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# Effects of 8-week High-Intensity Interval Training and Moderate-Intensity Continuous Training on Bone Metabolism in Sedentary Young Females

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## ABSTRACT

**Objective:** High-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) have been reported as effective exercise modes on bone metabolism. However, very few studies focused on young women with sedentary behavior. The purpose of this study was to investigate the effects of 8-week HIIT on bone metabolism in sedentary young women.

**Methods:** 26 healthy, sedentary female participants were randomly assigned to either the HIIT ( $n = 13$ , age  $23.2 \pm 2.9$  yr, weight  $59.2 \pm 7.2$  kg, height  $162.9 \pm 3.3$  cm, body mass index  $22.3 \pm 2.7$  kg/m<sup>2</sup>) or MICT ( $n = 13$ , age  $21.9 \pm 1.7$  yr, weight  $59.3 \pm 6.6$  kg, height  $160.9 \pm 4.4$  cm, body mass index  $21.6 \pm 2.4$  kg/m<sup>2</sup>) group. Both groups completed 8 weeks (3 sessions/week) of training on the treadmill, where the HIIT group were asked to complete  $6 \times 3$ -min bouts of running at the intensity of 80–90% maximum oxygen uptake ( $VO_{2max}$ ) separated by 2-min active recovery at 30–40%  $VO_{2max}$  and the MICT group completed 30-min continuous running at the intensity of 60–70%  $VO_{2max}$ . The body composition, bone mineral density (BMD), calcaneus quantitative ultrasound, bone turnover markers, and lower limb muscle strength were measured pre and post interventions.

**Results:** After 8-week interventions, 1) The total body BMD (HIIT, +8.5%; MICT, +5.5%) significantly increased ( $p < 0.05$ ) without difference between the two groups ( $p > 0.05$ ). The calcaneus broadband ultrasound attenuation (CUBA) (HIIT, +16.0%; MICT, +4.6%) and calcaneus stiffness index (CSI) (HIIT, +16.7%; MICT, +2.5%) significantly increased in HIIT group ( $p < 0.05$ ), but not in MICT group ( $p > 0.05$ ). 2) The 1,25-dihydroxyvitamin D<sub>3</sub> ( $1,25(OH)_2D_3$ ) (HIIT, +42.8%; MICT, +24.9%) level increased in both groups with significantly higher changes in HIIT ( $p < 0.05$ ). 3) The score of standing long jump (HIIT, +10.3%; MICT, +3.8%) and vertical jump (HIIT, +5.3%; MICT, +2.0%) increased in both groups with significantly higher changes in HIIT ( $p < 0.05$ ).

**Conclusions:** It suggested that 8-week HIIT and MICT interventions could improve bone metabolism. Compared with a similar workload of MICT, HIIT elicited superior benefits on bone metabolism.

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## 1. Introduction

Osteoporosis is an osteometabolic disease characterized by substantial loss of bone mass and mineral content, microarchitecture

deterioration of bone tissue, changes of bone shape alternations and geometry, affecting bone quality and strength, and increasing fracture risk.<sup>1–3</sup> It develops slowly over several years until a fall with a sudden bone fracture before clinical diagnosis.<sup>1</sup> Women are at more

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risk of osteoporosis, particularly under early menopause before 45 years old or ovaries removed.<sup>4</sup> Young women, however, may also develop osteoporosis when their bone mass density (BMD) falls below the Z-score for a given age and are unable to reach the level of peak bone mass at the corresponding age. Therefore, maximizing BMD at a young age appears to be an effective protective strategy in the prevention of osteoporotic fracture later in life.<sup>5,6</sup>

People who are less physically active throughout life are more likely to develop osteoporosis.<sup>7</sup> With advances in technology, screen time, including watching television, using a computer, and playing video games, is becoming a central component of daily life and the most common sedentary behavior.<sup>8</sup> Accumulating evidence of the link between sedentary behavior and adverse health indicators has perpetuated this interest.<sup>8–10</sup> Sedentary behavior is defined as activities that consume energy expenditures less than 1.5 metabolic equivalents (METs) while in a sitting or reclining posture.<sup>11</sup> Sedentary behavior leads to a lack of systemic muscle activity and a decrease in muscle strength; there is a low load to stimulate bone accretion, which accelerates the loss of BMD and damages bone health.<sup>12–14</sup>

