0.001$). *Post hoc* comparisons showed an increase in lactate pre to IP for WBV ($p=0.027$) and RE ($p=0.001$) but only RE had higher lactate concentrations from IP to 60P ($p=0.002$). For

TABLE 1 Participant characteristics (n=10) (means ± SD).

Age (yrs)	70.6	±	4.27
Height (cm)	159.6	±	6.19
Body Mass (kg)	64.51	±	12.47
Total Body Fat %	36.93	±	7.44
Total Body Fat Mass (kg)	24.48	±	8.54
Total Body BFLBM (kg)	37.89	±	4.75
Total Body BMC (g)	2068.0	±	187.1
ASMM (kg)	16.23	±	2.66
ASMM/height^2 (kg/m^2)	6.33	±	0.69
Osteoporosis/Osteopenia (%)	10%	/	70%
Calcium Intake (mg/day)	1174	±	446
BPAQ Past	58.3	±	28.1
BPAQ Current	4.2	±	10.3
BPAQ Total	31.3	±	15.1
IPAQ MET-min/week	6296	±	5670

BFLBM, Bone Free Lean Body Mass; BMC, Bone Mineral Content; ASMM, Appendicular Skeletal Muscle Mass; BPAQ, Bone-Specific Physical Activity Questionnaire; IPAQ, International Physical Activity Questionnaire; MET, Metabolic Equivalent.

plasma volume change, there was no significant interaction or modality difference, but there was a significant effect for time with the plasma volume decrease being greater for IP than 60P (p=0.041).

Bone resorption marker

There were no significant modality or modality × time interaction effects, but the time main effect was significant (p<0.001) for serum TRAP5b concentrations (Table 3). Effect sizes were medium for modality (ηp2 0.064) and the modality × time interaction (ηp2 0.117) and large for time (ηp2 0.736). TRAP5b significantly increased from Pre to IP (p=0.048) and decreased Pre to 24H post (p=0.007) for both modalities. Also, 24H post was significantly lower than both IP (p=0.001) and 60P (p=0.003). After correcting for plasma volume shifts, there was still a significant main effect for time (p=0.008) with TRAP5b concentrations decreasing from Pre to 24H post (p=0.007).

There were no significant modality or modality × time interaction effects, but there was a significant time effect (p<0.001) for TRAP5b percent changes from Pre. The effect sizes for modality (ηp2 0.086) and time (ηp2 0.773) were similar to the raw concentration analysis, however, the modality × time interaction effect size was small (ηp2 0.022). TRAP5b percent change was significantly different at 24H compared to IP (p=0.001) and 60P (p=0.001) and after correcting for plasma volume shifts, there was still a significant difference at 24H post compared to IP (p=0.035).

MiRNA responses

In total, 99 samples from the ten participants were analyzed for four miRNAs. MiR-21-5p was expressed in 99/99 samples (mean Cq=25.0). MiR-23a-3p was expressed in 99/99 samples (mean Cq=26.28). MiR-133a-3p was expressed in 98/99 samples (mean Cq=33.77). MiR-148a-3p was expressed in 99/99 samples (mean Cq=29.18).

MiRNA expression and effect sizes for the WBV and RE conditions are shown in Figure 1 and Table 4, respectively. There were no significant differences between WBV and RE for pre-exercise c-miR-21-5p, -23a-3p, -133a-3p, or -148a-3p (all p≥0.599). Based on the 5 time point repeated measures ANOVA, c-miR-21-5p was the only miRNA to show a significant response to the exercise protocols, although the majority of the effect sizes were medium to large for all c-miRNAs. There was a significant modality × time interaction (p=0.019) for c-miR-21-5p, which decreased 60P to 24H (p=0.036) for WBV but not for RE. There were no main effects or interaction effects for any of the miRNAs when analyzed for the 3 exercise time points or comparing the resting miRNA expressions over the 3 days (pre, 24H, 48H). None of the mean fold changes in miRNAs met the criteria for differential expression (≥ 2 for upregulation and ≤ 0.5 for down regulation) (Table 5). Adjusting miRNA expression and fold changes for plasma volume shifts had little effect on the results (Supplementary Tables S6, S7).

MiR-21-5p fold changes were negatively associated with absolute changes in TRAP5b for both exercise protocols (Figures 2A-C). Specifically, miR-21-5p FC IP was negatively

TABLE 2 Lactate and plasma volume changes (means ± SD).

	Time	WBV (n = 10)	RE (n = 10)
Lactate (mmol/L) §†	Pre	0.72 ± 0.23*	0.76 ± 0.32*
	IP	1.11 ± 0.47	2.73 ± 1.47
	60P	0.80 ± 0.42	0.84 ± 0.37*
PVΔ (%)	IP	-3.48 ± 4.6	-8.55 ± 3.89
	60P	-3.57 ± 6.69*	0.19 ± 7.78*

PVΔ, Plasma Volume Change; WBV, whole-body vibration; RE, resistance exercise.
§p ≤ 0.001 significant modality × interaction.
†p ≤ 0.05 modality difference.
*p ≤ 0.05 time difference from IP.

TABLE 3 Serum TRAP5b responses (means ± SD).

	Time	WBV (n = 9)	RE (n = 9)
TRAP5b (μ/L)	Pre	3.68 ± 1.04	3.74 ± 1.02
	IP*†	3.80 ± 0.99	3.98 ± 1.10
	60P†	3.72 ± 1.11	3.86 ± 1.04
	24H*	3.40 ± 0.97	3.60 ± 1.01
Corr TRAP5b (μ/L)	IP	3.73 ± 1.09	3.77 ± 1.01
	60P	3.69 ± 1.16	3.56 ± 1.77
	24H	3.40 ± 0.97	3.60 ± 1.01
% Change vs. Pre	IP†	4.00 ± 9.92	6.70 ± 6.85
	60P†	1.30 ± 5.81	3.56 ± 6.50
	24H	-7.56 ± 5.27	-3.44 ± 4.33
Corr % Change	IP†	0.89 ± 11.22	-0.67 ± 10.19
	60P	-1.67 ± 9.94	-6.70 ± 33.72
	24H	-7.56 ± 5.27	-3.44 ± 4.33

TRAP5, Tartrate-resistant acid phosphatase 5b; WBV, whole-body vibration; RE, resistance exercise; Corr, Corrected for plasma volume change; Pre, pre-exercise; IP, immediately post-exercise; 60P, 60 minutes post-exercise; 24H, 24 hours post-exercise.
*p ≤ 0.05 significant vs. Pre.
†p ≤ 0.05 significant vs. 24H.

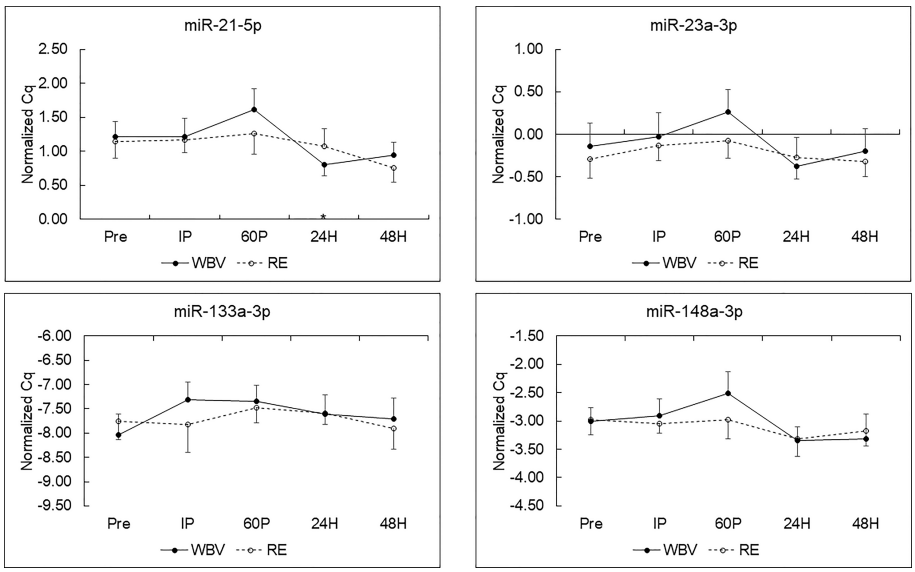

FIGURE 1
C-miRNA responses to resistance exercise (RE) and whole-body vibration (WBV) protocols (means ± SE) in postmenopausal women. *p < 0.05 vs. 60P for WBV; n=9 for miR-21-5p, -23a-p and -148a-3p; n=8 for miR-133a-3p.

correlated with absolute change in TRAP5b 60P (r=-0.642, p=0.045) for WBV, miR-21-5p FC 60P was negatively correlated with absolute change in TRAP5b 60P (r=-0.668, p=0.049) for RE, and miR-21-5p FC at 24H was negatively correlated with absolute change in TRAP5b at 24H (r=-0.724, p=0.027) for RE.

As shown in Supplementary Table S5, there were many significant correlations between baseline miRNA expression values (averaged over both WBV and RE test days) and bone characteristics. TRAP5b was significantly positively associated with miR-133a (r=0.758, p=0.01). Of note, miR-21-5p was significantly negatively correlated with tibia variables (r=-0.658 to -0.877, all p<0.05), miR-23a-3p was significantly correlated with left total hip BMD (r= -0.642, p=0.046) and 38% cortical area (r=-0.639, p=0.047), miR-133a-3p was positively correlated L1-L4 aBMD (r=0.666, p=0.036), and TRAP5b pre (r=0.758,

TABLE 4 Circulating miRNA relative expression two-way repeated measures ANOVA effect sizes.

	Effect Sizes (η_p^2)					
miRNA	**Modality**	**p**	**Time**	**p**	**Modality × Time**	**p**
miR-21-5p	0.047	0.546	0.197	0.125	0.300	0.019
miR-23a-3p	0.090	0.399	0.132	0.325	0.156	0.233
miR-133a-3p	0.174	0.231	0.101	0.379	0.084	0.427
miR-148a-3p	0.043	0.563	0.107	0.397	0.152	0.244

η_p^2 , partial eta squared.

TABLE 5 Circulating miRNA fold changes (vs. Pre).

	Fold Change Means (minimum - maximum)			
miRNA	**IP**	**60P**	**24H[a]**	**48H**
miR-21-5p				
WBV	0.94 (0.59-1.51)	1.31 (0.68-2.53)	0.75 (0.44-1.27)	0.82 (0.45-1.50)
RE	1.03 (0.66-1.60)	1.04 (0.71-1.52)	1.04 (0.60-1.81)	0.70 (0.36-1.37)
miR-23a-3p				
WBV	1.07 (0.65-1.79)	1.32 (0.73-2.36)	0.85 (0.42-1.73)	0.96 (0.43-2.18)
RE	1.12 (0.77-1.61)	1.12 (0.77-1.61)	1.04 (0.70-1.54)	0.90 (0.53-1.52)
miR-133a-3p				
WBV	1.65 (0.80-3.42)	1.60 (0.72-3.53)	1.33 (0.55-3.23)	1.10 (0.67-1.82) [b]
RE	0.95 (0.32-2.81)	1.54 (0.65-3.64)	1.33 (0.52-3.39)	0.79 (0.25-2.47)
miR-148a-3p				
WBV	1.08 (0.64-1.79)	1.42 (0.67-2.98)	0.79 (0.36-1.72)	0.81 (0.31-2.11)
RE	0.95 (0.57-1.61)	1.00 (0.61-1.65)	0.93 (0.46-1.89)	0.87 (0.69-1.96)

[a]n,9 for this time point; [b]n,8 for this time point; WBV , whole-body vibration; RE , resistance exercise; IP , immediately post-exercise; 60P , 60 minutes post-exercise; 24H , 24 hours post-exercise; 48H, 48 hours post-exercise.

p=0.011), and miR-148a-3p was significantly negatively correlated tibia variables (r=-0.635 to -0.821, all p<0.05).

Discussion

To our knowledge, this is the first study to investigate c-miRNA responses to acute bouts of resistance exercise and whole-body vibration in postmenopausal women. Unique findings were that c-miR-21-5p expression decreased 24 hours after WBV and miR-21-5p fold changes were negatively correlated with post-exercise absolute changes in TRAP5b for both exercise modalities. This is also the first known study to attempt to correct c-miRNA expression changes that may be due to plasma volume shifts. Serum TRAP5b concentrations increased immediately post-exercise then decreased below baseline by 24 hours for both WBV and RE, although the increase in TRAP5b immediately post-exercise was accounted for by plasma volume shifts. Generally, baseline c-miRNA expression was negatively associated with measures of bone metabolism but not with muscle variables.

Our findings support that miR-21-5p expression is responsive to mechanical stress imposed on the musculoskeletal system by WBV. Mechanical loading has been shown to change miRNA expression, which in turn, may be an important regulatory mechanism for the exercise-induced adaptations in bone metabolism (49). The lack of miRNA response to RE was unexpected and the underlying reasons for the different responses to WBV and RE are not clear. One possible explanation is that WBV poses a novel stimulus to bone in these physically active women. Also, the type of stresses imposed on the bone (e.g., shear stress, compression, torsional) may differ between WBV and RE. Cell line studies provide evidence that miR-21-5p is sensitive to mechanical loading as it was up-regulated in human periodontal stem cells by a stretch load (50) but down-regulated in MC3T3-E1 cells by fluid shear stress promoting osteogenic differentiation (51). In mice, miR-21-5p deficiency inhibited osteoclast function and bone resorption leading to increased trabecular bone accrual (52). Several clinical studies (53, 54) found that both serum and bone tissue miR-21-5p expression was higher in patients with osteoporotic fractures compared to normal controls. Taken together, these results

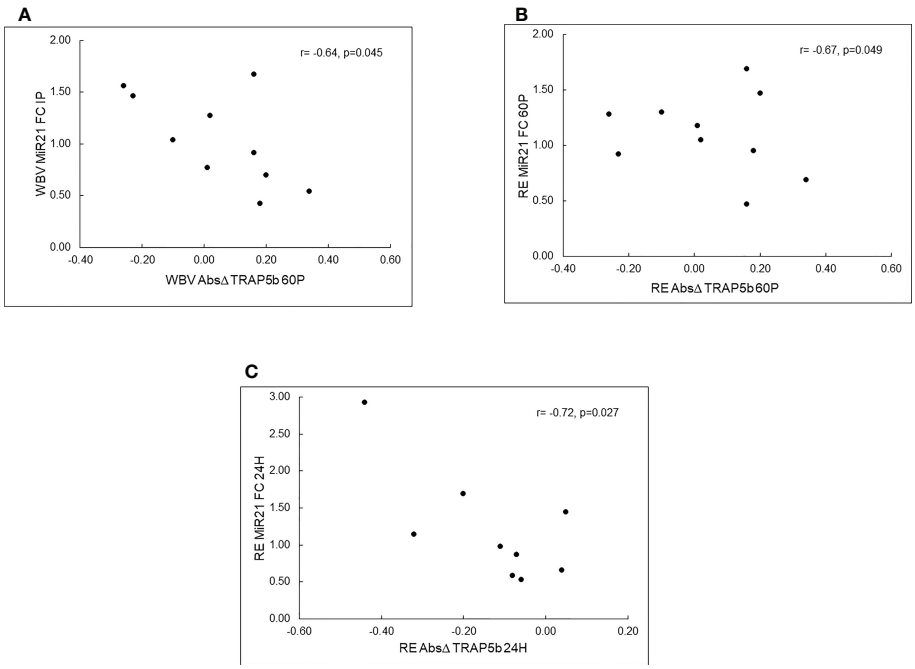

FIGURE 2
Pearson correlations between c-miRNA fold changes (FC) and TRAP5b absolute changes (Δ) for resistance exercise (RE) and whole-body vibration (WBV). Panel **(A)**–WBV c-miR-21-5p FC at IP vs. WBV TRAP5b Absolute Δ at 60P, Panel **(B)** – RE c-miR-21-5P FC at 60P vs. RE TRAP5b Absolute Δ at 60P, Panel **(C)**-RE c-miR-21-5p FC at 24H vs. RE TRAP5b Absolute Δ at 24H; IP - immediately post-exercise; 60P-60 minutes post-exercise; 24 hours post-exercise.

suggest the c-miR-21-5p decrease at 24 hours post-WBV we observed may be a favourable response for enhancing bone mass over time in postmenopausal women.

In addition to effects on bone, the miRNAs we selected have been shown to regulate skeletal muscle development (40, 55). MiR-21-5p, when overexpressed in denervated muscles, leads to enhanced muscle atrophy through targeting of YY1, eIF4E3, and PDCD10 (56). In mice, miR-23a-3p was shown to attenuate skeletal muscle atrophy through targeting of MAFbx/atrogin-1 (57). MiR-133a-3p targets SRF in muscle, leading to increased myocyte proliferation and miR-148a-3p promotes myogenic differentiation (40). Although we detected significant mean changes only for c-miR-21-5p, individual participants had biologically significant differential expression, such as upregulation (≥ 2 FC), while others showed downregulation (≤ 0.5 FC) for a given miRNA. Presently, the mediating factors that would explain these disparate responses are not clear.

Contrary to our hypothesis, the acute bout of resistance exercise did not elicit any changes in c-miRNA expression in our cohort of postmenopausal women. This lack of response contradicts previous reports of altered c-miRNA expression after acute bouts of RE in young men (58, 59). The type of resistance exercise protocol may modulate the c-miRNA responses. Cui et al. (58) compared c-miRNA expression to single bouts of maximal strength (4 sets, 6 reps, 90%1RM), muscular hypertrophy (3 sets, 12 reps, 70% 1RM), and strength-endurance protocols (3 sets, 15-20 reps, 40% 1RM) and found that c-miR-21-5p expression decreased immediately post exercise then returned to baseline levels by 60 minutes post exercise only for the muscular hypertrophy protocol. C-miR-133a-3p expression decreased immediately post both the muscular hypertrophy and maximal strength protocols. Although our resistance exercise protocol was similar to the muscular hypertrophy protocol used by Cui et al. (58), it did not stimulate c-miRNA responses in our postmenopausal women. Age and training status also affect miRNA profiles and responses to exercise. Age-associated declines in miRNA responses to an exercise stimulus may be partially attributed to dysregulation of miRNA expression. Margolis et al. (60) reported different patterns of c-miRNA expression in response to acute high intensity resistance exercise with aging as c-miRNA profiles were downregulated in older men but upregulated in younger men. Regarding training status, skeletal muscle miRNAs have been shown to be differentially expressed in power lifters compared to healthy controls as miR-133a-3p was lower and miR-23a-3p was elevated in powerlifters (61). C-miRNA resting profiles also were differentially expressed based on training status in young men (62) and women (63). The majority of our participants self-reported as being highly active, with 1 reporting exercising with light weights; however, they were not

resistance trained nor had any experience with WBV. Given their older age and physical activity status, the c-miRNA responses of our participants to resistance exercise may have been blunted.

TRAP5b, a marker of osteoclast cell number and bone resorption (64), significantly increased post-exercise then decreased below pre-exercise levels 24 hours later for both exercise modalities. Previously, we reported similar patterns of TRAP5b responses to single bouts of resistance exercise alone or combined with WBV exposures in young adults (65, 66). The TRAP5b decrease 24 hours post exercise suggests that bone resorption was decreased, which is supported by cell culture studies showing that osteoclast formation is inhibited by mechanical loading (18), and WBV treatment (19, 20). We hypothesized that TRAP5b would be positively associated with c-miR-133a-3p and -148a-3p since these miRNAs promote osteoclast differentiation (7). While baseline TRAP5b and c-miR-133a-3p were strongly positively correlated, absolute changes in TRAP5b were negatively correlated with c-miR-21-5p fold changes for both exercise modalities, thus increases in TRAP5b were associated with downregulation of c-miR-21-5p. The potential biological meaning of these relationships is not clear since miR-21-5p promotes both osteoblast and osteoclast differentiation through different target genes and signalling pathways. However, in a recent 30-day bedrest study, we documented that c-miR21-5p was upregulated, and c-miR-21-5p fold changes were positively correlated with serum calcium changes, suggesting that c-miR-21-5p was a useful biomarker for bone resorption in an unloaded condition (67). It is difficult to speculate on the underlying mechanisms for these correlations since we did not measure bone formation markers or target genes in this study.

C-miRNAs have potential to serve as biomarkers for age-related diseases such as osteoporosis and sarcopenia (3, 11, 68). There are several advantages for using c-miRNAs as biomarkers compared to other bone markers; miRNAs are stable in the blood, able to withstand multiple freeze-thaw cycles, and measurement with qPCR is a reliable and well-established method for molecular diagnostics (3). Pre-analytical factors such as stress, drugs, sleep, alcohol, smoking, and diet can affect miRNA expression (69) but can be controlled by the research design. To be useful as biomarkers for osteoporosis, c-miRNAs need to correlate with, and accurately predict, bone status. In a previous study, we reported that resting expression of c-miR-21-5p and -23a-3p were negatively correlated with hip and spine aBMD in elderly postmenopausal women (70). In this study, baseline c-miR-21-5p and -148a-3p were the predominant miRNAs showing significant negative correlations with tibia BMC, bone area, and bone strength variables, suggesting these miRNAs have potential as biomarkers for tibia bone status in postmenopausal women. In contrast, c-miR-23a-3p was negatively correlated only with total hip aBMD and tibia cortical area, and c-miR-133a-3p was positively correlated with TRAP5b and spine aBMD. Circulating miRNAs, including miR-133a-3p, also have shown promise as biomarkers for the muscle-related disease, Duchenne Muscular Dystrophy (71), and may have further utility for detecting sarcopenia. We did not find any significant correlations between the selected c-miRNAs and muscle variables. In contrast, Halper et al. (72) found that miR-21-5p had a low positive correlation with handgrip strength in 90 elderly women 65-92 years. The study mainly differed from the current by measuring absolute instead of relative expression and having an older mean population.

There are limitations to this study. The participants were mostly very physically active according to their IPAQ scores and may not reflect responses for sedentary or moderately active elderly postmenopausal women. This cohort was also fairly homogeneous in terms of bone and muscle status, with most of the participants meeting the T-score criteria for osteopenia or osteoporosis (48), and only two participants being classified as sarcopenic according to EWGSOP guidelines (47). We did not include a control day in this study, which would be helpful for determining circadian rhythms in the c-miRNAs (73). However, we did control for time of day and diet as all exercise tests were conducted in the morning (8:00-10:00 am) in a fasted condition, and the 24- and 48-hour blood draws were obtained at the same time of the morning as the exercise days. Another limitation is the miRNA assessment used. Some studies choose to include a discovery step to determine which miRNAs are expressed in their study population. This step is then repeated in a separate cohort to valid the miRNAs expressed. Due to the high cost of this approach, we instead chose to only target specific miRNAs that have been consistently and more frequently expressed in the literature and are involved in the regulation of bone metabolism. Normalization of miRNAs was limited to a single spike-in RNA control; while this method is acceptable for MIQE guidelines (42), there are assumptions during RNA isolation and cDNA synthesis that may have been violated (74). Inclusion of a larger number of miRNAs or measurement of endogenous controls would have provided the ability to use global mean normalization or geometric mean (74) and may reduce variability in relative expression.

Conclusions

Our findings suggest that whole-body vibration is a sufficient mechanical stimulus for altering c-miR-21-5p expression, whereas a high intensity resistance exercise protocol did not elicit significant c-miRNA responses in postmenopausal women. C-miRNAs may also serve as biomarkers to track the progression of adaptations from exercise stimuli, though more studies are needed to determine which miRNAs are sensitive enough to express changes and have targets affecting tissues of interest (28, 31). The few exercise studies with miRNA analysis in older populations have focused on men, therefore, more research is warranted focusing on the c-miRNA responses in postmenopausal women.

Data availability statement

The data used to support the findings of this study are restricted by the University of Oklahoma IRB 709 in order to protect participant privacy. Data may be available in aggregate form from the corresponding author upon request.

Ethics statement

The studies involving human participants were reviewed and approved by University of Oklahoma Health Sciences Center Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SB wrote the first draft of the manuscript, contributed to the conception and design of the study, conducted the data collection, performed the bone marker assays, and performed the data analyses for all dependent variables. RM revised the manuscript and assisted with data collection and data analyses. MN revised the manuscript and assisted with data collection and the bone marker assays. CB, JMK, and MB all revised the manuscript and contributed to the conception, design, and data analyses of the study. DB revised the manuscript, contributed to the conception and design of the study, supervised the data collection and the bone marker assays and contributed to the data analyses. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1038371/full#supplementary-material

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Glossary

ASMM	appendicular skeletal muscle mass
aBMD	Areal bone mineral density
BMP	Bone Morphogenetic Protein
BMPR/BMPR2	BMP receptor/type II
BPAQ	Bone-specific Physical Activity Questionnaire
BSI	bone strength index
cmiRNA	circulating microRNA
CTX-I	C-telopeptide of Type I collagen cross-links
CXCL11 C-X-C	Motif Chemokine Ligand 11
DXA	dual energy x-ray absorptiometry
eIF4E3	Eukaryotic translation initiation factor 4E family member 3
FASL	Fas Ligand
FC	fold change
HCT	haematocrit
IPAQ	International Physical Activity Questionnaire
KDM6B	Lysine Demethylase 6B
MAPK	Mitogen-Activated Protein Kinase
miRNA	microRNA
c-miRNA	circulating miRNA
KDM6B	Lysine Demethylase 6B
MAFB	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B
MAFbx	Muscle Atrophy F-box
MCSA	Muscle cross-sectional area
MMP13	Matrix Metalloproteinase 13
mRNA	messenger RNA
MuRF1	Muscle RING Finger 1
Nt	nucleotides
PDCD4	Programmed Cell Death Protein 4
PDCD10	Programmed Cell Death Protein 10
pQCT	peripheral quantitative computed tomography
pSSI	strength-strain index
% PVΔ	percent plasma volume change;
RE	resistance exercise
1 RM	One repetition maximum
RMS CV%	root mean square coefficient of variation percent
RNA	Ribonucleic Acid
ROCK1	rho associated coiled-coil containing protein kinase1
RUNX2	Runt-Related Transcription Factor 2
SMAD1	Small Mothers Against Decapentaplegic 1
SMAD3	Small Mothers Against Decapentaplegic 3
SMAD7	Small Mothers Against Decapentaplegic 7
SRF	serum response factor
TGF-b	Transforming Growth Factor-Beta
TRAP5b	Tartrate-resistant acid phosphatase 5b
vBMD	volumetric bone mineral density
WBV	whole-body vibration
Wnt	Wingless-Type MMTV Integration Site Family
YY1	Yin and Yang 1

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Moderate exercise protects against joint disease in a murine model of osteoarthritis

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Exercise is recommended as a non-pharmacological therapy for osteoarthritis (OA). Various exercise regimes, with differing intensities and duration, have been used in a range of OA rodent models. These studies show gentle or moderate exercise reduces the severity of OA parameters while high intensity load bearing exercise is detrimental. However, these studies were largely conducted in rats or in mouse models induced by severe injury, age or obesity, whilst destabilization of the medial meniscus (DMM) in mice has become a widely accepted model due to its lower variability, moderate progression and timescale. The present study was undertaken to provide insight into the effect of moderate exercise on early joint pathology in the DMM mouse model. Exercise was induced a week after induction by forced wheel walking for three or 7 weeks. Joints were analyzed by microcomputed tomography and histology. Assessment of skeletal parameters revealed that exercise offered protection against cartilage damage after 7 weeks of exercise, and a temporary protection against osteosclerosis was displayed after 3 weeks of exercise. Furthermore, exercise modified the metaphyseal trabecular microarchitecture of the osteoarthritic leg in both time points examined. Collectively, our findings corroborate previous studies showing that exercise has an important effect on bone in OA, which subsequently, at 8 weeks post-induction, translates into less cartilage damage. Thus, providing an exercise protocol in a surgical mouse model of OA, which can be used in the future to further dissect the mechanisms by which moderate exercise ameliorates OA.

KEYWORDS

exercise, osteoarthritis, bone, cartilage, destabilisation of medial meniscus model, subchondral bone

Introduction

Osteoarthritis (OA) affects ~80% of people aged over 50. It is characterized by structural and functional changes in articular joints, with concomitant pain and loss of joint mobility that significantly impairs quality-of-life. To delay rapid progression of OA, international guidelines recommend therapeutic exercise (Fernandes et al., 2013; McAlindon et al., 2014; Bannuru et al., 2019). Numerous studies have shown that exercise regimes, especially aerobic and strengthening, when monitored closely and performed regularly, lead to an improvement in joint movement, physical activity and pain (Fransen et al., 2015; Barton et al., 2021; Raposo et al., 2021). Exercise also induces weight loss, reduces inflammation (Messier et al., 2013; Onu et al., 2021) and has an important positive psychological impact in humans (Hurley et al., 2018; Wang and Ashokan, 2021). Whilst clinical studies consistently support exercise as a possible treatment for OA, there is a lack of understanding of how exactly exercise improves the joint.

To better understand the effects of exercise in the osteoarthritic joint, the last decade has seen an increase in studies of exercise on rodent OA *in vivo* models. The exercise regimes and the models of OA induction are varied (Table 1). In male rats, Iijima and others surgically induced OA *via* the destabilization of the medial meniscus (DMM) (Glasson et al., 2007), which in rodents results in progressive development of OA with cartilage damage, osteosclerosis, variable synovitis, ligament damage/calcification and osteophyte formation (Glasson et al., 2007; Jackson et al., 2014; Huesa et al., 2016). Studies utilizing DMM induction of OA on rats followed by treadmill exercise showed that 1) gentle treadmill walking prevented OA changes specially subchondral bone growth (Iijima et al., 2015), 2) longer rest before starting exercise was more beneficial (Iijima et al., 2016) and finally 3) that intense treadmill running is more detrimental to the joint (Iijima et al., 2017). Forced mobilization on a rotating cylinder in a rat transection of the anterior cruciate ligament (ACL-T) model induced increased cartilage degradation, subchondral plate failure and earlier subchondral bone sclerosis, suggesting that repetitive load-bearing exercise is detrimental (Appleton et al., 2007). However, a more severe model of OA on rats (ACL-T and DMM together) showed moderate to reduce progression of OA and this improvement was enhanced by reducing body weight load to 60%. In mice, exercise has also been explored, where OA was induced by high fat diet (Griffin et al., 2012; Hahn et al., 2021), age (Lapveteläinen et al., 1995), ACL rupture (Hsia et al., 2021), spaceflight/limb unloading (Kwok et al., 2021), ACL-T (Oka et al., 2021) or DMM exacerbated by restricted movement (Kim et al., 2013). Similar to the rat model, high intensity exercise resulted in aggravated OA whilst moderate treadmill or voluntary wheel exercise improved OA parameters in the joint.

One commonality of these mouse models is that the induction of OA is either very long or more severe than the standard, more commonly used, DMM. DMM on mice offers a widely accepted model due to its moderate progression, reproducibility, and timescale, better reflecting the course of a large proportion of human post-traumatic OA cases, as well as offering the availability of transgenics. In this study, we investigated the effects of moderate forced exercise in the mouse DMM model. To do so, we generated an exercise protocol that allows for a period of recovery after injury/induction before a type of exercise that mimics long daily walks. We then assessed joint osteoarthritic structural changes, such as cartilage damage, inflammation, and bone microstructure. In this study we sought to establish and characterize an exercise model of OA in mice that would facilitate investigation of the mechanisms underpinning the amelioration of OA by moderate exercise.

Methods

Animals, induction of OA and exercise

DMM (Glasson et al., 2007) was performed on 10-week-old male C57BL/6 mice weighing on average 26.0 ± 1.4 g. A total of 42 mice were purchased (Envigo, United Kingdom) and placed in plastic cages with sawdust bedding (4–five animals per cage) in a 12-h light/dark cycle at constant temperature. Animals were monitored daily, allowed to move freely in cages and provided free access to food, water and environmental enrichment. A week before surgery, all mice were tested for a few minutes on regulated rotating wheels (Campden Instruments Ltd. Loughborough) and those capable of using the wheels were selected for the exercise group. Exercise was set for 850m/day at a speed of 3.8 m/min, with 18s break every 4min. The total distance was divided in two sessions with a 2–3 h break in between. At surgery animals were given analgesics (Buprenorphine, 0.1 mg/kg). Exercise commenced 1-week post-surgery and continued 5 days/week for three or 7 weeks. Experimental groups are indicated in Supplementary Table S1. At endpoint, blood and tissues were collected. Legs were harvested for assessment *via* microcomputed tomography (μCT) and histology. Subcutaneous, gonadal, and brown fat pads, together with quadriceps and soleus muscles, were dissected and weighed (wet weight). The analysis was conducted blind; groups were only revealed at the end of all analysis. All procedures were in accordance with Home Office regulations and experimental design was pre-approved by the Ethical Review Committee at the University of Glasgow. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

TABLE 1 Published exercise and load studies on rodent models of osteoarthritis, summarizing the experimental set up and intensity of exercise, loading or unloading protocols and the effects these had in the joint.

Authors	Year of publication	Model	OA induction	Type of exercise	Speed	Duration	Result
Appleton et al.	2007	Male Rat	ACL-T and partial meniscectomy	Rotating cylinder	4 rpm	30 min 3 days/wk up to 20 weeks	Forced mobility accelerates OA onset and severity
Hao et al.	2021	Male rat	ACL-T + DMM	Treadmill running vs. Treadmill running with 60% body weight	15 m/min	30 min/d, 5 days/wk for 4 weeks	Exercise reduced progression of OA and this improvement was enhanced by reducing body weight load to 60%
Iijima et al.	2015	Male rat	DMM	Gentle treadmill walking	6 m/min	From day 2 for 1, 2 and 4 weeks 30 min 5 days/wk	Showed prevention of OA changes, especially in the subchondral bone
Iijima et al.	2016	Male rat	DMM	Moderate treadmill walking	12 m/min	from day 2 for 4 weeks, from week 4 through 8 weeks, or from day 2 through 8 weeks 30 min 5 days/wk	Longer rest from induction showed better prevention from OA changes
Iijima et al.	2017	Male rat	DMM	Moderate vs. high speed treadmill	12 or 24 m/min	From 4 weeks to 56 weeks 30 min 5 days/wk	Intense treadmill exercise is detrimental to the OA joint
Griffin et al.	2012	Male mice	High fat diet	Voluntary running wheel	N/A	from 20 to 24 weeks of age	Exercise reduced progression of OA.
Hahn et al.	2021	Male mice	High fat diet	Voluntary running wheel	N/A	from week 26–52	No improvement to articular cartilage and synovial fluid metabolite links to subchondral bone structure
Hsia et al.	2021	Female mice	Non-invasive ACL rupture	Hind limb unloading		One week after induction then resume normal activity up to 4 weeks	Less osteophyte formation in unloaded group
Kim et al.	2013	Male and female mice	DMM + restricted cage	Treadmill running	15 m/min	6 days/wk	Increased severity of OA lesions in exercise group
Kwok et al.	2021	Male mice	space flight or limb unloading	35 days of space flight or hind limb unloading followed by exercise 1) climbing or 2) treadmill	10.2 m/min	3 days/wk up to day 80 after unloading or space flight	Either spaceflight or unloading generated cartilage and menisci degradation. Exercise reduced cartilage degradation and improved thickness
Lapvetelainen et al.	1995	Male mice	Not induced	Treadmill running	13.3 m/min	From 2 months old up to 6, 10, 14 and 18 months old. 75 min 5 days/wk	Running increased the incidence and severity of OA.
Oka et al.	2021	Male mice	ACL-T	High speed treadmill	18 m/min	30 min 3 days/wk for 4 weeks	Deterioration of the cartilage due to exercise

Microcomputed tomography

Knees were fixed (4% paraformaldehyde) for 24 h and stored (70% ethanol). Joints were analyzed by μCT using the Skyscan 1272 (Bruker, Belgium; 0.5 aluminium filter, 50kV, 200μA, voxel size 4.57 μm, 0.5° rotation angle). Scans were reconstructed in NRecon software (Bruker, Belgium), with stacks analyzed: 1) osteophytes identified in three-dimensional reconstructions and volume measure by selecting a region of interest (ROI) in 2D stacks as previously described (Huesa et al., 2016) and 2) subchondral bone within the tibial epiphysis was selected (from the growth plate to subchondral plate) in a volume of interest (VOI) under the increased loading area (Das Neves Borges et al., 2014). 3) Tibial metaphyseal trabecular bone was analyzed in a stack of 200 slices taken ~230 μm from the lower end of the growth plate.

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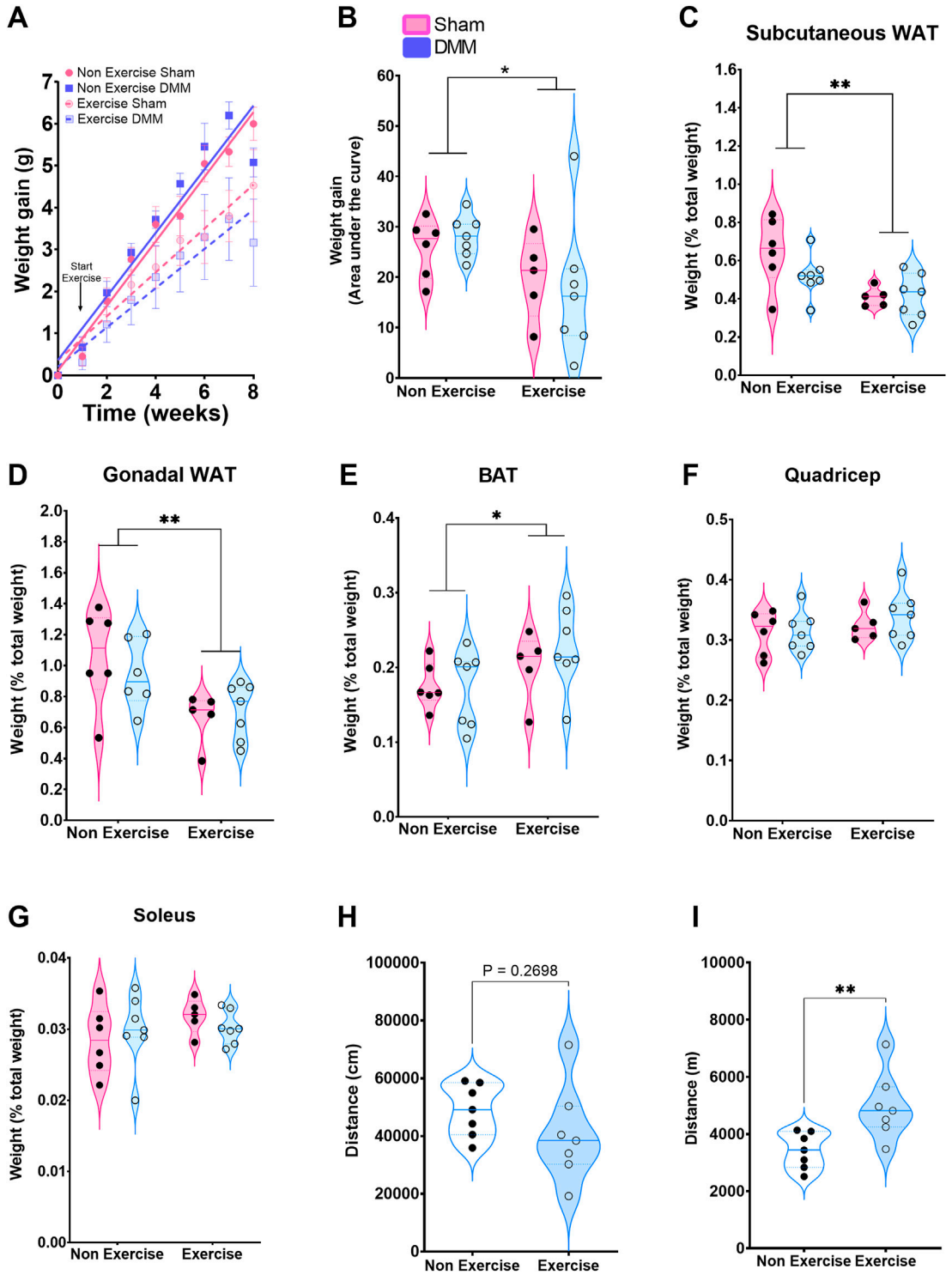

FIGURE 1
Overall effect of the exercise protocol on weight expressed as weight gain mean ± SEM **(A)** and area under the curve **(B)**. Subcutaneous **(C)**, gonadal **(D)** and brown **(E)** adipose tissue weight expressed as a percentage of total weight. Quadricep **(F)** and soleus **(G)** muscle weight as a percentage of total body weight. **(H)** Overnight distance travelled in DMM mice. **(I)** Calculated weekly distance based on overnight plus forced exercised distance. Arrow in **(A)** indicates start of exercise protocol. Weight gain was analyzed by mixed-effects model with time, surgery and exercise as factors. Time and exercise were significant ($p < 0.0001$). AuC, fat pad and muscle weight were analyzed with a 2-way ANOVA with Bonferroni correction. Movement in DMM groups was analyzed by Standard student t-test. *$p < 0.05$, **$p < 0.01$.

