Research Article

The effect of short-term sprint interval training on bone density of male Wistar rats under western diet

Alireza Tabatabaee[1], Mohammad Rahmani[1*], Maryam Khalesi[1]

Abstract

The aim of the present study was to investigate the effect of sprint interval training on markers of bone metabolism and bone density in Male Wistar Rats under unhealthy high fat, sugar, salt Diet. The study design was an 8-week protocol consisting of three groups: Control (CO), Western diet (WD) and Western diet+Sprint interval training (SIT) (WD/SIT). WD rats received a high-fat, sugar, and salt diet, while WD/SIT rats followed the same diet combined with sprint interval training. The one-way ANOVA revealed significant differences between groups for all variables ($p<0.05$). Effect sizes (η^2) ranged from 0.47 to 0.99, indicating large effects for bone density ($\eta^2=0.99$), ALP($\eta^2=0.77$), phosphorus ($\eta^2=0.74$), and calcium ($\eta^2=0.47$). Post-hoc analysis by LSD test showed that the WD group exhibited a significantly lower femur bone density percentage (24.09±2.32) compared to both the control (58.40±1.64) and WD/SIT (47.67±1.60) groups ($p<0.001$). Regarding bone metabolism markers, the WD/SIT group demonstrated significantly reduced serum ALP levels (195.67±20.83IU/L) compared to the control (248.33±29.30IU/L) and WD (253.17±38.46IU/L) groups ($p<0.001$). For serum phosphorus, the WD/SIT group (5.68±0.58 mg/dL) was significantly lower than the control (7.68±0.63 mg/dL) and WD (8.58±0.78mg/dL) groups ($p<0.001$). Furthermore, serum calcium levels in the control group (10.27±0.80 mg/dL) were significantly higher than in the WD/SIT (8.92±0.61 mg/dL) and WD (9.18±0.28 mg/dL) groups ($p<0.01$). These results indicate that a high-calorie, high-salt diet had a negative effect on bone metabolism. However, sprint interval training partially attenuated these adverse effects.

Key Words: Diet, High-fat, High calorie, Sodium, Exercise, Bone metabolism

Introduction

Bone tissue is a dynamic and unique structure. Maintaining the physiological function and homeostasis of bones is of great importance, as it not only provides structural integrity and protects vital organs, but also plays a vital role in mineral homeostasis and acid-base regulation (Vannucci et al., 2018). Normal bone homeostasis depends on a delicate balance between the activities of osteoclasts and osteoblasts, which are responsible for bone resorption and formation, respectively (Rondanelli et al., 2021). Therefore, the osteoclast/osteoblast balance is considered the main factor in maintaining proper physiological function and bone mineral density (BMD). The activity of osteoblasts can be assessed by measuring serum alkaline phosphatase (ALP) levels. Alkaline phosphatase is an enzyme secreted by osteoblast cells that catalyzes the hydrolysis of organic phosphates at alkaline pH. This enzyme is a biochemical indicator of bone metabolism (Chung, Ryu, & Kang, 2021). Calcium (Ca^{2+}) and phosphorus (P) are also important inorganic components of the bone matrix and serve as other key indicators of bone health (Naeimi, Sazvar, & Feyzi, 2022).

Although non-modifiable risk factors such as genetics play a key role (accounting for approximately 70% of variation in bone mass density), bone mineral density (BMD) and, more specifically, peak bone mass can be modified by lifestyle factors, especially physical activity and dietary habits (Wilson-Barnes, Lanham-New, & Lambert, 2022). The Framingham Study has demonstrated that some diets, particularly Western diets, which are widely consumed, are associated with reduced BMD and an elevated risk of bone fractures (Muñoz-Garach, García-Fontana, & Muñoz-Torres, 2020). Recent comprehensive reviews have further confirmed that Western dietary patterns adversely affect bone health through multiple interconnected mechanisms, including inflammation, oxidative stress, and altered calcium homeostasis (Clemente-Suárez, Beltrán-Velasco, Redondo-Flórez, Martín-Rodríguez, & Tornero-Aguilera, 2023).

1. Department of physical education and Sport Science, Faculty of Humanities, Shahed University, Tehran, Iran.

*Author for correspondence: rahmani@shahed.ac.ir

A T: 0000-0001-9521-9600, M R: 0000-0002-6014-1804, M K: 0000-0002-8852-9624

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Western diets are characterized by high calorie content (rich in fat and sugar) along with high salt intake. A recent comprehensive review by Liu et al. (2024) examined the effects of various dietary patterns on bone health and confirmed that Western dietary patterns consistently show negative associations with bone mineral density, while Mediterranean and other plant-based diets appear protective (X. Liu et al., 2024). According to studies, high fat intake directly impairs intestinal calcium absorption; indeed, just one week of a high-fat diet can alter bone metabolism in healthy men (Varley, James, Willis, King, & Clayton, 2022). Additionally, the accumulation of fat cells can inhibit osteoblast differentiation and bone formation (Paine et al., 2018) and, by stimulating pro-inflammatory factors such as TNF-α and IL-1, may contribute to decreased bone density (Wong et al., 2018). Excessive sodium intake is also associated with increased urinary calcium excretion (hypercalciuria), which leads to bone loss and impaired bone formation (Muñoz-Garach et al., 2020).