There is abundant evidence that physical exercise plays an essential role in maintaining or increasing BMD by improving bone metabolism.<sup>2,15</sup> However, reports have shown that muscle exercise without physical load is ineffective in preventing bone loss.<sup>16,17</sup> Exercises that stimulate osteogenic formation are usually characterized by dynamic exercise with high intensity and short stimulus duration.<sup>18,19</sup> High-intensity interval training (HIIT) has attracted increased attention as a possible time-effective alternative to traditional aerobic exercise.<sup>20</sup> HIIT is forecasted as the most popular trend in fitness, according to the American College of Sports Medicine (ACSM) Annual Fitness Trend Forecast.<sup>21</sup> HIIT refers to exercise characterized by relatively short bursts of vigorous activity at an intensity close to that which elicits maximum oxygen uptake ( $VO_{2max}$ ) (based on an intensity greater than 80%  $VO_{2max}$ ), interspersed by periods of rest or low-intensity exercise for recovery.<sup>22–24</sup> In contrast, moderate-intensity continuous training (MICT) is considered traditional aerobic exercise. It is performed as a continuous bout of moderate-intensity aerobic activity at a steady state for a set duration (typically between 20 and 60 min).<sup>20</sup> Studies have pointed out that repeated high-intensity exercises can stimulate the bone many times to reach the threshold of bone formation, and intermittent loading cycles determine a greater increase in bone formation than a single, even prolonged cycles; however, continuous stimulation desensitizes osteocytes.<sup>13,17,25</sup> Hence, HIIT could be a more efficient way to increase BMD and bone metabolism.

There are many indices for reflecting bone metabolism. Dual X-ray absorptiometry (DXA) is currently the most widely used technique for estimating BMD.<sup>26</sup> A meta-analysis indicated that quantitative ultrasound also has excellent sensitivity to assess exercise-induced changes in bone status, especially turnover rates in trabecular bone.<sup>27</sup> Calcaneus, essentially a trabecular structure, is more metabolically active than cortical bone and highly responsive to metabolic and mechanical stimuli for bone remodeling. It has been recommended as an appropriate skeletal site for assessing the impact of integrated physical activity on bone.<sup>28</sup> The calcaneus speed of sound (CSOS) was used to assess the elastic resistance of the bone correlated with the mineral and protein contents of the calcaneus. Calcaneus broadband ultrasound attenuation (CUBA) measures the loss of ultrasound energy due to absorption or dispersion, reflecting the spatial orientation of bone trabeculae. The calcaneus stiffness index (CSI) is a linear combination of CSOS and CUBA.<sup>28,29</sup> In addition, bone turnover markers reflect a generally dynamic process, including both resorption and formation of bones.<sup>30,31</sup> Studies have shown that serum osteocalcin (OC), as a

bone turnover marker,<sup>30</sup> was positively correlated with BMD. The catabolite pyridinoline (PYD) of ossein is a marker of bone resorption.<sup>32</sup> Furthermore, as a stimulant hormone, 1,25-dihydroxyvitamin D<sub>3</sub> (1,25 (OH)<sub>2</sub>D<sub>3</sub>) can promote the enhancement of osteoblast activity.<sup>33,34</sup>

Although it was suggested to use a 4–6 month intervention to observe bone remodeling, many studies with exercise interventions shorter than 4 months have increased bone mineral density and plasma bone turnover markers.<sup>35–37</sup> Additionally, very few studies have focused on young women with sedentary behavior, representing the most critical osteoporosis prevention population. Physical activity at this age can not only maximize peak bone mass but also carry over into later adulthood to lessen the age-associated decline in bone mass.<sup>38</sup> Therefore, the purpose of this study was to investigate the effects of an 8-week HIIT program on bone metabolism, including BMD, quantitative ultrasound of the calcaneus, and bone turnover markers, in sedentary young women. Assessing multiple indicators to understand bone metabolism better and discern the effects of HIIT compared with the MICT program was proposed. It was hypothesized that HIIT would have a better effect than MICT on bone metabolism in sedentary young women.