Histology and scoring

After μCT, joints were decalcified (Formical 2000; Decal Chemical, United States) overnight, embedded in paraffin wax and coronal sections (5 μm) cut, and stained with haematoxylin and Safranin-O/Fast-Green. Using a validated scoring system (Glasson et al., 2010) ranging from 0 (normal) to 6 (>80% loss of cartilage), the tibial quadrant in 8–10 sections from each mouse was graded by two scorers blinded to the specimens, with scores averaged. There was good agreement between scorers; intraclass correlation coefficient of 0.9 (95% CI 0.82–0.95), mean difference in score being 0.12 (95% CI 0.19–0.33). Synovitis was assessed using a validated scoring system (Jackson et al., 2014). This was modified to focus on pannus formation, synovial membrane thickening and sub-synovial hyperplasia. There was agreement between scorers; intraclass correlation coefficient of 0.88 (95% CI 0.79–0.94), mean difference in score of 0.002 (95% CI −0.07 to 0.35).

Nocturnal activity

Nocturnal activity was measured by placing a mouse in an activity cage (Activmeter, Bioseb, France). Activity monitoring was conducted in the last 2 weeks of the 8-week protocol. Cage activity measurements represent averaged total movements throughout 16 h of recording.

Statistics

Data were tested for normality with a Shapiro-Wilk test (GraphPad Prism, v9.4.1) and presented as mean ± standard deviation or showing each data point highlighting the mean/median. Differences were statistically analyzed by *t*-test or two-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Non-normal distribution or datasets too small to test for normality were compared by non-parametric tests such as Mann-Whitney test for un-paired data and Wilcoxson for paired data. Data is available upon request.

Results

Moderate exercise shows signs of physiological changes

Exercise induced a reduction in weight gain regardless of the type of surgery (DMM/Sham, two-way ANOVA, p = 0.006 Exercise vs. Non exercise), which was evident 5 and 6 weeks after initiation of exercise (Figure 1A&B). This was reflected in the loss of white adipose tissue (WAT), measured as percentage tissue weight to total body weight (Figure 1C&D). Subcutaneous and gonadal WAT were significantly lower in the exercise group 8-week post-DMM surgery, whilst inter-scapular brown adipose tissue (iBAT, Figure 1E) was significantly higher in the exercise group, regardless of surgical intervention. No changes in muscle mass were noted (Figure 1F&G). Recognizing that forced exercise might induce changes in the overall activity, we measured overnight activity in the DMM group comparing exercised to non-exercised (n = 6 per group). There was no significant difference in nocturnal distance travelled between the groups, and therefore forced exercise did not have a meaningful impact on the total amount of voluntary exercise/activity undertaken (Figure 1H). To calculate the weekly distance travelled we added 7 times the voluntary distance travelled to 5 times the calculated distance of the forced exercise (Figure 1I). This resulted in an increased mean weekly distance travelled (1.5 times higher) within the exercise group. Thus, exercise increased physical activity by 50%. We did not observe significant changes in pain behaviors as measured by dynamic weight bearing (Supplementary Figure S1).

Exercise reduces subchondral bone osteosclerosis at 4 weeks

Moderate exercise did not lead to any significant histological changes in articular cartilage damage (Figures 2A,B) or synovitis (Figure 2C) at the early 4-week time point. The number of osteophytes, measured as protruding bone formation on the medial side of the subchondral bone, was also not statistically significant at 4-week (Figure 2D). Despite this, 70% of exercise samples had two or more osteophytes whilst only one sample out of 7 (14%) in the non-exercise group had two or more osteophytes. A Fisher exact test where data was separated into two groups, 1) one osteophyte or less and 2) two osteophytes or more, indicated the exercise group was significantly different from the non-exercise group (p = 0.0498). This indicates increased osteophyte formation during the initial phase of the model, when the subchondral bone is adapting to the new loading resulting from the destabilization. This increase in osteophyte formation may be indicative of faster subchondral bone expansion, yet we found no changes in subchondral osteophyte volume at this time point (Figure 2E). Subchondral osteosclerosis, measured as the ratio-metric comparison of subchondral bone % BV/TV in the medial tibial compartment of the knee and the contralateral leg (SC % BV/TV, Figure 2F and Table 2), was evident in all DMM groups. Yet, osteosclerosis was significantly reduced in exercised mice (Table 2; Figure 2G). DMM also induced changes to metaphyseal trabecular bone, but only in the exercise group where the ipsilateral leg had less trabecular bone, due to a decrease in the number of trabeculae, which were also more plate-like (structural model index, SMI, Table 2).

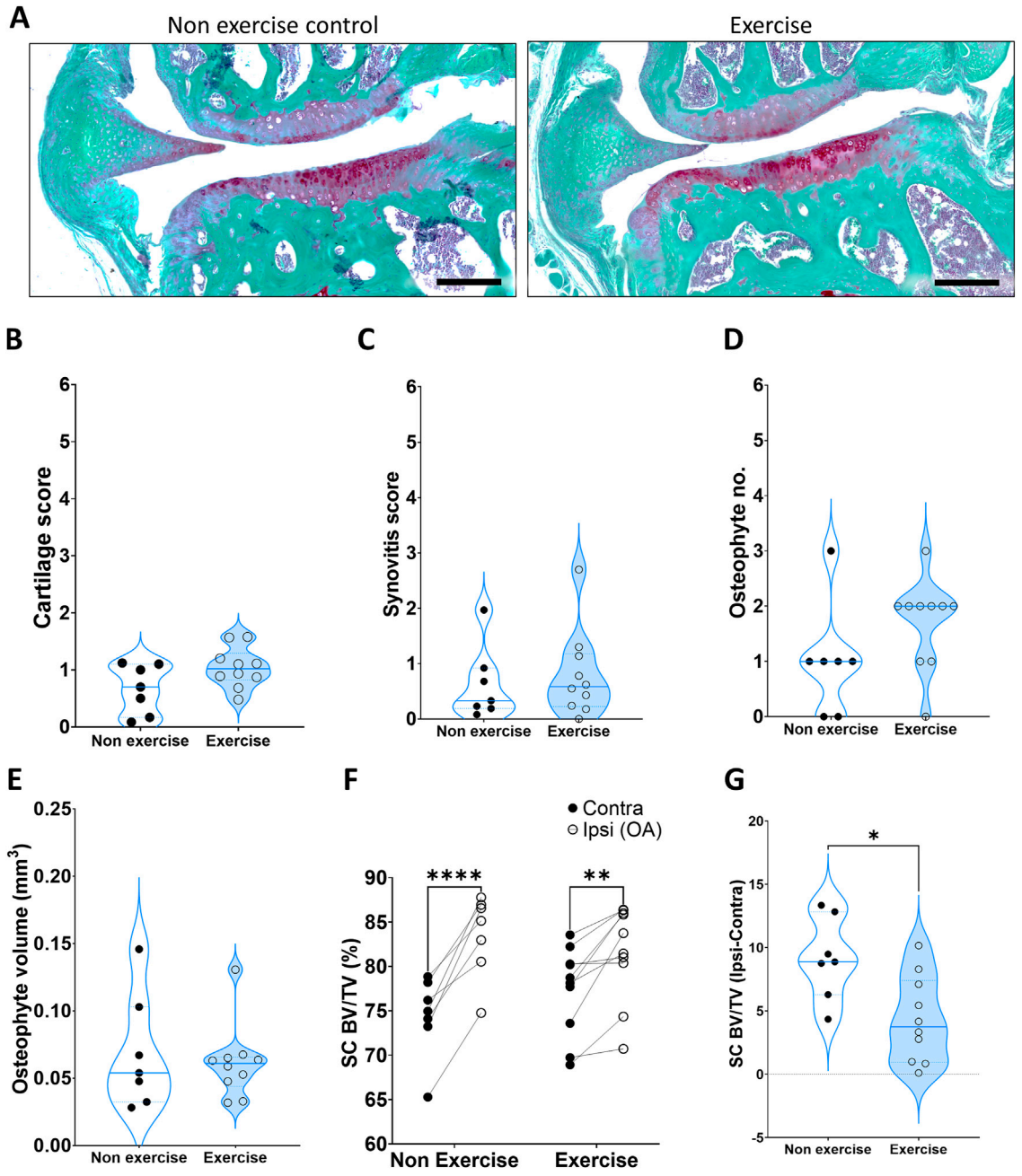

FIGURE 2
Disease parameters on mice 4 weeks after induction of OA. **(A)** Representative images of the DMM joint at 4 weeks, stained with SafraninO for cartilage and Fast Green for bone. **(B)** Cartilage score. **(C)** Synovitis score. **(D)** Osteophyte number. **(E)** Osteophyte volume. **(F)** Comparison of medial subchondral bone compartment density (% BV/TV) between the operated ipsilateral (Ipsi) and control contralateral (Contra) legs. **(G)** Change in tibial subchondral bone sclerosis (Ipsi–Contra). Comparison between exercise and non-exercise groups was done with a t-test unless data was not normally distributed, in which case it was then compared by a Mann-Whitney test. Paired comparisons were conducted via a paired t-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 2 MicroCT analysis of trabecular and subchondral bone changes in the DMM groups. BV/TV = Bone volume/Tissue volume. Tb.Th = trabecular thickness. Tb.No = Trabecular number. Tb.Sp. = Trabecular space. Tb.Pf = Trabecular pattern factor (connectivity). SMI = Structural model index (shape). DA = Degree of anisotropy (organization). SC = Subchondral. Bone scl = bone osteosclerosis. Each time point was analyzed with a Two-Way ANOVA, comparing relative changes to the contralateral leg and also interrogating the effect of exercise. Data was also compared between exercise and non-exercise within the DMM joint normalized against the contralateral leg (NE vs. E). *p* values under 0.05 were considered significant.

4 weeks	**Non exercise**							**Exercise**						**p**
	Contralateral			**Ipsilateral**			**p**	**Contralateral**			**Ipsilateral**		**p**	**Ne vs. E**
BV/TV (%)	**12.3**	**±**	**0.84**	**13.2**	**±**	**0.71**	**0.155**	**13.2**	**±**	**0.71**	**12.9**	**±**	**0.77**	**0.045**	**0.937**
Tb.Th (μm)	12.0	±	0.38	12.1	±	0.3	0.661	12.1	±	0.3	11.9	±	0.35	0.940	0.644
Tb.Sp (μm)	55.4	±	3.39	52.9	±	2.47	0.226	52.9	±	2.47	53.9	±	1.93	0.451	0.437
Tb.N (μm−1)	0.010	±	0.00051	0.011	±	0.00054	0.076	0.010	±	5 × 10-4	0.011	±	0.00045	0.026	0.894
Tb.Pf (μm−1)	0.097	±	0.00745	0.093	±	0.0049	0.392	0.093	±	0.005	0.093	±	0.0071	0.055	0.542
SMI	1.97	±	0.0663	1.94	±	0.0548	0.568	1.94	±	0.055	1.88	±	0.0584	0.008	0.113
DA	2.60	±	0.0937	2.76	±	0.778	0.292	2.76	±	0.778	2.65	±	0.0494	0.093	0.961
SC bone scl (%)	74.4	±	1.71	83.5	±	1.74	3 × 10-4	77.3	±	1.58	81.6	±	1.70	0.003	0.010
SC Tb.Th (μm)	28.0	±	1.23	33.1	±	1.14	0.003	28.7	±	1.24	34.5	±	1.58	5 × 10-6	0.536

8 weeks	**Non exercise**							**Exercise**						**p**
	Contralateral			**Ipsilateral**			**p**	**Contralateral**			**Ipsilateral**		**p**	**NE vs. E**
BV/TV (%)	**18.0**	**±**	**0.838**	**18.1**	**±**	**0.724**	**0.820**	**15.3**	**±**	**1.36**	**15.69**	**±**	**1.45**	**0.338**	**0.786**
Tb.Th (μm)	56.7	±	1.434	56.5	±	1.587	0.804	54.7	±	1.75	55.21	±	2.16	0.632	0.582
Tb.Sp (μm)	199.2	±	7.969	196.0	±	4.177	0.582	209	±	7.86	215	±	7.54	0.219	0.226
Tb.N (μm−1)	0.0032	±	0.00016	0.0032	±	0.00012	0.780	0.0028	±	2 × 10-4	0.0028	±	0.00021	0.362	0.946
Tb.Pf (μm−1)	0.0164	±	0.00073	0.0158	±	0.00067	0.480	0.0201	±	0.002	0.019	±	0.002	0.039	0.409
SMI	1.70	±	0.06386	1.64	±	0.047	0.328	1.8903	±	0.111	1.79	±	0.12	0.017	0.635
DA	2.22	±	0.05572	2.30	±	0.039	0.359	2.17	±	0.094	2.30	±	0.10	0.017	0.552
SC bone scl (%)	84.3	±	1.16	91.7	±	1.04	2 × 10-2	78.6	±	2.97	85.7	±	2.84	0.001	0.617
SC Tb.Th (μm)	30.2	±	1.05	38.4	±	1.24	2 × 10-5	28.6	±	1.65	33.84	±	1.80	0.002	0.038

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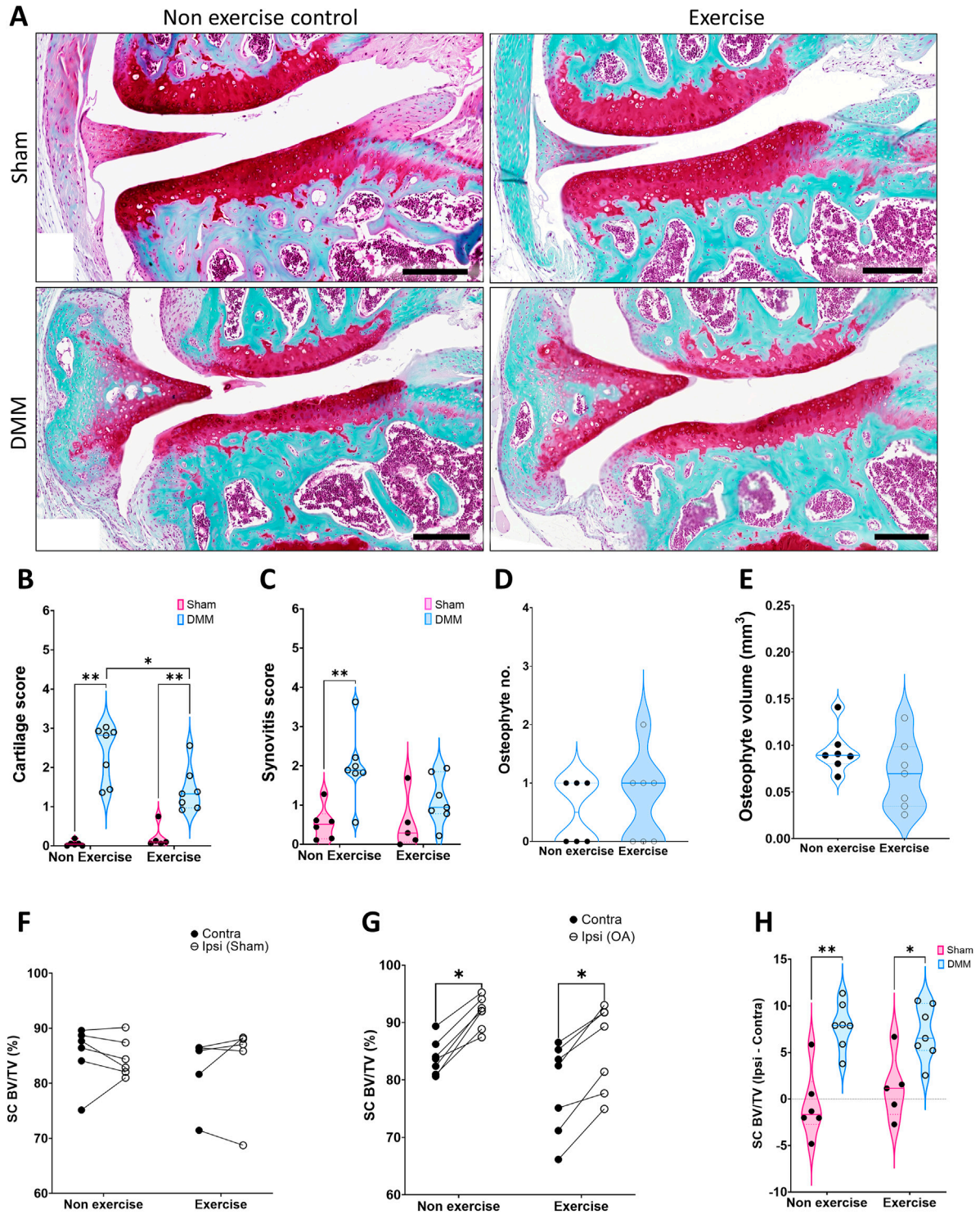

FIGURE 3
Disease parameters on mice 8 weeks after induction of OA. **(A)** Representative images of the joint at 8 weeks, stained with SafraninO for cartilage and Fast Green for bone. **(B)** Cartilage score. **(C)** Synovitis score. **(D)** Osteophyte number. **(E)** Osteophyte volume. **(F)** Comparison of medial subchondral bone compartment density (% BV/TV) between the operated ipsilateral (Ipsi) and control contralateral (Contra) legs in **(F)** sham and **(G)** DMM groups. **(H)** Change in tibial subchondral bone sclerosis (Ipsi−Contra). Comparison between exercise and non-exercise groups was done with a 2-way ANOVA with Bonferroni correction unless data was not normally distributed, in which case it was then compared by a Mann-Whitney test. Paired comparisons were conducted via a paired t-test. *$p < 0.05$, **$p < 0.01$.

Moderate exercise protects against osteoarthritis-related pathology at 8 weeks

While no difference was detected in cartilage damage between exercised and non-exercised groups at 4-week post-surgery, there was lower cartilage damage at 8-week in the exercised group (Figures 3A,B). DMM-driven synovitis was significantly higher than the sham control only in the non-exercise group (Figure 3C), however, this was not significant when comparing to the DMM exercise group ($p = 0.06$). 8 weeks after induction, osteophytes merge with the surrounding bone as the subchondral plate expands, and thus protruding osteophytes are difficult to discern. The outcome of this is that most DMM operated mice presented with one or less visible osteophytes (Figure 3D). Notably, at this time point subchondral bone expansion is clearly visible in 2D images, and quantification revealed that subchondral osteophyte volume was equivalent between the non-exercise and exercise group (Figure 3E).

Medial subchondral bone osteosclerosis was again evident in the DMM ipsilateral leg (Figures 3F,G), yet the change between ipsilateral and contralateral was similar in the non-exercise and exercise group (Figure 3H). However, the increase in trabecular thickness in the subchondral bone was significantly lower in the exercise group (Table 2). Furthermore, tibial trabecular bone of the operated leg was still structurally different only in the exercised DMM group when compared to the contralateral leg 8-week after surgical intervention. The trabecular bone was more connected (Trabecular pattern factor, Tb.Pf.) and more plate-like (structural model index, SMI), yet less organized (degree of anisotropy, DA, Table 2).

Discussion

In the present study, we used a moderate form of exercise requiring mice to walk 850 m a day, 5 days/week, which had no impact on the normal nocturnal activity. Hence, this did result in a 1.5 fold increase of physical activity in the exercised mice. This protocol allowed for recovery from surgical intervention before the start of forced exercise unlike other reported protocols which were initiated shortly after intervention or later when disease is established. Also, we induced OA by surgical DMM, which is a model of post-traumatic OA. DMM is milder in comparison to other more extreme forms of induction such as ACL-T,less variable than ageing, spontaneous or high fat diet models and resembles a proportion of human OA cases. We assessed whether the selected protocol exerted any physiological benefits. Exercise resulted in a decrease in weight gain and loss of WAT mass, indicating that this form of exercise, although moderate, exerted a physiological effect. This is an important aspect to consider, as it is well established that weight loss reduces risk of OA, as well as improving outcomes in established OA (Messier et al., 2013; Hunter et al., 2015; Panunzi et al., 2021).

Importantly, our induced moderate form of exercise resulted in protection against cartilage damage after 7-week of exercise. In addition to the significant changes in cartilage, evaluation of trabecular bone in the exercise DMM group revealed a more plate-like micro-structure with increased connectivity, similar to findings observed by Hahn et al. (2021), and known for offering higher bone strength (Teo et al., 2007). Moreover, there was an early, albeit temporary, improvement in subchondral bone osteosclerosis in the exercise group; expressed by the significantly smaller increase in bone density of the subchondral bone. It has been shown that increased bone density of the subchondral bone microarchitecture, as induced by PTH dosing, correlated with cartilage degeneration in mice (Orth et al., 2014). We observed a similar correlation where lower cartilage damage corresponded to lower subchondral bone density (Supplementary Figure S2). There was also an initial increase in osteophyte formation, which may indicate an acceleration of the subchondral bone expansion (Iijima et al., 2017) that ensues in the bone adaptation phase of the DMM model to dissipate the increased load. Quantification of the observed end-stage subchondral bone expansion (e.g. osteophyte volume) did not correspond with prior studies (Iijima et al., 2017) where an exercise-induced reduction was shown. This may be due to differences of DMM in rats in comparison to mice. Regardless of this inconsistency, the bone features we show in this study suggest that there is an improvement in the way the damaged joint is loaded in the exercised group. Notably, bone adapts to changes in mechanical loading and the DMM model substantially changes the way the joint is loaded. In essence, instead of the meniscus dissipating the load in the joint, this is transmitted primarily through cartilage and subchondral bone (Das Neves Borges et al., 2014). Thus, the delay in subchondral osteosclerosis we report in the exercise group, together with the change in the microarchitecture of the metaphyseal trabecular bone, suggest that exercise changes the way in which the load is dissipated throughout the joint. In explanation, it is conceivable that load is shifted to the metaphysis rather than subchondral bone. Furthermore, this delay in osteosclerosis might underpin the 8-week cartilage protection we observed. Indeed, it has previously been observed that subchondral bone changes occur rapidly, preceding significant cartilage damage in this OA model (Huesa et al., 2016). In addition to the observed bone changes, prior studies have demonstrated that DMM reduces muscle function four and 8-week post-surgery (van der Poel et al., 2016). It therefore has to be taken into consideration that exercise induced improvement in muscle strength, resulting in joint stabilization and altered load (Knoop et al., 2013; Nha et al., 2013). However, we did not observe any macroscopic changes in muscle mass, thus further studies are required to definitively address this question. Finally, going forward it is also important to consider that the effect of exercise may transcend load and fundamentally influence cellular signaling in the joint environment, which also contributes to the observed

pathological changes (Griffin et al., 2012; Hahn et al., 2021; Vadalà et al., 2020).

In summary, this study establishes a model of early moderate exercise that leads to reduced body weight gain, cartilage degradation, delays osteosclerosis, and changes trabecular microarchitecture on a widely used model of OA in mice, thus amenable to mechanistic studies utilizing transgenic animals. Such investigations may be particularly important as exercise programmes may be inappropriate for many patients, and low adherence to long term physiotherapy reduces effectiveness of prescribed exercise (Nicolson et al., 2018). It is important to note that the murine exercise protocol used simulates the situation of a human exercising shortly after sustaining a joint injury of a type likely to induce OA onset. The current study does not, however, address how this type of exercise regime would affect established OA; this is a key question that future studies should address. Furthermore, it will be important to conduct longer-term studies that would indicate if this form of moderate exercise affords long-term or merely transient benefit to the joint tissues.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by The Ethical Review Committee at the University of Glasgow.

Author contributions

Conceptualization WF, JL, CG, and CH. Methodology CH, WF, and JL Formal analysis CH Investigation CH, LD, KM, MF, AO, KM, SM, GL, AC, RV, WF, and JL Data Curation CH and LD Writing — Original Draft WF, JL, CG, and CH Writing — Review and Editing GL and AC,Visualization CH and RV Supervision WF, JL, CG, GL, AC, and CH Project administration WF, JL, CG, and CH Funding acquisition WF, JL, CG, and CH.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1065278/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Dynamic weight bearing as a measure of surrogate pain (Bioseb, France). Data expressed as the ratio of the load between the ipsilateral and contralateral load (Ipsi/Contra). Data expressed as Mean ± Standard deviation.

SUPPLEMENTARY FIGURE S2
Correlation of Cartilage damage and subchondral bone density 8 weeks after DMM induction, taking data from both exercise and non-exercise groups.

SUPPLEMENTARY TABLE S1
Experimental groups in the study.

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Sprint and upper limbs power field tests for the screening of low bone mineral density in children

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Background: The possibility of carrying out screening, with acceptable accuracy, of a child's bone mass status based on a physical fitness test can advance the concept of health-related physical fitness. In addition, the relevance of the applicability of this type of screening in educational environments is mainly due to the difficulty of direct assessments of bone health indicators. This study aimed to propose cut-off points for physical fitness tests based on children's bone health indicators.

Methods: This is a two-phase cross-sectional study. Phase-1: 160 children (6–11 years-old) performed the 20-m sprint test (20-mST) and the 2 kg medicine ball throw test (2 kgMBTT). Areal bone mineral density (aBMD) and content was assessed by DXA. The area under the ROC curve greater than 70% was considered valid. Phase-2: It was carried out a secondary analysis in a sample with 8,750 Brazilians (6–11 years-old). The percentile values (identified in phase-1) were used to identify the values of the cut-off points in the unit of measurement of the tests. The validation of the cut-off points found was by odds ratio values and $p \leq 0.05$.

Results: Phase 1: The areas under the ROC curve were 0.710, 0.712 (boys and girls–20-mST), 0.703, and 0.806 (boys and girls–2 kgMBTT) with total spine and pelvis aBMD as the outcome. Phase 2: From percentile values, we find valid cut-off points in the Brazilian sample (OR > 3.00; $p < 0.001$) for boys and girls. Values ranged between 5.22 s–4.00 s to 20-mST and between 125.0 cm–160.0 cm to 2 kgMBTT. Conclusion. The 20-mST and the 2 kgMBTT presented sufficient accuracy for the screening of children aged between 6 and 11 years with greater chances of having low aBMD in the total spine and pelvis, with valid cut-off points.

KEYWORDS

physical fitness, bones and bone tissue, physical fitness testing, child, bone, classification

1 Introduction

Globally, osteoporosis is one of the most relevant health problems, with high morbidity and mortality (Rizzoli et al., 2001). Because its development is related to low bone mass and marked deterioration of bone tissue, it is a consensus that optimizing the accumulation of bone mass during childhood and adolescence, through physical exercise, is an effective way to prevent osteoporosis and maintain adequate bone health in adulthood (Rutherford, 1999). In this way, three recent systematic reviews (Torres-Costoso et al., 2020; García-Hermoso et al., 2021; Mello et al., 2021) indicated the muscular strength, global physical fitness and vigorous physical activities practice as useful skeletal health markers during development and maturation.

Data from a 15-year longitudinal study showed that during adolescence and early adulthood, of all physical fitness variables, only power and isometric strength and speed were related to bone mineral density (BMD) in adulthood (Kemper et al., 2000). As Gómez-Bruton et al. (2017) showed that the increase in lower limb power through plyometric exercises (effects on muscle power) in children and adolescents causes positive effects on bone mineral content (BMC). As a consequence, the WHO 2020 guidelines on physical activity and sedentary behavior indicate for children and adolescents at least three days a week of vigorous-intensity activities that strengthen muscle and bone (in addition to 60 min a day of moderate-vigorous physical activity) (Bull et al., 2020).

In addition to interventions that include physical activity and exercise, national surveys that include measures of health-related physical fitness are an important prevention and monitoring strategy for population health indicators, as indicated in the 2012 American Institute of Medicine report (Committee on Fitness Measures and Health Outcomes in Youth et al., 2012). In this sense, a recent systematic review (Fraser et al., 2021) showed that there are only 13 articles that validated cut-off points for musculoskeletal physical fitness in young peopled. Furthermore, of the articles included in the review, only two (Baptista et al., 2016; Saint-Maurice et al., 2018) used bone health outcomes for cut-off point's propositions.

In this way, the possibility of carrying out screening, with acceptable accuracy, of a child's bone mass status based on a physical fitness test can advance the concept of health-related physical fitness. In addition, the relevance of the applicability of this type of screening in educational environments is mainly due to the difficulty of direct assessments of bone health indicators (Shepherd et al., 2011). These assessments are usually carried out in a clinical context by examining bone densitometry by dual-energy x-ray absorptiometry (DXA). In addition to the high cost of this exam, its application in children requires the use of software specifically developed to assess this audience, which in many services becomes an impediment to its applicability (Shepherd et al., 2011). In this sense, to make it possible for professionals working with human movement to have a simple tool for assessing bone mass indicators, this study aims to propose cut-off points for physical fitness tests based on children's bone health indicators.

2 Methods

2.1 Study design

This two-phase cross-sectional study with a quantitative approach was carried out after approval by Universidade Federal do Rio Grande do Sul Research Ethics Committee, under opinion 3.414.512 (CAAE-Brazil: 12222019.9.0000.5347, Brazil). In the first phase, field research was carried out with data collection related to the physical fitness and bone health of 160 students. Such information was used for performing the analysis of cut-off points proposition for the physical fitness variables. In the second phase, conducted to identify and validate the cut-off points proposed in phase 1, secondary analyses were carried out in a database consisting of a representative sample of Brazil with 8,750 children.

2.2 Phase 1

2.2.1 Research subjects and data collection procedure

In this phase, the convenience sample consisted of 160 children. These are students aged 6–11 years from the 1st to the 5th year of the public elementary school in the city of Porto Alegre, Brazil. The convenience of this sample is justified because the school has a formal partnership with the School of Physical Education, Physiotherapy and Dance at the Universidade Federal do Rio Grande do Sul, where the research was conducted.

To identify the test's power from the sample size of 160 children, *a posteriori* sample calculation was performed using the G-Power version 3.1 program. For that, we use the equation directed to the proposed association test. The calculation was performed for tests of the F family, considering that the research project foresees association analyses, carried out in another study (Mello, 2020). The alpha used was 0.05, the effect size used was $f^2 = 0.15$ (moderate) (Mello et al., 2021), and eight variables as predictors. From this protocol, considering the sample size of the present study, the power of the test (1-β) identified was 0.95.

For data collection, contact was made with the school's representatives. After signing the authorization terms, all children enrolled from the 1st to the 5th year of elementary school received an invitation to participate in the research and the consent terms. After this stage, a class period was scheduled with each class to carry out the physical tests. The DXA exam was

performed at the Exercise Research Laboratory (LAPEX) at the Universidade Federal do Rio Grande do Sul, Brazil. For this purpose, an evaluation time was scheduled with the parents/guardians. This procedure was carried out at the beginning of the academic years of 2017 and 2018.

2.2.2 Test variables

The procedures for collecting physical fitness variables were performed according to the PROESP-Br (Projeto Esporte Brasil) Guidelines for Measurements, Tests and Assessments (Gaya et al., 2021). The physical fitness variables tested were: 1) Sprint, assessed with the running test at a maximum speed of 20 m (20-m sprint test–20-mST); 2) Agility, assessed through the 4 × 4 meter square test (4x4-m square test); 3) Lower Limb Power, assessed using the horizontal jump test, and 4) Upper Limb Power, assessed using the 2 kg medicine ball throw test (2 kgMBTT). These tests also have validation and international use with good evidence (Davis et al., 2008; Bös and Schlenker, 2011; Calleja-González et al., 2015) and are widely used in Brazil (Pedretti et al., 2020).

For the 20-mST, the track was marked with three parallel lines on the ground as follows: starting line, timing line (20 m), and finishing line (2 m after the second line). The third served to avoid the deceleration before the timing line. The appraiser was positioned slightly beside the timing line. The adolescents performed the test twice and the evaluator noted the best time in seconds to two decimal places.

For 4 × 4-m square test, a four-meter square is marked on the side with a cone at each angle. The student started from the standing position. At the signal, the student should move at full speed and touch the cone located on the diagonal of the square. Then, you should run to touch the cone on your left (or right) and then the other diagonal. Finally, it should run towards the last cone. The appraiser was positioned slightly beside the start/finish cone. The adolescents performed the test twice and the evaluator noted the best time in seconds to two decimal places.

For the horizontal jump test, performed with a measuring tape fixed to the ground, perpendicular to a starting line. The adolescents were placed immediately behind the line, with feet parallel, slightly apart, knees semi-flexed, trunk projected in front. At the signal, the adolescents should jump as far as possible, landing with both feet simultaneously. The adolescents performed the test twice and the evaluator noted the best performance in cm to one decimal place.

For 2 kgMBTT, a measuring tape was fixed to the ground perpendicular to the wall. The student sits with knees outstretched, legs joined and back fully supported by the wall. The student holds a medicine ball (2 kg) next to the chest with your elbows bent. At the signal of the evaluator, the student throws the ball as far as possible, keeping his back against the wall. The result is recorded in centimeters to one decimal place.

2.2.3 Outcomes

BMC and areal bone mineral density (aBMD) were collected from the analysis of body composition according to the recommendations of the manufacturer of the DXA device of the GE Healthcare model, Lunar Prodigy (Madison, United States). A trained researcher and a laboratory technician qualified carried out the examinations and in the handling of the device. The device was calibrated once a day before the evaluation sessions. Calibration is performed automatically by the device after positioning the auxiliary calibration cubes. Children were instructed to remove any metal material and wear clothes without zips, buckles or buttons. The evaluator placed the subjects in the supine position and asked them to remain motionless during the measurement, for 5 min, while the equipment arm passed over the body in the head-foot direction.

The values were automatically calculated using the equipment's software. The values BMC (eg: 70.23 g) and aBMD (eg: 0.978 g/cm^2) have been described for the total body, total body less head, trunk, total spine, arms, pelvis and legs. These variables were categorized in each body segment was converted to Standard Deviation (SD) values adjusted for sex and age. After that, the variables were classified as "risk for low bone mass" (value ≤ -1 SD) and "normal bone mass" (value > -1 SD). We used the -1SD value because the sample size is not appropriate for using the children's recommendations (-2SD). This strategy was used by Gracia-Marco et al. (2011) in a study with 380 healthy Spanish adolescents about physical activity and bone mass.

2.2.4 Covariables

Due to the influence that bone mass indicators suffer from biological variables (Heaney et al., 2001), the total body fat percentage (TBF%) and maturity offset were considered covariates. The TBF% was made available during the DXA exam, along with bone variables. For maturity offset calculation, the following variables were required: height, body mass, sitting height, and length of lower limbs. The data collection for these variables and the calculation of maturity offset followed the recommendations proposed by Mirwald et al. (2002).

2.3 Phase 2

2.3.1 Research subjects and data collection procedure

The second phase was carried out based on a secondary analysis of a database. This study is part of the "Projeto Esporte Brasil" study (PROESP-Br). The PROESP-Br is a repeated cross-sectional surveillance study that was carried out since 1999. During 2003 and 2009, the Ministry of Sports through the National Secretariat of High-Performance Sports of Brazil

funded the PROESP-Br. It was designed to evaluate the anthropometry, sports practice and physical fitness levels of Brazilian children and adolescents using a standardized data collection protocol (Gaya et al., 2021) (the same as phase 1 of this study). Throughout the project (1999–2020), the data collection occurred over the years by previously trained volunteers. For all evaluation, children's parents/guardians authorized their participation signing the written informed. The Universidade Federal do Rio Grande do Sul originally obtained ethics approval for this project in 2000, under register number: 2008010.

For this study, we selected all children aged 6–11 years with valid data evaluated in the period between January 2011 and December 2020. The period of the last decade was chosen to represent the most current data of the project and suppress possible interference caused by the temporal effect on physical fitness levels (Sandercock and Cohen, 2019; Tomkinson et al., 2019). Following these procedures, the sample consisted of 8,750 Brazilian boys and girls.

2.4 Data analysis

2.4.1 Phase 1

A descriptive and exploratory analysis was carried out. In this procedure, the Kolmogorov-Smirnov test was performed and average values, SD, absolute and relative frequency and 95% confidence interval (95% CI) were calculated. Differences between sexes and ages were tested with the two-way ANOVA test adjusting for covariates. For the proposal of the cut-off points for the physical fitness variables, the analysis of Receiver Operating Characteristics (ROC) Curves was used. Firstly, the physical fitness variables were converted into SD values adjusted for sex and age (Gracia-Marco et al., 2011). After this procedure, the bone variables already classified were grouped, thus forming the outcome possibilities that would be tested in the analysis of ROC curves: 1) BMC of the total body less head, 2) aBMD of the total body less head, 3) BMC of the upper limbs and trunk, 4) aBMD of the upper limbs and trunk, 5) BMC of the total spine and pelvis, 6) aBMD of the total spine and pelvis, 7) BMC of the pelvis and lower limbs, 8) aBMD of the pelvis and lower limbs, 9) BMC of the upper limbs, trunk and pelvis, 10) aBMD of the upper limbs, trunk and pelvis, 11) BMC of the total spine, pelvis and lower limbs, and 12) aBMD of the total spine, pelvis and lower limbs.

After these procedures, the area under the ROC curves was calculated between the physical fitness variables (one by one, continuous variable) and the outcomes (one by one, categorical variable) stratified by sex and adjusted for maturity offset and body fat percentage. The outcome that presented the largest area under the ROC curve was chosen as the reference for bone health indicator. The analyses for the identification of the cut-off points were performed taking into account that the lower limit of the 95% CI was not less than 0.50. Acceptable values of area under the ROC curve were also considered to be greater than 70% and with significant p-values ($p \leq 0.05$). The cut-off points were defined based on the best balance between sensitivity and specificity. This balance was defined as the greatest sum between sensitivity and specificity, as long as the cut-off points were more sensitive than specific. Thus, percentage values were identified for boys and girls that represent greater chances of low bone mass based on the results of physical tests.

2.4.2 Phase 2

The percentile values (identified in phase 1) were used to identify the values of the cut-off points in the unit of measurement of the tests (e.g., 20-mST, cut-off point in seconds). For this, a descriptive analysis was carried out with the PROESP-Br database and the values corresponding to the percentiles were described for each sex and each age, these being the suggested cut-off points. To verify the validation of the cut-off points found, odds ratio (OR) calculations were used based on the binary logistic regression equation. In this analysis, the outcome variable was the bone health indicator that gave rise to the cut-off point. The independent variable was the physical fitness variable tested, already classified with the proposed cut-off point. The cut-off points that showed an indication of association were considered valid, verified through the 95% CI, the statistical probability value and the OR. Statistical programs IBM® SPSS 20.0 and Stata 13.0® (College Station, TX, United States) were used for all analyses.

3 Results

3.1 Phase 1

The sample of 160 children had an equivalent distribution between sexes and ages, in addition to having a normal distribution in all variables. Table 1 describes all anthropometric variables, physical fitness, fat percentage, maturity offset and age. The mean values of age, height and weight were similar ($p > 0.05$) by sex. However, maturity offset, TBF% and physical fitness variables showed differences ($p < 0.05$) between boys and girls. These results indicated that there is no need for the ROC curve analysis to be stratified by age, but only by sex.

Regarding BMC and aBMD in each analyzed body segment, even with adjustment, no differences ($p > 0.05$) between the sexes were identified (Supplementary Table S1). The percentage of low bone mass in the total sample varied between approximately 13% and 26% (Table 1). In the most of the outcomes, girls had a higher percentage of low bone mass when compared to boys in about 10 percentage points. There were between 5 and 8 cases of sample loss in the described variables.

For the ROC curve analysis, the physical fitness variables were converted into units of SD (Z score) adjusted for age and

TABLE 1 Description of physical fitness variables, bone outcomes and covariates of the sample used in phase 1 of the study (*n*: 160).