Along with an unhealthy diet, physical inactivity is another modifiable lifestyle factor that adversely affects bone tissue. Physical activity influences bone maintenance and formation, both directly through mechanical signals generated by muscle contraction and indirectly through the regulation of cytokines. Hormones and myokines released from muscles during exercise exert anabolic effects on bone tissue (Clemente-Suárez et al., 2023; Kang, Kim, Kim, & Kim, 2019). The impact of exercise on BMD depends on intensity, duration, and the type of training program employed. Studies show that short, intense weight-bearing exercises have the greatest effect on improving bone density (Liu et al., 2021; Nazari, Azarbayjani, Rahmati-Ahmadabad, & Guerra, 2022). Therefore, exercise specialists, recognizing the benefits of high-intensity exercise and the fact that time constraints are the most common barrier to achieving the preventive and protective effects of structured exercise programs (Sequeira, Cruz, Pinto, Santos, & Marques, 2011), have focused on developing high-intensity exercise interventions with minimal time requirements (Gibala, 2007). One such intervention that has recently gained attention is sprint interval training (≤10 seconds per bout), which requires no more than 15 minutes per session and improves key health markers (aerobic and anaerobic performance) to a similar or greater extent than traditional, longer-duration high-intensity continuous or interval protocols (Metcalfe et al., 2020).

Exercise is not only essential for maintaining the health of various body systems, including the skeletal system, but, as evidenced by numerous studies, it can also mitigate some risk factors, including those associated with the Western diet (Grant, 2024; Xu, Ma, Zhao, & Zhang, 2024). While modifying diet and adhering to traditional (time-consuming) exercise protocols may be challenging for many individuals due to the demands of modern life, several studies have demonstrated that sprint interval training, despite its short duration, can help prevent the adverse effects of other risk factors, including medications (Dias-da-Silva et al., 2024) and the Western diet (Y. Liu et al., 2024). Given the beneficial role of exercise in preventing bone resorption and the need for strategies to prevent or mitigate bone density loss induced by high-calorie, high-salt diets, this study aimed to investigate the effects of 8-week sprint interval training on bone density in rats fed a Western diet (high fat, sugar, salt).

Material and methods

Animals

This experimental study utilized 18 male Wistar rats (mean body weight of 140±5 g at baseline). The sample size was determined based on power analysis under defined assumptions and ethically/methodologically justified according to the 3Rs principles. Following a one-week adaptation period, the rats were randomly assigned to three equal groups: Control with normal diet (CO, n=6), Western diet (WD, n=6), Western diet + exercise (WD/SIT, n=6). Animals were maintained under standard conditions (12-h light/dark cycle, 45-50% humidity, 23±1°C) with free access to food and water. All procedures conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals (2011) and were approved by the research ethics committee at the university where the project was conducted (IR.Shahed.Rec.1400.005; Approval Date: May 27, 2021).

Diet

The rats had free access to food and water throughout the study period. The components of normal and western food rations used in the studied groups is presented in table No. 1.

General and specific familiarization with the exercise protocol

Table 1. Component of normal and western diet used in the studied groups.

Nutritional content	Fat (%)	Carbohydrates (%)		Protein (%)	Salt (%)	Caloric density (Kcal/g)
		sugar	other			
Normal ration	3-5	0	45-50	18-20	0.5	3.5
Western ration	24-25	20	20	15	1.5	4.8

After acclimatization to the laboratory environment and before the start of the SIT protocol, the WD/SIT group underwent a familiarization period consisting of both general and specific adaptation to the exercise protocol. General familiarization included five sessions of continuous running at 10–15 m/min on a rodent treadmill (Bionic Mobin 32588). The specific familiarization phase consisted of 10 second running intervals at a speed of 20–30 m/min, followed by 60 seconds of active recovery between intervals. This phase included four repetitions per session, performed three days per week.

Maximum Running Test (MRT): Following the familiarization period and before the start of the SIT protocol, the Maximum Running Test (MRT) was performed to estimate each rat's maximal running speed. The test was conducted only once, at the beginning of the study, to determine baseline training intensities. The test protocol was as follows: the initial treadmill speed was set at 10 m/min, and the speed was increased by 3 m/min every three minutes until exhaustion. Exhaustion was defined as the rat remaining on the shock grid for five consecutive seconds. The highest speed achieved during the test was recorded as the rat's maximal running speed and was considered 100% for determining individual training intensity. The relative intensity for each training session (MRT%) was then calculated as a percentage of this maximal speed. The MRT was not repeated during the training period; instead, training progression was applied based on the predefined protocol phases presented in Table 2 (Asadi, Rahmani, & Samadi, 2021).

Exercise protocol: The SIT protocol is presented in Table 2. This protocol was a modified version of the one used by Asadi et al (Asadi et al., 2021).