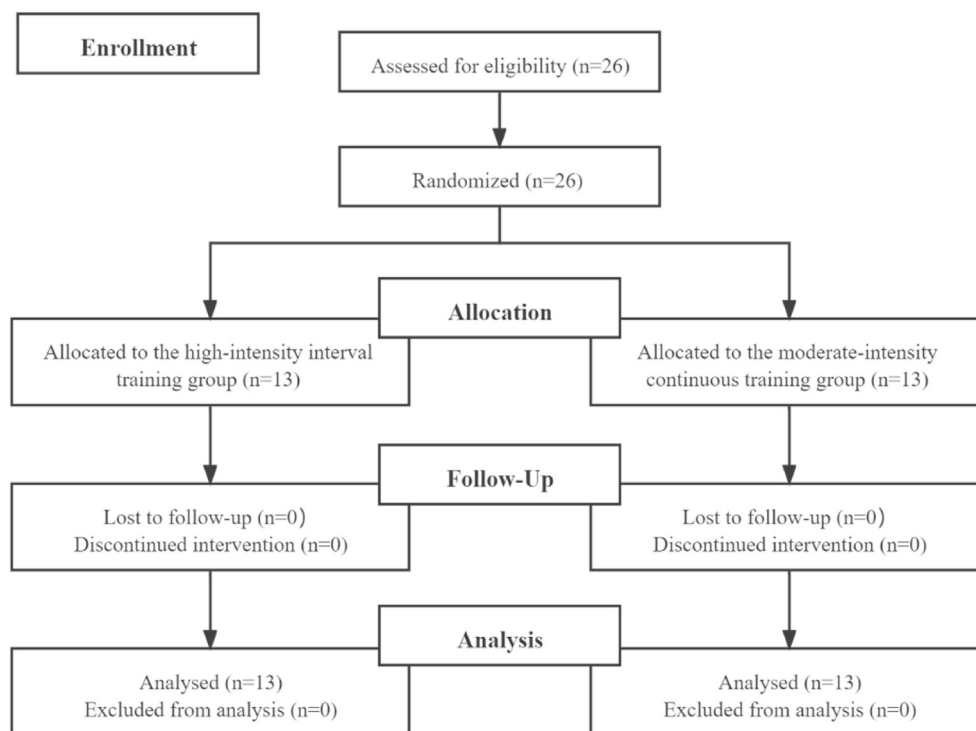
## 2. Materials and methods

### 2.1. Participants

Participants were recruited via posters and distribution of flyers on the campus of a local university. The inclusion criteria were as follows: 1) female age of 20–30 years, 2) no regular physical activity habits of moderate intensity (equal to 3.0 to 5.9 METs, such as brisk walking, as a rule of thumb, a person performing moderate-intensity aerobic activity can talk, but not sing, during the activity<sup>39</sup>) (30 min or more per day at least 3 days per week) during the 3-month period prior to the onset of this experimental protocol, 3) sedentary behavior with energy expenditure less than 1.5 METs with sitting or reclining posture for more than 6 h per day evaluated by acceleration sensor,<sup>11</sup> 4) negative responses to all questions on the Physical Activity Readiness Questionnaire, 5) no history of smoking, 6) medical examinations in the past 6 months revealed no abnormalities related to bone and mineral metabolism, no presence of any disease that affects bone metabolism and leads to secondary osteoporosis (e.g., hyperthyroidism and hyperparathyroidism), 7) no hormonal, orthopedic, or cardiovascular diseases, diabetes, hypertension, hyperlipidemia, and polycystic ovary syndrome, 8) no use of prescribed medication may alter bone metabolism (e.g., corticosteroids, estrogen, and thiazide diuretics), no hormonal contraceptives, no calcium, phosphorus, vitamin D, or K supplements. The present study was performed in accordance with the Helsinki Declaration and approved by the Ethical Committee for the Use of Human and Animal Participants in Research of the local university. Fully informed about this study's purpose, procedures, and potential risks, participants provided their written informed consent. During the interventions, no participants quit, all training sessions were completed by all participants, and the participants did not experience any adverse effects (CONSORT flow diagram as shown in Fig. 1).

### 2.2. Study design and procedures

This study was a randomized, controlled trial with two major procedures, experimental tests and 8-week interventions. Before the experimental tests, a three-axis acceleration sensor was used to screen participants' sedentary behavior for 7 days. Then, participants paid two visits to the laboratory. On the first visit, initial assessments, including body composition, BMD, and lower limb



**Fig. 1.** Consolidated Standards of Reporting Trials (CONSORT) diagram showing the flow diagram of participants.

muscle strength, were measured, and venous blood was drawn. Free exercise sessions were performed to accustom to the equipment, exercise intensity, and environment. On the second visit, participants performed graded exercise testing (GXT) on a treadmill to evaluate  $VO_{2max}$ . The exercise intensities for the interventions were determined accordingly. Each participant completed 8 weeks of HIIT and MICT protocols on the treadmill, 1 session per day, and 3 non-consecutive days per week. After 8 weeks of interventions, assessments, blood sampling, and GXT were performed again to estimate the effects of interventions.

All tests and interventions were conducted at the local sports science research center from April to July, with the air-conditioning temperature controlled at 20 °C and humidity controlled at 50%. Participants were asked to avoid strenuous exercise and alcohol the day before the laboratory visit and refrained from consuming carbonated drinks, caffeine, or other substances that could affect the GXT and blood test results within 2 h before. During the interventions, participants were also asked to avoid any other professional training regimen and nutritional supplement (e.g., calcium, phosphorus, vitamin D, and K) and to maintain their usual dietary intake, activities, and lifestyles.