	Total	Boys (85)	Girls (75)
	X ± SD	X ± SD	X ± SD
Age (years)	8.9 ± 1.56	8.25 ± 1.65	8.35 ± 1.50
Height (m)	1.33 ± 0.10	1.34 ± 0.10	1.33 ± 0.11
Weight (kg)	33.03 ± 10.14	32.80 ± 9.31	33.29 ± 11.03
Maturity-offset*	−3.02 ± 1.73	−4.01 ± 1.11	−1.95 ± 1.65
TBF%*	32.66 ± 8.53	30.47 ± 8.61	35.15 ± 7.77
Sprint (s)*	4.54 ± 0.85	4.45 ± 0.96	4.63 ± 0.70
Agility (s)*	7.91 ± 1.00	7.70 ± 1.01	8.13 ± 0.94
Lower limb power (cm)*	110.58 ± 24.04	117.09 ± 24.57	103.54 ± 21.48
Upper limb power (cm)*	184.23 ± 53.85	191.92 ± 51.83	175.90 ± 55.10
	n	%	n	%	n	%
BMC TBLH						
Normal bone mass	135	85.4	77	91.6	59	78.7
Risk for low bone mass	23	14.6	7	8.4	16	21.3
aBMD TBLH						
Normal bone mass	137	86.7	77	92.8	60	80.0
Risk for low bone mass	21	13.3	6	7.2	15	20.0
BMC upper limbs and trunk						
Normal bone mass	134	84.3	74	88.1	60	80.0
Risk for low bone mass	21	15.7	10	11.9	15	20.0
aBMD upper limbs and trunk						
Normal bone mass	124	78.0	67	79.8	57	76.0
Risk for low bone mass	35	22.0	17	20.2	18	24.0
BMC total spine and pelvis						
Normal bone mass	130	81.3	73	85.9	57	76.0
Risk for low bone mass	30	18.8	12	14.1	18	24.0
aBMD total spine and pelvis						
Normal bone mass	128	80.0	68	80.0	60	80.0
Risk for low bone mass	32	20.0	17	20.0	15	20.0
BMC pelvis and lower limbs						
Normal bone mass	130	81.3	73	85.9	57	76.0
Risk for low bone mass	30	18.8	12	14.1	18	24.0
aBMD pelvis and lower limbs						
Normal bone mass	124	77.5	67	78.8	57	76.0
Risk for low bone mass	36	22.5	18	21.2	18	24.0
BMC upper limbs, trunk and pelvis						
Normal bone mass	129	81.1	72	85.7	57	76.0

(Continued on following page)

TABLE 1 (*Continued*) Description of physical fitness variables, bone outcomes and covariates of the sample used in phase 1 of the study (*n*: 160).

	n	%	n	%	n	%
Risk for low bone mass	30	18.9	12	14.3	18	24.0
aBMD upper limbs, trunk and pelvis						
Normal bone mass	117	73.6	61	72.6	56	74.7
Risk for low bone mass	42	26.4	23	27.4	19	25.3
BMC total spine, pelvis e lower limbs						
Normal bone mass	126	78.8	70	82.4	56	74.7
Risk for low bone mass	34	21.3	15	17.6	19	25.3
aBMD total spine, pelvis e lower limbs						
Normal bone mass	121	75.6	66	77.6	55	73.3
Risk for low bone mass	39	24.4	19	22.4	20	26.7

*Denote a significant difference ($p < 0.05$). *n*: absolute value; X ± SD: average value and standard deviation; %: percentage value; TBF%: total body fat percentage; BMI: body mass index; BMC: bone mineral content; aBMD: areal bone mineral density; TBLH: total body less head.

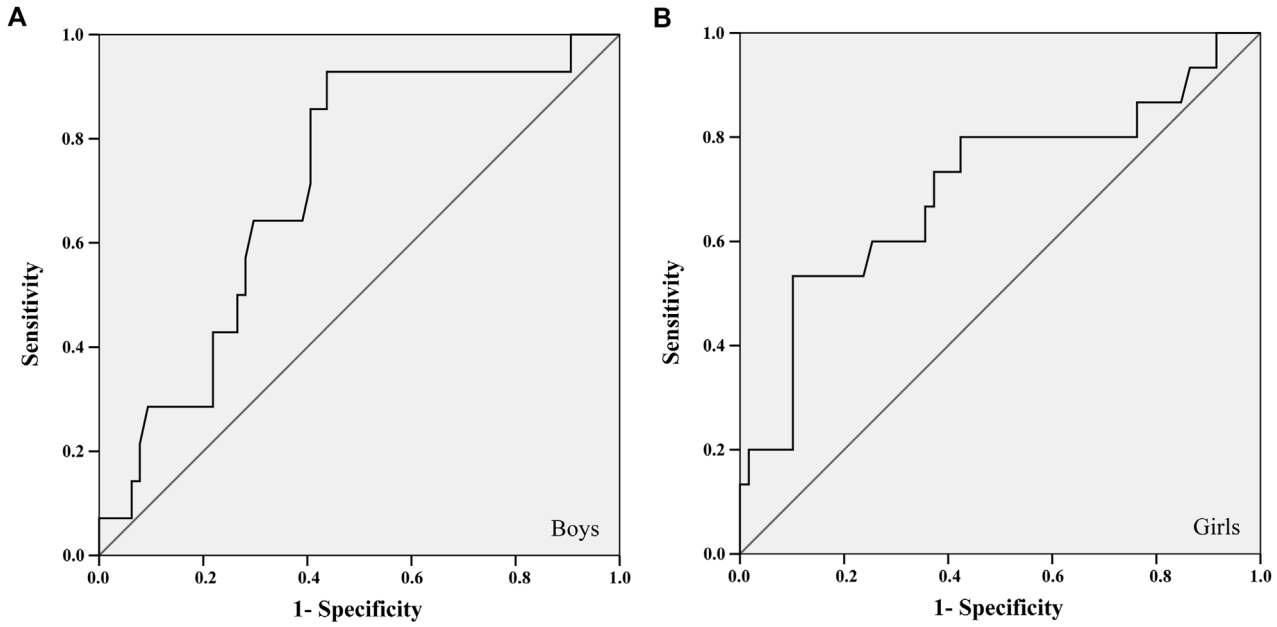

FIGURE 1
ROC curve between the 20-m sprint test and the areal bone mineral density of the total spine and pelvis of boys **(A)** and girls **(B)**.

stratified by sex. Thus, it was possible to position the points of each child on the probability distribution curve without disregarding some differences identified between ages and sexes (Supplementary Tables S2, S3). The largest areas under the ROC curves in all tests were found about the aBMD of the total spine and pelvis, for both sexes. Two tests met all the criteria: 20-mST and 2 kgMBTT (Figures 1, 2).

The 20-mST and 2 kgMBTT showed an area under the ROC curve greater than 0.700 with significant statistical probability value and acceptable confidence intervals for identifying cut-off points (Table 2). The better adjustment point between sensitivity and specificity was identified in the cut-off list provided by the ROC curve analysis (Table 2). For the 2 kgMBTT, the cut-off points for both sexes were those with the highest sum between sensitivity and specificity. In the 20-mST, the cut-off point for boys was the highest sum. For girls, we choose the fourth-highest sum, because the previous three values were more specific than sensitive. From these cut-off values, the percentile corresponding to the standardized value of the tests (Z score) in each sex was identified in the sample.

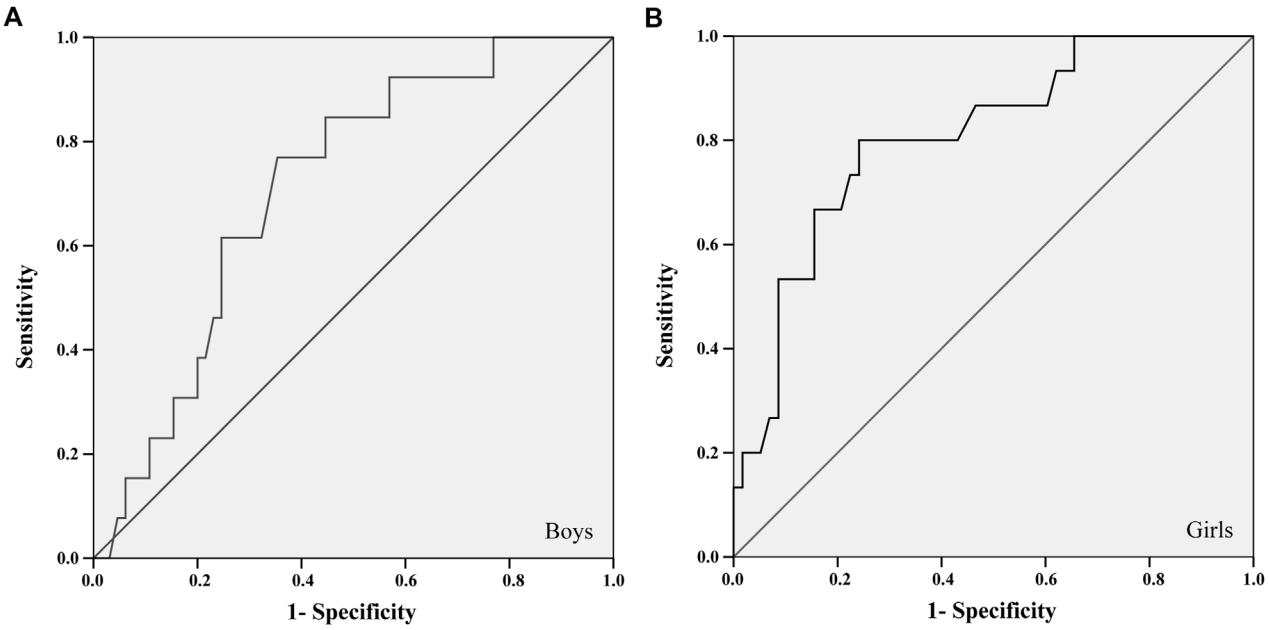

FIGURE 2
ROC curve between the 2 kg medicine ball throw test and the areal bone mineral density of the total spine and pelvis of boys **(A)** and girls **(B)**.

3.2 Phase 2

From the secondary analysis of the PROESP-Br database, the values that represent the cut-off points (in the test measurement unit) equivalent to the percentiles described are shown in Table 3.

In the validation analysis (Table 4), it was possible to see that children classified as the Risk for low bone mass for upper limb power and sprint are about four times more likely to have a low aBMD in the total spine and pelvis than their no-classified peers. When stratified by sex, associations remain with high odds ratios, ranging from 2.88 to 6.63 times more likely to have a low aBMD in the total spine and pelvis.

4 Discussion

The main evidence from this study indicates that for children aged 6–11 years, the 2 kgMBTT and 20-mST were accurate enough to identify cut-off points for screening children with greater chances of having a low aBMD in the total spine and pelvis. From these results, it was possible to establish valid cut-off points for these tests from a secondary analysis of a national database. Thus, we emphasize that the main practical application of this study is the proposition of cut-off points for physical fitness tests that can be applied in the school environment, training, pediatric care, or any place that deals with the child's health (Fraser et al., 2021). The use of physical fitness tests for the initial screening of the bone health profile of children is a simple strategy, cheap and does not require the use of sophisticated equipment in primary healthcare. Such information may help Physical Education teachers, for example, to carry out screening in the school environment, since the assessment of bone mineral density has a high cost. Therefore, greater care could be devoted to children who are not in the healthy test zone.

4.1 Physical fitness and bone health

Some studies have shown that the level of physical activity is associated with bone development variables (Tan et al., 2014; Gómez-Bruton et al., 2017; García-Hermoso et al., 2021), in addition, some studies specifically address the relationship between physical fitness and bone health indicators in childhood. Current evidence (Torres-Costoso et al., 2020) supports that muscular strength is a marker of skeletal health in children and adolescents, besides, available evidence from longitudinal studies demonstrates that only muscle strength and speed in childhood are related to BMD in adulthood (Kemper et al., 2000; Barnekow-Bergkvist et al., 2006).

Our study proposed an association, based on ROC curve analyses, between physical fitness and bone health indicators. The organization of outcomes took into account the potential regional effect of physical exercise on bones (McKay et al., 2000; Fuchs et al., 2001; Vicente-Rodriguez et al., 2004; Lu et al., 2009). However, it is noteworthy that literature data on the association between physical fitness and bone mass are still controversial.

TABLE 2 Synthesis of areas under the ROC curves between the physical fitness variables and the areal bone mineral density outcome of the total spine and pelvis.

Test variable	Area under ROC curve	*p*-value	95% CI
		Boys	
20-m sprint	0.710	0.014	0.572–0.848
4 × 4 m square	0.664	0.056	0.504–0.824
Horizontal jump	0.652	0.076	0.513–0.790
2 kg medicine ball throw	0.712	0.016	0.579–0.846
		Girls	
20-m sprint	0.703	0.016	0.538–0.867
4 × 4 m square	0.641	0.093	0.477–0.806
Horizontal jump	0.539	0.643	0.385–0.693
2 kg medicine ball throw	0.806	0.000	0.686–0.927

	Cut-off point (Z score)	Sensibility	Specificity	Percentile value
20-m Sprint test				
Boys	−0.173	0.929	0.563	47.5
Girls	−0.038	0.800	0.576	50.0
2 kg medicine ball throw test				
Boys	−0.208	0.769	0.646	42.4
Girls	−0.233	0.800	0.759	35.8

Bold *p*-values denote a significant difference ($p < 0.05$); *p*-value: statistical probability value; 95% CI: 95% confidence interval.

TABLE 3 Bone health-related cut-off points for the 20-m sprint tests and 2 kg medicine ball throw test for children 6–11 years old.

	2 kg medicine ball throw test (centimetres)		20-m sprint test (seconds)	
Age	Boys	Girls	Boys	Girls
6	147.0	125.0	4.81	5.22
7	168.7	140.0	4.52	4.88
8	190.0	158.1	4.31	4.66
9	210.0	175.0	4.25	4.58
10	232.0	202.0	4.09	4.44
11	260.0	228.0	4.00	4.36

Although longitudinal studies have pointed out specific physical capacities as influencing factors in long-term osteogenesis (Kemper, 2000; Vicente-Rodríguez et al., 2008; Khawaja et al., 2019), other studies have shown that only muscle fitness (Sayers et al., 2011; Torres-Costoso et al., 2020) is associated with BMD, although others have found a relationship with cardiorespiratory fitness (Dib et al., 2005; Ginty et al., 2005; Vicente-Rodríguez et al., 2008).

The associations between bone mass and physical fitness variables can be based on evidence on the osteogenic effect in childhood of some types of physical exercise (Rutherford, 1999; Vicente-Rodríguez, 2006). Different controlled trials in school and non-school settings demonstrate that although the intensity is important, the type of activity also has an important influence on the osteogenic effect (Tan et al., 2014; Larsen et al., 2018; García-Hermoso et al., 2021; Mello et al., 2021). This influence takes place through the actions of the reaction force of the different activities.

4.2 Physical fitness and screening aBMD

Some studies suggest that the greater the ground reaction force in activities, the greater the osteogenic stimulus (Rutherford, 1999; Kontulainen et al., 2002; Gómez-Bruton et al., 2017). Thus, if children perform jumping activities, the lower limbs and lumbar spine tend to have a greater osteogenic effect, unlike ball receptions, punches and grips,

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TABLE 4 Association of the speed and upper limbs power with the areal bone mineral density of the total spine and pelvis of children.

	OR	95% CI	*p*-value
Total sample			
Sprint			
Health zone	1	–	–
Risk for low bone mass	4.25	1.80–10.0	0.001
Upper limb power			
Health zone	1	–	–
Risk for low bone mass	3.95	1.46–7.86	0.004
Boys			
SPRINT (s)			
Health zone	1	–	–
Risk for low bone mass	4.26	1.26–14.40	0.020
Upper limb power			
Health zone	1	–	–
Risk for low bone mass	3.75	1.062–13.23	0.040
Girls			
SPRINT (s)			
Health zone	1	–	–
Risk for low bone mass	4.21	1.26–14.04	0.019
Upper limb power			
Health zone	1	–	–
Risk for low bone mass	3.59	1.10–11.68	0.034

Bold *p*-values denote a significant difference ($p < 0.05$); OR: odds ratio; 95% CI: 95% of confidence interval.

which would suffer the action of reaction forces from other bodies.

Even so, it is necessary to be cautious when interpreting associations, taking into account the physical fitness test that was performed and what it might represent in the child's usual activities (Bull et al., 2020; Gómez-Bruton et al., 2020). In this sense, our results indicated that the 20-mST and 2 kgMBTT had acceptable accuracy for screening children with increased chances of low aBMD of total spine and pelvis. There is a strong point in using these two tests because together they represent different reaction force possibilities–ground or not–by the lower and upper limbs.

The 20-mST assesses the child's speed, but it also represents the ability to high-intensity run, which tends to reflect their usual activities. High-intensity runs are multiple jumps with a specific pattern of ground reaction force, which can lead the test to represent an osteogenic effect that has already occurred in children for up to months (Janz et al., 2003; Sutter et al., 2019). Similarly, the 2 kgMBTT assesses the power of the child's upper limbs (Davis et al., 2008). Furthermore, it represents the muscle's ability to produce force (Harris et al., 2011), which can occur naturally (hormonal production) or through stimuli for activities that require strength (adaptation through the neural pathway in the case of prepubertal children). It is also assumed that the result of this test may be a reflection of the child's usual activities.

Both tests mentioned have adequate validity criteria for children (Davis et al., 2008; Calleja-González et al., 2015), in addition to being described in national (Pedretti et al., 2020) and international studies (Davis et al., 2008; Harris et al., 2011). Even so, the proposition of cut-off points for muscle speed and power tests based on a BMD variable is in line with the results reported by longitudinal studies discussed above (Kemper et al., 2000; Barnekow-Bergkvist et al., 2006). In agreement with our results, current evidence (Gómez-Bruton et al., 2020) demonstrated that upper and lower limb muscle strength, in addition to speed/agility predicted all measured bone variables, except for volumetric BMD. The authors strongly suggest that global fitness is an essential determinant of bone structure and strength in preschool children. Furthermore, they indicate that physical fitness testing could provide useful information related to bone health in children.

4.3 Limitations and strengths

Our results, despite being innovative, must be analysed based on the knowledge of the study's limitations. The children's level of physical activity and sports practice was not controlled, so the explanations for the relationship between physical fitness and bone outcomes may be due to other interferences. As all the children were in the same maturity offset group (they did not reach the growth peak), even with an adjustment for maturation, the analysis is still fragile and future studies must include children at different maturational levels in the sample. The use of -1SD value for the first classification of bone health (phase 1) is a good strategy for a small sample size but is not a clinical recommendation. A future study with a larger number of children and considering vitamin-D intake may be able to better explore the tangent relationships with physical capacities that were not related to this study. Finally, it is natural that the results of cross-sectional studies be analysed with caution, as it is not possible to attribute a cause-and-effect relationship.

Despite the aforementioned limitations, it is important to consider that this study has strengths that should be highlighted. The results described show applicability in different contexts related to primary care for children. Another important fact is that the gold standard assessment of body composition was used to assess the main outcomes, showing the reliability of the results. Finally, phase 2 of this study, where the cutoff points were identified and validated, was carried out with a database of more than 8,000 children from all regions of Brazil, adding characteristics of representativeness to the sample and the results found.

5 Conclusion

The 20 mST and the 2 kgMBTT presented sufficient accuracy for the screening of children aged between 6 and 11 years with greater chances of having low aBMD in the total spine and pelvis. From these physical fitness tests, it was possible to identify valid cut-off points for boys and girls. In this way, these results have important practical applicability, in which we suggest the use of tests and cut-off points in all primary healthcare settings, schools, schools and sports clubs, in addition to pediatric clinics.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: proesp.ufrgs.br.

Ethics statement

The studies involving human participants were reviewed and approved by Universidade Federal do Rio Grande do Sul Research Ethics Committee (3.414.512); (CAAE-Brazil: 12222019.9.0000.5347). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JM contributed to conceptualization, methodology, data collection, data analysis, data curation, and writing the original draft preparation. AP contributed to methodology, data collection, data curation and writing the original draft preparation. GB contributed to methodology, results' discussion and reviewed it critically for important intellectual content. AG contributed to data collection, conceptualization, methodology, and results' discussion and reviewed it critically for important intellectual content. EU-G contributed to data collection, conceptualization, methodology, and results' discussion and reviewed it critically for important intellectual content. AG had guided the development of the research question and data analysis, to drafting the manuscript, reviewed it critically for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1066462/full#supplementary-material

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Prolonged treadmill training is not able to prevent ovariectomy-induced bone loss

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Introduction: Exercise is widely recognized as prophylaxis for osteoporosis. However, exactly which type of exercise is best to prevent loss of bone mass remains undefined. To find an appropriate form of treadmill exercise that would ameliorate postmenopausal loss of cortical and trabecular structures, we compared various training regimen in ovariectomized (OVX) C57BL/6J mice.

Methods: Common to all regimen were training durations of 14 weeks including five 30 min-sessions per week. Two groups—one sham operated, one OVX—served as controls that did not perform any training. Three OVX groups ran at constant speed, either without any incline or at 20° in- and 20° decline, respectively. An additional OVX group ran an interval training, an alternation between intensive tempo sections and so-called slower regeneration phases. Femoral and humeral bone structures were assessed *via* micro-computed tomography (μCT), biomechanical stability of the femora *via* 3-point bending test, muscle volumes of the posterior extremities *via* magnetic resonance imaging (MRI), and bone metabolic parameters *via* ELISA on peripheral blood.

Result: OVX resulted in loss of bone mass and stability and a transient rise in the N-terminal collagen type I pro-peptide (PINP). Training resulted in increased muscle volumes of the heart and the lower extremities as well as increased running velocities. However, none of the exercise programs was able to prevent ovariectomyinduced loss of bone mass.

Discussion: These data therefore suggest that axial loading and tensile strain do not suffice as prophylaxis for postmenopausal osteoporosis yet may need to be complemented by low dose pharmaceutics or dietary supplements.

KEYWORDS

treadmill, ovariectomy, osteoporosis, physical training, mouse, micro-computed tomography, bone turnover markers

1 Introduction

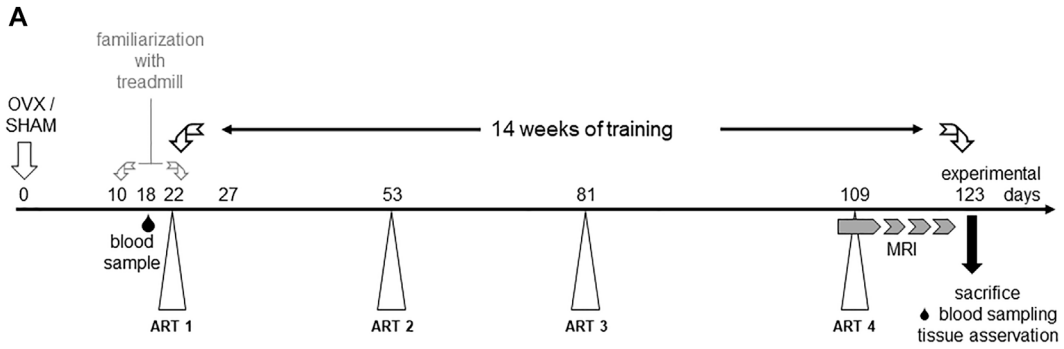
Osteoporosis is the most common bone disease in humans, and it is estimated that worldwide, one in three women and one in five men over the age of 50 will experience an osteoporotic fracture. Postmenopausal osteoporosis is a form of primary osteoporosis. The exact aetiology is unknown. However, the decline in estrogen levels after menopause appears to be one of the possible causative factors (Hernlund et al., 2013). It is defined as "a [silent] skeletal disease characterized by reduced bone strength leading to an increased risk of fracture. Bone strength reflects the integration of two main characteristics: bone density and bone quality" (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). These fractures can have serious impacts on quality of life and even death. In the context of demographic change, a further significant increase in the number of osteoporosis cases and associated fractures is expected (Camacho et al., 2020). Pharmacological therapy of osteoporosis largely relies on bisphosphonates and these have been associated with serious side effects (Khan 2008). Measures to reduce or even replace pharmacological measures for prevention of osteoporosis are therefore gaining attention.

Leading international guidelines define immobility as a risk factor for the development of osteoporosis and therefore recommend exercise as a preventive measure. Along these lines, an Australian study showed that high-intensity resistance and impact training (HiRIT) had a significant positive effect on bone structure in postmenopausal women (Watson et al., 2018). However, the exact type and amount of physical activity that will prevent deterioration of the bone are yet ill defined (Hernlund et al., 2013; Kanis et al., 2019; Camacho et al., 2020). Based on positive experience of our research group in using mice for the model of postmenopausal osteoporosis, we deliberately decided to use C57BL/6J mice. However, the literature on exercise studies in mice is still inconclusive. For example, four and 8 weeks of sprint interval training (SIT) in young and healthy female C57BL/6J mice did not result in a significant difference between untrained and trained mice—neither for cortical nor trabecular bone masses. In male C57BL/6J experimental animals, SIT even resulted in a significant loss of cortical bone mass, consistent with a site-specific reduction of cortical bone mass (Hollinski et al., 2018). Similarly, a total of 8 weeks of high-intensity interval training with different gradient angles (-10/0/10°) had no effect on cortical and trabecular bone parameters of female STR/ort mice (Koenen et al., 2017). Furthermore, low-to-moderate intensity interval training for 5 weeks failed to prevent significant cortical and trabecular bone loss in ovariectomized C57BL/6J mice (Latza et al., 2020). In contrast, 4 weeks of endurance training in female Hsd:ICR mice led to significant improvements in cortical bone structures of femur and tibia (Wallace et al., 2015). Also, significant improvements in bone mass and quality were observed in male C57/BL6 mice after 5 weeks of endurance training with an incline of 5° (Zhang et al., 2020). Overall, the scientific literature shows very contradictory results, especially when considering the model of postmenopausal osteoporosis. Furthermore there is simply a lack of scientific data on a significantly longer training period and its effects on bone structure. In addition, we here introduced 20° inclinations and declinations, in order to investigate the impact of tensile strain and axial load, respectively. To do justice to the consideration of postmenopausal osteoporosis as a systemic disease, in addition to the pure morphology of the bone, its stability and metabolism were also methodically investigated.

2 Materials and methods

2.1 Study design

A total of 42 7–9 weeks old female C57BL/6J mice were obtained from Charles River (Charles River Laboratories, Sulzfeld, Germany). Initially, the experimental animals had a week to acclimate to the new environment. Animals were housed in groups of six animals per cage under a 12h/12 h day and night cycle, were given water and food *ad libitum* and materials for enrichment. Randomization of animals to the six experimental groups was performed using a random number generator (Microsoft Excel 2010; Microsoft, Redmond, WA, United States). Group 1 underwent SHAM surgery and the remaining groups II-VI were ovariectomized (Figure 1). After that, the animals had 1 week to recover from the surgery. At the end of the postoperative recovery phase, groups II-IV were familiarized with the treadmill for 2 weeks. Before starting the treadmill training on experimental day 18, blood samples of the animals were collected by puncture of the great saphenous vein. Mice from groups II-VI performed a first augmentation-run-test (ART) to determine the maximum speed (Vmax), individual animals were capable of running. During the following 14 weeks of treadmill training, three more ARTs (day 53, 81, and 109) were performed to monitor changes in Vmax (Høydal et al., 2007) and to adapt the interval training accordingly (group VI). In the last 3 weeks of training, muscle volumes of the lower extremities were measured by 7 T small animal MRI. After 14 weeks of training, the animals were euthanized by cervical dislocation, blood, organs and bones of the lower extremities were harvested and preserved for post-mortem analyses. All experiments were performed in accordance with the current guidelines and scientific findings of the Society for Laboratory Animal Science (GV-SOLAS). Permission was granted by local authorities, i.e. the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Germany and was registered under 722.13-1-055/19.



B

group	I	II	III	IV	V	VI
procedure	SHAM	OVX	OVX	OVX	OVX	OVX
endurance training (ET)	-	-	no incline	20° incline	20° decline	-
intervall training (IT)	-	-	-	-	-	+

FIGURE 1
Experimental scheme **(A)** and study groups **(B)**. OVX: ovariectomy, sham: sham operation, ART: Augmentation Run Test, MRI: magnetic resonance imaging.

2.2 Ovariectomy

Animals were anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg ketamine and 5 mg xylazine/kg body weight) and depth of anaesthesia was ensured. Before surgery, panthenol ophthalmic ointment was applied to protect eyes from dehydration, and mice were positioned on a warm plate (37°C) to protect them from hypothermia. After abdominal positioning, the lower back was shaved in the area of the surgical field followed by skin incision and opening of the peritoneum to expose uterus, tubes, and ovaries. Removal of tubes and ovaries under microscopic view (Leica Biosystems, Dusseldorf, Germany) was performed by electrocautery, followed by suturing of peritoneum and skin (Ström et al., 2012). Metamizol was added for 5 days post-surgery to the drinking water and daily scoring was used to assess animal distress. During post-mortem organ removal, uterine weight was determined in relation to body weight to verify successful ovariectomy. One mouse had to be excluded from further analyses due to incomplete ovariectomy.

2.3 Augmentation Run Test

Augmentation Run Tests were performed to determine the maximum velocity of experimental groups II-VI (Hollinski et al., 2018; Latza et al., 2020) and to adapt the interval training to the measured maximum speed of the group (Høydal et al., 2007). In detail, ART started at a speed of 0.17 m/s for 3 minutes. Subsequently, the speed was slowly increased to 0.2 m/s over an interval of 2 min and held for another 3 min. Thereafter, the speed was increased to 0.25 m/s over a period of 2 min and again held constant for 3 min. Subsequently, the speed was again increased by 0.05 m/s over a period of 3 min and held for 2 min before the next cycle started. The maximum speed reached without external assistance was defined as Vmax (Ingalls et al., 1996). Over the whole experimental period, four augmentation run tests were completed to monitor the effect of the training on the animals' maximum velocity (Figure 1).

2.4 Training modalities

Treadmill training was performed 5 days a week from Monday to Friday for 30 min each on a six-track treadmill (TSE Systems Inc., MO, United States). Endurance training of groups III-V was accomplished at a constant speed of 0.2 m/s, but with different inclination angles of the treadmill. Thus, group III trained without any gradient, group IV trained at 20° of incline and group V at 20° of decline. Group IV also trained without any gradient yet underwent interval training adapted to their maximum velocity. In detail, mice ran at 40% of their Vmax for the first 6 min and then for four cycles of 80% of Vmax for 3 min followed by 40% of Vmax for 1.5 min.

2.5 Magnetic resonance imaging

To quantify the training effects on the muscle volume, MR-images of the abdomen, pelvis and lower extremities were taken.

In order to ensure high quality images, the animals were sedated using isoflurane, restrained on the MRI table, continuously warmed and breath monitored. For muscle imaging T2 weighted Turbo Rapid Acquisition with Relaxation Enhancement sequences (TurboRARE) (BioSpec 70/30, Bruker, Ettlingen, Germany) were used on a 7 T MRI with the following settings: TE/TR 25.25/3227 m, field of view: 28 µm × 21 mm, matrix size 233 × 175, slice thickness 0.85 mm, resolution 120 µm × 120 µm, and RARE factor: 8, 72 mm volume resonator. All measurements were respiration triggered. Since muscle volume is also affected by individual mouse size, it was related to femur length for statistical comparison. Based on the number of slices in µCT imaging, the length of each femur was calculated using CT Analyzer (Bruker, Billerica, MA, United States). Lower extremity muscles were manually marked slice-wise using ITK SNAP software (version 3.8.0) and muscle volume was also automatically calculated by the program (Feng et al., 2014).

2.6 Micro-computed tomography

Ex vivo µCT images of right femora and right humeri were taken to evaluate the cortical and trabecular bone structure (Bruker, Billerica, MA, United States) Software Version 4.2, 0.5 mm Al-filter, integration time of 1.5 s, isotropic voxel size of 9 µm at 49 kV and 200 µA, rotation step of 0.5°, and averaging frame of 3). Femora and humeri were fixed in 4% PFA solution, and preserved in ethanol. To prepare the specimens for µCT imaging, bones were washed three times with distilled water and stored in 0.9% sodium chloride solution at 4°C over night for rehydration. During each measurement procedure, two hydroxide apatite phantoms of known density (0.25 and 0.75 g/cm^3) were analysed in parallel in order to calibrate for the determination of bone mineral density (BMD). The CT scans were generated in a 360° scan to allow high quality and avoid artefacts. Using NRecon software (Bruker, Billerica, MA, United States), primary processing of the micro-CT data was accomplished using a Gaussian filter with a smoothing parameter of 2, x-ray hardness correction of 30%, ring artefact reduction by a factor of six and defect pixel masking of less than 20%. Homogeneous and adequate orientation of the bones for subsequent analyses was achieved using the DataViewer (Bruker, Billerica, MA, United States) software. Once the reconstruction was complete, the actual analysis of femora and humeri followed, using CT Analyser software and appropriate algorithms. In order to define regions of interest (ROI) for femora the distal metaphyseal growth plate and the fusion zone of the greater trochanter and caput femori were used as lower and upper reference levels, respectively. To define the ROI in humeri, the distal metaphyseal growth plate and the fusion zone between the greater tuberosity and caput humeri were used. Finally, the most proximal and distal 20% of the ROI were subjected to 3D bone architecture analysis to investigate trabecular structures. The 3D algorithms were used in CT Analyser to calculate the following parameters: trabecular number (Tb.N.), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th) and bone volume fraction (BV/TV). For the subsequent assessment of cortical structures, areas between upper and lower reference levels were divided into 10 sections. The cortical ROI was located in the middle of the diaphysis and contained 10% of the upper and 10% of the lower reference levels, respectively. Afterwards, the diaphyseal area was subjected to a 2D analysing algorithm to measure the parameters cortical thickness (Ct.Th) and cortical area fraction (Ct.Ar/Tt.Ar.) (Bouxsein et al., 2010; Behrendt et al., 2016). In order to eventually determine bone mineral density (BMD) in the diaphysis, attenuation coefficient of the phantoms of known density (0.25 and 0.75 g/cm^3) were used and the BMD was calculated automatically by algorithms.

2.7 Blood sampling and analyses of bone turnover markers

Blood samples were taken by saphenous vein puncture before the start of the training phase for later determination of basal values (Figure 1). For this purpose, the mice were fixed as previously described (PerkinElmer, Massachusetts, United States) (Hem et al., 1998). The thigh was shaved distally to the knee, the veins were tourniquetted by manual compression, punctured by a 25G needle (B. Braun SE, Melsungen Germany) and blood was collected into an EDTA microvette (Sarstedt, Nümbrecht, Germany). Samples were temporarily stored on ice. Subsequently, plasma was separated *via* centrifugation (Eppendorf SE, Germany) and stored at −80°C until further analysis. At the end of each training phase, mice were anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg ketamine and 5 mg xylazine/kg body weight) for final blood sampling. In detail, blood was collected by puncturing the retrobulbar venous plexus, plasma was separated and preserved as described above. Before removal of organs and bones, the animals were euthanized *via* cervical dislocation. To assess bone metabolism, TRACP5b, as a marker of bone resorption and osteoclast activity, and PINP, as a marker of bone formation, were analysed *via* an ELISA for TRAcP 5b (Mouse Trap, Immunodiagnostic Systems Ltd., Boldon, United Kingdom) and an EIA for PINP (Rat/Mouse PINP EIA, Immunodiagnostic Systems Ltd., Boldon, United Kingdom). Both kits were used according to the manufacturer's instructions. Due to a limited sample volume, the tests were only performed once. Photometric measurements of TRAcP ELISA and PINP EIA were performed using the Infinite M200 automated plate reader running with the iControl software v1.9 (Tecan Trading AG, Switzerland) at an absorbance of 405 nm with reference at 650 nm. Subsequently, the concentrations of our samples were estimated for TRAcp5b

using a 3-parameter logistic regression model for calibration. In order to extrapolate concentration for high absorbance values readings of absorbance at 450 were naturally logarithmized (ln) and the resulting linear regression was used for further estimations. Back transformation using e^ln(conc) was used to calculate the concentrations of PINP in ng/mL. In both cases, RStudio 1.2.5033 software (version 3.5.1, RStudio Inc. Boston, MA, United States) and Microsoft Excel (Microsoft, Redmond, WA, United States) were used.

2.8 Three-point bending test

After removal, the left femora were soaked in 0.9% NaCl and wrapped, placed in Eppendorf tubes and frozen at −20°C until further analyses. 24 h before the measurements, samples were thawed and continuously kept moist using 0.9% sodium chloride solution to protect them from exsiccation. First, the exact dimensions of the bones were collected using a caliper gauge (Vogel GmbH, KevelaerGermany) for the subsequent calculations. The femora were then placed in the three-point bending machine with the condyles facing downwards so that the femoral mid-shaft was centred with a span of 6 mm on the test punch. The three-point bending machine was equipped with a 500N pressure load cell (zwickiLine Z2.5, Zwick GmbH, Ulm, Germany) and gradually increased the bending force applied to the femur by 1 mm/min during the course of the test. The increase of the bending force was either stopped manually or automatically by the measuring unit when the bone broke. Subsequently, the recorded load-displacement curves could be used to calculate bending strength (MPa), maximum load (N), breaking load (N) and Young`s modulus (MPa), taking into account the bones' elliptical cross-section (Grote und Feldhusen 2007).

2.9 Statistics

The statistical analyses in our experiments were performed using IBM SPSS Statistics 27 software (IBM, NY, United States). The Shapiro-Wilk test was used to examine the normal distribution of the data. Due to small sample sizes we used non-parametric tests only. Comparisons between two groups (OVX vs. non-OVX or trained vs. untrained) were performed using Mann-Whitney U-tests and Kruskal–Wallis with *post hoc* tests for multiple comparisons. Pairwise comparisons (TRAcP concentrations at experimental day 6 vs. at sacrifice) were done *via* Wilcoxon Signed-Rank-test. Values of p lower than 0.05 were considered significant. Finally, the experimental results were presented graphically using SigmaPlot 13.0 (Systat Software, CA, United States).

3 Results

3.1 Treadmill training led to increased running speeds

Four augmentation run tests (ART) dispersed over the length of the experiments served to adapt the interval training and to evaluate the impact of the training. Individual maximum running velocities (Vmax) before the start of the training period (ART1) ranged between 0.22 and 0.37 m/s and were comparable between all groups (Table 1). Likewise, at any given ART thereafter, mean Vmax between groups were comparable as shown by *p*-values ranging from 0.3691 to 0.9288. However, all groups increased their mean Vmax from ART1 to ART4. While this increase was only marginal for groups IV (20° incline) and VI (interval training), it was significant for groups III (no decline/incline) and V (20° decline). The highest results across all running groups was achieved by group V. In summary, all training regimes led to increased running speeds and did so with comparable efficiency.

3.2 Treadmill training led to significant increases in heart weight

In order to assess any training effect on the cardiovascular system, heart weights were measured post-mortem and were calculated in relation to the respective body weight. While median ratios of heart to body weight were 0.5% for the untrained groups I-II, they were 0.6% for the trained groups III-VI. A Mann-Whitney test comparing trained and untrained animals resulted in a *p*-value of 0.0037 confirming that 14 weeks of treadmill training led to significant gains in heart weight (Figure 2).

3.3 Treadmill training significantly increased the muscle volumes of the lower extremities

In order to assess any training effect on the musculature, MRI scans of the lower extremities and pelvic regions were performed (Figure 3A). In detail, the scans served to assess the muscle volumes of the lower extremities which were then—in order to compensate for different sizes - calculated in relation to the respective femur length. The results yielded cross sectional areas and Figure 3B summarizes the results for all experimental groups. Comparing the cross sectional areas between the untrained groups I and II (median of 64.2 mm^2) and the trained groups III-VI (median of 76.3 mm^2) resulted in a statistically significant difference (Figure 3B).

TABLE 1 Treadmill training led to increased running speeds

group	Vmax-ART 1 mean ± SD [m/s]	Vmax-ART 2 mean ± SD [m/s]	Vmax-ART 3 mean ± SD [m/s]	Vmax-ART 4 mean ± SD [m/s]	*p*-value [H-test]
III: endurance training no incline (n = 8)	0.274 ± 0.04	0.289 ± 0.03	0.315 ± 0.04	0.35 ± 0.03	**0.006**
IV: endurance training 20° incline (n = 9)	0.284 ± 0.05	0.302 ± 0.06	0.322 ± 0.05	0.336 ± 0.05	0.152
V: endurance training 20° decline (n = 6)	0.280 ± 0.05	0.325 ± 0.03	0.308 ± 0.04	0,372 ± 0.03	**0.009**
VI: interval training (n = 6)	0.290 ± 0.05	0.315 ± 0.03	0.322 ± 0.04	0.352 ± 0.04	0.111
p-value [H-test]	0.967	0.375	0.665	0.404	

Bold values are statistically significant ($p < 0.05$).

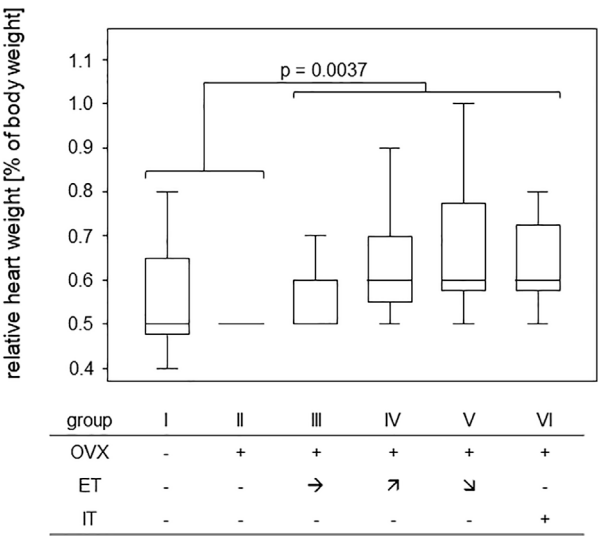


FIGURE 2
Treadmill training led to significant gains in heart weight. Box plots show the ratios of heart to body weight for the various training groups. Comparison between untrained (groups I and II) and trained groups (groups III-VI) was performed *via* Mann-Whitney U-Test and statistically significant difference is indicated by the *p*-value.

3.4 Treadmill training did not compensate for OVX-induced loss of bone stability and elasticity

In order to assess the efficiency of OVX, post-mortem uterus weights were taken and compared between all experimental groups. Results are presented in Figure 4 and show a significant difference between the sham operated group and all others confirming successful procedure. For analysis of bone stability, the femora were subjected to three-point bending tests. The results presented as bending strength and Young's modulus are summarized in Figure 5. While the sham group I (no OVX, no training) exhibited highest means for bending strength and Young's modulus, respectively, OVX resulted in a significant reduction for both. However, there were no significant differences between any of the trained groups and the non-trained OVX control. In summary, OVX led to a reduction of both, flexural strength and elasticity, and neither of the training regimens managed to prevent loss of bone integrity.

3.5 Treadmill training did not prevent OVX-induced bone loss

Measurements of cortical bone parameters were collected in the diaphyses of both, femora and humeri and the results are summarized in Figure 6A: The highest mean bone mineral density (BMD) for both extremities were found in group I (no OVX, no training). OVX resulted in a significant reduction in BMD, but there was no statistically significant difference between the trained animals (group III-VI) and group II. Likewise, cortical thicknesses of femur and humerus were highest in the SHAM group with median values of 0.23 and 0.22 mm, respectively (Figure 6A). Again, there was no difference between any of the trained groups and group II.

Figure 6B summarizes the results for the trabecular bone parameters assessed in the distal metaphyses of femora and humeri. As with the cortical parameters, highest means for BV/TVs were observed in the SHAM group with medians of 16.0% for femora and 3.9% for humeri, respectively. All OVX groups yielded significantly lower means with none of the trained groups differing from control group II (Figure 6B). Additional measurements obtained by CT are available in the supplements. In summary, OVX led to a loss of cortical and trabecular bone parameters and none of the treadmill training regimen was able to prevent this process.

3.6 Treadmill training did not impact on bone metabolism

To investigate how OVX and treadmill training impacted on the metabolic activity of osteoclasts and osteoblasts, serum

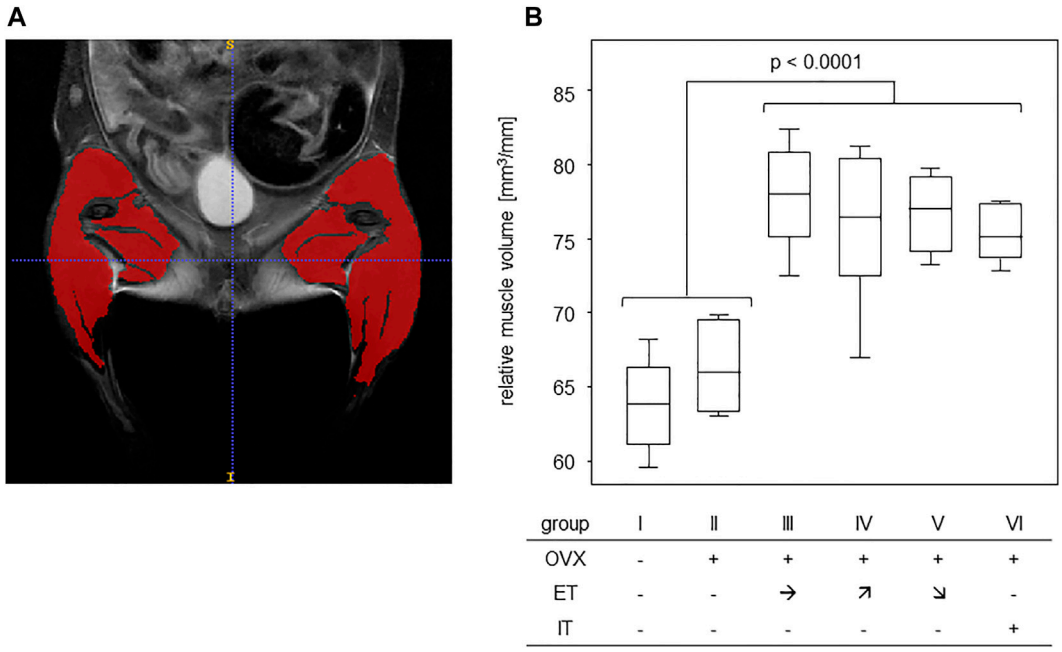


FIGURE 3
Treadmill training increased muscle volumes of the lower extremities. **(A)** shows an exemplary T2 TurboRARE illustration of an abdomen, pelvis and lower extremities and marks the muscle tissues of the lower extremities by ITK snap. **(B)** presents the muscle volumes in relation to femur lengths of groups that were either untrained (groups I-II) or trained (groups III—VI). Statistics were calculated by Mann-Whitney U-test and statistically significant difference is indicated by the *p*-value.

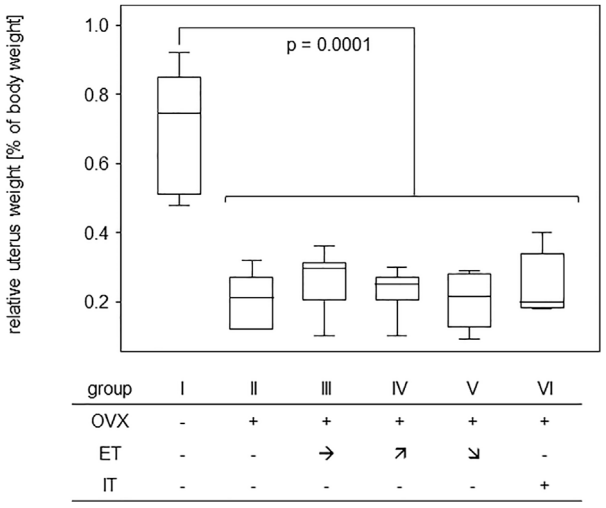


FIGURE 4
Effective OVX was confirmed by significant weight reduction of uteri. Box plots represent uterus weights compared to body weights for all groups. Statistics were calculated by Mann-Whitney U-test and statistically significant difference is indicated by the *p*-value.

TRAcP and PINP were analysed. In detail, we compared experimental day 18–after OVX yet before the start of the treadmill training - to the endpoint of the experiments at sacrifice (see Figure 1). Figure 7 summarizes our results. While TRAcP-values were comparable among all experimental groups at both time points, values did differ significantly between the early and late time points (left panel). At the early time point, serum values for TRAcP 5b ranged from means of 9.2 U/L in group VI to 12.2 U/L in group V however, they dropped significantly to means ranging between 5.5 (group V) and 7.3 U/L (group I) at the experimental end point. *p*-values describing these differences pairwise were 0.028 (group I); $p = 0.08$ (group II) 0.017 (group III) $p = 0.011$ (group IV), 0.028 and $p = 0.046$ (group VI). In summary, osteoclast metabolic activity was affected by age, but unaffected by OVX or treadmill training.

The situation was different for PINP. OVX led to an almost 10-fold and significant rise in PINP levels within 18 days following surgery (right panel). As the treadmill training had not begun yet, all OVX mice were calculated as one group and showed a statistically significant increase from a median 1.2 ng/ml in group I to 11.6 ng/ml for all OVX mice. The *p*-value resulting from a Mann-Whitney test was 0.019. At sacrifice, serum PINP concentrations were again comparable among all groups with a median value of 1.7 ng/ml (Figure 7). In summary, osteoblast metabolic activity was transiently elevated by OVX but was unaffected by treadmill training.

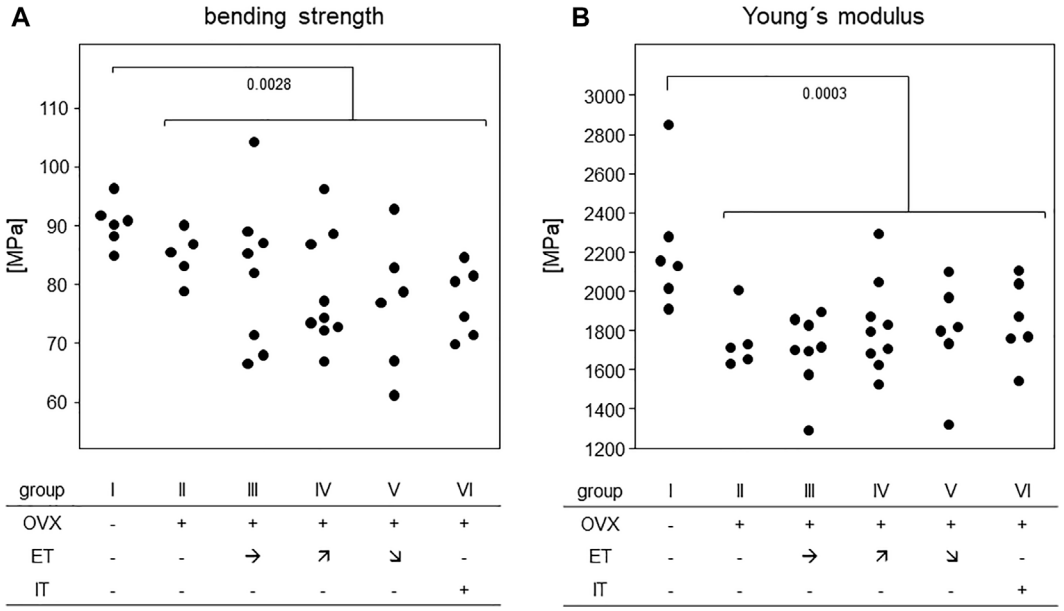


FIGURE 5
Treadmill training did not compensate for OVX-induced loss of bone stability. Dot blots summarize **(A)** the bending strength and **(B)** Young`s Modulus. Statistics were calculated by Mann-Whitney U-test and statistically significant differences are indicated by *p*-values.

4 Discussion

We here explored treadmill training for its capacity to prevent OVX induced bone loss. In detail, we compared increased axial loading *via* a 20° decline of the treadmill, to increased tensile forces *via* a 20° incline to endurance training with no gradient to high intensity interval training. We confirmed efficient OVX followed by deterioration of bone microstructures and mechanical properties and we validated our training *via* improved running velocities and increased muscle volumes of both, heart and lower extremities. These validations are in line with previous publications (Kaplan et al., 1994; Gibb et al., 2017; Latza et al., 2020). A distinctive feature of our work is the CT-based analysis of femora and humeri in order to control for our experimental animals being quadrupeds.

In short, the analysis of bone mechanical stability following treadmill training was rather disillusioning. Despite an extended training period of 14 weeks, we here confirmed earlier results with a shorter training period from our own group demonstrating that treadmill training by itself did not suffice to prevent OVX-induced deterioration of bending strength and Young's modulus (Latza et al., 2020). Considering that bone stability is mainly dependent on cortical parameters (Holzer et al., 2009), these findings are in line with our observation that cortical bone parameters of front and hind legs deteriorated in all OVX mice, again without any benefits from treadmill training. Moreover, our CT analyses confirmed that OVX also led to a loss of trabecular bone which could not be prevented by treadmill training. Negative as these results are, they are in line with a number of previous experiments that aimed to improve bone parameters in young and healthy mice (Koenen et al., 2017; Hollinski et al., 2018) or prevent OVX induced bone loss (Latza et al., 2020). Our research group so far analysed different mouse strains, compared purely aerob to more strenuous training regimen with anaerob peaks, investigated varying training durations and assessed the impacts of axial loading vs. tensile strain by introducing various inclines and declines. However, irrespective of whether training periods lasted 5 or 14 weeks, we never observed any clear cut positive effect on the bone that would immediately be translated into a suggestion for osteoporosis prone patients. Our results suggest that increases in muscle volume of the heart and the extremities are not paralleled by the retention of bone integrity. We are aware though that the literature holds conflicting results as to training effects on the bone and can only speculate about these discrepancies (Iwamoto et al., 1998; Wu et al., 2001). Among the arguments at hand are different genetic backgrounds, diverse experimental set-ups and varying read-outs (Wallace et al., 2015; Zhang et al., 2020).

Comparable to structural and mechanical bone characteristics, metabolic bone parameters were also unaffected by any of the training programs. Nevertheless both, PINP as a marker of bone formation and TRAcP-5b as a marker for bone resorbing osteoclast activity, showed altered expressions over the course of the experimental phase. In detail, PINP was transiently increased after OVX and again down to baseline at the end of the experiments, 14 weeks later. Likewise, Rissanen et al. previously described that PINP in rats significantly increased during the first 2 weeks after OVX and returned to sham level at 8 weeks (Rissanen et al., 2008).

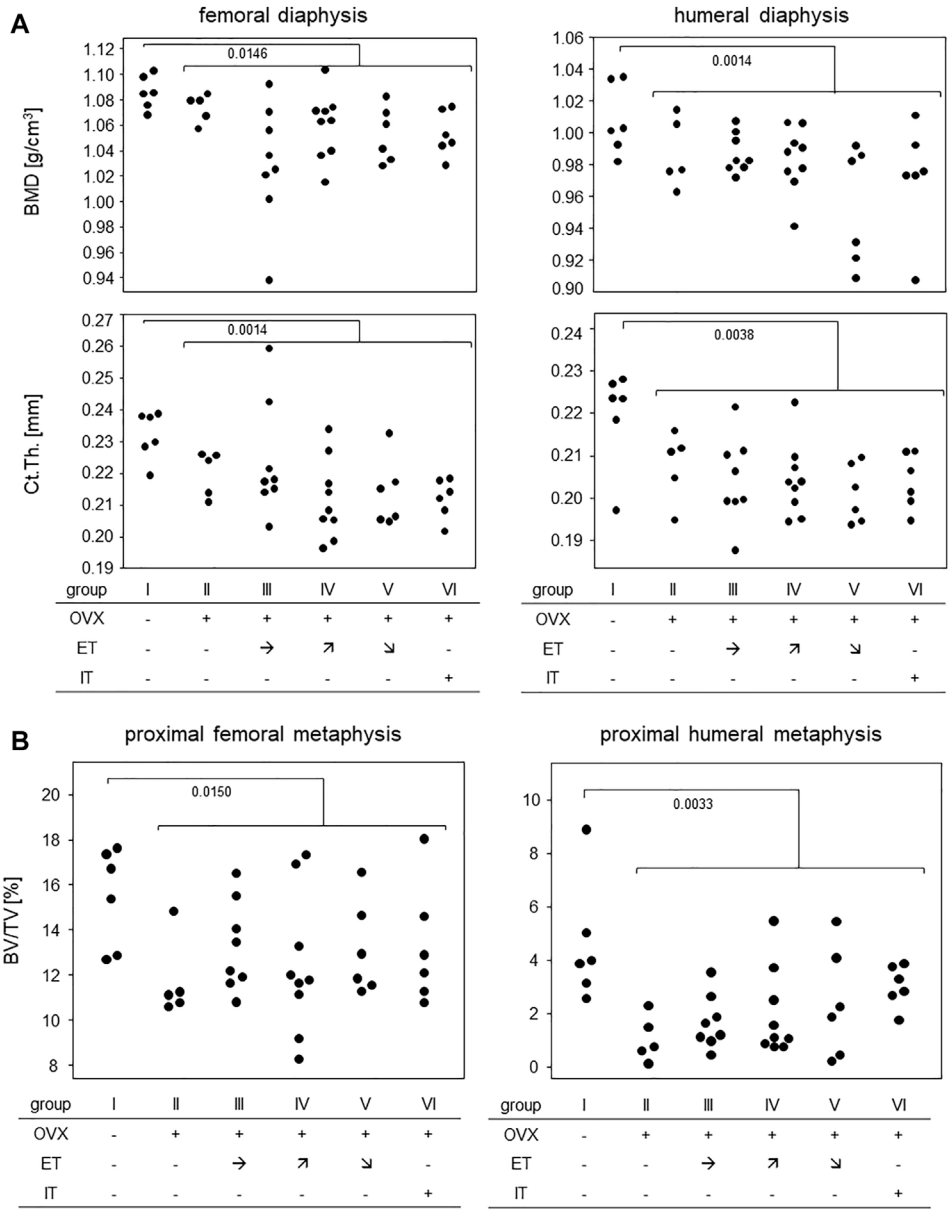


FIGURE 6
Treadmill training did not prevent OVX-induced bone loss. **(A)** shows bone mineral density and cortical thickness of the femoral (left panels) and humeral (right panels) diaphysis. **(B)** shows BV/TV in the proximal metaphyses of femora (left panel) and humerus (right panel). Statistics were calculated by Mann-Whitney U-test and statistically significant differences are indicated by *p*-values.

Collectively, these findings foster the expectation of a transient increase in bone mass rather than a decrease. However, Rissanen et al. also described an OVX induced and permanent increase in carboxy-terminal collagen crosslinks (CTX) that reflects ongoing bone resorption and holds the potential for disturbing the bone remodeling balance towards resportion. In contrast, our TRAcP levels were unaffected by OVX and even decreased over the course of the experiments. These findings are conflicting as transiently elevated PINP and decreasing TRAcP-levels cannot be reconciled with a net loss of bone mass, deterioration of bone integrity and osteoporosis. The possibility remains though, that serum measurements only inaccurately reflect processes at the bone surface and that metabolic requirements for homeostasis in the light of an osteoporotic bone mass are difficult to calculate.

In summary, our results challenge the idea that treadmill training by itself is suitable to stop OVX-related bone loss. For instance, combining endurance and athletic exercises may turn out more beneficial for the bone. Indeed, the HiRiT program of an Australian

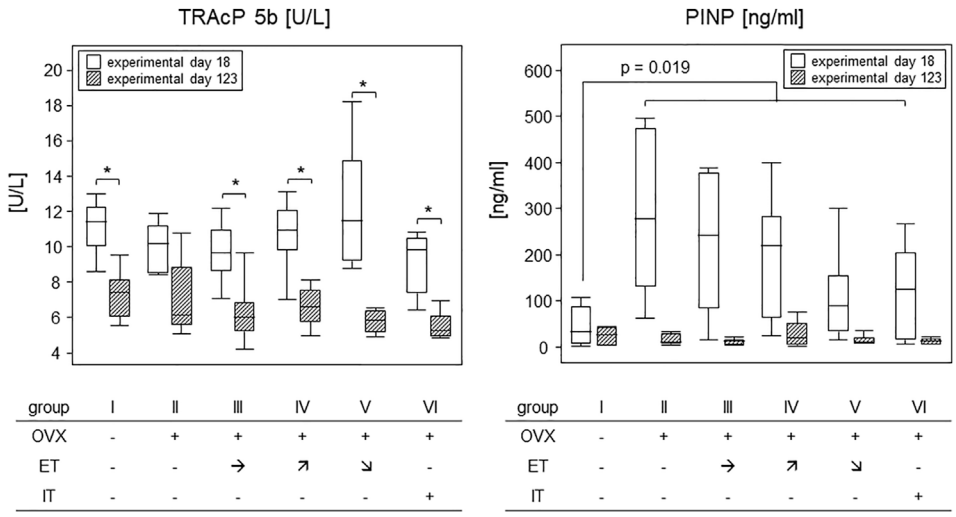


FIGURE 7
Treadmill training did not impact on bone metabolism. Box plots compare serum TRAcP 5b (left panel) and PINP (right panel) concentrations on experimental day 18 and at sacrifice. To describe changes in TRAcP concentrations (left panel), statistics were calculated by Wilcoxon signed-rank test, asterisks indicate statistically significant differences (*$p < 0.05$). For changes in PINP concentrations on experimental day 6 (right panel), the sham group I was compared to all OVX groups (II—VI) *via* Mann-Whitney U-test. For details see text.

study included deadlifts, overhead presses, squats and pull-ups with drop landings and led to a significant improvement of the bone structure (Watson et al., 2018). Even though encouraging, these experiments are difficult to model in animal experiments. Alternatively, the diet could be supplemented with extra calcium and vitamin D in order to investigate if the combination with treadmill training will favour bone accrual. Finally, as an additional preventive measure, treadmill training may allow for a lower minimum therapeutic dose and an associated reduced risk of adverse drug reactions, in addition to the overall improvement in cardiovascular risk profile.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Germany.

Author contributions

TMi and BM-H contributed to the conception and design of this study. MM, JV, TL and MW contributed to the experimental work. TMa and KW contributed most to the experimental work. TMi is guarantor. BM-H drafted the article. All authors contributed to the analysis of the data. All authors contributed to the critical revision of the article and approved the final manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1078857/full#supplementary-material

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Twelve weeks of a diet and exercise intervention alters the acute bone response to exercise in adolescent females with overweight/obesity

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Introduction: Exercise and consumption of dairy foods have been shown to improve bone mineralization. However, little is known about the magnitude and timing of their synergistic effects on markers and regulators of bone metabolism in response to acute exercise in adolescent females with obesity, a population susceptible to altered bone metabolism and mineral properties. This study examined the influence of twelve weeks of exercise training and nutritional counselling on the bone biochemical marker response to acute exercise and whether higher dairy consumption could further influence the response.

Methods: Thirty adolescent females (14.3 ± 2.0 years) with overweight/obesity (OW/OB) completed a 12-week lifestyle modification intervention involving exercise training and nutritional counselling. Participants were randomized into two groups: higher dairy intake (RDa; 4 servings/day; n = 14) or low dairy intake (LDa; 0-2 servings/d; n = 16). Participants performed one bout of plyometric exercise (5 circuits; 120 jumps) both pre- and post-intervention. Blood samples were taken at rest, 5 min and 1 h post-exercise. Serum sclerostin, osteocalcin (OC), osteoprotegerin (OPG), receptor activator nuclear factor kappa B ligand (RANKL), and C-terminal telopeptide of type 1 collagen (βCTX) concentrations were measured.

Results: While there was an overall increase in sclerostin pre-intervention from pre to 5 min post-exercise (+11% $p = 0.04$), this response was significantly decreased post-intervention (–25%, $p = 0.03$) independent of dairy intake. The OPG:RANKL ratio was unresponsive to acute exercise pre-intervention but increased 1 h post-exercise (+2.6 AU; $p < 0.001$) post-intervention. Dairy intake did not further influence these absolute responses. However, after the 12-week intervention, the RDa group showed a decrease in the relative RANKL post-exercise response (–21.9%; $p < 0.01$), leading to a consistent increase in the relative OPG:RANKL ratio response, which was not the case in the LDa group. There was no influence of the intervention or dairy product intake on OC, OPG, or βCTX responses to acute exercise ($p > 0.05$).

Conclusion: A lifestyle modification intervention involving exercise training blunts the increase in sclerostin and can augment the increase in OPG: RANKL ratio to acute exercise in adolescent females with OW/OB, while dairy product consumption did not further influence these responses.

KEYWORDS

exercise, dairy, adiposity, sclerostin, OC (osteocalcin), CTX (C-terminal telopeptide of type 1 collagen), OPG (osteoprotegerin), RANKL (receptor activator for nuclear factor k B ligand)

1 Introduction

During adolescence, there is a high rate of bone remodelling, characterized by bone resorption and subsequent formation. The balance of these two processes determines bone growth/development and the peak bone mass that one will achieve in their lifetime. Higher achievement of peak bone mass following adolescence is associated with a reduced risk of fragility fractures with age (Boreham and McKay, 2011; Gordon et al., 2017), highlighting the importance of the adolescent time period for the prevention of low bone mass-induced fractures (Hernandez et al., 2003). Further, understanding factors (e.g., nutrient intake) and stimuli (e.g., loading and unloading) that influence bone metabolism [i.e., increased muscle force/contractions predictably drive bone growth (Bass et al., 2005)] are critical when designing lifestyle modification strategies to augment bone accrual in youth.

It has been previously thought that individuals with overweight or obesity (OW/OB) have higher bone mass (Fassio et al., 2018), but this point has recently been challenged, as adolescents with OW/OB have a lower bone mineral density (BMD) and bone quality compared to normal weight females when body mass is controlled for (Janicka et al., 2007; Pollock et al., 2007; Sioen et al., 2016). Additionally, lifestyle modification strategies that aim only to reduce body mass (i.e., through caloric restriction) may put adolescent females at risk of not only losing lean mass but also blunting bone growth and negatively influencing bone mineral properties (Rourke et al., 2003; Kaulfers et al., 2011; Chaplais et al., 2020). Thus, designing lifestyle interventions for adolescents with OW/OB still undergoing linear growth that involves elements of weight management (not necessarily weight loss), including healthy eating and exercise, are critical for not only improving body composition and associated cardiometabolic risk but also for optimizing bone mass accrual (Rajjo et al., 2017).

Dairy products have been shown to increase bone accrual in adolescent youth (de Lamas et al., 2019; Wallace et al., 2021), a finding repetitively confirmed by whole-body BMD measures (Chan et al., 1995; Cadogan et al., 1997; Du et al., 2004; Lau et al., 2004) or specific regions [pelvis (Lau et al., 2004), trochanter (Merrilees et al., 2000), and tibia (Vogel et al., 2017)], and act synergistically with exercise training to improve whole-body BMD compared to each alone (Gómez et al., 2021). We recently conducted a randomized controlled trial in adolescent females with OW/OB that involved a 12-week lifestyle modification intervention consisting of nutritional counselling and thrice-weekly supervised exercise training (Calleja et al., 2020). Those that were given 4 servings/d of dairy products [i.e., the recommended dairy group (RDa)] had an increased daily intake of bone-supporting nutrients, including protein, vitamin D, calcium, phosphorous, and potassium, while those that maintained a low dairy intake [0–2 servings/d; i.e., the low dairy group (LDa)] did not. The RDa group also had reductions in fat mass and increased lean mass that were both greater than the LDa group (Calleja et al., 2020), suggesting a beneficial effect of increased dairy intake for body composition. Following the intervention, the RDa group had reductions in resting levels of the bone resorption marker C-terminal telopeptide of type 1 collagen (βCTX) compared to the LDa group, which is likely related to the increased intake of nutrients that support bone growth found in dairy products (Josse et al., 2020).

Acute exercise typically leads to a transient increase in markers of bone resorption (e.g., βCTX) and upstream catabolic osteokines (e.g., sclerostin), with generally a lack of change in markers of bone formation [e.g., N-terminal propeptide of type 1 procollagen (P1NP)] and bone turnover [i.e., osteocalcin (OC)], as well as upstream anabolic osteokines [i.e., osteoprotegerin (OPG)]. This temporal response in bone biochemical markers to acute exercise is thought to be essential for improving bone mineral properties by initiating resorption of old damaged bone and a subsequent, slightly delayed (i.e., not immediate), replacement with new osteoid/bone (Dolan et al., 2020; Dolan et al., 2022). Therefore, alterations in the regulation of this process may prevent bone mass accrual. Indeed, the acute responses of some bone biochemical markers have also been shown to be negatively influenced by adiposity (Kurgan et al., 2020). While our lab found no difference in the βCTX response to acute exercise between adolescent females with normal weight and OW/OB, we did observe a larger and more sustained increase in sclerostin in adolescent females with OW/OB (Kurgan et al., 2020). This post-exercise increase in sclerostin, which was only seen in adolescents with OW/OB, is similar to what is typically seen in adults. Concomitantly, this study also demonstrated that adolescents with OW/OB had consistently lower OC compared to adolescents with normal weight (Kurgan et al., 2020), possibly

indicating lower turnover rates (Dolan et al., 2020; Dolan et al., 2022). Indeed, previous studies report that obesity in adolescence may accelerate bone maturity at the expense of peak bone mass (De Leonibus et al., 2014; de Groot et al., 2017) and may even oppose the long-term bone benefits observed with exercise training in adolescents. Recent research has also demonstrated that long-term exercise training can blunt the acute exercise transcriptional program in muscle in normal weight adults (Norrbom et al., 2022). Similar adaptations may occur with bone. In contrast, exercise training may augment the bone response to acute exercise in adolescents with OW/OB given that they appear to have lower bone quality than adolescents with normal weight (Janicka et al., 2007; Dimitri et al., 2010). Thus, using a sub-group of participants from our main study (Calleja et al., 2020; Josse et al., 2020; Skelly et al., 2021), the present study aimed to determine whether the response of bone markers and osteokines, including βCTX, sclerostin, OC, OPG, and receptor activator of nuclear factor kappa B Ligand (RANKL), to acute exercise is altered after a 12-week lifestyle intervention involving nutrition counselling and exercise training in adolescent females with OW/OB, and whether increased dairy consumption could further influence the response. Understanding the impact of exercise training and dietary considerations on the short-term regulation of bone metabolism to an acute bout of exercise will provide insight into long-term adaptations to bone growth and development.

2 Materials and methods

2.1 Participants

This study represents a secondary analysis that answered a novel question from a previously published intervention (Calleja et al., 2020; Josse et al., 2020; Skelly et al., 2021). The present study includes the available blood samples from 30 (mean age 14.3 ± 2.0 years; range 10–18 years) out of 63 adolescent females who took part in a lifestyle modification, weight management parallel randomized controlled intervention trial entitled, "*Improving Diet, Exercise And Lifestyle (IDEAL) for Adolescents*," which was registered at ClinicalTrials.gov (NCT02581813), and was approved by our institution's Research Ethics Board (BREB file # 14-284). The *IDEAL for Adolescents* Study was a 12-week, diet and exercise intervention study in adolescent girls with OW/OB carried out in different waves from June 2016–October 2018. The primary purpose of the *IDEAL for Adolescents* Study was to assess the effect of consuming the recommended 4 servings/day of dairy products vs. a low dairy product diet (0–2 servings/day), along with mixed-mode exercise training as part of a weight management intervention, on body composition in female adolescents (aged 10–18 years) with OW/OB. The effects of the intervention on fasted, resting concentrations of selected markers of bone remodelling before and after the intervention have been published (Josse et al., 2020); however, the analyses presented herein on potential changes in the acute post-exercise response of bone markers and osteokines has not been previously published.

The *IDEAL for Adolescents* Study participants were recruited from the Niagara Region, Ontario, Canada. Eligibility required participants to be menarcheal, between the ages of 10 and 18 years, overweight (OW; ≥85–96 percentile BMI) or obese (OB; ≥97 percentile BMI) based on World Health Organization growth charts, low dairy consumers (0–2 servings per day), minimally active (activity 0–2 times per week), and otherwise healthy. Participants were excluded if they reported an allergy to dairy foods or lactose intolerance, were taking medications related to a chronic condition or one that affected bone health or were consuming vitamin or mineral supplements not prescribed by a physician. All eligible participants and their parents/guardians were invited to Brock University, where they provided informed assent and consent, respectively. The main CONSORT flow diagram has been previously published (Josse et al., 2020).

On their first visit to the lab, participants completed a general health questionnaire to document medical history and medication use. After all entry criteria were met, participants were stratified by BMI percentile (either OW/OB) and were randomly assigned (using a random number generator) to one of three different groups: recommended dairy (RDa), low dairy (LDa), or a no-intervention control using an unblocked random allocation ratio of 2:2:1. The no-intervention control group was not included in this study due to low sample size for the specific outcomes of interest (i.e., the bone markers' responses to acute exercise).

2.2 Study design and procedures

Details on the design and procedures of the *IDEAL for Adolescents* Study were previously published in Calleja et al. (2020). Briefly, before commencing the intervention, participants visited the laboratory for baseline testing. Tests included anthropometric measurements and the first set (i.e., baseline) of blood samples taken at rest and in response to an acute exercise bout (described in detail in the following section). Participants were also instructed on how to properly complete a 7-day food record, which was used to examine their habitual diet/nutrient intakes. After the initial visit, participants returned to the laboratory for an exercise introduction session, where the parent/guardian and participant met their personal trainer, reviewed the exercise program, and outlined the exercise schedule for the next 12 weeks. After the exercise introduction session, participants had their first diet consultation with a registered dietitian who reviewed the baseline 7-day food

record with them and their parent/guardian and gave instructions for beginning the dietary protocol. Participants in the RDa group were then provided with dairy products and general guidance regarding their consumption during this visit and every 2 weeks for the 12-week intervention.

2.3 Acute exercise session and blood sampling

The acute exercise bout was meant to examine the bone response to exercise; thus, we utilized a protocol that involves high-impact, weight-bearing loads, as we have done previously in adolescents (Falk et al., 2016; Dekker et al., 2017; Klentrou et al., 2018; Kurgan et al., 2020). Participants began the exercise session with a warm-up that included 5 min of low-intensity cycling (40 W of resistance) on a cycle ergometer. Once the warm-up was completed, participants were given a comprehensive explanation and demonstration of each of the circuits. The protocol included 120 jumps organized into five circuit training stations, which included box jumps (jump height was set at 25 cm), lunge jumps, tuck jumps, single-leg hopping and jumping jacks (MacKelvie et al., 2003). Each station included three sets of eight repetitions and participants were allowed 3 min of recovery between sets. In addition to the comprehensive explanation before beginning the exercise session, before beginning each station, participants were shown how to perform each exercise and familiarized themselves with each circuit type to ensure each jump was done with the correct technique during the trial to avoid injury, and promote full effort for consistency across trials. Each plyometric testing protocol was carried out by the same two researchers to ensure safety, technique, and effort were monitored.

Blood sampling occurred in the morning hours (between 0800 and 1100 h) to minimize any circadian rhythm variation in the serum biomarkers. Participants were asked to come to the laboratory fasted (~10–12 h) and to avoid any vigorous or high-impact exercise for at least 24 h before testing. Upon arrival to the laboratory, if requested, a topical anesthetic cream, Emla (25 mg/g lidocaine +25 mg/g prilocaine), was applied to the antecubital fossa of the participant's arm before blood sampling. Subsequently, height, weight and body composition were measured. Participants then sat down and rested for 10 min. This was followed by a rested, pre-exercise, fasted venous blood sample, which was drawn by a phlebotomist using a standard venipuncture procedure with a 23G butterfly needle and vacutainers [serum separator tubes (SST)] (cat#: 367983-1, BD, Mississauga, ON). Vacutainer tubes sat to clot (~20 min) before being centrifuged at 1400 RCF (xg) for 15 min at 4°C. Serum was separated and aliquoted into 1.5 ml polyethylene cryotubes that were stored at −80°C for analysis upon study completion. Following the resting blood sample, participants were provided with a standardized light breakfast (one small granola bar, one banana, and water). The breakfast was provided to ensure participants had adequate energy during the exercise session. It was also low in protein and calcium to not allow for the acute intake of these nutrients to influence the bone turnover response to acute exercise (Scott et al., 2012; Shams-White et al., 2017; Bhattoa, 2018). Within 20 min, they began the 30 min plyometric exercise protocol followed by two more blood samples at 5 min and 1 h post-exercise.

2.4 Exercise intervention

Participants in both groups (RDa and LDa) completed a structured, supervised exercise training program over the 12 weeks (×3/week). The exercise intervention was individualized and based on the principles of progressive loading whereby the exercise trainers would change exercise variables (i.e., load/reps/duration/speed) to maintain a constant exercise stimulus. Each session lasted between 60–90 min and began with a plyometric-based (jumping) warm-up for 5–10 min, followed by 20–30 min of aerobic training (on either a treadmill, cycle ergometer, elliptical or rowing ergometer) and either 20–30 min of resistance training using free weights and machines (2× per week), or plyometric exercises (1× per week). Upon completion, participants cooled down by stretching and walking. Participants consumed a drink immediately after each training session. The RDa group drank one cup (250 ml) of 1% chocolate milk, and the LDa group drank one cup of a non-dairy, vitamin D- and calcium-free, carbohydrate-based, electrolyte drink. On the days participants did not receive formal exercise training, they were encouraged to increase their physical activity to achieve a predetermined number of steps during their leisure time.

2.5 Dietary intervention

Dietary counselling by a registered dietitian was provided five times during the study (weeks 0, 2, 4, 8, 12) to each participant and their parent/guardian, individually. Energy requirements/expenditures were calculated for each participant using predictive equations from the Institute of Medicine for girls with OW/OB (Institute Of Medicine Food And Nutrition Board, 2005). This was used to prescribe a diet for weight maintenance (and not weight loss) based on the participant's age, height, and body mass. Participants were provided with an eating plan outlining how many servings from each food group they should consume [according to Canada's 2007 Food Guide (Health Canada, 2007)]. All participants were counselled on consuming a healthy diet of fruit, vegetables, high fibre foods, whole grains, lean meats, and meat alternatives. Participants were also asked to avoid processed foods, foods high in "bad" fats (trans and

TABLE 1 Age, anthropometric, body composition, and nutrient intake at pre- and post-intervention for the higher dairy intake (RDa) and the low dairy intake (LDa) groups.

Variable	RDa (n = 14)			LDa (n = 16)			p-values		
	Pre-Intervention	Post-Intervention	Δ	Pre-Intervention	Post-Intervention	Δ	Group	Intervention	Interaction
Age (years)	13.8 ± 1.4	—	—	14.5 ± 2.0	—	—	0.3	—	—
Age from PHV (years)	2.0 ± 0.9	2.2 ± 0.9	0.2	2.3 ± 1.0	2.4 ± 1.0	0.1	0.5	<0.001	0.6
Height (cm)	165.7 ± 6.2	166.3 ± 6.3	0.6	164.1 ± 6.3	164.6 ± 6.3	0.5	0.5	0.002	0.9
Body mass (kg)	78.4 ± 13.9	77.8 ± 11.7	−0.6	78.0 ± 13.7	77.9 ± 14.2	−0.1	>0.9	0.5	0.7
Fat Mass (kg)	29.4 ± 7.4	27.9 ± 6.1	−1.5	28.7 ± 7.3	27.8 ± 7.8	−0.9	0.9	0.004	0.5
Energy Intake (kcal)	1,729 ± 393	1,755 ± 253	26	1,575 ± 400	1,410 ± 239	−165	0.01	0.4	0.2
Protein intake (g·kg bm^{-1}·d^{-1})	0.90 ± 0.3	1.18 ± 0.3$^{*,\#}$	0.28	0.84 ± 0.3	0.90 ± 0.2	0.06	0.1	<0.001	0.008
Carbohydrate intake (g·kg bm^{-1}·d^{-1})	2.85 ± 0.9	2.72 ± 0.8	−0.13	2.57 ± 0.9	2.25 ± 0.7	−0.32	0.2	0.1	0.5
Fat intake (g·kg bm^{-1}·d^{-1})	0.92 ± 0.3	0.88 ± 0.2	−0.04	0.85 ± 0.4	0.74 ± 0.3	−0.11	0.3	0.2	0.5
Vitamin D (μg·d^{-1})	2.99 ± 1.4$^{\#}$	5.34 ± 1.2$^{*,\#}$	2.35	1.69 ± 1.1	1.67 ± 1.1	−0.02	<0.001	<0.001	<0.001
Calcium (mg·d^{-1})	794 ± 271$^{\#}$	1,306 ± 165$^{*,\#}$	512	513 ± 240	448 ± 162	−65	<0.001	<0.001	<0.001
Phosphorous (mg·d^{-1})	908 ± 368	1,477 ± 216$^{*,\#}$	569	771 ± 193	860 ± 174	89	<0.001	<0.001	<0.001
Potassium (mg·d^{-1})	1,697 ± 673$^{\#}$	2,413 ± 554$^{*,\#}$	716	1,588 ± 477	1,778 ± 402	190	0.03	<0.001	0.02

Data are reported as mean ± SD. Δ = post-intervention—pre-intervention in their respective units. A two-way RMANOVA, was used to examine main effects for group, intervention, and their "interaction" = group*intervention. *Bonferroni* correction was used for pairwise comparisons; * = p< 0.001 compared to pre-intervention for RDa, # = p< 0.05 for RDa, compared to LDa, at pre- or post-intervention; ANCOVA, was used for fat mass with change in body weight as the covariate.

some sources of saturated fat), sugar-sweetened beverages, pastries, and confections, and they were instructed not to take any vitamin or mineral supplements or fortified juices/drinks during the study.

The study was designed such that the RDa and LDa groups differed primarily in the source of protein they consumed, as the RDa group consumed half of their daily protein (~20% of total energy intake) from dairy sources. Specifically, for the duration of the intervention (12 weeks), the RDa group was provided with 4 servings/day of mixed dairy products including two cups of 1% milk (white and chocolate), 2 × 100 g cartons of 0% or 2% MF Greek yogurt (any flavour) and 42 g of full-fat cheddar or marble cheese. The LDa group maintained their low dairy intake of 0–2 servings/day and continued to consume protein from other sources including meat, egg, fish, chicken, legumes, and grains. They were also asked to refrain from consuming calcium-fortified beverages/foods. Thus, as per the study design (i.e., due to the provision of dairy foods), the RDa group should consume greater levels of bone-supporting nutrients, namely protein, vitamin D, potassium, phosphorus, magnesium, and calcium than the LDa group. Of note, the intakes of some of these nutrients were already higher in RDa compared to LDa at pre-intervention (Table 1). Energy intake was also higher in RDa which likely contributed to the greater micronutrient intakes at baseline.

2.6 Adherence

Adherence was calculated separately for the exercise and dairy components of the study as detailed in (Calleja et al., 2020). Briefly, adherence to the exercise training component of the intervention was calculated by comparing the number of exercise sessions attended to the number of sessions that were scheduled

and converting it to a percentage. Adherence to the consumption of the dairy products (or not) was based on self-reported average daily servings of dairy products consumed by the participants at weeks 4, 8, and 12. Consumption was monitored and verified by the dietitian using specific daily checklists. RDa participants were considered adherent if they consumed ≥3 of the 4 prescribed servings/day. LDa participants were considered adherent if dairy servings intake was ≤2/day.