### 2.2.1. Sedentary behavior assessment

The three-axis acceleration sensor (ActiGraph wGT3X-BT, ActiGraph, USA) was used to evaluate participants' daily energy expenditure. Data were collected at 60 Hz sampling rate and the time interval set to 1 epoch. All participants wore the accelerometer on their waists for 7 days, except for bathing and sleeping.<sup>40</sup>

### 2.2.2. $VO_{2max}$

All participants performed GXT on the treadmill (H/p/cosmos Pulsar 4.0, H/p/cosmos Sports and Medical gmbh, Nussdorf-Traunstein, Germany) to assess  $VO_{2max}$ . Participants completed a 5-min warm-up at 6 km/h and then initially ran at 8 km/h. The velocity was increased by 1 km/h every 3 min until the velocity

reached 12 km/h. Then, the gradient was increased by 1% every 3 min until termination.<sup>41–43</sup> Participants were strongly encouraged verbally to ensure maximum efforts were achieved throughout the test. Oxygen uptake ( $VO_2$ ) was measured breath-by-breath using a gas analyzer (MAX-II, Physio-Dyne Instrument, New York, USA) and subsequently averaged over 15-sec intervals throughout the test. Heart rate (HR, Polar T34, Polar, Finland) was monitored during the test with an HR transmitter paired with the gas analyzer. The criteria for determinant  $VO_{2max}$  included the following: 1) HR reached within  $\pm 10 \text{ min}^{-1}$  of the predicted maximal HR ( $220 - \text{age}$ )<sup>44</sup> or entered the plateau phase with increasing load; 2) respiratory exchange ratio reached or remained 1.1; 3) the variation range of  $VO_2$  did not exceed 5% or 150 ml/min or 2 ml/min/kg; 4)  $VO_2$  did not increase with the increase of load, and the participants voluntarily stopped the treadmill when they were exhausted.  $VO_{2max}$  was determined when at least 3 of the above criteria were met. The value of  $VO_{2max}$  was calculated as the highest 15-s value. During the test, if participants had shortness of breath and consciousness, they stopped immediately.

Following the  $VO_{2max}$  test, the corresponding velocities in the HIIT and MICT groups were estimated from the linear relationship of steady-state oxygen consumption versus running velocity.

### 2.2.3. Interventions

All training sessions were conducted on the treadmill. The standardized 5-min warm-up and 10-min final relaxation and cool-down were identical in both groups. For the HIIT group, 6 × 3-min bouts of running at the intensity of 80–90%  $VO_{2max}$  (corresponding running velocity:  $9.2 \pm 0.3$  to  $10.0 \pm 0.4$  km/h) separated by 2-min active recovery at 30–40%  $VO_{2max}$  ( $5.5 \pm 0.8$  to  $6.2 \pm 0.6$  km/h).<sup>45–47</sup> For MICT group, 30-min continuous running at the intensity of 60–70%  $VO_{2max}$  ( $8.0 \pm 0.6$  to  $8.6 \pm 0.5$  km/h). The participants trained at the lower intensity border for the first 2 weeks before increasing towards the upper border.

## 2.3. Measurements

### 2.3.1. Body composition

Body composition was measured using the bioelectrical impedance method (Inbody 230, Biospace Company, Seoul, Korea). During the measurement, participants were required to fast and to avoid carrying any metal objects. Participants were required to extend their arms and remain still at an angle of 30° with the torso, put their fingers on the electrodes, and step on the metal electrodes with bare feet. The percentages of body fat and lean muscle mass were recorded.

### 2.3.2. Total, lower body and calcaneus BMD

BMD was measured at the total and lower body by one registered technician using DXA (XR-46, Norland, Wisconsin, USA) with a standardized procedure. Between pre- and post-intervention measurements, the calibration of the densitometer was performed by standard calibration blocks daily. The instrument should be preheated and calibrated before every use. During the test, participants were required to fast and avoid carrying any metal objects and electronic equipment.

Calcaneus BMD, CSOS, CBUA, and CSI were measured using a calcaneus ultrasound densitometer (Achilles EXP11, GE Healthcare, USA) with a standardized procedure. The machine should be preheated and calibrated before every use. Participants were required to be in the sitting position, then put their left foot (without shoes and socks) in the apparatus with coupling agent on the ankle and probe part, and kept motionless.