2.7 Anthropometrics, body composition and maturity

Height (cm), seated height (cm), body mass (kg), and body composition (lean mass and fat mass) were assessed for each participant at weeks 0 and 12 by the same investigator. Standing and seated height were measured using a stadiometer (Seca 213 Portable Stadiometer, CME Corp., Warwick, RI) to the nearest 0.1 cm with light clothing and no shoes. Body mass was assessed using a standard scale (Digital Physician Scale, Rice Lake Weighing Systems, Rice Lake, WI). Body composition was measured as previously described (Calleja et al., 2020), using the BodyMetrix (BMX; BodyMetrix System, BX-2000, IntelaMetrix, Inc., Livermore, CA), a handheld device that utilizes amplitude-mode ultrasound technology to measure fat thickness. The somatic maturity offset (years from peak height velocity) was estimated using a sex-specific regression equation (Mirwald et al., 2002). This is a simple, non-invasive method of assessing somatic maturity in children using known differential growth measures of height, seated height, and leg length.

2.8 Food records

Participants provided 7-day food records at weeks 0 and 12 and 3-day food records at weeks 2, 4, and 8 before each dietetic counselling session to assess dietary intake, to track compliance with the nutrition protocol, and to provide guidance moving forward. Food records were analyzed using the Food Processor Diet analysis software program (ESHA Research, Inc. Salem, OR).

2.9 Serum bone biochemical markers

The βCTX (β-CrossLaps; cat#: 11972308 122) was measured from serum at the Mount Sinai Hospital Core Laboratory (Toronto, Ontario) using a Roche Cobas e602 automated analyzer. Lower and upper detection limits were 0.010–6.00 ng/ml (quality control standard CV: 4.8%). Serum concentrations of sclerostin, OC, OPG and RANKL were measured in duplicate with intra-assay and inter-assay coefficients of variation (CVs) measured in house. Sclerostin was measured using an enzyme-linked immunosorbent assay (ELISA; cat# DSST00; R&D, Minneapolis, MN). OC and OPG were measured using a microbead multiplex kit (cat# HBNMAG-51K-08, EMD Millipore, Darmstadt, Germany) and RANKL was measured using a microbead single-plex kit (cat# HRNKLMAG-51K-01, EMD Millipore, Darmstadt, German). The average intra-assay CVs for sclerostin, OC, OPG, and RANKL were 5.4, 5.6, 4.8, and 6.1%, respectively. The average inter-assay CVs for sclerostin, OC, OPG, and RANKL were 5.7, 6.4, 5.1, and 4.1%, respectively.

2.10 Statistical analysis

Results are presented as mean ± standard deviation (SD) for all Tables and Figures. For age, anthropometrics, and nutrient/energy intake variables, two-way repeated-measures ANOVAs (RMANOVA) were used to examine main effects for group (differences between RDa and LDa) and intervention (pre- and post-intervention response), as well as their interaction (group*intervention) to examine if the groups responded differently to the intervention. In case of a significant interaction, a *Bonferroni* correction was applied accounting for the four *post hoc* pairwise comparisons. For fat mass, a 2-way repeated measures ANCOVA was used with weight change as a covariate as we have previously done (Calleja et al., 2020). Biochemical data were assessed for normality using skewness and kurtosis. For absolute concentrations of biochemical markers, sclerostin, βCTX, and OC were normally distributed, while RANKL and OPG:RANKL were not, but normality improved following log transformation. Of a total of 174–180 samples, missing datapoints (n = 3) were replaced with the group, intervention, and timepoint-specific mean value. In addition, individual outlying datapoints were identified (>±3 SD) and replaced with the corresponding group, intervention, and timepoint-specific upper or lower 3 SD limit, which resulted in 0/180, 4/180, 0/180, 11/174, 4/174, and 0/180 replaced datapoints (174–180 total datapoints in the dataset) for sclerostin, OC, OPG, RANKL, OPG:RANKL, and βCTX concentration, respectively.

Three-way RMANOVAs were performed to examine main effects for group (differences between RDa and LDa), intervention (pre- and post-intervention response), and the time response of bone biochemical markers (absolute concentrations and relative change from pre-exercise) to acute exercise and their interactions. For main effects in the three-way RMANOVA, short-term response to the acute exercise bouts was the main effect of time, comparison between pre- and post-intervention was the main effect of intervention, and the difference between groups (RDa vs. LDa) was the main effect of group. We also examined their interactions, which included

TABLE 2 Serum concentrations of bone biochemical markers and osteokines at rest (pre-exercise) and in response to acute plyometric exercise at pre- and post-intervention in the higher dairy intake (RDa) and the low dairy intake (LDa) groups.

Protein	Group	Pre-intervention acute exercise response			Post-intervention acute exercise response			Significant interactions
		Pre	5 min	1 h	Pre	5 min	1 h	
Sclerostin (pg·ml^{-1})	RDa (n = 14)	153.8 ± 43.4	177.0 ± 58.1$^{\$}$	164.0 ± 43.8$^{\$}$	155.4 ± 31.5	152.7 ± 46.09$^{\&}$	140.8 ± 31.3$^{\#}$	intervention*time p = 0.008
	LDa (n = 16)	134.0 ± 49.6	175.4 ± 52.2$^{\$}$	160.7 ± 48.8$^{\$}$	151.0 ± 46.0	168.0 ± 52.3$^{\&}$	144.4 ± 35.3$^{\#}$	
Osteocalcin^{b} (ng·ml^{-1})	RDa (n = 14)	19.8 ± 8.0	18.5 ± 7.0	18.3 ± 7.3	20.6 ± 8.5	20.0 ± 8.2	20.2 ± 8.7	
	LDa (n = 16)	22.5 ± 12.2	19.9 ± 10.6	19.0 ± 9.2	22.2 ± 9.4	21.9 ± 9.5	20.5 ± 8.9	
OPG^{b,c} (pg·ml^{-1})	RDa (n = 14)	265.2 ± 84.5	271.6 ± 81.6	240.5 ± 53.4	281.6 ± 75.5	282.5 ± 64.7	272.3 ± 51.7	
	LDa (n = 16)	291.8 ± 105.9	284.7 ± 116.4	260.9 ± 79.6	272.6 ± 86.2	272.9 ± 88.1	272.6 ± 104.1	
RANKL^{b,c} (pg·ml^{-1})	RDa (n = 13)$^{\$}$	101.5 ± 147.9	116.6 ± 169.2	101.3 ± 149.1	113.9 ± 147.9	117.2 ± 157.6	96.0 ± 128.3	
	LDa (n = 16)	64.4 ± 57.3	61.3 ± 56.6	58.7 ± 59.9	63.4 ± 51.0	60.1 ± 44.4	51.7 ± 48.2	
OPG:RANKL (AU)	RDa (n = 13)$^{\$}$	5.0 ± 2.7	4.3 ± 2.2	4.6 ± 2.6	5.2 ± 5.5	4.9 ± 4.8	7.5 ± 9.5$^{\$\%}$	intervention*time p = 0.03
	LDa (n = 16)	7.5 ± 5.4	8.1 ± 6.2	9.7 ± 8.7	7.1 ± 6.1	6.7 ± 4.9	10.0 ± 8.6$^{\$\%}$	
βCTX^{a,b,c} (pg·ml^{-1}	RDa (n = 14)	941.6 ± 221.8	1,026.2 ± 219.5	804.2 ± 233.3	821.0 ± 217.8	912.7 ± 252.6	728.1 ± 208.7	
	LDa (n = 16)	942.1 ± 391.2	963.6 ± 395.3	702.5 ± 370.1	964.0 ± 419.8	988.8 ± 361.0	762.1 ± 311.3	

Data are reported as mean ± SD. OPG, osteoprotegerin; RANKL, receptor activator nuclear factor kappa B ligand, βCTX, C-terminal telopeptide of type 1 collagen. § = No serum available for one participant to measure RANKL. A three-way RMANOVA, was used to examine main effects for group, time, intervention and their interactions. When a main effect of time is present, pairwise comparisons across timepoints are shown for groups and pre-/post-intervention values combined: a = p< 0.05 5 min post-exercise compared to pre-exercise, b = p< 0.05 1 h post-exercise compared to pre-exercise, c = p< 0.05 1 h post-exercise compared to 5 min post-exercise independent of intervention and group. When an Intervention*Time interaction is present, pairwise comparisons for both groups at either pre- or post-intervention: $ = p< 0.05 compared to pre-exercise, % = p< 0.05 compared to 5 min post-exercise, & = p< 0.05 compared to 1 h post-exercise. # = p< 0.05 difference at that timepoint compared to the same timepoint at pre-intervention.

time*intervention, time*group, intervention*group, and time*intervention*group. Following significant main effects or interactions, pairwise comparisons using a *Least Significant Difference (LSD)* correction were assessed. For all statistical tests, significance was assumed at an alpha level of <0.05. Sphericity was assumed at p > 0.05, and if <0.05, the Greenhouse-Geisser correction was used. Analyses were performed using SPSS version 26.0 for Windows (SPSS, Chicago, Illinois, United States) and graphs were made in GraphPad Prism 9 (San Diego, CA, United States).

3 Results

Adherence to the scheduled exercise sessions of the RDa group was 86% ± 9% and the LDa group 79% ± 11%. All (100%) of the RDa participants reported consuming ≥3 servings/day of dairy products and 100% of the LDa participants reported consuming ≤ 2 servings/day of dairy products. Specifically, the combined dietary intake data from weeks 4, 8 and 12, showed RDa reported 3.8 ± 0.4 servings/day and LDa reported 0.3 ± 0.3 servings/day.

Table 1 presents pre- and post-intervention data for demographic, anthropometric, body composition, and nutrient intakes between LDa and RDa groups. There was a significant main effect for group in energy intake with no intervention effect or interaction, which was a result of the RDa group having higher energy intake compared to the LDa group both at pre-intervention (mean difference = +249 kcal) and post-intervention (mean difference = +344.8). While there was no difference between groups at either pre- or post-intervention in carbohydrate and fat intake, there was a group*intervention interaction for protein intake. This was due to RDa increasing their protein intake from pre-to post-intervention (+31%), while LDa had no difference. This increase resulted in the RDa group having a higher (p = 0.02) protein intake post-intervention compared to the LDa group (mean difference = +0.28). Based on our design, there were also group*intervention interactions showing that RDa increased intakes of micronutrients that are known to support bone growth in adolescents including vitamin D (+79%), calcium (+64%), phosphorous (+63%), and potassium (+42%) from pre-to post-intervention, while LDa had no changes in the intakes of these nutrients.

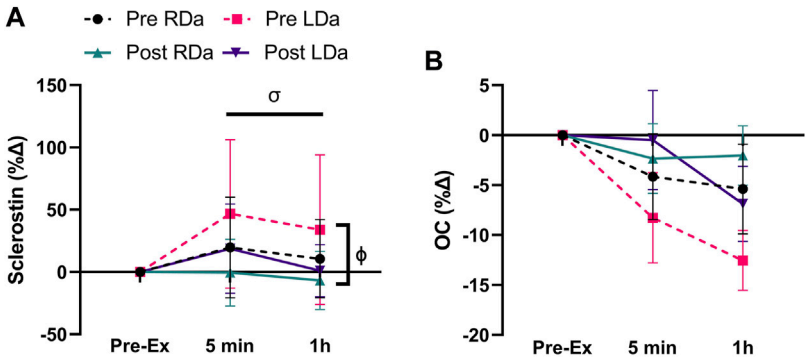

FIGURE 1
Serum sclerostin and osteocalcin (OC) percent change to acute exercise at pre- and post-intervention in both RDa and LDa groups: percent change of serum sclerostin **(A)** and OC **(B)** at 5 min and 1 h post-exercise relative to pre-exercise in both RDa (n = 14) and LDa (n = 16) groups. σ = main effect for time ($p < 0.05$) independent of intervention or group. ϕ = main effect for intervention ($p < 0.05$) independent of group. Data are reported as mean ± SD.

In terms of the biochemical markers (Table 2), there was an intervention*time interaction ($p = 0.008$), but no other significant interactions for sclerostin. Specifically, at pre-intervention, sclerostin increased from pre-exercise to 5 min ($p = 0.004$, +33.3 pg ml^{-1}) and remained higher than its pre-exercise level at 1 h post-exercise ($p = 0.02$, +20.4 pg ml^{-1}), while at post-intervention, there was a decrease in sclerostin from 5 min to 1 h post-exercise ($p = 0.02$, −19.2 pg ml^{-1}) irrespective of group (Table 2). For sclerostin percent change, there was no significant main effect for group and no interactions. However, main effects were found for intervention ($p = 0.03$, −25%), and time ($p = 0.04$, +11% from pre to 5 min post-exercise), reflecting an overall decrease in the relative post-exercise sclerostin response pre-to post-intervention (Figure 1A).

For total OC, a main effect was found for time ($p = 0.005$) but no significant main effects for intervention or group, and no significant interactions. Thus, the time effect reflects a small but significant decrease from pre-to 1 h post-exercise ($p = 0.001$, −1719.9 pg ml^{-1}) in both groups and pre/post-intervention combined (Table 2). OC percent change showed no significant main effects or interactions (Figure 1B).

We found no significant main effects for intervention or group for OPG, and no significant interactions (Table 2). There was a main effect for time ($p = 0.01$), reflecting a decrease in OPG at the 1 h post-exercise timepoint compared to pre-exercise ($p = 0.02$, −16.2 pg ml^{-1}) and 5 min post-exercise ($p = 0.02$, −16.4 pg ml^{-1}) in both groups and pre-/post-intervention combined (Table 2). OPG percent change showed no significant main effects or interactions (Figure 2A).

RANKL showed a significant main time effect ($p < 0.0001$) but no intervention or group effects, and no significant interactions (Table 2). Pairwise comparisons for time identified no difference in RANKL between pre- and 5 min post-exercise, while it was lower at 1 h post-exercise compared to both pre-exercise ($p < 0.001$, −8.9 pg ml^{-1}) and 5 min post-exercise ($p < 0.001$, −11.9 pg ml^{-1}) in both groups and pre-/post-intervention combined (Table 2). However, RANKL percent change showed an intervention*group interaction ($p = 0.005$), which was the result of RDa having a relatively positive percent change following acute exercise compared to LDa at pre-intervention ($p = 0.003$, +25%). However, the relative responses of RDa and LDa at post-intervention were not different. Specifically, the RANKL percent change response to acute exercise had a net decrease from pre-to post-intervention in the RDa group ($p = 0.003$; −22%) whereas LDa's percent change response to acute exercise was unchanged. There was also a main effect for time ($p < 0.001$, −19%), but with no other significant interactions (Figure 2B).

We found an intervention*time interaction ($p = 0.03$) for the OPG:RANKL ratio reflecting no changes in OPG:RANKL from pre to post-exercise at pre-intervention, while the ratio was higher 1 h post-exercise compared to pre-exercise ($p < 0.001$, +2.6 AU) and 5 min post-exercise ($p < 0.001$, +2.9 AU) post-intervention in both groups (Table 2). No other significant interactions were found. However, the OPG:RANKL ratio percent change showed a main effect for time ($p < 0.001$, +20%) and an intervention*group ($p = 0.003$) interaction, but no other significant interactions. Pairwise comparisons for the intervention*group interaction showed that at pre-intervention, the relative OPG:RANKL ratio was lower in the RDa compared to LDa group ($p = 0.01$, −22%) but was not different post-intervention, and that the relative acute response of the OPG:RANKL ratio to exercise increased post-intervention only in RDa ($p = 0.001$; +21%) whereas it did not change in LDa (Figure 2C).

Finally, βCTX concentration showed a main time effect ($p < 0.001$) but no significant main effects for intervention or group and no significant interactions (Table 2). Pairwise comparisons for time identified an increase from pre- to 5 min post-exercise ($p = 0.001$, +63.5 pg ml^{-1}), while 1 h post-exercise βCTX was lower than both pre-exercise ($p < 0.001$, −187.1 pg ml^{-1}) and

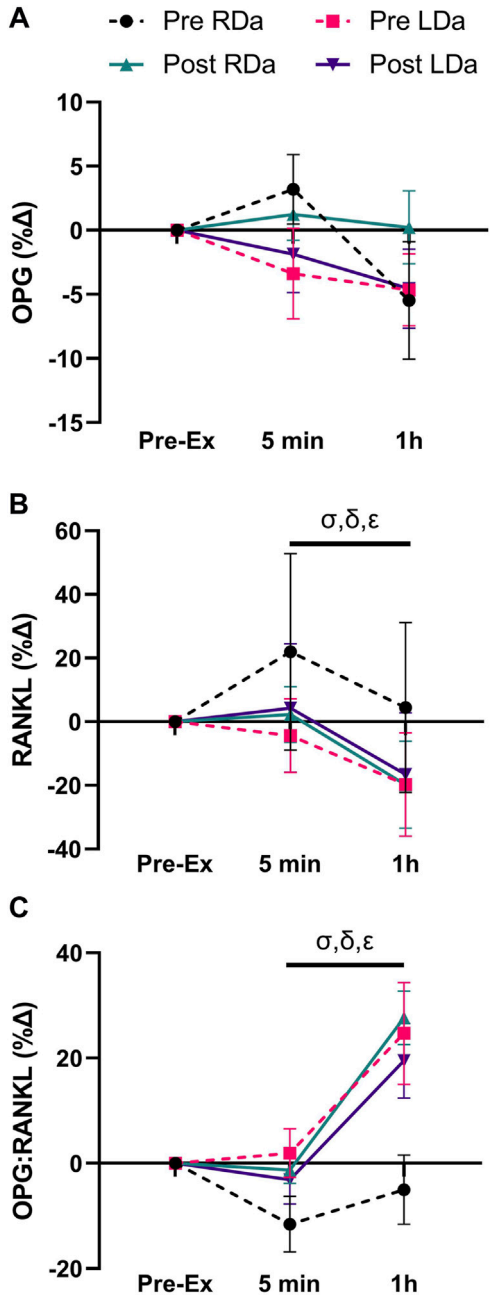

FIGURE 2
Serum osteoprotegerin (OPG), receptor activator nuclear factor kappa B ligand (RANKL), and OPG:RANKL ratio percent change to acute exercise at pre- and post-intervention in both RDa and LDa groups: percent change of serum OPG **(A)**, RANKL **(B)**, and OPG:RANKL ratio **(C)** at 5 min and 1 h post-exercise relative to pre-exercise in both RDa (n = 14, 13 and 13, respectively) and LDa (n = 15 for all markers) groups. σ = main effect for time ($p < 0.05$) independent of intervention or group. Following the intervention*group interaction, δ = pairwise comparison between RDa and LDa at pre-intervention ($p < 0.05$), ε = pairwise comparison between pre- and post-intervention in RDa only ($p < 0.05$). Data are reported as mean ± SD.

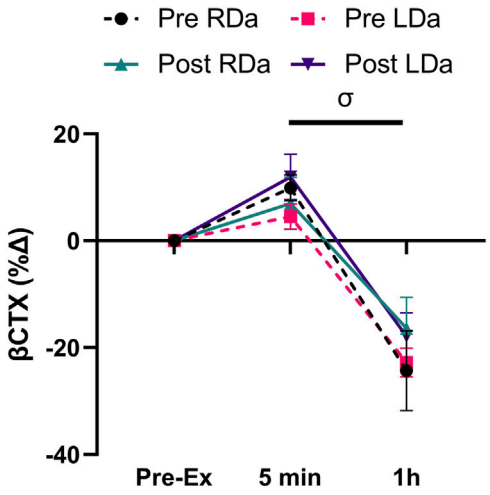

FIGURE 3
Serum C-terminal telopeptide of type 1 collagen (βCTX) percent change to acute exercise at pre- and post-intervention in both RDa and LDa groups: percent change of serum βCTX at 5 min and 1 h post-exercise relative to pre-exercise in both RDa (n = 14) and LDa (n = 16) groups. σ = main effect for time ($p < 0.05$) independent of intervention or group. Data are reported as mean ± SD.

5 min post-exercise ($p < 0.001$, −250.6 pg ml^{-1}) in both groups and pre-/post-intervention combined. For βCTX percent change, there was a main effect for time ($p < 0.001$, −28.4%) but no main effects for intervention or group and no significant interactions (Figure 3).

4 Discussion

This is the first study to compare the influence of a 12-week lifestyle modification (dietary counselling and exercise training) intervention on the response of bone biochemical markers and regulators to acute exercise, and to specifically isolate the effect of increased dairy intake during the intervention. The 12-week intervention, independent of dairy consumption, reduced the sclerostin response and augmented the OPG:RANKL ratio response to acute exercise. Dairy intake during the intervention influenced the relative RANKL and OPG:RANKL ratio responses to exercise in the RDa group, bringing this group's RANKL response closer to the LDa post-exercise response, which was lower at pre-intervention. Aside from this, increased dairy consumption, and subsequently increased habitual intake of bone-supporting nutrients, during the intervention did not further influence the regulation of bone markers in response to acute exercise. Thus, our results suggest that a lifestyle intervention of healthy eating and exercise training

for weight management in adolescents with OW/OB may not influence the short-term response of bone turnover markers (βCTX and OC) to acute exercise but may influence upstream regulators of bone turnover (i.e., osteokines including sclerostin and OPG:RANKL).

In this study, we showed that sclerostin increased immediately post-exercise and remained elevated at 1 h post-exercise at pre-intervention in adolescent females with OW/OB. The pre-intervention response was similar to our previous findings (Kurgan et al., 2020), which showed adolescent females with OW/OB had a transient post-exercise increase in sclerostin that is more characteristic of the adult bone response (Kouvelioti et al., 2019), while normal weight age-matched controls had no response in sclerostin to acute exercise. However, following 12 weeks of a lifestyle intervention, the transient post-exercise increase in sclerostin, which we initially observed in those with OW/OB, was blunted, independent of dairy intake. This new finding is important because sclerostin is known to inhibit bone formation (Poole et al., 2005), suggesting that during adolescence, when peak bone mass is accrued, the transient post-exercise increase in sclerostin is likely a maladaptation related to OW/OB. Thus, our 12-week intervention involving diet modification and exercise training, corrected this perturbation/maladaptation in our group of adolescent females with OW/OB. It has been previously shown *in vivo* (Robling et al., 2008) and *in vitro* (Spatz et al., 2015) that osteocyte sclerostin expression decreases with long-term mechanical loading. This finding is likely why other researchers have observed a negative association of circulating sclerostin with long-term exercise training in humans (Amrein et al., 2012) and potentially why we see a difference in the response to acute exercise in this study. Our lifestyle intervention also induced a decrease in fat mass (and an increase in lean mass) in both groups. Indeed, observational studies have found circulating sclerostin to be positively correlated with fat-free mass in adult females (Sheng et al., 2012). Additionally, while we found no response in whole-body measures of insulin resistance in our main IDEAL Study cohort [reported elsewhere (Skelly et al., 2021)], it is important to note that serum sclerostin levels appear to be elevated in individuals with prediabetes and correlate with insulin resistance in skeletal muscle, liver, and adipose tissue (Daniele et al., 2015). Additionally, in our previous assessment of adolescent females, we found those with OW/OB had a higher and more sustained increase of insulin following consumption of a high carbohydrate breakfast and acute plyometric exercise compared to those with normal weight and this difference mimicked the differential response of sclerostin between groups (Kurgan et al., 2020). Therefore, tissue-specific changes in metabolism/insulin sensitivity, not detected by our whole-body resting measures, throughout this study may also explain the changes we observed in sclerostin regulation to exercise. We propose that alterations in fat mass and the subsequent metabolic and biochemical changes that occur in tandem can govern sclerostin content within the bone microenvironment and influence the post-exercise response. Further studies are needed to identify the factors regulating the changes in the response of sclerostin to acute exercise and the biological significance of this adaptation (e.g., long-term influence on tissue growth and metabolism).

Given sclerostin is a critical regulator of osteoblast activity, we next examined OC, an enzymatic marker of bone turnover and osteoblast activity (Lee et al., 2007). OC has previously been shown to transiently decrease in response to acute exercise, suggesting that osteoblasts contribute less to the acute bone response to exercise (Dolan et al., 2020). Findings from this study provide evidence of no influence of a lifestyle modification intervention involving diet modification and exercise training on the regulation of OC following acute exercise. This finding was expected, as previous studies in normal-weight adults (Scott et al., 2010) and children (Pomerants et al., 2008; Theocharidis et al., 2020) rarely detect increases in bone formation (i.e., P1NP) following acute exercise (Dolan et al., 2020). In addition, this analysis only included a measure of total OC, and not the assessment of other proteoforms of OC (e.g., undercarboxylated) which are known to influence metabolism (Mizokami et al., 2013; Rehder et al., 2015; Tsuka et al., 2015; Gao et al., 2016; Guo et al., 2017; Mukai et al., 2021). Taken together, our study does not support the effect of a diet and exercise intervention influencing OC's response to acute exercise in adolescent females with OW/OB.

The bone resorption marker, βCTX, increased immediately after acute exercise with a subsequent dip at 1 h post-exercise, which was not influenced by the intervention or level of dairy intake. This response of βCTX mimics data from adults (Dolan et al., 2020; Dolan et al., 2022) and contrasts our previous analysis that found a progressive reduction at 5 min and 1 h post-exercise in adolescents with either normal weight or OW/OB (Kurgan et al., 2020). Furthermore, we also did not find an effect of dairy consumption on the response of βCTX to acute exercise, despite RDa having increased habitual protein, vitamin D, calcium, phosphorous, and potassium intake while LDa had no change. This suggests that exercise training and increasing habitual intake of bone anabolic nutrients, at least in the shorter term (12-weeks) and in this cohort, does not influence the immediate response of βCTX to acute exercise.

Absolute concentrations of OPG and RANKL decreased slightly in response to acute exercise and this response was not influenced by either the intervention or dairy intake. However, our percent change data identified RANKL's acute response to exercise adapted differently in the RDa and LDa groups, with only the RDa group having a lower relative RANKL response to acute exercise at post-intervention compared to pre-intervention indicating that dairy intake during the intervention led to a lesser bone resorptive response post-

intervention in RDa. Likewise, we observed an effect of dairy intake on the percent change of OPG:RANKL, reflecting that the RDa group increased their relative anabolic response of this pathway from pre-to post-intervention. However, this interaction resulted from a pre-intervention difference in the percent change response between RDa and LDa. Thus, we acknowledge that it is also possible that the differential response between RDa and LDa to the intervention could be driven by the pre-intervention difference rather than the influence of dairy during the intervention.

Our study had several limitations. A significant limitation is that the control group was not included in the analysis (due to a very low number of control participants completing the two acute exercise bouts). Despite the short duration (12 weeks) of the intervention, the exclusion of the control group has implications for the current data as we are unable to account for the effect of general growth and maturation on our outcomes. There were also several statistical trends in main effects and interactions, including those for RANKL, OPG:RANKL, and βCTX (not described herein), which were likely a result of the small sample size. We also lack timepoints that extend past 1 h post-exercise. Indeed, some of these markers are transiently sensitive days following acute exercise (Sale et al., 2015), while we have previously shown that others return to baseline levels around 1 h post-exercise (Falk et al., 2016; Dekker et al., 2017; Kouvelioti et al., 2019; Kurgan et al., 2020). While we endeavoured to include a 24 h timepoint, ~30% of participants did not return for sampling at 24 h resulting in too many missed data points for this timepoint to be included in our analysis. An intrinsic limitation of this study (and other similar studies measuring osteokines in human blood) is that we cannot decipher the extent to which sclerostin, OPG and RANKL measured in serum reflect bone tissue protein levels (it is not possible or feasible to obtain bone biopsies). Lastly, due to specific concerns in this population, we provided them with a small, consistent breakfast high in carbohydrates before their acute exercise session, which is known to blunt bone turnover (Sale et al., 2015; Townsend et al., 2017). This may have reduced some of the acute responses and prevented the identification of key differences (Sale et al., 2015; Townsend et al., 2017). It is important to note that we have used this same pre-exercise breakfast across studies within our lab (Falk et al., 2016; Dekker et al., 2017; Kouvelioti et al., 2019; Kurgan et al., 2020) to allow for appropriate comparison between studies. While there are several limitations, this study provides unique insight into bone metabolism by examining the response of bone biochemical markers to acute exercise both pre- and post-intervention in a group of adolescent females with OW/OB. This unique, complex, study design led to the identification of proteins that adapted to the acute stress of exercise following our lifestyle modification intervention that the assessment of resting values may not otherwise identify (Josse et al., 2020). Future studies should assess the response of bone turnover biomarkers and upstream osteokine regulators to acute exercise pre- and post-exercise training across different age groups, levels of adiposity, levels of fitness (e.g., sedentary to high-performance athletes), and in disease/clinical populations (e.g., metabolic syndrome) to provide further context to our findings.

5 Conclusion

This study utilizes a unique design to add to a body of literature examining the effects of exercise and nutrition (specifically, dairy intake) on bone remodelling and metabolism in adolescent females with OW/OB. We found that our lifestyle modification intervention of healthy dietary counselling and exercise training (3×/week) blunted the increase in sclerostin and augmented the increase in OPG:RANKL following acute exercise, while dairy product consumption did not further influence these responses. To our knowledge, there is no previous study designed to investigate the influence of a lifestyle modification intervention for weight management with higher dairy intake vs. low dairy intake on the acute bone metabolic response to exercise, particularly in this demographic. These findings are critical to furthering our understanding of the influence of OW/OB on bone metabolism in adolescent females, which dietary factors influence bone metabolism and the mechanisms that lead to improved bone growth and achievement of peak bone mass with multiple bouts of acute exercise (i.e., exercise training).

Data availability statement

The raw data supporting the findings of this study are available from the corresponding author ARJ, upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by the Brock University Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AJ and PK conceived and designed the study and obtained funding. NK and IL collected and organized the samples. NK performed blood analysis. NK and LS performed data analysis. All authors contributed to the interpretation of the results. NK drafted the manuscript and did the subsequent revisions. All authors edited and revised the manuscript and approved the final version submitted for publication.

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Conflict of interest

AJ and PK report grants for this research from Dairy Farmers of Canada, US National Dairy Council (Dairy Management Inc.), and non-financial support from Danone and Parmalat during the conduct of the study. LS reports some salary support from the US National Dairy Council. AJ reports consultant/speaker fees from Dairy Farmers of Canada Grant Review Board outside of this work. AJ and LS report additional grant funding from Dairy Farmers of Canada and non-financial support from Lactalis Canada outside of this work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effects of Tai Chi on physical function and safety in patients with rheumatoid arthritis: A systematic review and meta-analysis

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Background: Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease that results in the destruction of joints, connective tissues, muscle, tendons and fibrous tissue. Until now, there are no cure therapies.

Objective: We aimed to assess the effectiveness of Tai Chi (TC) on RA patients by meta-analysis.

Methods: The PubMed, Cochrane Library, EMBASE, web of science, China National Knowledge Infrastructure and Google Scholar were searched up to January 2023. We included randomized controlled trials (RCTs) or controlled clinical trials (CCTs) comparing TC to control conditions for RA patients. Review Manager (Version 5.3) software was used to analyze outcomes of time to walk 50 feet, joint tenderness, number of swollen joints or tender joints, handgrip strength, pain, the Health Assessment Questionnaire (HAQ) and withdraws overall.

Results: A total of 351 patients with RA from six RCTs and three CCTs were included for meta-analysis. TC could also significantly decrease withdrawals overall in studies (OR = 0.28, 95% CI 0.12 to 0.67, p = 0.002). No significant treatment effects of physical function were identified of the other outcomes.

Conclusion: Our findings indicated that TC was safe to RA patients, but it cannot improve physical function and pain. However, there is still lack of more evidence.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=367498], identifier [CRD42022367498].

KEYWORDS

physical exercise, arthritis, pain, joint tenderness, swollen joints, health assessment questionnaire

Abbreviations: CCTs, controlled clinical trials; CIs, confidence intervals; HAQ, health assessment questionnaire; MD, mean difference; OR, odds ratio; RA, rheumatoid arthritis; RCTs, randomized controlled trials; TC, Tai Chi.

Introdution

Rheumatoid arthritis (RA) is a prevalent disease with incidence by 8.2% (Finckh et al., 2022). RA presents a systemic inflammatory autoimmune disease that destroys the joints, connective tissues, muscle, tendons and fibrous tissue. The accurate aetiology of RA is still ambiguous, but it is well known that the development of RA is associated with genetic susceptibility, environmental factors and immune response (Scherer et al., 2020; Testa et al., 2021). RA is often progressive and primarily involves the pain, stiffness and swelling of joints (Han et al., 2004). Some extra-articular manifestations also usually happen, such as cardiovascular disease, respiratory disease, central and peripheral nervous system (Figus et al., 2021). When compared to the general population, those with RA have a 50% greater risk of cardiovascular death (Finckh et al., 2022). RA brings a substantial burden for both the individual and society, because of decline in physical function, quality of life, work capacity and societal participation, and major direct medical costs (Hsieh et al., 2020). Current therapeutic approaches for RA includes pharmacological and non-pharmacological approaches. Pharmacological methods refer to disease-modifying antirheumatic drugs, non-steroidal anti-inflammatory drugs, glucocorticoids and biological drugs (Fraenkel et al., 2021). Regarding non-pharmacological approaches, such as exercise, education, psychological and self-management therapies for RA patients were found to be beneficial in improving non-inflammatory symptoms (mainly functional disability, pain and fatigue) (Roodenrijs et al., 2021). However, no cure is currently available for RA (Nagy et al., 2022).

Recently, several clinical studies and systematic reviews suggested that physical activity attenuates inflammation, cardiovascular risk, psychological health and sleep in RA patients (Metsios et al., 2015; McKenna et al., 2017; Pope, 2020). As a mitigatory therapeutic exercise, Tai Chi (TC) has been practiced for centuries as a martial art in China. At the same time, it has been drawn more and more attention. After introduced to Europe and America, the viewpoints of TC shifted and it is nowadays well-known as a kind of exercise to treat patients with knee osteoarthritis (Wang et al., 2016). TC consists of a series of slow and purposeful movements that involve turning, shifting one's weight from one leg to the other one, bending and unbending the legs with various arm movement, which is benefit for balance, flexibility, strength and function of human beings (Wu et al., 2004).

In RA, TC appears safe (Christie and Fongen, 2005) and improves pain and functional status of RA (Kirsteins et al., 1991; Wang et al., 2005; Wang, 2008). A review in year of 2004 by Han (Han et al., 2004) suggests that TC is beneficial on lower extremity range of motion for RA patients. However, in Han's review the three included studies were only up to December 2003. Another review in year of 2019 by Mudano (Mudano et al., 2019) showed that it was uncertain whether TC had any effect on joint pain, activity limitation or function in RA, and important effects cannot be confirmed or excluded since all outcomes had very low-quality evidence. Nevertheless, an overview of systematic reviews suggests that clinical improvement of TC is achieved, although not statistically significant with regard to pain and disease pattern (Imoto et al., 2021). Additionally, a clinical study published in 2020 is not included in any systematic reviews or meta-analysis (Liang, 2020). Thus, the effectiveness of TC for RA is still considered unproven, because of lack of enough convincing evidence. Therefore, the aim of this study was to conduct a systematic review and meta-analysis for exploring effectiveness of TC and summarizing the existing literature.

Materials and methods

The work was reported in line with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Page et al., 2021) and registered in PROSPERO (registration identification: CRD42022367498; website: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=367498).

Search strategy

The search strategy was made by two reviewers (HYW and QW). They searched the following electronic databases (up to January 2023): PubMed, Cochrane Library, EMBASE, web of science, China National Knowledge Infrastructure and Google Scholar. The search strategy included "Tai Chi," "Tai-Chi Chuan", "Taiji" and "rheumatoid arthritis". HYW manually screened conference proceedings (such as the International League of Associations for Rheumatology, the Chinese Rheumatology Association, and Chinese Journal of Rheumatology) and files from our department as supplemental material. Details of the English search strategy were shown in the Supplementary Appendix S1.

Inclusion criteria

All studies searched were imported into Endnote X9. Firstly, two reviewers (HYW and QW) screened the titles and abstracts relevant to TC for patients suffering from RA independently. Then still independently these two reviewers read full articles and identified whether the study to be included or not according to the following inclusion criteria. Disagreements were solved by JHW. All the reviewers were trained together to fully understand the inclusion criteria, exclusion criteria and using method of Endnote software before starting selection.

Participants

Participants were adults (16 years of age and older) suffering from RA. Patients were diagnosed by rheumatologists or clinicians in the department of rheumatology.

Intervention and comparison

The eligible trials should be TC therapy which compared with no therapy, usual care, sham therapy or any active treatment. Different types of TC protocol and co-interventions were allowed. Additionally, there were no limitations of the frequency of TC exercise, time of every intervention or the duration of trials.

Outcomes

1 Main outcomes (physical function): Time to walk 50 feet, joint tenderness, number of swollen joints or tender joints, handgrip strength, pain and HAQ.

2 Additional outcome (safety): Withdrawals overall.

Study design

Randomized controlled trials (RCTs) and controlled clinical trials (CCTs) were considered whether published or not in this review. Studies were included without language limitations.

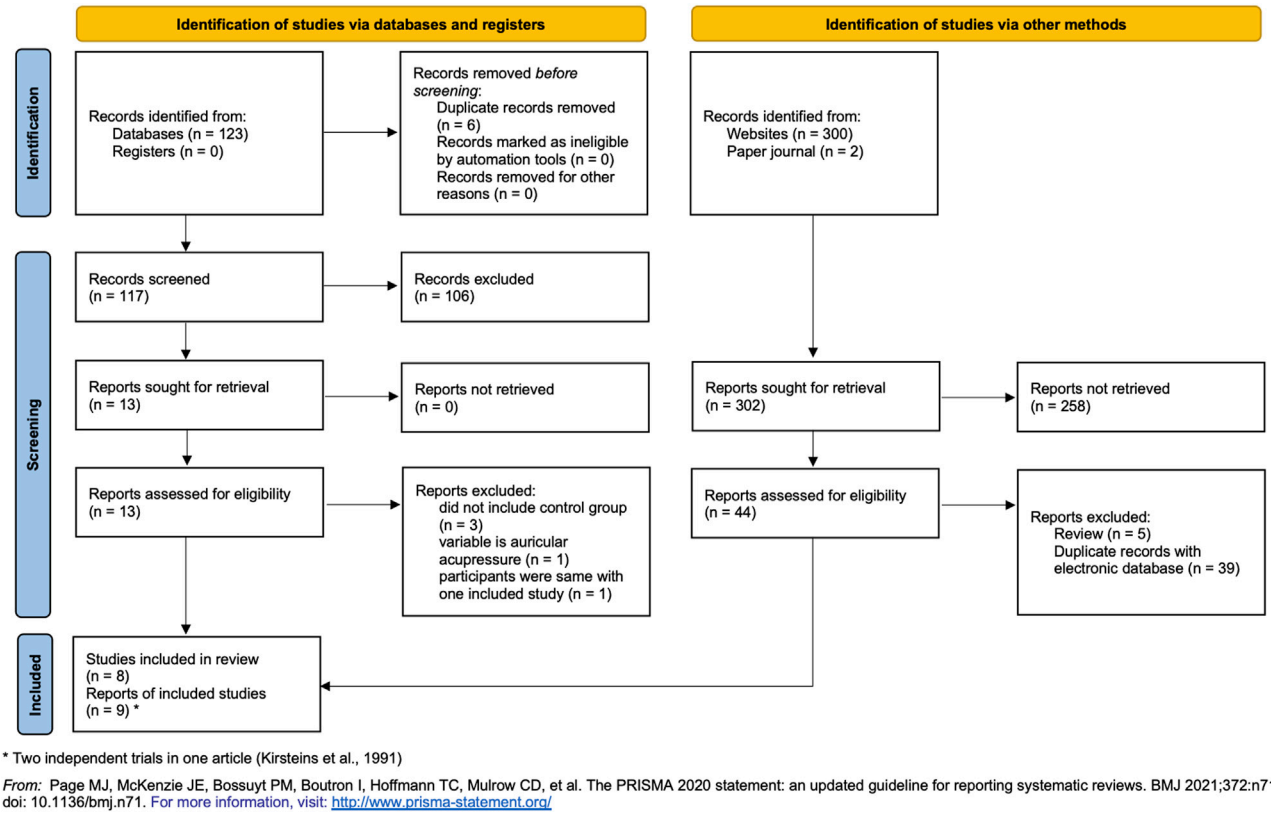

FIGURE 1
Flowchart of trial selection process. * Two trials in one article (Kirsteins et al., 1991).

Risk of bias and quality assessment

The risk of bias was assessed using Review Manager software (Version 5.3.5, The Nordic Cochrane Centre, Copenhagen; available from: http://community.cochrane.org) and the 2011 revised Guidelines and Handbooks for Systematic Reviews in the Cochrane Back Review Group (Cumpston, 2011) by two reviewers (HYW and GWW). This handbook recommended seven quality criteria, each of which was rated with yes, no or unclear. Details of seven quality criteria were as follows: Random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias. Disagreements were solved by a third party (YRW). A study would not be excluded even with a high risk, but it might degrade our confidence to recommend this cure strategy.

Data extraction and meta-analysis

Two reviewers (HYW and QW) extracted data from the included studies independently by a pre-pilot standardized form, which included first authors' last names, publication years, types of studies, characteristics of interventions and participants (included TC and comparison groups), outcome measures of effectiveness (efficacy of functional and clinical outcomes) and safety (withdrawals overall), methodological qualities, allocation concealments and durations of studies. Disagreements were solved by a third investigator (JHW) with discussion.

The extracted data were divided into two parts: characteristics of studies were shown in a table, outcome measures of effectiveness and side effects were imported into the Review Manager software for performing meta-analysis. The outcomes of effectiveness data in the TC and control groups were used to estimate the mean difference (MD) and 95% confidence intervals (CIs). The outcomes of safety data were in terms of odds ratio (OR). All reported values were two sided and $p < 0.05$ was considered to be statistically significant. All the data was performed on the Review Manager software by one reviewer (HYW).

Regarding the methodological (methodology of included studies) and clinical (clinical characteristics of the participants) heterogeneity, we evaluated as not homogeneous due to different intervention periods and various countries of subjects. Based on these, random-effect model was used to perform the analysis.

Results

Study selection

After searching the electronic databases, websites (Google Scholar) and paper sources, we collected 425 articles. However, in the electronic databases 106 articles were excluded based on titles and abstracts after duplicates removed, only 13 records were

TABLE 1 Characteristics of included studies.

Author	Design	Participants	Interventions	Comparison	Outcomes
Kirsteins 1991-1	CCT	47 adults (age 37–70 years, 42 females and 5 males) with RA. 25 patients in TC group and 22 in control group	Series of 15 movements extracted from Yang Style TC	Usual activities without TC	Joint tenderness, written functional, number of swollen joints, time to walk 50 feet, handgrip strength, safety
		Inclusion criteria: ambulatory adults with RA after age 18 and on a stable regimen of medications for a sufficient time for maximal results	Frequency: Once per week for 10 weeks, for 60 min sessions		
Kirsteins 1991-1	CCT	28 adults (age 38–72 years, 21 females and 7 males) with RA. 18 patients in TC group and 10 in control group	Series of 15 movements extracted from Yang Style TC	Usual activities without TC	Joint tenderness, written functional, number of swollen joints, time to walk 50 feet, handgrip strength, safety
		Inclusion criteria: Ambulatory adults with RA after age 18 and on a stable regimen of medications for a sufficient time for maximal results	Frequency: Twice per week for 10 weeks, for 60 min sessions		
Lee 2005	RCT	31 adults (age >30 years, all females) with RA. 16 patients in TC group and 15 in control group	Frequency: Once per week for 6 weeks, for 60 min sessions	Usual activities without TC	Pain (VAS) Mood (Profile of Mood State)
		Inclusion criteria: diagnosed RA in Dong-A University			Fatigue
Lee 2006	CCT	61 adults (All married females) with RA. 32 patients in TC group and 29 in control group	Frequency: Once per week for 12 weeks, for 50 min sessions	Usual activities without TC	Pain (VAS)
		Inclusion criteria: diagnosed RA in Dong-A University, no movement restrictions			Fatigue
Liang 2020	RCT	20 adults (age 30–65 years, 16 females and 4 males) with RA. 10 patients in TC group and 10 in control group	Frequency: Once everyday for 12 weeks, for 50 min sessions	Usual oral medicine treatment	HAQ, ESR, and CRP, number of swollen joints
		Inclusion criteria: Diagnosed RA according to 2010 ACR criteria			
Shin 2015	RCT	43 adults (age>50 years) with RA. 29 patients in TC group and 14 in control group	Twelve Movement TC	Received information about lifestyle modification and advice about appropriate regular exercises	Number of swollen joints and tender joints, HAQ, ESR, and CRP
		Inclusion criteria: more than 50 years old, sedentary lifestyle (no participation in structured exercise for the preceding 6 months), and stable disease (no changes in disease-modifying anti-rheumatic drugs or steroid in the last 3 months)	Frequency: Once per week for 3 months, for 60 min sessions		
Van Deusen 1987	RCT	33 adults (age 29–80 years) with RA. 17 patients in TC group and 16 in control group	TC ROM Dance program (including health education)	Rested at home, received a brochure which explained the program but no specific instructions	Shoulder flexion, shoulder internal and external rotation, wrist extension and flexion, ankle plantar flexion, lower extremity flexion, safety
		Inclusion criteria: ambulatory adults with RA who had medical recommendations for home rest and exercise and no prior ROM Dance experience	Frequency: Once per week for 8 weeks, for 90 min sessions		
Wang 2008	RCT	20 adults (age > 18 years) with RA. 10 patients in TC group and 10 in control group	Yang style TC	Usual physical activities, but not to participate in additional strength training other than class stretching exercises	ACR 20 response criterion, functional capacity, health-related quality of life and depression index
		Inclusion criteria: adults with functional class I or II RA (ACR criteria)	Frequency: Twice per week for 12 weeks, for 60 min sessions		
Zhu 1999	RCT	68 adults (age 16–56 years) with RA. 35 patients in TC group and 33 in control group	Oral San Bi recipe and exercise (slow running, walk, gymnastics and TC)	oral San Bi recipe in the same way but no exercise	safety
		Inclusion criteria: adults diagnosed with RA (ACR criteria)	Frequency: Once a day for 2 months, for 60 min sessions		

CCT, non-randomized controlled clinical trial; yrs, years; RA, rheumatoid arthritis; TC, Tai Chi: RCT, randomized controlled trial.

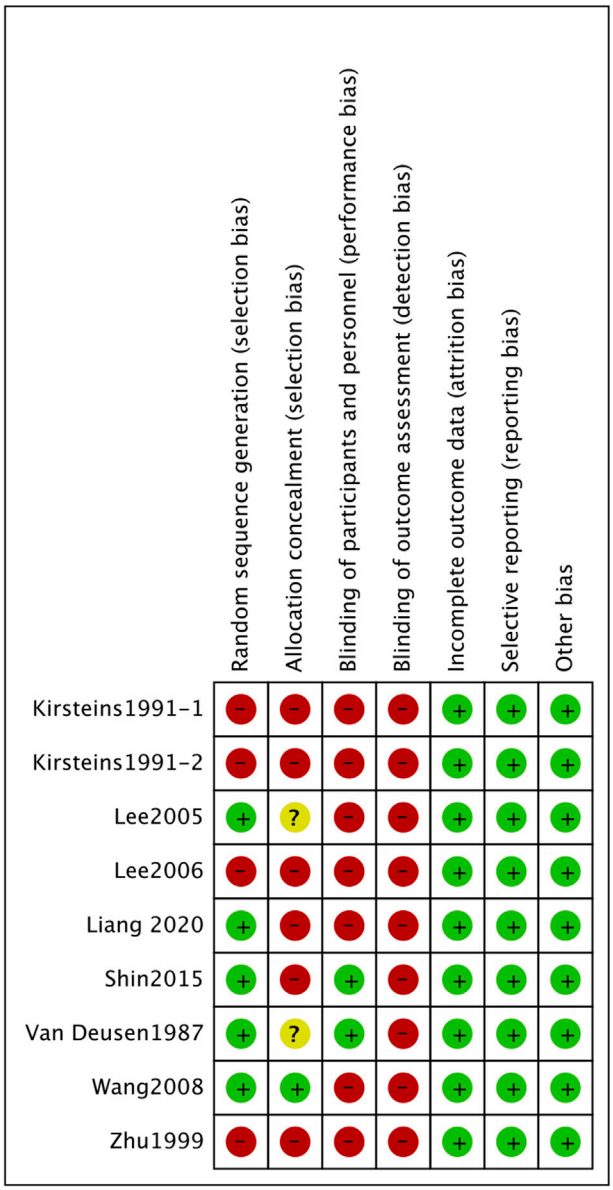

FIGURE 2
Risk of bias graph. Review authors' judgements about each risk of bias item presented as percentages across all included studies.

screened by reading full texts. Among these, three studies did not include control group (Uhlig et al., 2005; Uhlig et al., 2010; Waite-Jones et al., 2013), the variable is auricular acupressure in one study (Lee et al., 2012), and participants were same in one study (Wang et al., 2005) with another included study (Wang, 2008). Regarding the websites results, the first three hundred records were evaluated, but there were no studies that could be included. In addition, two studies were found in paper journals, but did not meet the inclusion criteria. Finally, as two independent CCTs in the same article (Kirsteins et al., 1991), nine trials from eight articles included were analyzed (Van Deusen and Harlowe, 1987; Kirsteins et al., 1991; Zhu et al., 1999; Lee, 2005; Lee and Jeong, 2006; Wang, 2008; Shin et al., 2015; Liang, 2020). The difference lies in the frequency of TC intervention (details in Figure 1).

Description of studies

The recruited articles were published from 1987 to 2020 years. The sample size ranged from 20 (Wang, 2008; Liang, 2020) to 68 (Zhu et al., 1999). All studies were single-center studies, while only one study was a multicenter one (Kirsteins et al., 1991). 351 RA participants were analyzed in this review. All patients satisfied the American College of Rheumatology 1987 revised classification criteria for RA. The frequency of TC was twice weekly (Kirsteins et al., 1991; Wang, 2008), once a week (Van Deusen and Harlowe, 1987; Kirsteins et al., 1991; Lee, 2005; Lee and Jeong, 2006; Shin et al., 2015) or once a day (Zhu et al., 1999; Liang, 2020). The duration of TC was 6 weeks (Lee, 2005), 8 weeks (Van Deusen and Harlowe, 1987; Zhu et al., 1999), 10 weeks (Kirsteins et al., 1991) and 12 weeks (Wang, 2008; Shin et al., 2015; Liang, 2020).

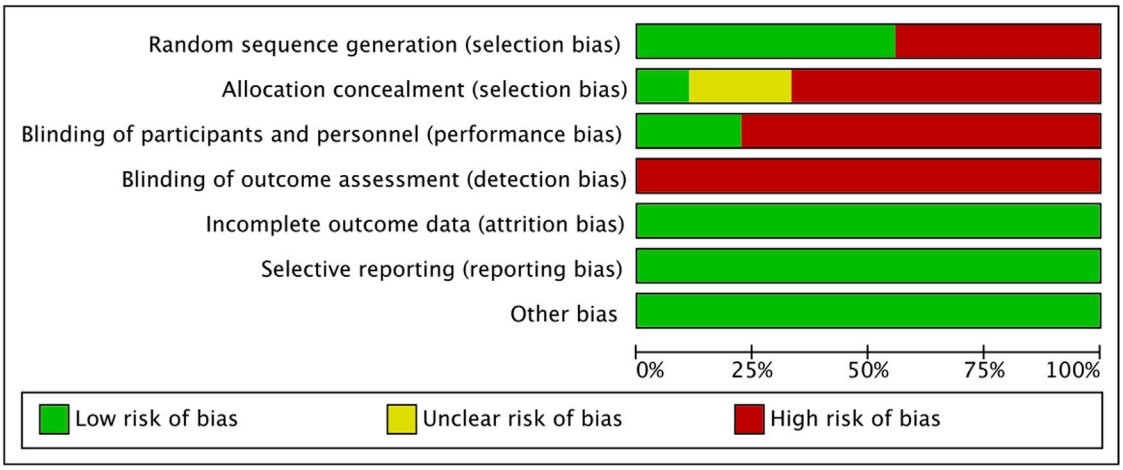

FIGURE 3
Risk of bias summary. Review authors' judgements about each risk of bias item for each included study.

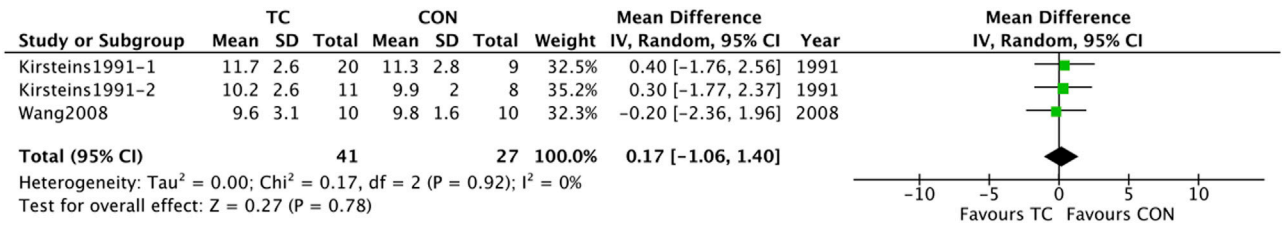

FIGURE 4
A Forest plot of the meta-analyses compared TC group with control group for changing in time to walk 50 feet.

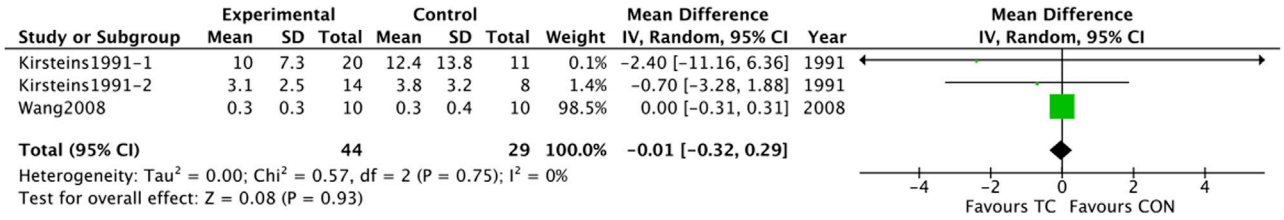

FIGURE 5
A Forest plot of the meta-analyses compared TC group with control group for changing in joint tenderness.

The control groups were adopted usual activities without TC, advice about lifestyle, rest at home or oral the same medicine of TC group. The time to walk 50 feet was described in three studies (Kirsteins et al., 1991; Wang, 2008), joint tenderness in three studies (Kirsteins et al., 1991; Wang, 2008), the number of swollen joints in four studies (Kirsteins et al., 1991; Wang, 2008; Shin et al., 2015; Liang, 2020), the number of tender joints in two studies (Wang, 2008; Shin et al., 2015), handgrip strength in three studies (Kirsteins et al., 1991; Wang, 2008), pain in three studies (Lee, 2005; Lee and Jeong, 2006; Wang, 2008), HAQ in two studies (Wang, 2008; Shin et al., 2015; Liang, 2020), withdrawals overall during the study (Van Deusen and Harlowe, 1987; Kirsteins et al., 1991; Zhu et al., 1999). No studies described patients' cost (details in Table 1).

Risk of bias and quality

The final results were shown in the form of summary (Figure 2) and graph (Figure 3). All studies had low risks of attrition bias, reporting bias and other bias. Selection bias of random sequence generation was high in four studies (Kirsteins et al., 1991; Zhu et al., 1999; Lee and Jeong, 2006) and was low in the other five studies (Van Deusen and Harlowe, 1987; Lee, 2005; Wang, 2008; Shin et al., 2015;

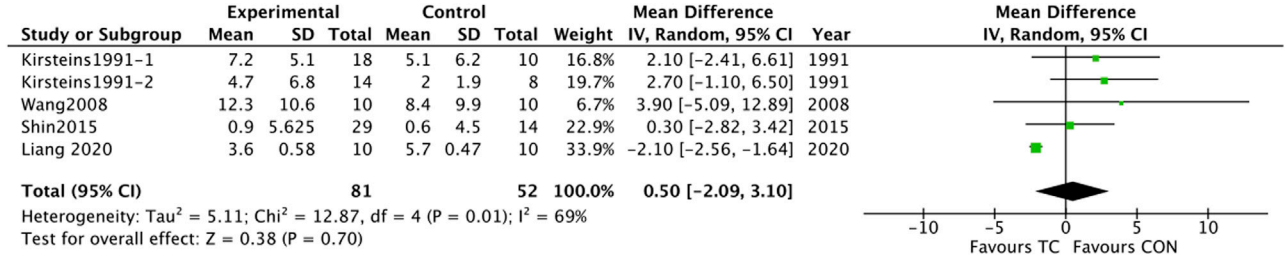

FIGURE 6
A Forest plot of the meta-analyses compared TC group with control group for changing in number of swollen joints.

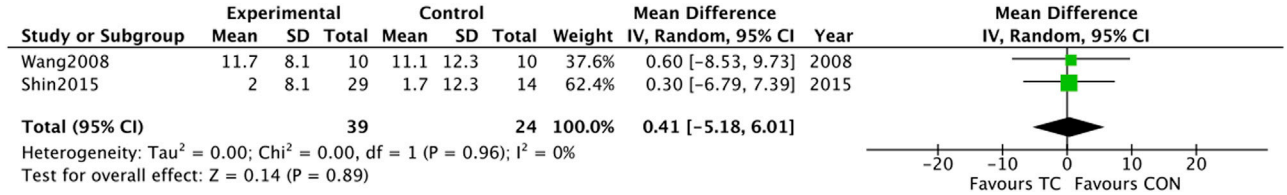

FIGURE 7
A Forest plot of the meta-analyses compared TC group with control group for changing in number of tender joints.

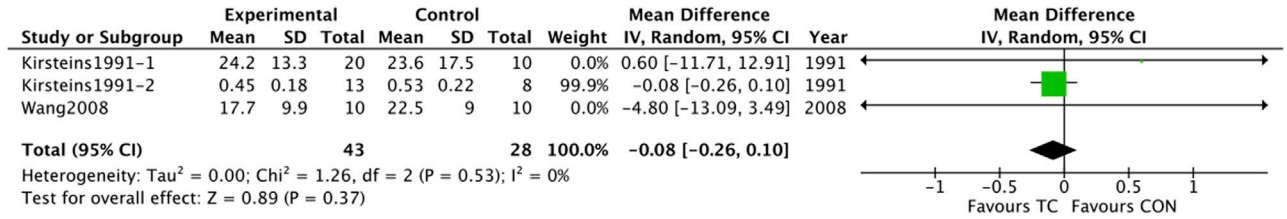

FIGURE 8
A Forest plot of the meta-analyses compared TC group with control group for changing in handgrip strength.

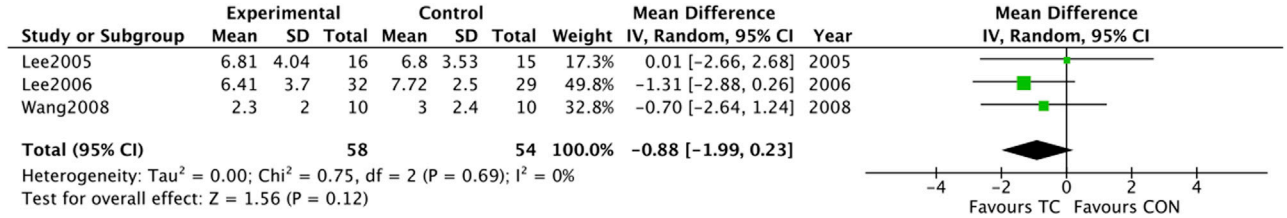

FIGURE 9
A Forest plot of the meta-analyses compared TC group with control group for changing in pain.

Liang, 2020). Selection bias of allocation concealment was high in six studies (Kirsteins et al., 1991; Zhu et al., 1999; Lee and Jeong, 2006; Shin et al., 2015; Liang, 2020), unclear in two studies (Van Deusen and Harlowe, 1987; Lee, 2005) and low in one study (Wang, 2008). Performance bias of blinding of participants and personnel was high in seven studies (Kirsteins et al., 1991; Zhu et al., 1999; Lee, 2005; Lee and Jeong, 2006; Wang, 2008; Liang, 2020) and was low in the other two studies (Van Deusen and Harlowe, 1987; Shin et al., 2015). Detection bias blinding of outcome assessment was high in all the included studies.

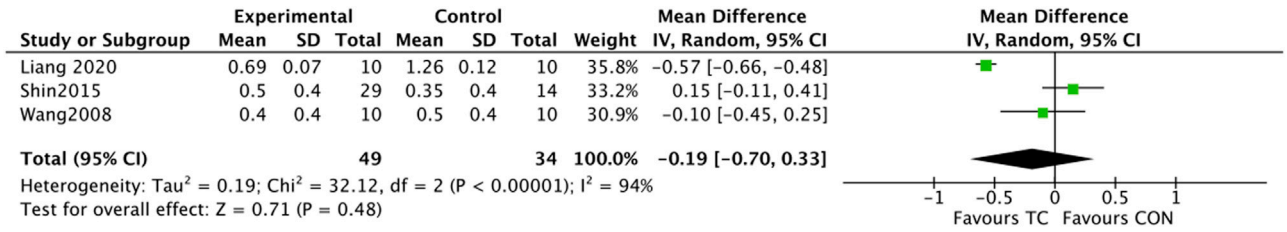

FIGURE 10
A Forest plot of the meta-analyses compared TC group with control group for changing in HAQ.

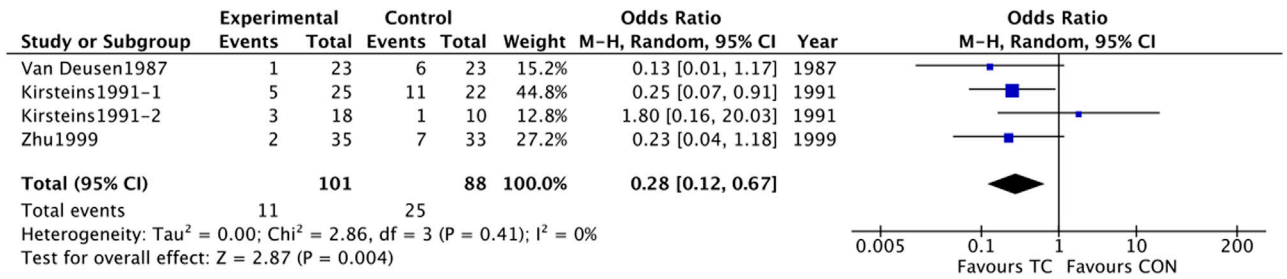

FIGURE 11
A Forest plot of the meta-analyses compared TC group with control group for changing in withdrawals overall.

Regarding the risk of bias of individual studies, four trials (tow trials from one study) were considered with high risk (Kirsteins et al., 1991; Zhu et al., 1999; Lee and Jeong, 2006). In contrast, two studies were rated as medium risk (Lee, 2005; Liang, 2020) and three studies as low risk (Van Deusen and Harlowe, 1987; Wang, 2008; Shin et al., 2015).

Outcomes and analysis

Time to walk 50 feet

We collected the data from three studies (Kirsteins et al., 1991; Wang, 2008) together and acquired evidence that TC therapy could not significantly improve time to walk 50 feet, with MD 0.17 (95% CI −1.06–1.40) in a random effect model (Figure 4). Tow independent CCTs were from one article (Kirsteins et al., 1991).

Joint tenderness

The data from three studies (Kirsteins et al., 1991; Wang, 2008) were collected together and evidence was acquired that TC therapy could not significantly improve joint tenderness, with MD -0.01 (95% CI −0.32 to 0.29) in a random effect model (Figure 5). Tow independent CCTs were from one article (Kirsteins et al., 1991).

Number of swollen joints

The data were collected from five studies (Kirsteins et al., 1991; Wang, 2008; Shin et al., 2015; Liang, 2020) suggested that TC therapy could not significantly improve number of swollen joints, with MD 0.50 (95% CI −2.09 to 3.10) in a random effect model (Figure 6). Tow independent CCTs were from one article (Kirsteins et al., 1991).

Number of tender joints

The data from two studies (Wang, 2008; Shin et al., 2015) together indicated that TC therapy could not significantly improve number of tender joints, with MD 0.41 (95% CI −5.18 to 6.01) in a random effect model (Figure 7).

Handgrip strength

After the collection of the data from three studies (Kirsteins et al., 1991; Wang, 2008), the results showed that TC therapy could not significantly improve handgrip strength, with MD −0.08 (95% CI −0.26 to 0.10) in a random effect model (Figure 8). Tow independent CCTs were from one article (Kirsteins et al., 1991).

Pain

The data from three studies (Lee, 2005; Lee and Jeong, 2006; Wang, 2008) showed that TC therapy could not significantly improve pain, with MD −0.88 (95% CI −1.99 to 0.23) in a random effect model (Figure 9).

HAQ

After the collection of the data from three studies (Wang, 2008; Shin et al., 2015; Liang, 2020), the results showed that TC therapy could not significantly improve HAQ, with MD −0.19 (95% CI −0.70 to 0.33) in a random effect model (Figure 10).

Withdrawals overall

The data from four studies (Van Deusen and Harlowe, 1987; Kirsteins et al., 1991; Zhu et al., 1999) was combined and provided evidence that TC therapy could significantly improve withdrawals overall during the study, with OR 0.28 (95% CI 0.12–0.67) in a random effect model (Figure 11). Tow independent CCTs were from one article (Kirsteins et al., 1991).

Discussion

351 participants were included in this meta-analysis from nine trials. Three of them were CCTs and six were RCTs in total. All patients were diagnosed by rheumatologists or clinicians in department of rheumatology. We used the collective data to perform a meta-analysis and found that TC could significantly improve the withdrawals overall during the study. Available data suggested that TC was not linked closely with serious adverse events. However, TC cannot improve physical functions of RA patients. Additionally, the included studies were assessed as having a relative high risk of bias. Four trials with high risk might greatly reduces the credibility of the results. Two studies rated with medium risk and three studies with low risk might have relatively small impact on the confidence of the results. Therefore, the confidence in the findings were seriously reduced.

RA is a second common form of arthritis. However, treating strategy is limited and medications are frequently toxic (Nagy et al., 2022). Therefore, RA patients turn to complementary and alternative therapies often (Zhao et al., 2017). The value of regular physical activity is well documented in the management of RA (Hu et al., 2021; Roodenrijs et al., 2021). Physical activity for patients with RA needs to be sustainable and enjoyable, however most of them have less physically active than the general population in fact (Hu et al., 2021). In addition, A systematic review about efficacy of occupational therapy-related interventions for adults with RA concluded strong evidence to support the use of aerobic exercise, such as TC (Siegel et al., 2017).

Recently, TC has been applied with substantial benefits in patients with RA. Intensity in TC is low and equivalent to walking 6 km/h and produces a secondary increase in heart rate (Jin, 1992), which comprised rhythmic movements and emphasis on body balance and coordination (Song et al., 2010). There are different kinds of actions, such as bend knees slightly, keep arms below the shoulder level, forward or backward strides, and turn around while shifting the center of gravity (Song et al., 2007). Although TC has lots of styles and flexible action details, it can be assumed that the major function of TC is similar. TC is considered safe in patients with RA, especially long-standing and dramatically physically inactive individuals (Kirsteins et al., 1991). This is the same with the withdraw overall outcome in our meta-analysis. TC could decrease the percentage of dropouts in trials.

Studies had demonstrated a favorable effect or tendency to improve physical function (Chen et al., 2016). A study indicated that the positive effects of TC were attributed to increases in the muscle strength and endurance of the lower extremity (Song et al., 2010). It may also help to improve body balance and stabilize the weighted joints thereby reducing the risk of falling (Wang, 2009). Additionally, another review about TC treating RA concluded that there were positive effects on a selected range of motion outcomes (Han et al., 2004). However, investigators thought that TC had no effectiveness of TC treating RA in another meta-analysis (Lee et al., 2007). Our results also showed TC cannot improve physical function of RA patients.

The primary limitation of this review is the small total number of eligible trials. Therefore, the results of the studies might or might not apply to the majority of RA patients; there were not enough studies for conclusive judgment, especially the side effects of TC. TC only could be assumed with a low risk of injury as a treatment method. In addition, we tried our best to search relevant articles in different ways, but we could not make sure that all the relevant studies were included. So, the bias from selecting the studies for inclusion in a meta-analysis could not be avoided.

Conclusion

The results of our systematic review and meta-analysis have provided the newest evidence on TC for the treatment of RA. It suggests that TC is a safe method to exercise for RA patients as the lower withdrawals overall. However, TC cannot improve physical function of RA patients. In addition, as the high risk of bias of included studies, the confidence in the findings was seriously reduced. More high-quality clinical studies are needed to further update the results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

Write manuscript, HW; search articles, HW and QW; assess risk of bias, HW and GW; finish the Table and Figures, QW; data analysis, HW and QW; solve disagreements, JW and YW; study design, YW.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1079841/full#supplementary-material

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Speed, agility, and musculoskeletal fitness are independently associated with areal bone mineral density in children

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Background: There is still little understanding of the associations between physical fitness variables and bone health in children taking into account key confounders.

Aim: The aim of this study was to analyze the associations between performance in tests of speed, agility, and musculoskeletal fitness (power of the upper and lower limbs) with bone mass of different regions in children, considering the adjustment to maturity-offset, lean percentage, and sex.

Methods: Cross-sectional study design: the sample consisted of 160 children aged 6–11 years. The physical fitness variables tested were 1) speed, assessed with the running test at a maximum speed of 20 m; 2) agility, assessed through the 4×4-m square test; 3) lower limb power, assessed using the standing long jump test, and 4) upper limb power, assessed using the 2-kg medicine ball throw test. Areal bone mineral density (aBMD) was obtained from the analysis of body composition by dual-energy X-ray absorptiometry (DXA). Simple and multiple linear regression models were performed using the SPSS software.

Results: In the crude regression analyses, the results indicated a linear relationship between all the physical fitness variables and aBMD in all body segments, but maturity-offset, sex, and lean mass percentage seemed to have an effect on these relationships. Except for the upper limb power, the other physical capacities (speed, agility, and lower limb power) were associated with aBMD in at least three body regions in the adjusted analyses. These associations occurred in the spine, hip, and leg regions, and the aBMD of the legs presented the best association magnitude (R^2).

Conclusion: There is a significant association between speed, agility, and musculoskeletal fitness, specifically the lower limb power and aBMD. That is, the aBMD is a good indicator of the relationship between fitness and bone mass in children, but it is essential to consider specific fitness variables and skeletal regions.

KEYWORDS

physical fitness, bone tissue, physical conditioning, school, child

Introduction

The bone mass acquisition during adolescence is related to several factors [e.g., genetic (Slemenda et al., 2009), maturational (Theintz et al., 1992), and lifestyle (Bachrach, 2001)]. However, there is evidence that sex hormones are important modulators of bone mass in the period of maturation (in other periods too), suggesting an estrogenic effect on the trabecular and cortical bone (Arabi et al., 2004; Yilmaz et al., 2005). Thus, it is assumed that optimizing peak bone mass in childhood and adolescence may be the main strategy to compensate for the decline in bone density associated with advancing age and to promote long-term bone health (Weaver et al., 2016).

In this sense, the systematic reviews by Mello et al. (2021) and García-Hermoso et al. (2021) demonstrate that interventions with physical exercise and physical activity with vigorous intensity (both with a positive effect on physical fitness) promote gains in bone mass in children and adolescents [e.g., bone mineral content (BMC) and bone mineral density (BMD)]. As Gómez-Bruton et al. (2017) have shown that increase in lower limb power through plyometric exercises (effects on muscle power) in children and adolescents causes positive effects on general BMC.

However, it is noteworthy that the strong literature data are about the cause–effect relationship between physical activity interventions and bone mass, called the osteogenic effect (Gómez-Bruton et al., 2017; García-Hermoso et al., 2021; Mello et al., 2021). Some longitudinal studies (Kemper, 2000; Vicente-Rodríguez et al., 2008) have already demonstrated that in the long term, some physical capacities that are developed from activities with constant impacts, strong muscle contractions, and constant osteotendinous tensions may be associated with osteogenesis. The physical capacities described in the longitudinal studies are musculoskeletal fitness (i.e., muscular strength, power, and endurance), agility, and speed.

Recently, Gómez-Bruton et al. (2020) investigated the association between global physical fitness (such as cardiorespiratory fitness, agility, speed, and musculoskeletal fitness) and bone health indicators (BMD in different body sites, e.g., the spine, hip, and neck femur) in a sample of 92 Spanish children aged between 3 and 5 years. The results indicated that relative upper and lower limb muscle strength and speed/agility predicted all measured bone variables, except for BMD. The authors also point out that the global physical fitness results are determinants for bone structure and strength and that, consequently, the performance of physical fitness tests could provide useful information related to bone health in children. It should be noted that although these children are very young, the associations can be expected in the other stages of childhood as well due to the uninterrupted process of bone growth.

Thus, experimental evidence points to a possible relationship between physical fitness and bone health indicators in childhood. But this possible relationship is not clear when we talk about children and adolescents in the growth peak for example. Some studies have strongly suggested that musculoskeletal fitness, mainly power and strength, is related to good bone health (evidence from the osteogenic effect of exercises) (Kemper et al., 2000; Gracia-Marco et al., 2011; Janz et al., 2015; Gómez-Bruton et al., 2017; Gómez-Bruton et al., 2020; Henriques-Neto et al., 2020). Although there is less evidence, a review (Mello et al., 2021) has also indicated the relationship between speed and agility with bone health—specifically with the hip and spine bones—and development in children, however it had not evaluated the influence of maturation.

Therefore, the aforementioned studies suggest that there is still little understanding of the associations between physical fitness variables and bone health indicators in children and adolescents; however, they are promising in pointing out some directions. In this sense, the aim of this study is to analyze the associations between performance in tests of speed, agility, and musculoskeletal fitness (power of the upper and lower limbs) and the bone mass of different regions of the body in children, considering the adjustment to maturity-offset, lean and fat percentage, and sex.

Materials and methods

Study design

This cross-sectional study with a quantitative approach and non-probabilistic sample was carried out after approval of the Universidade Federal do Rio Grande do Sul Research Ethics Committee (number: 3.414.512; Brazilian system CAAE: 12222019.9.0000.5347).

Research subjects and data collection procedure

The sample consisted of 160 children aged 6–11 years from the first to fifth year of the public elementary school in the city of Porto Alegre, Brazil. To identify the test's power from the sample size of 160 children, *a posteriori* sample calculation was performed using the G-Power version 3.1 program, and for this, the equation directed to the proposed association test was used. The calculation was performed for tests of the F family, considering that the research project foresees the association analyses those were conducted in another study (Mello et al., 2021). The alpha used was 0.05; the used effect size for differences was $f^2 = 0.15$ (moderate); and eight variables were used as predictors. From this protocol, considering the sample size of the present study, the power of the test $(1 - \beta)$ was identified as 0.95.

For data collection, contact was made with the school. After signing the authorization terms (school authorization form), all the children who were enrolled from the first to fifth year of the elementary school received an invitation to participate in the research with the consent terms (parents/guardian and children consent and assent forms). After this stage, a class period was scheduled to carry out the physical tests. The dual-energy X-ray absorptiometry (DXA) exam was performed at the Exercise Research Laboratory (LAPEX) at the Universidade Federal do Rio Grande do Sul, Brazil. For this purpose, an evaluation time was scheduled with the parents/guardians. All tests and examinations were performed by previously trained researchers. This procedure was conducted at the beginning of the academic years 2017 and 2018.

Physical fitness

The procedures for collecting physical fitness variables were performed according to the PROESP-BR (*Projeto Esporte Brasil*) Guidelines for Measurements, Tests, and Assessments (Gaya et al., 2021) and have been described in detail in previous studies (Mello et al., 2015; Mello et al., 2016; Pedretti et al., 2020). The physical fitness variables tested were 1) sprint, assessed with the running test at a maximum speed of 20 m; 2) agility, assessed through the 4×4-m square test; 3) lower limb power (LLP), assessed using the standing long jump

test, and 4) upper limb power (ULP), assessed using the 2-kg medicine ball throw test. These tests have international use and validation with good evidence (Bös and Schlenker, 2011; Calleja-González et al., 2015) and are widely used in Brazil (Pedretti et al., 2020).

Bone outcomes

Areal bone mineral density (aBMD) was collected from the analysis of body composition according to the recommendations of the manufacturer of the DXA device of the GE Healthcare model, Lunar Prodigy (Madison, United States). A trained researcher and qualified laboratory technician carried out the examinations and handling of the device, respectively. The device was calibrated once a day before the evaluation sessions. Children were instructed to remove any metal material and wear clothes without zips, buckles, or buttons. Before the evaluation, the guardians were instructed that DXA uses X-rays emitted at two different energy levels to allow for distinguishing the bone tissue from its surrounding soft tissue and that radiation exposure is low with DXA (less than 5 mrem/scan). The evaluator placed the subjects in the supine position and asked them to remain motionless during the measurement, for approximately 5 min, while the equipment arm passed over the body in the head–foot direction. The values were automatically calculated using the equipment's software (Encore version 14.1, Madison, United States). The values of aBMD (e.g., 0.978 g/cm^2) have been described for the total body, total body less the head, and body segments such as the trunk, spine, arms, hip, and legs.

Covariates

Due to the influence that bone mass indicators suffer from biological variables (Bachrach, 2001), the lean mass percentage and maturity-offset were considered covariates. The total body lean mass percentages were made available during the DXA exam, along with the bone variables. For maturity-offset calculation, the following variables were required: height, body mass, sitting height, and length of the lower limbs.

The data collection for these variables and the calculation of maturity offset followed the recommendations proposed by Mirwald et al. (2002). The children performed the measurements in light clothes (e.g., physical education class clothes) and without shoes. All anthropometric procedures followed are as described in PROESP-BR Guidelines for Measurements, Tests, and Assessments (Gaya et al., 2021). For the measurement of body mass, a portable scale with an accuracy of up to 100 g was used. During the assessment, the children and adolescents remained standing with their elbows extended and close to their bodies. For the measurement of height and sitting height, a portable stadiometer or measuring tape with a precision of up to 2 mm was used. For the sitting height, a bench with a standard size of 40 cm was used, and the zero point of the measuring tape was on the bench.

Data analysis

For the treatment of data, a descriptive analysis was first performed. In this analysis, the mean value and standard deviation were identified for continuous variables. In the next step, an exploratory analysis was conducted to identify the normality parameters in the physical fitness variables. In this procedure, the Kolmogorov–Smirnov test was performed.

After these steps, the association analyses were performed to estimate variabilities of bone health indicators (outcomes) from the physical fitness variables (analyzed separately). At this stage, a correlation matrix was created between the physical fitness variables and all the bone variables; this procedure is a prerequisite for linear regression. Then, simple and multiple linear regression equations were used. Collinearity was checked for the variables using the variance inflation factor (VIF) and tolerance levels. In the multiple regression analysis, the equation was adjusted for covariates (sex, maturity-offset, and lean mass percentage) after the multicollinearity test. For these analyses, we have applied the Bonferroni correction for multiple testing and assumed a significant p-value below 0.007, which results from dividing the alpha value (0.05) by the number of dependent variables, in our case 7. All statistical analyses were performed using the SPSS for Windows version 24.0. For all analyses, an alpha value of 0.05 was considered.

Results

Descriptive analysis and correlation

Table 1 describes information on the distribution and central tendency of anthropometric variables, maturity-offset, physical fitness, lean mass percentage, age, and aBMD in each body region. The mean values of the variables age, height, and weight were similar in the total sample and when stratified by sex. The other variables had different mean values.

The correlation between the physical fitness variables and bone mass variables is described in Table 2. Several associations showed minimal correlation ($r < 0.5$); however, due to the lack of regularity between the associations, all bone variables were included in the linear regression analyses. Regarding collinearity, there was a strong correlation between age and maturity-offset ($r > 0.7$), thus the multiple analyses were adjusted only by sex, lean and fat percentage, and maturity-offset.

Association analysis—simple and multiple regression

Sprint

The association of sprint with aBMD presented a magnitude that varied between −0.043 (arms) and −0.108 (legs) in the crude analyses (Table 3). When the co-variables were included in the analyses (adjustment), some variables lost their statistical significance. Considering the constant co-variables, the sprint was associated with aBMD in the total body (for every 1 s more in the 20 m run test, a 0.028 g/cm^2 reduction in the aBMD was estimated), total body less head (0.021 g/cm^2 of reduction per second), hip (0.032 g/cm^2 of reduction per second), and legs (0.035 g/cm^2 of reduction per second). The value of the coefficient of determination (R^2) in all analyses was high (>65), indicating that sprint, sex, lean mass, and maturity-offset forms an important set of variables to explain the variability of aBMD in some body regions.

TABLE 1 Distribution and central tendency of anthropometric variables, maturity-offset, physical fitness, lean and fat percentage, age and aBMD.