### 2.3.3. Bone turnover markers

A venous blood sample (5 ml) was extracted from the antecubital vein after participants fasted for one night (>10 h). Blood samples were coagulated and balanced at room temperature and then centrifuged at 3000 g for 15 min. The obtained serum sample was stored at -80 °C for subsequent analysis. The biomarkers of bone formation and resorption OC were measured with commercial assay kits (Cloud-Clone Corp, Houston, USA), as well as PYD and 1,25(OH)<sub>2</sub>D<sub>3</sub> (Shanghai EK-Bioscience Biotechnology Co., Shanghai, China) in a microplate reader (SYNERGY H1, BioTek Company, Vermont, USA).

### 2.3.4. Lower limb muscle strength

The standing long jump test was measured on a special gym mat. The participants were instructed on the correct technique prior to the test, and they performed several jumps after a warm-up. The participants were required to jump forward vigorously as far as possible from a standing position during the test. Each of them completed 3 effective trials interspersed by a 1-min rest period, and the best performance was included in the analysis. The performance was measured with 1 cm precision.

The vertical jump test was measured in a special vertical jump height instrument. The participants were instructed on the correct technique prior to the test, and they performed several jumps after a warm-up. During the test, the participants were asked to stand barefoot on the mat, naturally separate their feet, bend their legs and squat, and jump up vigorously as high as possible from a standing position while touching the test strip. Each of them completed 3 effective trials interspersed by a 1-min rest period, and the best performance was included in the analysis. The performance was measured with 1 cm precision.

## 2.4. Statistical analysis

Power analysis was conducted by G\*Power version 3.1.9.2 (Universitat Kiel, Germany) to estimate the target sample size.

Using an ANOVA, repeated measures within-between interaction design, 12 participants per group was required as the smallest sample size for an effect size (ES) of 0.53<sup>48</sup> based on the main outcome (osteocalcin), alpha of 0.05, and 80% power. Finally, 26 participants were included in the initial assessment and then divided into two groups (HIIT or MICT) using the computer-generated random numbers by IBM SPSS Statistics for Windows (v21.0; Armonk, NY).

Data are presented as the means ± standard deviation (SD). Log-transformation was applied prior to further analysis.<sup>49</sup> An independent sample T test was used to assess the difference in age, height, and METs pre-intervention between the HIIT and MICT groups. Two-way repeated-measure ANOVA was performed to assess the interaction between time (pre- and post-intervention) and group (HIIT and MICT) with post hoc Bonferroni tests when appropriate. Partial eta square ( $\eta^2$ ) group × time interaction ES were calculated and interpreted as follows: < 0.06 as small, < 0.14 as moderate, and ≥ 0.14 as large.<sup>50</sup> The absolute value of each test result was used to calculate the ES for comparisons, represented as Cohen's d. It was interpreted according to the following thresholds: < 0.2 as trivial, 0.2–0.6 as small, 0.6–1.2 as moderate, 1.2–2.0 as large, and > 2.0 as very large.<sup>51</sup> The level of significance was set at  $p < 0.05$  for all tests. Statistical analysis was performed by IBM SPSS Statistics for Windows (v21.0; Armonk, NY).

## 3. Results

All 26 participants completed every training session of 8-week intervention. There were no significant differences in age (HIIT vs MICT: 23.2 ± 2.9 vs 21.9 ± 1.7 years,  $p > 0.05$ ), height (162.9 ± 3.3 vs 160.9 ± 4.4 cm,  $p > 0.05$ ), averaged energy expenditure (1.43 ± 0.03 vs 1.41 ± 0.05 METs,  $p > 0.05$ ), and other assessments pre-intervention listed in [Table 1](#).

After 8-week intervention, VO<sub>2max</sub> (HIIT, +18.8%; MICT, +17.8%) significantly increased in both groups without between-group difference. The total body BMD (HIIT, +8.5%; MICT, +5.5%) significantly increased in both groups without between-group difference. The CBUA (HIIT, +16.0%; MICT, +4.6%) and CSI (HIIT, +16.7%; MICT, +2.5%) significantly increased in HIIT group, but not in MICT ([Table 1](#)).

Changes in bone turnover markers are summarized in [Table 1](#). The serum level of 1,25(OH)<sub>2</sub>D<sub>3</sub> (HIIT, +42.8%; MICT, +24.9%) increased in both groups with significantly greater changes in HIIT ([Table 1](#)).

Changes in indicators of lower limb muscle strength are summarized in [Table 1](#). After 8-week interventions, the score of standing long jump (HIIT, +10.3%; MICT, +3.8%) and vertical jump (HIIT, +5.3%; MICT, +2.0%) increased in both groups with significantly greater changes in HIIT.

## 4. Discussion

Our findings suggested that both supervised 8-week HIIT and MICT could improve BMD, bone turnover markers, and lower limb muscle strength in sedentary young women; moreover, HIIT did better than MICT in improving bone metabolism.

Available human data show that the magnitude of benefit on bone from exercise is inconsistent and is often influenced by safety concerns (such as “high impact/ground reaction” imposed by HIIT), leading to conservatively prescribed training loads.<sup>52–54</sup> However, in our study, all 26 participants completed every training session, and no injury or adverse events were reported. It may be that our study adopted a progressive approach (i.e., from lower intensity border to upper border) which might help minimize the risk of



**Table 1**  
All obtained indexes data in the pre- and post-intervention.

	HIIT				MICT				Interaction effect			
	pre	post	Changes (%)	p	ES	pre	post	Changes (%)	p	ES	p	Partial $\eta^2$
Weight (kg)	59.22 ± 7.24	57.37 ± 6.64	-3.0 ± 3.1	0.002	0.27	59.27 ± 6.64	57.70 ± 6.38	-2.6 ± 2.7	0.005	0.24	0.76	0.00
BMI (kg/m <sup>2</sup> )	22.33 ± 2.73	22.99 ± 3.23	-0.9 ± 0.0	0.001	0.22	21.63 ± 2.42	22.37 ± 3.07	-0.9 ± 0.0	0.006	0.27	0.70	0.00
Body fat (%)	31.43 ± 5.65	28.85 ± 5.52	-8.2 ± 6.0	<0.001	0.46	33.15 ± 4.48	31.48 ± 4.73	-5.0 ± 6.8	<0.001	0.36	0.17	0.08
Lean Mass (kg)	40.31 ± 3.17	40.55 ± 2.27	0.6 ± 3.1	0.585	0.09	39.37 ± 2.27	39.30 ± 2.57	-0.3 ± 3.6	0.876	0.03	0.62	0.01
VO <sub>2max</sub> (ml/min/kg)	37.45 ± 3.69	44.35 ± 4.72	18.8 ± 9.6	<0.001	1.12	35.56 ± 3.91	42.84 ± 4.49	17.8 ± 11.9	<0.001	1.73	0.78	0.00
HR <sub>max</sub> (min <sup>-1</sup> )	184.00 ± 16.44	173.85 ± 8.82	-5.1 ± 6.3	0.002	0.77	189.23 ± 9.35	179.23 ± 8.82	-5.2 ± 4.0	0.002	1.10	1.00	0.00
Total body BMD (g/cm <sup>2</sup> )	0.936 ± 0.050	1.014 ± 0.052*	8.5 ± 3.7	<0.001	1.53	0.947 ± 0.046	1.000 ± 0.053*	5.5 ± 3.3	<0.001	1.07	0.03	0.18
Lower limb BMD (g/cm <sup>2</sup> )	1.010 ± 0.079	1.058 ± 0.063	4.8 ± 4.1	<0.001	0.67	0.992 ± 0.077	1.039 ± 0.096	4.4 ± 3.8	<0.001	0.54	0.92	0.00
Calcaneus BMD (g/cm <sup>2</sup> )	0.962 ± 0.067	0.971 ± 0.079	0.8 ± 2.3	0.144	0.13	0.968 ± 0.054	0.973 ± 0.054	0.3 ± 1.3	0.552	0.09	0.53	0.02
CSOS (m/s)	1601.42 ± 35.15	1598.88 ± 34.39	-0.2 ± 1.1	0.876	0.07	1593.13 ± 37.19	1599.01 ± 41.62	0.4 ± 2.2	0.533	0.15	0.58	0.01
CBUA (dB/MHz)	121.70 ± 9.47	140.40 ± 9.17*#	16.0 ± 11	<0.001	2.01	112.27 ± 20.94	116.55 ± 17.92	4.6 ± 7.7	0.093	0.22	0.01	0.30
CSI (%)	108.77 ± 10.47	126.38 ± 11.92*#	16.7 ± 10.2	<0.001	1.57	99.46 ± 21.24	101.54 ± 19.89	2.5 ± 3.2	0.224	0.10	0.01	0.50
OC (ug/L)	6.82 ± 1.25	10.01 ± 3.37	50.8 ± 53.7	0.005	1.26	5.74 ± 2.02	7.36 ± 1.40	46.5 ± 63.6	0.019	0.93	0.70	0.01
PYD (nmol/L)	29.93 ± 7.00	25.42 ± 6.79	-15.4 ± 7.4	<0.001	0.65	31.64 ± 6.22	25.13 ± 5.39	-20.4 ± 8.7	<0.001	1.12	0.16	0.08
1,25(OH) <sub>2</sub> D <sub>3</sub> (ug/L)	12.10 ± 2.59	17.19 ± 3.44*#	42.8 ± 13.3	<0.001	1.67	12.08 ± 2.11	14.80 ± 2.01*	24.9 ± 22.9	<0.001	1.32	0.01	0.23
Standing long jump (m)	1.61 ± 0.78	1.77 ± 0.83*#	10.3 ± 2.2	<0.001	0.20	1.59 ± 0.13	1.65 ± 0.14*	3.8 ± 2.1	<0.001	0.44	0.01	0.75
Vertical jump (m)	2.30 ± 0.05	2.42 ± 0.05*#	5.3 ± 1.8	<0.001	2.40	2.33 ± 0.04	2.37 ± 0.04*	2.0 ± 1.5	<0.001	1.00	0.01	0.47