	Total		Boys		Girls	
	n	$\dot{X} \pm SD$	n	$\dot{X} \pm SD$	n	$\dot{X} \pm SD$
Anthropometry, maturity offset, and physical fitness						
Age (year)	160	8.90 ± 1.50	85	8.20 ± 1.60	75	8.30 ± 1.50
Height (m)	155	1.33 ± 0.10	85	1.34 ± 0.10	74	1.33 ± 0.11
Weight (kg)	155	33.03 ± 10.14	85	32.80 ± 9.31	74	33.29 ± 11.03
Maturity offset (year)	152	−3.02 ± 1.73	79	−4.01 ± 1.11	73	−1.95 ± 1.65
LM%	160	21.73 ± 4.84	85	22.27 ± 4.31	75	21.12 ± 5.34
Sprint (s)	154	4.54 ± 0.85	80	4.45 ± 0.96	74	4.63 ± 0.70
Agility (s)	153	7.91 ± 1.00	79	7.70 ± 1.01	74	8.13 ± 0.94
Lower limb power (cm)	154	110.58 ± 24.04	80	117.09 ± 24.57	74	103.54 ± 21.48
Upper limb power (cm)	152	184.23 ± 53.85	79	191.92 ± 51.83	73	175.90 ± 55.10
Bone mass variables						
aBMD (g/cm^2)						
Total body	159	0.847 ± 0.091	84	0.849 ± 0.086	75	0.845 ± 0.096
TBLH	159	0.717 ± 0.098	84	0.714 ± 0.089	75	0.722 ± 0.108
Arms	159	0.559 ± 0.075	84	0.555 ± 0.072	75	0.565 ± 0.078
Trunk	160	0.677 ± 0.090	85	0.670 ± 0.077	75	0.684 ± 0.103
Spine	160	0.728 ± 0.100	85	0.718 ± 0.081	75	0.739 ± 0.116
Hip	160	0.715 ± 0.106	85	0.706 ± 0.094	75	0.724 ± 0.118
Legs	160	0.831 ± 0.124	85	0.832 ± 0.117	75	0.830 ± 0.133

n, sample number; $\dot{X} \pm SD$, mean and standard deviation; m, meter; kg, kilogram; LM%, lean mass percentage; aBMD, areal bone mineral density; TBLH, total body less head.

TABLE 2 Correlation matrix between physical fitness and bone mass variables.

	Sprint		Agility		Lower limb power		Upper limb power	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
aBMD								
Total body	−0.54	<0.001	−0.47	<0.001	0.36	<0.001	0.56	<0.001
TBLH	−0.52	<0.001	−0.44	<0.001	0.30	<0.001	0.60	<0.001
Arms	−0.38	<0.001	−0.30	<0.001	0.25	0.001	0.45	<0.001
Trunk	0.44	<0.001	0.37	<0.001	0.21	0.007	0.55	<0.001
Spine	−0.35	<0.001	−0.29	0.002	0.10	0.200	0.50	<0.001
Hip	−0.51	<0.001	−0.41	<0.001	0.30	<0.001	0.52	<0.001
Legs	−0.58	<0.001	−0.50	<0.001	0.37	<0.001	0.63	<0.001

r, correlation value; *p*-value, value of statistical significance; aBMD, areal bone mineral density; TBLH, total body less head.

Agility

The association of agility with aBMD presented a magnitude that varied between −0.023 (arms) and −0.064 (legs) in the crude analyses (Table 4). When the co-variables were included in the analyses (adjustment), some variables lost their statistical significance. Considering the constant co-variables, agility was associated with aBMD in the total body (for every 1 s more in the 4×4-m square test, a 0.014 g/cm^2 reduction in aBMD was estimated) and legs (0.020 g/cm^2 of reduction per second). The value of the coefficient of determination (R^2) in all analyses was high (>65), indicating that agility, sex, lean mass, and maturity-offset forms an important set of variables to explain the variability of aBMD in some body regions.

Lower limb power

The association of lower limb power with aBMD presented a magnitude that was −0.001 for all body regions, except for the legs (−0.002 for legs) in the crude analyses (Table 5). When the co-variables were included in the analyses (adjustments), some variables lost their statistical significance. Considering the constant co-variables, the lower limb power was associated with aBMD in the legs (for every 1 cm more in the standing

TABLE 3 Estimation of the variability of aBMD in different body segments from sprint and covariates.

	Crude analysis			Adjusted analysis*			
	B	95% CI	*p*-value	R^2	β	95% CI	*p*-value
aBMD							
Total body	−0.074	−0.093 to −0.056	**<0.001**	0.67	−0.028	−0.043 to −0.012	**0.001**
TBLH	−0.078	−0.098 to −0.057	**<0.001**	0.78	−0.021	−0.034 to −0.007	**0.004**
Arms	−0.043	−0.059 to −0.026	**<0.001**	0.52	−0.007	−0.022 to −0.008	0.374
Trunk	−0.061	−0.081 to −0.041	**<0.001**	0.69	−0.011	−0.026 to −0.004	0.149
Spine	−0.053	−0.076 to −0.030	**<0.001**	0.61	−0.001	−0.019 to 0.019	0.978
Hip	−0.082	−0.104 to −0.060	**<0.001**	0.67	−0.032	−0.050 to −0.013	**0.001**
Legs	−0.108	−0.133 to −0.083	**<0.001**	0.80	−0.035	−0.051 to −0.018	**<0.001**

*R^2: adjusted coefficient of determination by sex, lean percentage, and maturity offset.
β, regression unstandardized coefficient value; 95% CI, confidence interval of 95%; *p*-value, value of statistical significance; aBMD, areal bone mineral density; TBLH, total body less head.
p-values below 0.007 were considered as significant after applied Bonferroni correction for multiple testing.

TABLE 4 Estimation of the variability of aBMD in different body segments from the agility and covariates.

	Crude analysis			Adjusted analysis*			
	β	95% CI	*p*-value	R^2	B	95% CI	*p*-value
aBMD							
Total Body	−0.043	−0.056 to −0.030	**<0.001**	0.65	−0.014	−0.025 to −0.004	**0.006**
TBLH	−0.044	−0.058 to −0.030	**<0.001**	0.77	−0.010	−0.019 to −0.001	0.034
Arms	−0.023	−0.034 to −0.011	**<0.001**	0.51	−0.001	−0.011 to 0.009	0.859
Trunk	−0.034	−0.048 to −0.020	**<0.001**	0.69	−0.004	−0.014 to 0.005	0.388
Spine	−0.030	−0.046 to −0.014	**<0.001**	0.59	0.000	−0.012 to 0.003	0.947
Hip	−0.045	−0.060 to −0.029	**<0.001**	0.64	−0.013	−0.025 to 0.001	0.037
Legs	−0.064	−0.081 to −0.047	**<0.001**	0.79	−0.020	−0.030 to −0.009	**<0.001**

*R^2, adjusted coefficient of determination by sex, lean percentage, and maturity-offset.
β, regression unstandardized coefficient value; 95% CI, confidence interval of 95%; *p*-value, the value of statistical significance; aBMD, areal bone mineral density; TBLH, total body less head.
p-values below 0.007 were considered as significant after applying Bonferroni correction for multiple testing.

TABLE 5 Estimation of the variability of aBMD in different body segments from the lower limb power and covariates.

	Crude analysis			Adjusted analysis*			
	β	95% CI	*p*-value	R^2	β	95% CI	*p*-value
aBMD							
Total body	0.001	0.001–0.002	**<0.001**	0.64	<0.001	0.000–0.001	0.032
TBLH	0.001	0.001–0.002	**<0.001**	0.77	<0.001	0.000–0.001	0.178
Arms	0.001	0.001–0.002	**<0.001**	0.51	<0.001	0.000–0.001	0.407
Trunk	0.001	0.000–0.001	**0.007**	0.69	<0.001	0.000–0.001	0.554
Spine	0.001	0.000–0.001	0.217	0.62	0.001	0.000–0.001	0.018
Hip	0.001	0.001–0.002	**<0.001**	0.65	<0.001	0.000–0.001	0.114
Legs	0.002	0.001–0.003	**<0.001**	0.72	0.001	0.001–0.002	**0.006**

*R^2, adjusted coefficient of determination by sex, lean percentage, and maturity offset.
β, regression unstandardized coefficient value; 95% CI, confidence interval of 95%; *p*-value, value of statistical significance; aBMD, areal bone mineral density; TBLH, total body less head.
p-values below 0.007 were considered as significant after applied Bonferroni correction for multiple testing.

long jump test, it was estimated to increase by 0.001 g/cm^2 of aBMD). The value of the coefficient of determination (R^2) in all analyses was high (>60), indicating that lower limb power, sex, lean mass, and maturity-offset forms an important set of variables to explain the variability of aBMD in some body regions.

Upper limb power

The association of upper limb power with aBMD presented a magnitude that was −0.001 for all body regions in crude analyses (Table 6). When the co-variables were included in the analyses (adjustments), all variables lost their statistical significance.

Discussion

The aim of this study was to analyze the associations between performance in tests of speed, agility, and musculoskeletal fitness (power of upper and lower limbs) with the bone mass of different regions of the body in children, considering the adjustment to

TABLE 6 Estimation of the variability of aBMD in different body segments from the upper limb power and covariates.

	Crude analysis			Adjusted analysis*			
	β	95% CI	*p*-value	R^2	β	95% CI	*p*-value
aBMD							
Total body	0.001	0.001–0.001	**<0.001**	0.64	0.000	0.000–0.001	0.285
TBLH	0.001	0.001–0.001	**<0.001**	0.77	0.000	0.000–0.001	0.198
Arms	0.001	0.001–0.001	**<0.001**	0.50	0.000	0.000–0.000	0.994
Trunk	0.001	0.001–0.001	**<0.001**	0.69	0.000	0.000–0.000	0.319
Spine	0.001	0.001–0.001	**<0.001**	0.60	0.000	0.000–0.001	0.479
Hip	0.001	0.001–0.001	**<0.001**	0.65	0.000	0.000–0.001	0.567
Legs	0.001	0.001–0.002	**<0.001**	0.78	0.000	0.000–0.001	0.075

*R^2, adjusted coefficient of determination by sex, lean percentage, and maturity-offset.
β, regression unstandardized coefficient value; 95% CI, confidence interval of 95%; *p*-value, value of statistical significance; aBMD areal bone mineral density; TBLH, total body less head.
p-values below 0.007 were considered as significant after applied Bonferroni correction for multiple testing.

maturity-offset, lean mass percentage, and sex. Thus, the main evidence of this study points to a relationship between speed, agility, and lower limb power, the capacities those require constant ground impact plus large muscle contraction, with the aBMD in different skeletal regions (TBLH, spine, hip, and specially legs) of children regardless of the level of lean mass, sex, and distance to the peak of their growth.

For both speed and agility, it was not possible to clearly observe associations in the correlation matrix. However, in these results, the highest correlations (later confirmed in the regression models) were with the aBMD of the legs. This relationship seems logical since the greatest muscle demand and the greatest bone overload for sprint and agility activities are in the legs (Douma-van Riet et al., 2012).

The physical activities that potentially develop speed and agility are high-intensity running (with or without changing the direction). Earlier studies (Castro-Piñero et al., 2010; Chaouachi et al., 2014) have indicate that children who have higher performances in different running tests are those who routinely practice activities with this characteristic. In this way, as Gómez-Bruton et al. (2017) have already demonstrated, our results confirm that activities that have a high volume of stress on the bones (high-intensity running) seem to be beneficial for their development.

Running activities are characterized by a succession of jumps, and the ground reaction force directly impacts the bones with each touch of the foot on the ground (Završnik et al., 2017). The force impressed on the ground by a child during a high-intensity run is approximately two–three times their body weight (Anliker et al., 2011). In this sense, a force passes through the bones of the legs, hips, and lumbar spine specifically, causing the piezoelectric effect (passage of electric currents through the interior of the bones). Evidence indicates that the piezoelectric effect is one of the main reasons responsible for the osteogenic effect of these activities (Theintz et al., 1992).

In the same sense, the lower limb power was strongly related to all bone variables on the basis of the correlation analyses. In the same logic, the muscular power of the lower limbs mainly responds to the activities of different jumps, which also take advantage of the benefits of the ground reaction force (jumps can have 3.5–5 body weights in ground reaction force (Gómez-Bruton et al., 2017). However, for this physical capacity specifically, rapid muscle contraction is more required that causes the tendons to also overload the bones, thereby enhancing the osteogenic effect.

Although lower limb power mainly responds to activities with higher osteogenic potential, when the associations were adjusted for sex, maturation, and lean mass, only the aBMD of the spine, total body, and legs remained associated. This demonstrates that although there is a tendency for more powerful children to have a better profile of bone mass and lean mass, the maturational stage has to be evaluated as well. Our suggestion for future studies is that this association can be explored by considering mediation and moderation analyses of these covariates.

The physical capacity that had lost the significant association, after adjustment, was the power of the upper limbs. As discussed earlier, we expected that because of the traction exerted by the tendons, the associations would also occur with this variable. However, we understand that the test does not completely isolate muscle power and may mechanically benefit children and adolescents with longer, but not necessarily more powerful, arms (Ikeda et al., 2009).

Furthermore, the evident lack of activities that generate a continuous impact on the upper limbs may be the explanation for the results found. Studies with adolescent fighters showed that the BMD was higher than that in non-fighters (Ciaccioni et al., 2019), mainly due to the characteristics of the activities performed. Furthermore, lean mass, as found in other studies (Vicente-Rodríguez et al., 2008; Anliker et al., 2011), influenced the power performance level of children and adolescents, a factor that was not considered in the present study.

This study has strong points that deserve to be highlighted. The evidence presented comes largely from the gold standard assessments. Furthermore, the physical tests that were used have great national repercussion and have acceptable validity criteria (see the Materials and methods section). Furthermore, as far as we know, the analysis proposal has not yet been presented for the age group studied, making the study unprecedented. However, it is important that the readers' interpretations are carried out knowing the limitations of this study. The development of physical fitness is different in boys and girls, and the ideal was a stratified analysis; however, the sample size did not allow us to do this. The maturity-offset proposed by Mirwald et al. (2002) does not include children aged between 6 and 7 years, while in this case, the maturity-offset is just an estimate and have to be analyzed carefully. Furthermore, more specific bone mass assessments such as the femoral neck and radioulnar epiphysis would be very appropriate for children, but the DXA device used did not have specific software for this assessment. We found that the adjustment analysis could have

the considered physical activity levels but could not access this variable in all participants.

Conclusion

Collectively, the results indicated that speed, agility, and musculoskeletal fitness, specifically lower limb power, are associated with aBMD in different body regions. These associations occur in the total body, spine, hip, and legs when adjusted for sex, maturity-offset and lean body mass, and aBMD of the legs having the best magnitude of association (R^2) with these three physical fitness components. Furthermore, sprint ability appears to be associated with both legs and hip aBMD, being a great predictor of bone health. In summary, the aBMD is a good indicator between fitness and bone mass relationship in children, but it is important to consider what fitness variable and what skeletal region.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Universidade Federal do Rio Grande do Sul Research Ethics Committee. Written informed consent to participate in this study was provided by the participants and their legal guardian/next of kin.

Author contributions

JM and FR have the same importance as the first authors working on all stages of this manuscript, statistical analysis, drafting, and argumentative structure. LG reviewed the draft, statistical analysis, and final version. JP performed the data collection, discussed the results, and drafted the manuscript. ARG and AG coordinated the development of the research project and reviewed the final version of the manuscript. All authors have reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1080091/full#supplementary-material

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Effect of exercise on bone health in children and adolescents with cancer during and after oncological treatment: A systematic review and meta-analysis

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Background: Although regular physical activity and exercise programs might improve bone health caused by oncological treatment and the disease itself, it remains unknown the pooled effect of exercise interventions following frequency, intensity, time and type prescriptions.

Objective: This systematic review and meta-analysis aimed to synthesise evidence regarding the effectiveness of exercise interventions on bone health in children and adolescents with cancer during and after oncological treatment.

Methods: A systematic search was conducted in the MEDLINE (*via* PubMed), Web of Science and Scopus databases from November 2021 to January 2022. Randomised controlled trials (RCTs) and non-RCTs reporting pre-post changes of the effectiveness of exercise interventions on DXA-measured bone parameters in young population (1–19 years) during or after oncological treatment were included. Pooled (ESs) and 95% confidence intervals (95%CIs) were calculated. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed.

Results: A total of eight trials with 341 participants were included. The meta-analyses did not reveal a statistically significant increase in whole body areal bone mineral density (ES = 0.10; 95%CI: −0.14, 0.34), lumbar spine (ES = 0.03; 95%CI: −0.21, 0.26) or femoral neck (ES = 0.10; 95%CI: −0.37, 0.56). Similarly, during the oncological treatment phase the ES was 0.04 (95%CI: −0.17, 0.25) and after the ES was 0.07 (95%CI: −0.20, 0.33).

Conclusion: To date, exercise interventions have been inappropriate and therefore, ineffective to illustrate any beneficial effect on bone health in children and adolescents with cancer during and after oncological treatment.

Systematic Review Registration: PROSPERO registration number: CRD42022310876

KEYWORDS

bone mineral density, dual-energy x-ray absorptiometry, impact-loading exercise, paediatrics, physical activity, weight-bearing exercise

Highlights

- Radiotherapy and chemotherapy, and cancer itself can affect bone mass between 20% and 50% of paediatric cancer patients through endocrine complications, such as gonadal dysfunction, growth hormone deficiency, and altered body composition.
- Exercise interventions to date have not been effective at improving bone health of children and adolescents with cancer during or after oncological treatment.
- There is a need of implementing well-designed exercise interventions in RCTs specifically focused on improving bone health in children and adolescents diagnosed with cancer.

1 Introduction

Paediatric cancer survival has experienced an unparalleled increase because of the advances in cancer detection and treatment (Miller et al., 2020). The current overall 5-year survival rate has risen up to 85% in children and adolescents (Trama et al., 2016; Siegel et al., 2023). However, all oncological treatments and the disease itself can decrease bone mass through endocrine alterations, such as gonadal dysfunction, growth hormone deficiency, and altered body composition (Marcucci et al., 2019). This is shown by a decreased bone formation and increased bone resorption in cancer-treated children (Kelly and Pottenger, 2022). Research has shown that between 20% and 50% of paediatric cancer patients present impaired bone mass (Wilson and Ness, 2013; Marcucci et al., 2019). Moreover, paediatric cancer occurs during a critical phase for bone development and bone strengthening, since up to 95% of the adult bone mass may be accrued by the end of adolescence (Bailey et al., 1999; Harel et al., 2007; Rizzoli et al., 2010). Therefore, implementing feasible strategies to counteract cancer-related bone loss are vital to optimize skeletal health during growth and reduce the risk of osteoporosis later in life.

Although acquiring the peak bone mass strongly depends on genetics (Davies et al., 2005; Bonjour et al., 2007) regular physical activity and exercise programmes may contribute to achieve it (Weaver et al., 2016). Evidence has shown that exercise is safe during and after paediatric oncological treatment, even during the most aggressive phases (i.e., hematopoietic stem cell transplantation) (Campbell et al., 2019; Morales et al., 2021) and hence, it might contribute to preserve bone health in paediatric cancer patients during and after oncological treatment (Marcucci et al., 2019; Rodd et al., 2022). Weight-bearing impact exercise of high intensity including strains in different axes and multiple rest periods is known to improve bone mass (Ubago-Guisado et al., 2016; Ubago-Guisado et al., 2019). Interestingly, a systematic review showed that plyometric jump training causes improvements in areal bone mineral density (aBMD), bone mineral content (BMC) and structural properties in healthy children and adolescents (Gómez-Bruton et al., 2017). In adolescent males, a jump-based intervention enhanced bone parameters in those engaged in non-osteogenic sports and with poorer bone health (Vlachopoulos et al., 2018a; Vlachopoulos et al., 2018b). However, there is limited evidence of the effects of exercise on bone parameters in paediatric cancer patients, the reported findings are inconsistent (Hartman et al., 2009; Waked and Albenasy, 2018) and some of the studies have been carried out in a very small sample of participants (Müller et al., 2014; Dubnov-Raz et al., 2015). The interest in exercise oncology has sharply risen during the last decade and therefore, there is a need to know the pooled effect of exercise interventions on bone health in young paediatric cancer patients.

Therefore, the aims of this systematic review and meta-analysis were to (i) determine the pooled effect of exercise interventions from randomised controlled trials (RCTs) and non-RCTs in children and adolescents with cancer during and after oncological treatment on bone health and (ii) explore factors influencing the response of the exercise intervention. We hypothesised that (i) exercise would have a positive effect on bone health in this population when compared with control non-exercise groups, and (ii) enhancements will be greater in studies with longer interventions that involve weight-bearing and impact exercises of high intensity.

2 Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary Appendix S1) (Page et al., 2021; Ardern et al., 2022).

2.1 Search strategy

This systematic review and meta-analysis were registered in the International Prospective Register for Systematic Reviews (PROSPERO; registration number CRD42022310876). The recommendations of the Cochrane Collaboration Handbook for conducting systematic reviews and meta-analyses were strictly followed (Higgins et al., 2022). A systematic search of the literature was conducted in various electronic databases: MEDLINE (*via* PubMed), Web of Science and Scopus databases from November 2021 to January 2022. Intervention studies addressing the change in aBMD and BMC after exercise programmes in paediatric cancer participants in the childhood and adolescence periods were eligible. This systematic search was

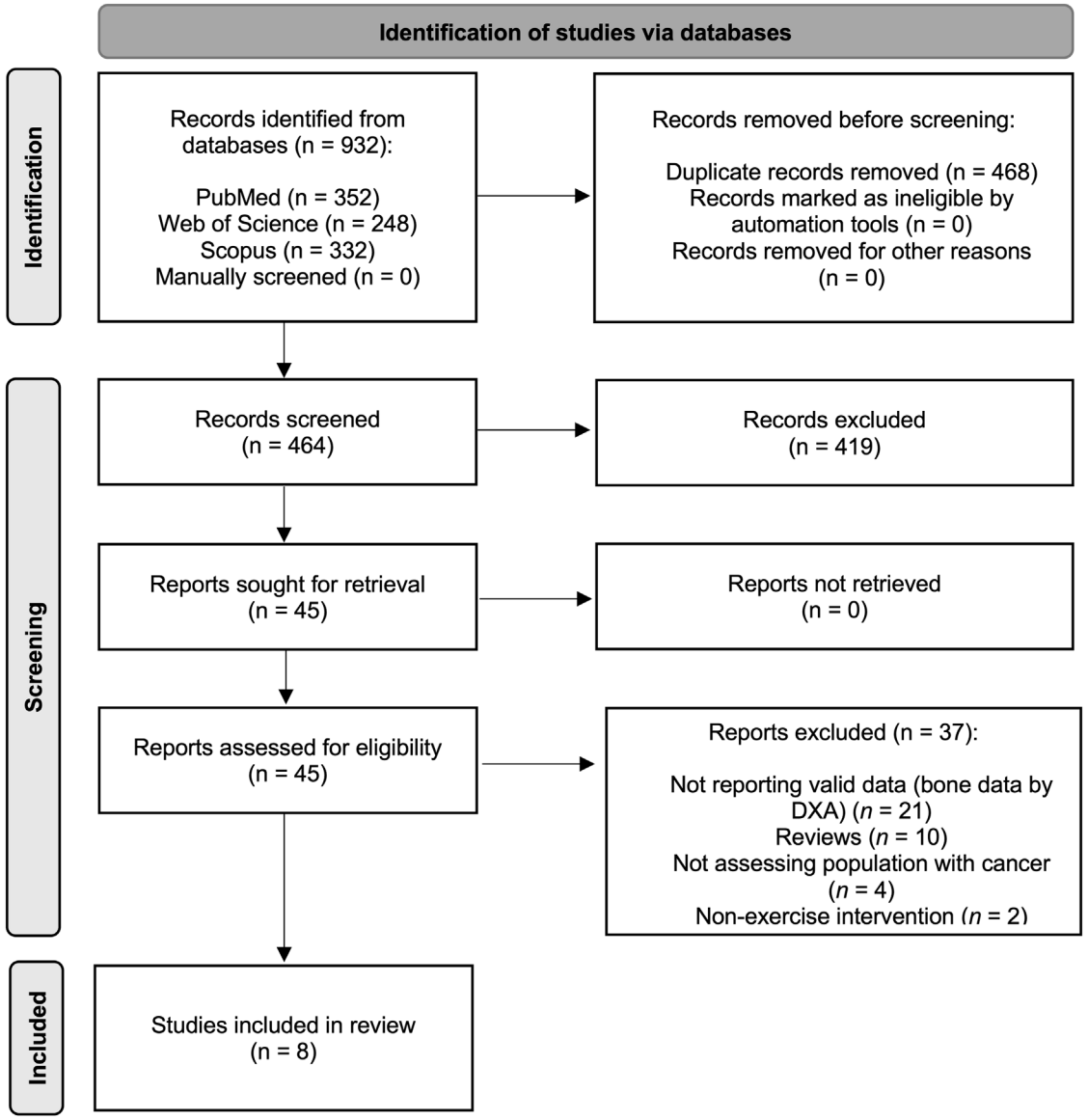

FIGURE 1
Literature search Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) consort diagram.

only restricted by language, solely including those studies published in English. We also manually screened other sources for additional records (i.e., references from previous reviews) and contacted authors for missing information when necessary. No studies were included from manual screenings. Combinations of the following keywords were used in the search (Supplementary Appendix S2): exercis*, move*, moving, sport*, train*, "physical activity", weightbear*, "high impact", running, walk*, strength*, "physical fitness", step*, gymnastic, balance, bone, cancer, onco*, myelo*, leukaemia, leukemia, neoplasm*, lympho*, carcinoma, tumor, tumour, sarcoma, child*, adolescen*, young*, boy*, girl*, pediatric*, paediatric*, trial*, random, intervention*, program* and rehabilitation. The literature search was complemented by reviewing references of the articles considered eligible.

2.2 Study selection

Study inclusion criteria were as follows: (i) participants: Paediatric cancer population (aged 1–19 years) during and after oncological treatment irrespective of the type of the treatment at any time point; (ii) study design: Intervention studies based on exercise programmes (RCT and non-RCTs) with a non-exercising control group; (iii) exposure: Exercise programmes with a minimum of 1 month of duration without restrictions on the setting, resistance, aerobic, walking, gymnastic, yoga, whole-body vibration and balance interventions were included, no minimal adherence required and the concomitant exposure to other treatment such as nutritional supplementation with calcium or vitamin D to both groups was allowed; and (iv) outcome: aBMD and BMC assessed using dual-energy X-ray absorptiometry

TABLE 1 Descriptive characteristics of included studies.

Reference	Country	Design	Population characteristics at baseline							Outcomes	
			Age, years	Sample size [n (% male)]	BMI, kg/m^2	Height, cm/m	Weight, kg	Cancer-type	Treatment phase/Type	Method	Baseline bone	Follow-up
Hartman et al. (2009)	Netherlands	Prospective randomised study	**Exercise group: Mean (range)** 5.3 (1.3–15.6) **Control group: Mean (range)** 6.2 (1.7–17.1)	**Exercise group:** 20 (56%) **Control group:** 21 (62%)	**Exercise group: SDS**-0.33 **Control group: SDS**-0.38	**Exercise group: SDS**-0.11 **Control group: SDS**-0.10	**Exercise group: SDS**-0.40 **Control group: SDS**-0.09	**Exercise group**: 25 Acute lymphoblastic leukemia **Control group**: 26 Acute lymphoblastic leukemia	During treatment/Chemotherapy	X-ray absorptiometry (DXA; Lunar DPX-L, Madison, WI)	**Exercise group: SDS WB aBMD** (g/cm^2): −0.10 **LS aBMD** (g/cm^2): −0.42 **LS BMAD** (g/cm^2): 0.14 **Control group: SDS WB aBMD** (g/cm^2): −0.18 **LS aBMD** (g/cm^2): −0.96 **LS BMAD** (g/cm^2): −0.48	**Exercise group: SDS WB ΔaBMD** (g/cm^2): 0.42 **LS ΔaBMD** (g/cm^2): 0.10 **LS ΔBMAD** (g/cm^2): 0.12 **Control group: SDSWB ΔaBMD** (g/cm^2): 0.35 **LS ΔaBMD** (g/cm^2): 0.14 **LS ΔBMAD** (g/cm^2): −0.04
Müller et al. (2014)	Germany	Non-randomised interventional study	**Exercise group: Mean ± SD** 15.2 ± 2.0 **Control group: Mean ± SD** 12.2 ± 2.6	**Exercise group:** 10 (40%) **Control group:** 11 (45%)	**Exercise group: Mean ± SD** 19.9 ± 2.9 **Control group: Mean ± SD** 18.2 ± 3.9	**Exercise group: Mean ± SD** 1.71 ± 0.10 **Control group: Mean ± SD** 1.54 ± 0.08	**Exercise group: Mean ± SD** 57.9 ± 7.2 **Control group: Mean ± SD** 44.0 ± 12.6	**Exercise group**: 7 osteosarcomas and 3 Ewing sarcoma **Control group**: 7 osteosarcomas and 4 Ewing sarcoma	During treatment/Surgery and/or radiotherapy	X-ray absorptiometry (DXA), Lunar Prodigy system (enCore 2006; Software version 10.51.006, GE Healthcare)	**Exercise group: Mean (SEM) LS (L2-L4) vBMD** (g/cm^3): 0.348, (0.020) **LS (L2-L4) aBMD** (g/cm^2): 1.074 (0.054) **LS (L2-L4) BMC** (g): 42.06 (2.58) **FN vBMD** (g/cm^3): 0.418 (0.024) **FN aBMD** (g/cm^2): 1.103 (0.043) **FN BMC** (g): 4.85 (0.27) **Control group: Mean (SEM) LS (L2-L4) vBMD** (g/cm^3): 0.322 (0.019) **LS (L2-L4) aBMD** (g/cm^2): 0.961 (0.050) **LS (L2-L4) BMC** (g): 35.47 (2.43) **FN vBMD** (g/cm^3): 0.381 (0.023) **FN aBMD** (g/cm^2): 0.898 (0.040) **FN BMC** (g): 4.06 (0.25)	**Exercise group: Mean (SEM) LS (L2-L4) vBMD** (g/cm^3): 0.347, (0.018) **LS (L2-L4) aBMD** (g/cm^2): 1.068 (0.055) **LS (L2-L4) BMC** (g): 41.23 (2.76) **FN vBMD** (g/cm^3): 0.406 (0.027) **FN aBMD** (g/cm^2): 0.998 (0.052) **FN BMC** (g): 4.72 (0.30) **Control group: Mean (SEM) LS (L2-L4) vBMD** (g/cm^3): 0.294 (0.017) **LS (L2-L4) aBMD** (g/cm^2): 0.875 (0.051) **LS (L2-L4) BMC** (g): 32.97 (2.60) **FN vBMD** (g/cm^3): 0.332 (0.027) **FN aBMD** (g/cm^2): 0.791 (0.052) **FN BMC** (g): 3.63 (0.30)
Cox et al. (2018)	United States and Canada	RCT	NR	**Exercise group:** 35 (64.2%) **Control group:** 40 (66.7%)	NR	NR	NR	**Exercise group**: 53 Acute lymphoblastic leukemia Control group: 55 Acute lymphoblastic leukemia	During treatment/Chemotherapy	Dual-energy X-ray absorptiometry (DEXA) using the GE Lunar Prodigy (Atlanta and Toronto) or the Hologic (SJCRH and MDA)	**Exercise group: LS (L1-L4): Z-score (SEM)** −0.21 (±1.27) **Control group: LS (L1-L4) Z-score: Z-score (SEM)** −0.62 (±1.14)	**Exercise group: LS (L1-L4) Z-score: Z-score (SEM)** −0.55 (±0.86) **Control group: LS (L1-L4) Z-score: Z-score (SEM)** −0.78 (±1.11)
Waked and Albenasy (2018)	Saudi Arabia	RCT	**Exercise group: Mean ± SD** 9.26 ± 2.39 **Control group: Mean ± SD** 9.91 ± 2.09	**Exercise group:** 23 (65.2%) **Control group:** 23 (78.3%)	**Exercise group: Mean ± SD** 18.15 ± 1.79 **Control group: Mean ± SD** 19.12 ± 1.56	**Exercise group: Mean ± SD** 124.13 ± 11.95 **Control group: Mean ± SD** 129.30 ± 10.8	**Exercise group: Mean ± SD** 28.52 ± 7.39 **Control group: Mean ± SD** 32.26 ± 6.57	**Exercise group**: 23 Acute lymphoblastic leukemia **Control group**: 23 Acute lymphoblastic leukemia	During treatment/Chemotherapy	Dual Energy X-ray Absorptiometry (DEXA) (DXA, Lunar DPXL/PED, Madison, Wisconsin, United States).	**Exercise group: Mean (SD) WB aBMD** (g/cm^2): 0.811 ± 0.072 **LS (L2-L4) aBMD** (g/cm^2): 0.727 ± 0.059 **Control group: Mean (SD) WB aBMD** (g/cm^2): 0.814 ± 0.071 **LS (L2-L4) aBMD** (g/cm^2): 0.712 ± 0.050	**Exercise group: Mean (SD) 6 months WB aBMD** (g/cm^2): 0.842 ± 0.076 **LS (L2-L4) aBMD** (g/cm^2): 0.778 ± 0.035 **12 months WB aBMD** (g/cm^2): 0.869 ± 0.069 **LS (L2-L4) aBMD** (g/cm^2): 0.808 ± 0.058 **Control group: Mean (SD) 6 months WB aBMD** (g/cm^2): 0.805 ± 0.056 **LS (L2-L4) aBMD** (g/cm^2): 0.716 ± 0.040 **12 months WB aBMD** (g/cm^2): 0.797 ± 0.055 **LS (L2-L4) aBMD** (g/cm^2): 0.724 ± 0.032

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TABLE 1 (*Continued*) Descriptive characteristics of included studies.

Reference	Country	Design	Population characteristics at baseline							Outcomes	
			Age, years	Sample size [n (% male)]	BMI, kg/m^2	Height, cm/m	Weight, kg	Cancer-type	Treatment phase/Type	Method	Baseline bone	Follow-up
Dubnov-Raz et al. (2015)	Israel	Interventional trial	**Exercise group: Mean (range)** 11.1 (7.8–13.8) **Control group: Mean (range)** 11.8 (9.0–12.8)	**Exercise group:** 10 (40%) **Control group:** 11 (50%)	**Exercise group: Mean (range)** 19.6 (17.6–3.9) **Control group: Mean (range)** 18.7 (17.1–21.2)	**Exercise group: Mean (range)** 144 (130–152) **Control group: Mean (range)** 148 (127–158)	**Exercise group: Mean (range)** (33.0–52.8) **Control group: Mean (range)** 40.6 (29.1–54.9)	**Exercise group**: 5 Acute lymphoblastic leukemia, 1 Burkitt lymphoma, 1 acute myeloid leukemia, 1 acute promyelocytic leukemia, 1 juvenile myelomonocytic leukemia and 1 neuroblastoma **Control group**: 3 Acute lymphoblastic leukemia, 2 Burkitt lymphoma, 2 Hodgkin lymphoma, 1 medulloblastoma, 1 rhabdomyosarcoma, 1 Wilms' tumor, 1 severe aplastic anemia and 1 Wiskott–Aldrich syndrome	After treatment/Chemotherapy and/or steroids and/or bone marrow transplantation	Dual energy X-ray absorptiometry with Lunar DPX software version 3.6 (Lunar Prodigy; General Electric Healthcare, Madison, Wisconsin, United States)	**Exercise group: Median (IQR) B aBMD** (g/cm^2): 0.95 (0.87–1.01) **WB BMC:** (g): 1435 (1117–2051) **LS (L1-L4) aBMD** (g/cm^2): 0.84 (0.78–0.92) **FN aBMD** (g/cm^2): 0.85 (0.75–0.89) **Control group: Median (IQR) WB aBMD** (g/cm^2): 0.90 (0.87–0.99) **WB BMC** (g): 1293 (1124–2069) **LS (L1-L4) aBMD** (g/cm^2): 0.75 (0.63–0.82) **FN aBMD** (g/cm^2): 0.82 (0.70–0.97)	**Exercise group: Median (IQR) WB aBMD** (g/cm^2): 0.97 (0.86–1.03) **WB BMC** (g): 1631 (1076–1993) **LS (L1-L4) aBMD** (g/cm^2): 0.88 (0.79–0.97) **FN aBMD** (g/cm^2): 0.89 (0.82–0.95) **Control group: Median (IQR) WB aBMD** (g/cm^2): 0.91 (0.90–1.03) **WB BMC** (g): 1445 (1222–2139) **LS (L1-L4) aBMD** (g/cm^2): 0.79 (0.69–0.85) **FN aBMD** (g/cm^2): 0.86 (0.72–0.97)
Braam et al. (2018)	Netherlands	RCT	**Exercise group: Mean ± SD** 13.4 ± 3.1 **Control group: Mean ± SD** 13.1 ± 3.1	**Exercise group:** 26 (53%) **Control group:** 33 (55%)	NR	**Exercise group: Mean ± SD** 158.9 ± 16.5 **Control group: Mean ± SD** 154.5 ± 17.2	**Exercise group: Mean ± SD** 51.6 ± 16.0 **Control group: Mean ± SD** 49.2 ± 16.9	**Exercise group**: 8 Acute lymphoblastic leukemia, 12 acute myeloid leukemia or Hodgkin lymphoma or non-Hodgkin lymphoma or chronic myeloid leukemia or Burkitt, 1 central nervous system/brain tumor and 9 solid tumors **Control group**: 12 Acute lymphoblastic leukemia, 13 acute myeloid leukemia or Hodgkin lymphoma or non-Hodgkin lymphoma or chronic myeloid leukemia or Burkitt, 6 centrals nervous system/brain tumor and 7 solid tumors	After treatment/Chemotherapy and/or radiotherapy	Dual-energy-X-ray absorptiometry (DXA)-scanner. (Hologic DXA scanner with the same software) + Lunar	**Exercise group: Mean (SD) LS (L1-L4) aBMD** (g/cm^2): 0.78 (±0.21) **Control group: Mean (SD) LS (L1-L4) aBMD** (g/cm^2): 0.75 (±0.18)	**Exercise group: Mean (SD)** Post Short-term **LS (L1-L4) aBMD** (g/cm^2): 0.78 (±0.20) Post Long-term **LS (L1-L4) aBMD** (g/cm^2): 0.83 (±0.23) **Control group: Mean (SD)** Post Short-term **LS (L1-L4) aBMD** (g/cm^2): 0.76 (±0.20) Post Long-term **LS (L1-L4) aBMD** (g/cm^2): 0.78 (±0.21)
Mogil et al. (2016)	United States	Prospective, double-blind, placebo-controlled trial	**Exercise group: Mean ± SD** 13.6 ± 3.7 **Control group: Mean ± SD** 13.6 ± 2.9	**Exercise group:** 22 (56.2%) **Control group:** 26 (51.5%)	NR	NR	NR	NR	After treatment/Unspecified	X-ray absorptiometry (DEXA, 4500 QDR-A/ Discovery fan beam; Hologic	NR	**Exercise group: Mean change (SD) WB BMC/height, total, %:** 1.71 (9.01) **WB BMD/height, total, %:** 6.56 (7.64) **LS BMC/height, total, %:** 3.70 (21.20) **LS BMD/height, total, %:** 4.91 (10.34) **LS vBMD, %:** 5.64 (10.83) **Control group: Mean change (SD) WB BMC/height, total, %:** 3.99 (8.97) **WB BMD/height, total, %:** 3.45 (7.60) **LS BMC/height, total, %:** 2.54 (21.06) **LS BMD/height, total, %:** 5.01 (10.29) **LS vBMD, %:** 5.30 (11.06)

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TABLE 1 (*Continued*) Descriptive characteristics of included studies.