Values were summarized as mean ± standard deviation. HIIT = high-intensity interval training, MICT = moderate-intensity continuous training, BMI = body mass index, VO<sub>2max</sub> = maximum oxygen uptake, HR<sub>max</sub> = maximum heart rate, BMD = bone mineral density, CSOS = calcaneus speed of sound, CBUA = calcaneus broadband ultrasound attenuation, CSI = calcaneus stiffness index; OC = osteocalcin, PYD = pyridinoline, 1,25(OH)<sub>2</sub>D<sub>3</sub> = 1,25-dihydroxyvitamin D<sub>3</sub>.

\*indicates a significant difference from pre-to post-intervention (*p* < 0.05).

#indicates a significant difference (*p* < 0.05) vs. MICT-group.

adverse events. Thus, our HIIT program may be safe for sedentary female participants.

In our study, the total body BMD value increased over 8 weeks in both groups. This is because physical activity forces can be exerted on bones through ground reaction forces and the contractile activity of muscles,<sup>55</sup> resulting in a gain of BMD in both groups. Our findings were similar to those observed in other studies. Basat et al.<sup>30</sup> reported that 6-month high-impact exercise increased BMD in the lumbar spine and femoral neck regions. There have been few studies on young women, but a study pointed out that high-impact football has a higher BMD among pubertal girls than other sports.<sup>56</sup> In addition, young female international football players had a 23% higher total body BMD than their untrained and age-matched counterparts.<sup>57</sup> Furthermore, although our intervention lasted for only 8 weeks, the optimal single exercise duration should be longer than 6 months before significant changes in BMD occur.<sup>52</sup> It might be that our participants had been sedentary, bones became more sensitive to mechanical loading for sedentary individuals. When their lifestyles were changed, BMD increased more rapidly and significantly during exercise than in the rest of the population. The study showed that people who exercise regularly had greater total body BMD than sedentary individuals.<sup>56</sup> Additionally, individuals with a low BMD baseline would benefit more from any bone-stimulating activities. Although the average baseline total body BMD level of participants was within the normal range, it was less than 1 g/cm.<sup>2</sup> Ravnholt et al.<sup>35</sup> demonstrated that 7 weeks of the high-intensity intermittent running of untrained adults was effective in increasing BMD. However, after 12 weeks of intensive training, the subjects' total bone mass remained unchanged in the high-intensity training,<sup>58</sup> which may be related to the small amount of exercise (40 min per week). Regrettably, there was no difference between both the HIIT and MICT groups. It might be that the BMD represents the static state of bone minerals,<sup>59</sup> and our intervention was only 8 weeks and was unable to make a significant difference between the two groups. However, the time threshold for the significant difference between the two groups of BMD was not known, and future research can focus on this aspect.

Although there was no difference between the two groups in total body BMD, the CBUA and CSI closely significantly increased in

the HIIT group but not in the MICT group. The CBUA and CSI could accurately reflect bone mineral status by abating the influence of soft tissue thickness variations.<sup>28</sup> The study showed that CUBA and CSI were excellent sensitives for assessing exercise-induced changes in bone status.<sup>27,29,60</sup> The study also highlighted that a high-impact exercise regimen could significantly increase CBUA, while no significant changes were observed in the control group.<sup>61</sup> Therefore, HIIT was more effective in improving bone status.

In this study, 1,25(OH)<sub>2</sub>D<sub>3</sub>, a bone formation marker, increased significantly in the HIIT groups, and bone metabolism improved after 8 weeks of intervention. Notably, the 1,25(OH)<sub>2</sub>D<sub>3</sub> concentrations in HIIT were significantly higher than those in the MICT group. Impact force is a relevant element in the stimulation of bone metabolism.<sup>30</sup> Under mechanical loading, the bones remodel themselves to repair the microdamage and increase density to prevent any future damage to the same loading sites. In other words, bone increases the threshold of stress tolerance when encountered with high impact mechanical loading.<sup>62</sup> High-impact physical activities involving ground reaction forces and joint reaction forces have greater loading stimulus on bone, thus promoting bone metabolism and increasing BMD to a greater extent.<sup>63</sup> However, 1,25(OH)<sub>2</sub>D<sub>3</sub>, a dynamic indicator of bone metabolism, can evaluate the dynamic fluctuations in bone turnover and better reflect the dynamic changes of bones.<sup>59</sup> One previous study pointed out that 7 and 11 weeks of treadmill running exercise increased the OC and 1,25(OH)<sub>2</sub>D<sub>3</sub> levels,<sup>64</sup> although no significant changes in OC were found in our study. Basat et al.<sup>30</sup> studied 42 postmenopausal women and divided them into three groups (strengthening exercise; high-impact exercise; no exercise); after 6 months, OC increased only in the high-impact group, and the bone resorption marker significantly decreased. Similarly, Ravnholt et al.<sup>35</sup> demonstrated that a 7-week high-intensity interval training elevated the bone formation marker OC. Altogether, high-intensity exercise could improve bone formation marker levels.<sup>35</sup> Thus, our findings suggested that HIIT was more osteogenic than MICT. It is known that exercise stimulates bone formation and suppresses bone resorption, resulting in an increased demand for minerals that are satisfied by an increase in serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels and increased intestinal absorption of calcium. HIIT had a greater

impact on bones and muscles than MICT, consistent with the increases in 1,25(OH)<sub>2</sub>D<sub>3</sub> observed in the present study.

Efficient muscle strength also affects bone mass.<sup>65</sup> Sports with high ground reaction forces produce high muscle forces. Exercise activities that combine the mechanical stimulus from ground reaction forces and the tension produced by intense muscle contractions are considered better for bone stimulation.<sup>29</sup> Studies have confirmed that muscle strength, significantly lower limb muscle strength, has a significant positive effect on BMD in young women and has become a predictor of BMD.<sup>66,67</sup> Improving lower limb strength in young women could help increase BMD and prevent osteoporosis later in life.<sup>67</sup> This study suggested that both types of training could significantly improve lower limb muscle strength, and the HIIT group was superior to the MICT group. Therefore, HIIT elicited superior benefits on bone metabolism. Increased muscle strength also protects the bones and reduces the risk of falls and fractures.<sup>2</sup>

This study had several limitations. First, although participants were required to avoid other nutritional supplements during the study, we did not record all food and fluid intake during the study period. Therefore, it was recommended that future studies perform a full assessment of participants' diets. Second, the period of bone remodeling required at least 4–6 months, and the intervention period of our study was 8 weeks, which had already had an impact on bone turnover markers or BMD. It might be that the effect of sedentary on bone health is different from the mechanism of bone remodeling. This study was encouraged to repeatedly observe the effect of different intervention periods on bone metabolism and further explore the mechanism of bone metabolism. In addition, our study used a relatively small sample of only sedentary female participants, which limits the generalizability to other populations and the ability to draw some conclusions about the relative potency of HIIT versus that of MICT. Therefore, caution should be taken when generalizing to other populations; simultaneously, we would encourage duplication of this study's protocol with other more populations in future research. Finally, many indicators can be used to assess bone metabolism, such as parathyroid hormone, insulin, OC, 1,25(OH)<sub>2</sub>D<sub>3</sub>, PYD, cortisol, procollagen type 1 amino-terminal propeptide, carboxy-terminal cross-linking telopeptide of type I collagen, and N-telopeptides of type I collagen. However, we only selected three of them, and there were limitations in the selection of indicators. Future research should focus on more indicators and comprehensively analyze bone metabolism.

## 5. Conclusions

Our data suggested that 8-week HIIT and MICT interventions could improve bone metabolism. Compared with similar workload MICT, HIIT elicited superior benefits on bone metabolism.

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## Author statement

MLu, MLi, and FLi wrote the manuscript. MLu, MLi, LY, TJ and JQ conceived and designed the study. MLu, MLi, FLi and TJ was responsible for data collection and data interpretation. MLu, MLi, LY, FLi, LF, TJ, YZ and JQ assisted in revision of the manuscript.

## Declaration of competing interest

The authors declare no conflict of interest.

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