Reference	Country	Design	Population characteristics at baseline							Outcomes	
			Age, years	Sample size [n (% male)]	BMI, kg/m^2	Height, cm/m	Weight, kg	Cancer-type	Treatment phase/Type	Method	Baseline bone	Follow-up
Elnaggar and Mohamed (2021)	Saudi Arabia	Prospective, single-blinded quasi-experimental study	**Exercise group: Mean ± SD** 13.33 ± 3.13 **Control group: Mean ± SD** 12.87 ± 2.56	**Exercise group: 15 (73.3%) Control group: 15 (53.3%)**	**Exercise group: Mean ± SD** 22.53 ± 1.40 **Control group: Mean ± SD** 21.89 ± 1.57	**Exercise group: Mean ± SD** 145 ± 14 **Control group: Mean ± SD** 149 ± 0.13	**Exercise group: Mean ± SD** 48.20 ± 10.86 **Control group: Mean ± SD** 49.80 ± 11.54	**Exercise group**: 15 Acute lymphoblastic leukemia Control group: 15 Acute lymphoblastic leukemia	After treatment/Unspecified	Lunar DPX-L pediatric software and dual-energy x-ray absorptiometry (DEXA) device (GE-Lunar)	**Exercise group: Mean (SD) LS (L_2 through L_5 segment) aBMD** (g/cm^2): 0.64 ± 0.10 **LS (L_2 through L_5 segment) vBMD** (g/cm^3): 0.32 ± 0.04 **LS BMC (L_2 through L_5 segment)** (g): 33.91 ± 7.12 **FN aBMD** (g/cm^2): 0.59 ± 0.06 **FN vBMD** (g/cm^3): 0.31 ± 0.04 **FN BMC** (g): 32.35 ± 6.69 **Control group: Mean (SD) LS (L_2 through L_5 segment) aBMD** (g/cm^2): 0.61 ± 0.06 **LS (L_2 through L_5 segment) vBMD** (g/cm^3): 0.30 ± 0.03 **LS (L_2 through L_5 segment) BMC** (g/cm): 30.63 ± 5.92 **FC aBMD** (g/cm^2): 0.62 ± 0.05 **FN vBMD** (g/cm^3): 0.30 ± 0.04 **FN BMC** (g): 32.88 ± 6.16	**Exercise group: Mean (SD) LS (L_2 through L_5 segment) aBMD** (g/cm^2): 0.70 ± 0.06 **LS (L_2 through L_5 segment) vBMD** (g/cm^3): 0.36 ± 0.03 **LS (L_2 through L_5 segment) BMC** (g): 37.46 ± 4.59 **FN aBMD** (g/cm^2): 0.67 ± 0.07 **FN vBMD** (g/cm^3): 0.34 ± 0.03 **FN BMC** (g): 37.76 ± 5.65 **Control group: Mean (SD) LS (L_2 through L_5 segment) aBMD** (g/cm^2): 0.64 ± 0.07 **LS (L_2 through L_5 segment) vBMD** (g/cm^3): 0.32 ± 0.03 **LS (L_2 through L_5 segment) BMC** (g): 33.29 ± 4.14 **FC aBMD** (g/cm^2): 0.63 ± 0.05 **FN vBMD** (g/cm^3): 0.31 ± 0.04 **FN BMC** (g): 34.45 ± 5.02

Abbreviations: RCT, randomised controlled trial; WB, whole body; LS, lumbar spine; FN, femoral neck; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BMAD, bone mineral apparent density; BMC, bone mineral content; SDS, standard deviation scores; NR, not reported.

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TABLE 2 Intervention characteristics of included studies.

Reference	Frequency (F)	Volume (V)	Supervision (S)
	Intensity (I)	Progression (P)	Place of exercise program (PEP)
	Time (TM)	Intervention duration (ID)	Control group (CG)
	Type (TP)	Attendance (A)	Other characteristics (OC)
Hartman et al. (2009)	F: 1/6W (educational sessions), 7/W (functionality maintenance EX) and 2/D (stretching and jumping EX) I: - TM: - TP: Education regarding possible motor problems resulting from chemotherapy, EX to maintain hand and leg function and stretching EX to maintain ankle dorsiflexion mobility and short-burst high-intensity EX to prevent reduction in BMD	V: - P: - ID: 2 years A: -	S: No (it was only supervised by their parents) PEP: Home CG: Standard care for the CG included neither an initial session nor any prescheduled follow-up sessions with the hospital-based physiotherapist OC: Parents were supplied with an EX list, enabling them to select EX most appropriate for their child's age and also to vary EX
Müller et al. (2014)	F: During hospital stays: preferably every second day. However, patients had the opportunity to work-out on a daily basis, except for the weekends I: Moderate to vigorous (according to Borg's ratings of perceived exertion of 13–16) TM: 15–45 Min TP: RT	V: 1–3 sets x 6–12 reps P: - ID: 6 M A: Patients participated in 34.5 ± 8 training sessions on average, corresponding to an adherence rate of 77%, based on the recommendation of training every other day	S: Yes PEP: - CG: Received standard physiotherapeutic treatment based on their disability and as prescribed by the attending physician daily on workdays and included mobilization techniques of 20–30 Min duration OC: All patients received the same standard physiotherapeutic treatment than the CG. Additionally, sports games like football, basketball or table tennis were offered especially for younger children who could hardly be encouraged for the structured workouts
Cox et al. (2018)	F: 2/W (1st W—4th W), 1/W (5th W—8th W) and 1/M (9th W—135th W) I: - TM: - TP: Supporting motivation sessions about relatedness, competency, and autonomy	V: - P: No ID: 2.5 years A: There were no differences between the groups relative to APN ($p = 0.12$) missed appointments (intervention, missed APN visits, mean = 4.39, SD = 5.41; usual care, missed APN visits, mean = 2.49, SD = 3.60	S: No (it could have been supervised by their parents) PEP: Home CG: Usual-care attention control (advanced practice nurse inquired in a neutral manner on the same schedule as for the intervention group) OC: It was emphasized the volitional nature of participation in the program and avoided coercive language)
Waked and Albenasy (2018)	F: 2/W (1st-6th M), 1/W (7st-12th M) I: Light to moderate (according to Borg's ratings of perceived exertion of 3–6 out of 10) TM: 30–45 Min TP: Mixed-modality EX program: 1) AE such as walking or stationary cycling 2) RT using resistance bands 3) Flexibility training such as static stretching	V: - P: Progression of EX for each patient depended on patient tolerance ID: 12 M A: -	S: Yes PEP: - CG: Each patient in CG was advised to be active as much as possible OC: Necessary written instructions and tools such as resistance bands for prescribed EX were given to each child
Dubnov-Raz et al. (2015)	F: 3/W I: Moderate TM: 55–60 Min TP: Strength and endurance EX using bands, balls, games, free-weights and various EX machines in the gym	V: - P: - ID: 6 M A: -	S: No PEP: Go-Active gym chain in Israel CG: They were asked to continue with their usual lifestyle habits OC: Adherence to the program was verified by telephone calls to the participants every two W and by periodic visits to the EG
Braam et al. (2018)	F: 2/W I: 66%–77% of HRpeak (1st W—4th W), 77%–90% of HRpeak (5th W—8th W) and 90%–100% of HRpeak (9th W—12th W) TM: 45 Min TP: AE and weight-bearing EX performed in a circuit training-setting with balls, hoops, and running activities	V: - P: The intensity of the physical EX training program gradually increased ID: 12 W A: The median adherence was 24 sessions (interquartile range (IQR): 20–24). 20 out of 30 children (67%) attended all physical EX training sessions within 12–16 W. 13% dropped-out mainly due to recurrence of the disease (7/9)	S: Yes PEP: Local physical therapy practice CG: Usual care according to local guidelines and preferences OC: 10 children (33%) performed some of the EX at a lower heart rate than described
	F: At least 3/W (7th W—12th W) I: High intensity TM: 11 Min TP: Weight-bearing EX	V: - P: No ID: 6 W (from 7th W) A: -	S: No PEP: Home CG: Usual care according to local guidelines and preferences OC: N
	F: 1/W I: - TM: 60 Min TP: Psycho-education and cognitive-behavioral techniques including items on expression of feelings, self-perception and coping skills	V: - P: Yes ID: 12 W A: The psychosocial training intervention was completed by 27 children (90%)	S: Yes PEP: - CG: Usual care according to local guidelines and preferences OC: After each individual session home EX on the topic of this specific session could be given to the patient if the psychologist considered it necessary

(Continued on following page)

TABLE 2 (*Continued*) Intervention characteristics of included studies.

Reference	Frequency (F)	Volume (V)	Supervision (S)
	Intensity (I)	Progression (P)	Place of exercise program (PEP)
	Time (TM)	Intervention duration (ID)	Control group (CG)
	Type (TP)	Attendance (A)	Other characteristics (OC)
Mogil et al. (2016)	F: Twice daily I: The mechanical signal (0.3 g at 32–37 Hz) produced a subtle, sinusoidal, vertical translation less than 100 μm *via* a linear electromagnetic actuator TM: 10 Min TP: Standing on an active platform	V: - P: - ID: 1 year A: Median (interquartile range) values of 70.1% (35.4%–91.5%) in the intervention and 63.7% (33.3%–86.5%) in the placebo group (p = .40)	S: No PEP: Home CG: The placebo group stood on a device identical in appearance to the active platform. The placebo device emitted a 500-Hz audible hum but did not deliver the signal OC: Received calcium (800–1200 mg/d) and vitamin D supplements (cholecalciferol, 400 IU/d)
Elnaggar and Mohamed (2021)	F: 3/W I: Weight-bearing TM: 45 Min TP: Lower-body plyometric EX program	V: 10 lower-body Aqua-PLYO EX: 1st W—4th W: from 1 set x 4 reps to 3 sets x 10 reps 5th W—8th W: from 1 set x 15 reps to 3 sets x 15 reps 9th W—12th W: from 2 sets x 10 reps to 5 sets x 10 reps P: The training volume or intensity was increased as the W progressed in three blocks (specifically, every 4 W) ID: 12 W A: The median and interquartile range (IQR) of adherence-to-treatment was 91.67% (IQR 91.67% and 95.83%) in the Aqua-PLYO group and 95.83% (IQR 95.83% and 100%) in the CG	S: Yes PEP: 3 × 4 m water pool CG: Usual physical therapy OC: The water depth was waist-leveled, and the room and water temperature were regulated at 26°C–28°C and 30°C–31°C, respectively

Abbreviations: AE, aerobic exercise; EX, exercise; EG, exercise group; HRpeak, Heart Rate Peak; IQR, interquartile range; M, Month(s); D, day; Min, Minutes; "-", not reported; RT, resistance training; SD, standard deviation; W, week.

(DXA). Exclusion criteria were as follows: (i) studies including individuals older than 19 years old; (ii) non-eligible publication types, such as review articles, editorials, comments, guidelines or case reports; (iii) assessment of aBMD and BMC using other methods (i.e., computed tomography); and (iv) studies published in any language other than English. Based on the selection criteria, all studies were independently screened for inclusion by two reviewers and disagreements were solved by consensus or involving a third researcher. A total of potential manuscripts were identified following database examination (Figure 1), eight of them met the inclusion criteria and were, therefore, included in the meta-analysis.

2.3 Data extraction and risk of bias assessment

All articles retrieved from the respective databases were exported and handled in an EndNote library (Endnote version X7). After removing the duplicated articles, two researchers independently read the titles and abstracts to screened out the irrelevant articles according to the inclusion/exclusion criteria, and finally screened the articles by reading the full text. Any conflicts were solved by consensus with a third researcher.

The following data were retrieved from the original reports: (i) first author and year of publication; (ii) country from which the data were collected; (iii) study design; (iv) sample characteristics (age, sample size, body mass index, height, weight, and type of cancer); and (v) the method used for measuring bone measurement characteristics (aBMD, volumetric BMD, bone mineral apparent density and BMC, including values for whole body, lumbar spine, and femoral neck) at baseline and at end of follow-up. Besides, data concerning exercise programmes were extracted from the original manuscripts: (i) frequency, (ii) intensity, (iii) time, (iv) type, (v) volume, (vi) progression, (vii) intervention duration, (viii) attendance, (ix) supervision, (x) home exercise programmes, (xi) control group and (xii) other characteristics.

Two researchers independently assessed the risk of bias and any discrepancies were resolved by a third reviewer. The Cochrane Collaboration's tool for assessing the risk of bias (RoB2.0) was used to assess the certainty of the evidence of the RCT studies (Sterne et al., 2019). This tool covers bias in five domains: Randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. According to this assessment tool, the studies were rated as "low risk of bias" (if all domains were judged as "low risk"), "some concerns" (if there was at least one domain rated as having "some concerns"), or "high risk of bias" (if there was at least one domain judged as "high risk"). The Joanna Briggs Institute Critical Appraisal Tool (Tufanaru et al., 2017) for Quasi-Experimental Studies were used to assess the certainty of the evidence of the non-randomised experimental studies. According to this assessment tool, the studies were rated as good (i.e., most criteria met, with a low risk of bias), fair (i.e., some criteria met, with a moderate risk of

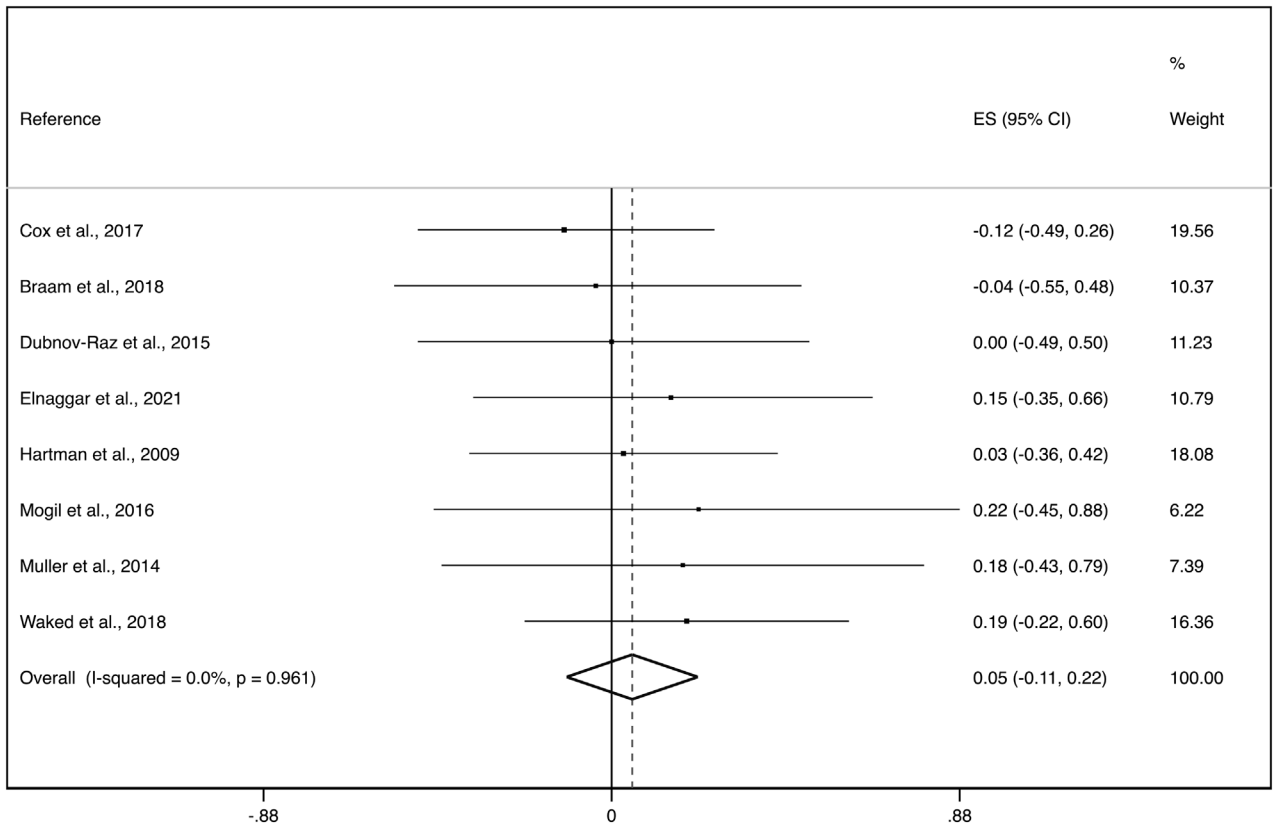

FIGURE 2
Forest plot of the effect size for the change in total aBMD. CI: confidence interval, ES: effect sizes.

bias), or poor (i.e., few criteria met, with a high risk of bias). No studies were excluded based on the quality appraisal.

2.4 Statistical considerations

The inverse-variance-weighted method used to compute the pooled effect size (ES) estimate and the 132 respective 95% confidence interval (95%CI). An ES was calculated for the pre-post aBMD mean values or the mean value change using Sn's d index. ES values of 0.2 were considered a weak effect, values of 0.5 were considered a moderate effect, values of 0.8 were considered a strong effect, and values larger than 1.0 were considered a very strong effect. When studies reported means and standard errors (SE) or 95% CI we used the formulas, standard deviation (SD) = sqrt (sample size) * SE and SD = sqrt (sample size) * [(upper limit 95% CI—lower limit 95% CI)/3.92], to convert to SD. Additionally, when studies reported pre-post aBMD mean values or the mean value change data in graphs, the online tool WebPlotDigitizer (https://apps.automeris.io/wpd/) was used to extract the data for the ES calculation.

Heterogeneity of results across studies was assessed using the I^2 statistic (Higgins and Thompson, 2002). I^2 values were considered as follows: might not be important (0%–40%), may represent moderate heterogeneity (30%–60%), may represent substantial heterogeneity (50%–90%), or considerable heterogeneity (75%–100%); the corresponding *p* values also were considered. Finally, we calculated the statistic τ2 to establish the size and clinical relevance of heterogeneity. A τ2 estimate of 0.04 can be considered as low, 0.14 as moderate, and 0.40 as a substantial degree of the clinical relevance of heterogeneity (Stettler et al., 2008).

Exploratory subgroups analyses were conducted according to the type of aBMD region (whole body, lumbar spine or femoral neck) and patient status (during oncological treatment or surviving patients). Furthermore, sensitivity analyses (systematic reanalysis while removing studies one at a time) and subgroup analyses were conducted to assess the robustness of the summary estimates. The results of the sensitivity analyses were considered meaningful when the resulting estimates were modified beyond the CIs of the original summary estimate. In addition, sensitivity analyses provided insight into whether any study or special condition included in the studies accounted for a large proportion of the heterogeneity among the ES pooled estimations, based on the change in I^2 values (and associated categories previously reported).

Finally, small-study effects and publication bias were examined using the Doi plot and the Luis Furuya–Kanamori index (LFK index). No asymmetry, minor asymmetry or major asymmetry were considered with values of one, between one and two, and two, respectively (Furuya-Kanamori et al., 2018). Statistical analyses were performed using STATA SE software, version 14 (StataCorp, College Station, Texas).

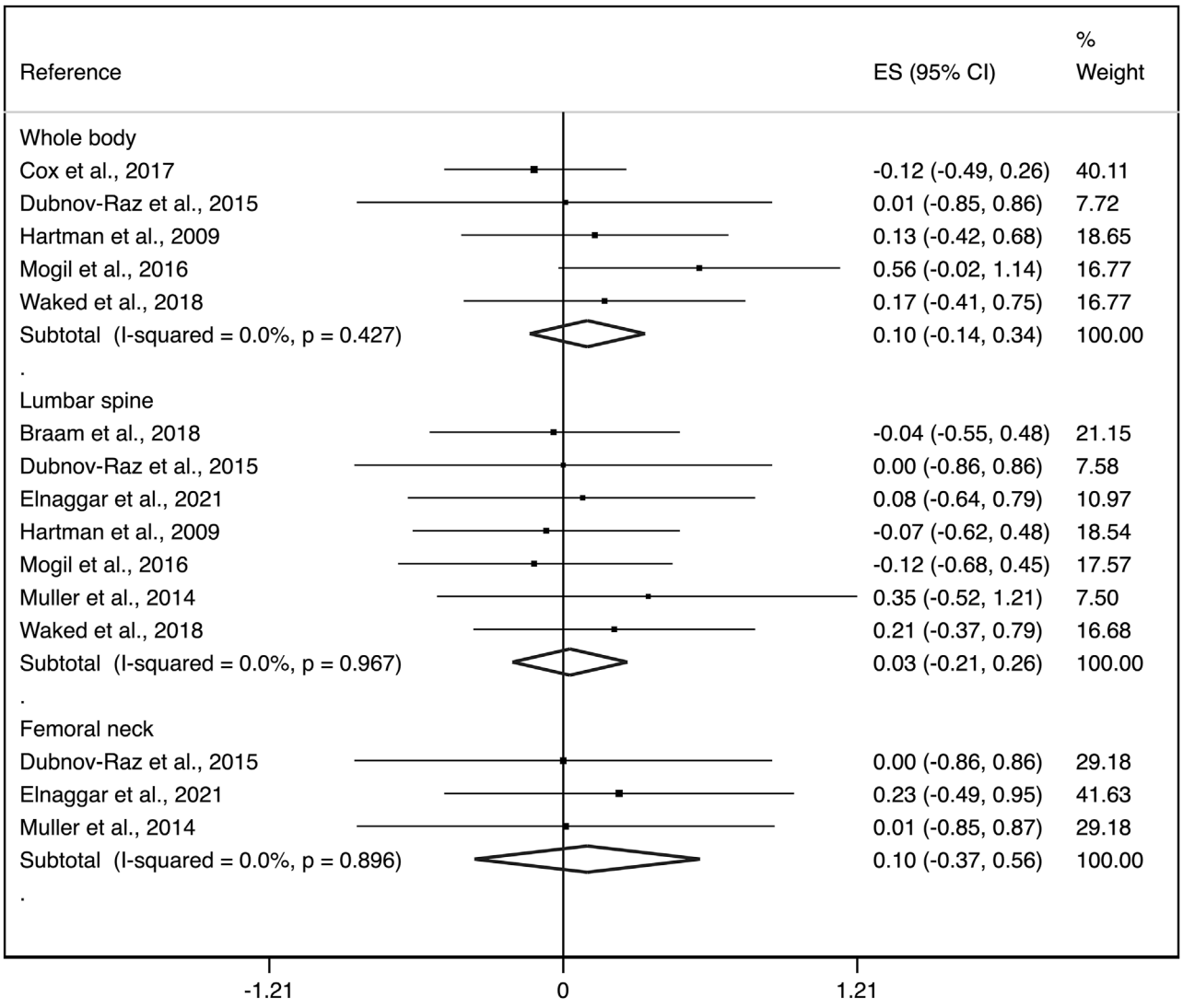

FIGURE 3
Forest plots of the effect size for the change in aBMD at the whole body, lumbar spine and femoral neck. CI: confidence interval, ES: effect sizes.

3 Results

The PRISMA flow diagram for the systematic search and study selection is shown in Figure 1.

3.1 Level of evidence and risk of bias of the studies

The overall risk of bias for RCTs showed two studies with low risk (40%) and three studies with some concerns (60%) (Supplementary Appendix S3). Regarding the specific domains, in the randomisation process, missing outcome data, and measurement of outcome domains, all the studies (n = 5, 100%) were scored as low risk. In the deviations from intentional interventions and selection of the reported results domains, two studies (40%) were scored as some concerns and three studies as low risk (60%).

The risk of bias for non-randomised experimental studies showed two studies with high quality (66,67%) and one study with medium quality (33,33%). When the studies were analysed by individual domains, all the studies (n = 3, 100%) made clear what the 'cause' was and what the 'effect' was, had a control group, had multiple measurements of the outcome both pre and post the intervention/exposure, adequately described and analysed any differences between groups in terms of their follow up, and measured in the same way the outcomes of included participants and in a reliable way. In addition, two studies included similar participants (66.67%), and one study (33.3%) included participants in any comparisons receiving similar treatment/care other than the exposure or intervention of interest, and used an appropriate statistical analysis (Supplementary Appendix S4).

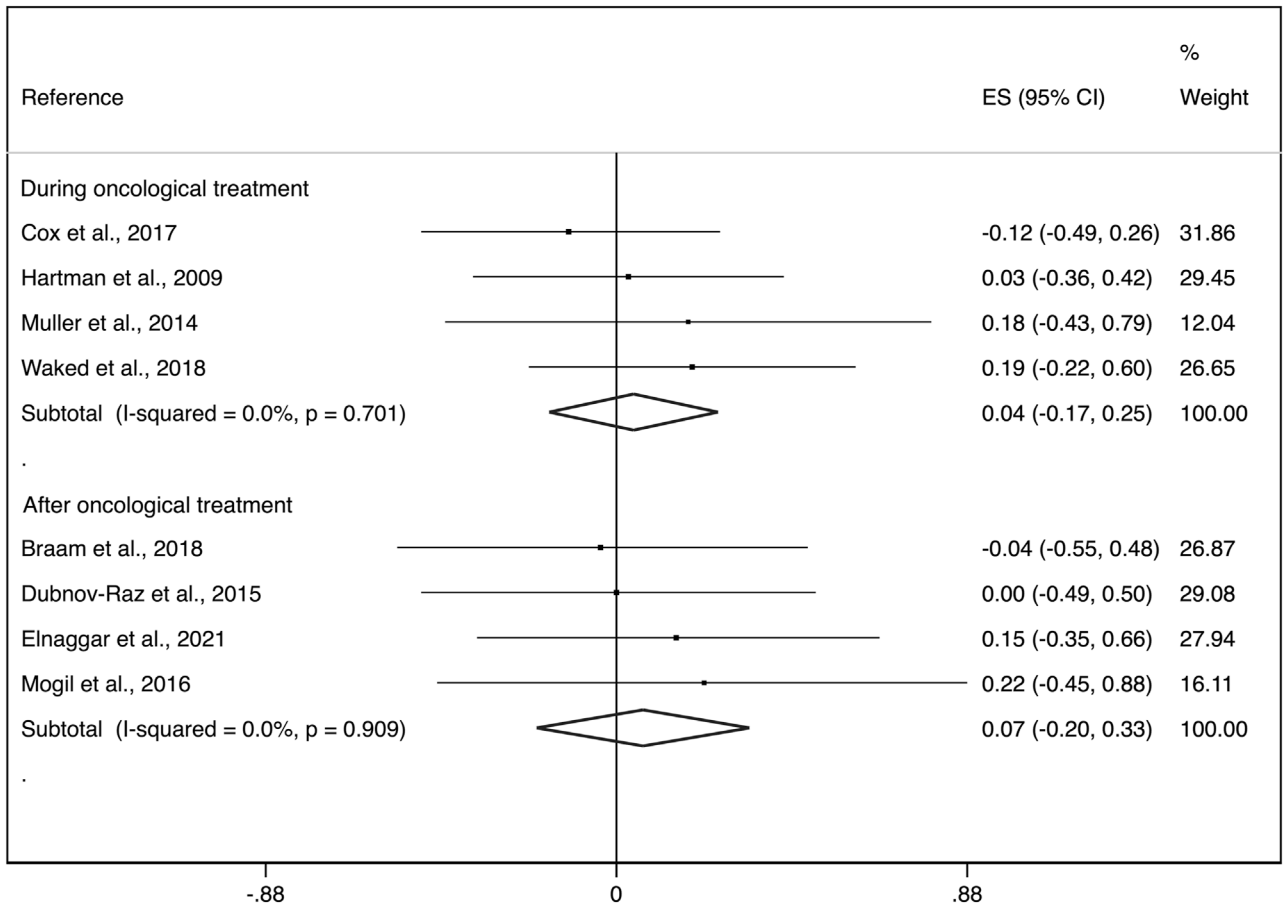

FIGURE 4
Forest plots of the effect size for the change in aBMD at the whole body, lumbar spine and femoral neck by groups (during cancer treatment and surviving patients). CI: confidence interval, ES: effect sizes.

3.2 Characteristics of the participants and assessment methods selected

Table 1 shows the participants characteristics of the eight studies included in this meta-analysis. Participants age ranged from 1.3 to 18 years old, with sample sizes ranging from 21 to 75 participants (mean = 48 participants, total = 341). The type of cancer included acute lymphoblastic leukaemia, acute myeloid leukaemia, Hodgkin lymphoma or non-Hodgkin lymphoma, chronic myeloid leukaemia or Burkitt, central nervous system/brain tumour, solid tumour, neuroblastoma, Wiskott–Aldrich syndrome or osteosarcomas and Ewing sarcoma. Concerning the assessment methods carried out in the studies, five studies used the Lunar Prodigy, one study used the Hologic, one study used both the Lunar Prodigy or the Hologic and one study used both the Hologic and Lunar Prodigy.

3.3 Characteristics of the studies selected

These eight studies reported aBMD and BMC changes after exercise interventions in paediatric cancer survivors during (n = 4) and after (n = 4) oncological treatment (Table 1) (Hartman et al., 2009; Müller et al., 2014; Dubnov-Raz et al., 2015; Mogil et al., 2016; Braam et al., 2018; Cox et al., 2018; Waked and Albenasy, 2018; Elnaggar and Mohamed, 2021), compared with a non-exercising control group. They were published between 2009 and 2021 and were carried out in six different countries: two studies conducted in Netherlands, two in Saudi Arabia, one in United States, one in United States and Canada, one in Israel and one in Germany.

There were five RCTs (Hartman et al., 2009; Mogil et al., 2016; Braam et al., 2018; Cox et al., 2018; Waked and Albenasy, 2018) and 3 non-RCTs (Müller et al., 2014; Dubnov-Raz et al., 2015; Elnaggar and Mohamed, 2021). Table 2 shows the eight screened studies highlighting their FITT interventions. The characteristics of the interventions are as follows: (i) Frequency, ranged 1 from 1.5 to 7 days a week (mean = 3 days a week); (ii) Intensity, was differently reported depending on the type of exercise in terms of heart rate peak (HRpeak), mechanical stimulation from a platform, Borg's scale, weight-bearing, light-to-moderate, moderate-to-vigorous and high intensity, while two studies did no describe the intensity target; (iii) Time per session, ranged from 10 to 60 min (mean = 36 min) but one study did not report it and time per intervention, ranged from 3 to 30 months (mean = 13 months); and (iv) Type, four

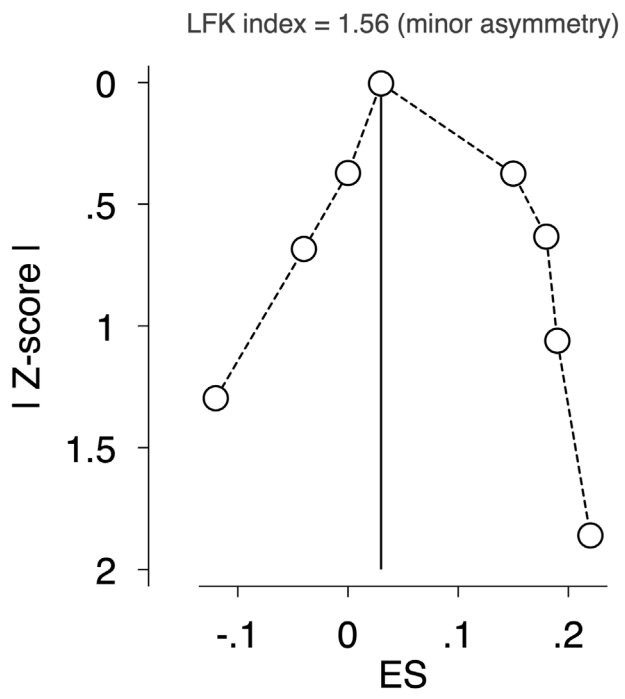

FIGURE 5
Assessment of potential publication bias by LFK index.
Abbreviations: RCT, randomised controlled trial; WB, whole body; LS, lumbar spine; FN, femoral neck; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BMAD, bone mineral apparent density; BMC, bone mineral content; SDS, standard deviation scores; NR, Not reported.

studies conducted a concurrent exercise intervention (resistance and endurance training), three studies implemented a resistance training intervention and one study carried out a low-magnitude, high-frequency mechanical stimulation. Control groups did not receive an exercising treatment.

3.4 Meta-analysis

The eight studies reporting aBMD changes after exercise interventions in paediatric cancer survivors during (n = 4) and after (n = 4) oncological treatment were included in this meta-analysis with a total of 341 participants. The pooled ES of exercise interventions showed no evidence of an effect on aBMD (ES = 0.05; 95%CI: −0.11, 0.22) with not important heterogeneity (I^2 = 0.0%, p = 0.961; $\tau 2 = 0.000$). (Figure 2).

Exploratory subgroup analyses by aBMD region showed an ES of: i) 0.10 (95%CI: −0.14, 0.34) with not important heterogeneity (I^2 = 0.0%, p = 0.427; $\tau 2 = 0.000$) for whole body, ii) 0.03 (95%CI: −0.21, 0.26) with not important heterogeneity (I^2 = 0.0%, p = 0.967; $\tau 2 = 0.000$) for lumbar spine and, iii) 0.10 (95%CI: −0.37, 0.56) with no evidence of important heterogeneity (I^2 = 0.0%, p = 0.896; $\tau 2 = 0.000$) for femoral neck (Figure 3).

Additionally, during the treatment phase the ES was: i) 0.04 (95%CI: −0.17, 0.25) with not important heterogeneity (I^2 = 0.0%, p = 0.701; $\tau 2 = 0.000$) and after the treatment phase, ii) 0.07 (95%CI: −0.20, 0.33) with not important heterogeneity (I^2 = 0.0%, p = 0.909; $\tau 2 = 0.000$) (Figure 4).

The pooled ES estimate for exercise interventions was not modified in aBMD when studies were removed from the analysis one at a time to examine the impact of individual studies. There was a minor asymmetry of small-study effects for exercise interventions, as evidenced by visual inspection of the Doi plot and LFK index (1.56) (Figure 5).

4 Discussion

The findings of the present systematic review and meta-analysis suggest that previous studies are inappropriate to illustrate any beneficial effect on improving bone parameters in children and adolescents during and after oncological treatment. Results should however be interpreted with caution due to the low number of the studies included and the low homogeneity of the intervention characteristics. To the best of our knowledge, this is the first systematic review and meta-analysis synthesising the evidence on the effect of exercise on bone health in children and adolescents during and after oncological treatment.

During oncological treatment, there are no studies showing a beneficial effect of exercise on bone parameters in children and adolescents. First and foremost, one of the most common side effects during oncological treatment is cancer-related fatigue (Lucía et al., 2003; Ng et al., 2005). This may be reflected by the poor adherence of participants to the exercise intervention as in the study of (Hartman et al., 2009), in which 36% of participants exercised less than once a week. This could have been an important barrier to achieve the required exercise intensity to effectively stimulate the bone and to obtain bone adaptations. As an example, previous research in healthy adolescents showed that those who did 28–32 min of vigorous physical activity per day had optimal aBMD at key regions within the hip (Gracia-Marco et al., 2011). Secondly, the prescribed exercise type might not be appropriate to bone adaptations in some studies. For instance, despite weight-bearing and impact exercises of high intensity significantly contribute bone development, this type of exercise was not chosen in the studies of (Müller et al., 2014) and Waked (Cox et al., 2018; Waked and Albenasy, 2018), and when included, the intensity required to modify bone parameters were not achievable as mentioned in the study of (Cox et al., 2018). The latter intervention was proven not to be feasible during the early oncological treatment phase owing to the child's responses to the disease and the treatment. Interestingly, this exercise intervention was the longest (30 months) in comparison with the rest of studies. Finally, it is important to mention that half of the exercise interventions were unsupervised (Hartman et al., 2009; Cox et al., 2018), which does not concur with the International Pediatric Oncology Exercise Guidelines which recommend that a qualified exercise professionals should implement supervised exercise programmes throughout cancer continuum (Wurz et al., 2021).

To sum up, the most updated research in children and adolescents during oncological treatment suggests that there is no evidence of an effect of exercise at inducing meaningful bone adaptations. Overall, the potential cancer-related fatigue sequel, the selection of the inappropriate exercises to improve bone parameters (e.g., cycling, lack of weight-bearing impact exercises of high intensity, unsupervised exercise interventions) and the unachievable intensity of the interventions are important factors that have hindered the required stimulus in the bones. The use of behaviour change techniques (i.e., gamification) in long-

lasting interventions with growing population is recommended (Muntaner-Mas et al., 2017; Sailer et al., 2017) and could have helped to increase the low adherence rate reported (Hartman et al., 2009; Cox et al., 2018).

Shortly after oncological treatment, there is no evidence of positive effects of exercise interventions aimed at improving bone parameters. One of the potential factors could be the short duration as half of the interventions lasted for only 3 months (Braam et al., 2018; Elnaggar and Mohamed, 2021). The bone remodelling process takes approximately 5 months and therefore, shorter interventions could not reflect true bone adaptations (Kenkre and Bassett, 2018). In addition, the type of exercise has not been the most appropriate to improve bone parameters in some cases (Dubnov-Raz et al., 2015) did not include weight-bearing impact exercises of high intensity yet participants reported to be mentally and physically healthier than those in previous studies during oncological treatment (Cox et al., 2018). Likewise, Elnaggar et al. (Elnaggar and Mohamed, 2021) included lower-body plyometric exercises in a swimming pool, that is, in a microgravity environment, which is not effective at increasing bone parameters (Gómez-Bruton et al., 2013). Nevertheless, (Mogil et al., 2016) implemented an intervention including standing on an active vibration platform emitting low-magnitude high-frequency mechanical stimulation, considered a type of weight-bearing physical activity as it requires muscles and bones to work against gravity (Cardinale and Bosco, 2003; Cardinale and Wakeling, 2005). From the included studies, the latter was the only intervention that observed a borderline significant increase in total body aBMD ($p = 0.05$). The timing of the intervention (i.e., after oncological treatment), the frequency (twice per day) and adequate intervention duration (1 year) could explain the findings. However, their intervention type was clearly ineffective at increasing lumbar spine aBMD outcomes. As stated by the authors, this might have been caused by the potential loss of vibratory energy as the signal travelled from the distal lower extremity to the trunk. This agrees with a recent systematic review and meta-analysis in children and adolescents with motor disabilities that found no pooled effect of similar interventions on lumbar spine aBMD (Li et al., 2022). Lastly, some studies did not exclude participants receiving growth hormone, corticosteroids or bisphosphonates (Dubnov-Raz et al., 2015), or even included participants during the remaining oncological treatment period (Braam et al., 2018), which might have affected the results.

In conclusion, there is no evidence of an effect of exercise interventions conducted after oncological treatment at increasing bone parameters in children and adolescents. There are several reasons that may explain this lack of effect: the short duration of the interventions, the type of the exercises (i.e., lack of weight-bearing exercises or in a microgravity environment) and inclusion of participants undergoing maintenance treatment that affects bone parameters.

Remarkably, the exercise interventions were not delivered by exercise professionals in 75% of the included studies. This sets a potential barrier and limitation for the intervention to succeed. There is a need of exercise professionals with a high qualification and robust background in exercise oncology. Similar thoughts have been shared by (Adams et al., 2021) who stated that oncologic healthcare providers working in cancer care system did not feel confident when prescribing exercise and therefore, they should not be responsible for prescribing it. According to the International Pediatric Oncology Exercise Guidelines, qualified exercise professionals should be part of standard care and therefore should facilitate programme implementation and uptake throughout the cancer continuum (Wurz et al., 2021).

The present systematic review and meta-analysis has several limitations. The main limitation is the availability of published studies and well-designed RCTs aiming at investigating bone changes in children and adolescents diagnosed with cancer. Additionally, the data reported were exclusively taken from the manuscripts included in this work and not from the clinical trials registries. In most of the cases, the interventions were not designed to meet the aim of improving bone health. Thus, these findings should be viewed with caution. Nevertheless, it shows the current evidence on exercise paediatric oncology and bone health and should be viewed as a starting point for researchers to think of the best approach for designing their exercise interventions. To date, only two systematic reviews and meta-analyses have been conducted with the same purpose in adult cancer patients during and after oncological treatment with promising positive results (Rose et al., 2022; Singh and Toohey, 2022).

5 Conclusion

Our systematic review and meta-analysis indicate that the exercise interventions were inappropriate and therefore, ineffective to illustrate any beneficial effect on bone of children and adolescents with cancer during and after oncological treatment. Several limitations in the design of the interventions have been identified. There is a need of implementing well-designed exercise RCTs specifically focused on improving bone health in children and adolescents diagnosed with cancer due its scientific and clinical importance. Early intervention strategies to optimize bone health through effective tailoring of osteogenic exercise programmes are of vital importance.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AM-P conceptualised and designed the study with the support of EU-G and LG-M. AM-P drafted the initial manuscript and, along with EU-G and LG-M, approved the final manuscript as submitted. AM-P, EU-G, and LG-M designed the data collection instruments, and coordinated and supervised data collection. AM-P, EU-G, AR-S, JG-C, VM, IC-R, JR, and LG-M were involved in the analysis and interpretation of data, and reviewed and revised the manuscript, approving the final manuscript as submitted.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1088740/full#supplementary-material

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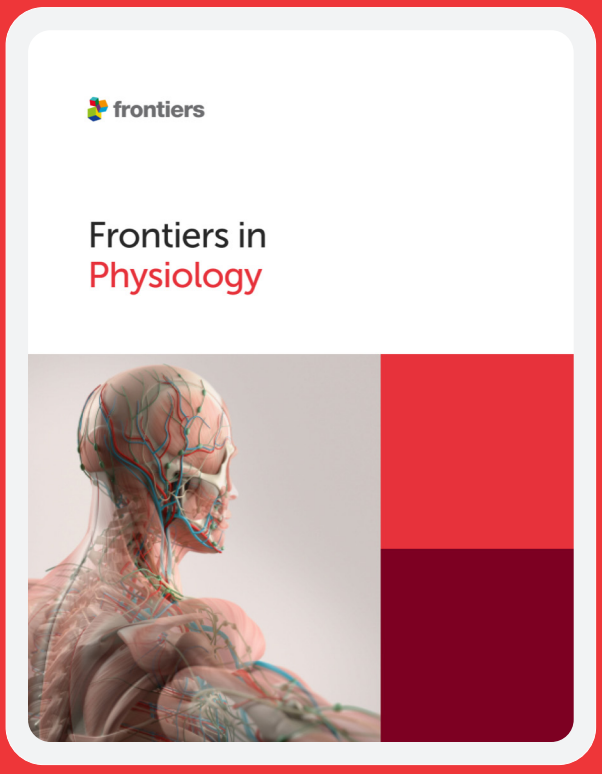

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