At the end of the eighth week and 48 hours after the last training session, the rats were anesthetized and sampled. Blood samples were collected via cardiac puncture, and the femurs were excised and fixed in 10% formalin solution. For serum separation, blood samples were centrifuged at 3000 rpm for 10 minutes. The serum was then transferred to sterile microtubes and stored at −80°C until laboratory analysis. Serum levels of Ca^{2+}, P, and ALP were measured using Pars Azmoun kits and an autoanalyzer.

Histological Analysis: Bone tissue was stained with alizarin red and examined under a light microscope. Alizarin red staining is a sensitive and complementary method for detecting calcium crystals in tissue samples, particularly in bone. This technique utilizes Alizarin Red S dye, an organic sodium salt with high specificity for calcium pyrophosphate crystals.

The Alizarin red test involves preparation steps for tissue molding and coloring. In this study, the steps were as follows: First, to prepare the tissue, water was removed from the tissue samples. For this purpose, bone samples were placed in a graded series of ethanol solutions (70%, 80%, 90%, and 100%) for 50 minutes each, allowing ethanol to replace tissue water. Subsequently, xylol solution was used to harden and prepare the bone tissue for molding. Before molding, the samples were fixed in paraffin to preserve the bone cells. Following fixation, very thin sections of the paraffin-embedded bone samples were cut with a microtome and mounted on slides. For the staining procedure, a solution of 2 grams of Alizarin Red S dye powder (Merck, Cat. No.1.06278) in 100 mL of distilled water was prepared. The pH of the solution was adjusted with 10% ammonium hydroxide (Sigma, Cat. No.1336-21-6). After preparation of the staining solution, deparaffinized tissue sections on slides were counterstained with the alizarin solution for 1–5 minutes and then examined under a light microscope.

For quantitative analysis, five non-consecutive sections per animal were examined. Digital images were captured using a light microscope (Olympus BX-51, Japan) at 40× magnification. The percentage of bone area (mineralized tissue) was measured using ImageJ software (Version 1.54, NIH, USA). The mineralized region was selected using the color threshold tool, and the bone density percentage was calculated as the ratio of the Alizarin Red-positive area to the total tissue area. All analyses were performed by a single investigator who was blinded to the experimental groups to prevent observer bias.

Table 2. SIT protocol

Phase (Week)	Sessions per week	Repetition Interval	Interval duration (seconds)	Speed (m/min^{-1})	MRT [a] (%)	Active recovery (seconds)	Active rest Speed (m/min^{-1})
1	3	4	10	50-55	118-130	60	15-20
2	3	4	10	55-60	130-141	60	15-20
3	3	5	10	55-60	130-141	60	15-20
4	3	5	10	60-65	141-153	60	15-20
5	3	5	10	60-65	141-153	60	15-20
6	3	7	10	65-70	153-165	60	15-20
7	3	8	10	65-70	153-165	60	15-20
8	3	9	10	65-70	153-165	60	15-20

a MRT (%), percentage of speed in the maximal running test

Table 3. Mean and standard deviation of serum ALP, P, Ca^{2+} levels and bone density in the study groups.

Group	Co	WD	WD/SIT
ALP (IU/L)	248.33± 29.30	253.17±38.46	195.67±20.83
P (mg/dl)	7.68±0.63	8.58±0.78	5.68±0.58
Ca^{2+} (mg/dl)	10.27±0.80	9.18±0.28	8.92±0.61
Bone mineral density (%)	58.40±1.64	24.09±2.32	47.67±1.60

Table 4. Results of one-way ANOVA

	Bone mineral density (%)	ALP (IU/L)	P (mg/dl)	Ca^{2+} (mg/dl)
MSB	1848.52	6103.39	13.22	3.07
MSW	3.54	923.97	0.45	0.37
$F_{(2, 15)}$	522.95	6.10	29.58	8.41
Sig.	< 0.001	< 0.01	< 0.001	< 0.005
η^2	0.99	0.47	0.80	0.53

Bone density percentage quantified using ImageJ software. MSB: Mean Square Between, MSW: Mean Square Within

Statistical analysis

Descriptive statistics, including mean and standard deviation (SD), were used to report the data. The normality of data distribution was confirmed using the Shapiro–Wilk test ($p>0.05$), and the homogeneity of variances was verified by Levene's test ($p>0.05$). Consequently, a one-way analysis of variance (ANOVA) was employed to determine significant differences among the experimental groups. When a significant difference was detected, Fisher's Least Significant Difference (LSD) post hoc test was applied for multiple comparisons. The alpha level for statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS software (Version 24). Graphs were generated using Microsoft Excel (Version 1903).

Results

The initial and final body weights of the rats in each experimental group were recorded. In the control group, body weight increased from 284.3±11.8 g to 370.2±14.8 g. Conversely, in the Western Diet (WD) group, body weight decreased from 286.3±24.2 g to 213.3±22.4 g. Similarly, the Western Diet plus Exercise (WD/SIT) group showed a decrease from 283.7±22.0 g to 223.2±44.4 g. Table 3 presents the descriptive statistics (mean±SD) for the main research variables, including bone mineral density (BMD), serum alkaline phosphatase (ALP), serum phosphorus (P), and serum calcium (Ca^{2+}).

Based on these results, the control group (normal diet; CO) exhibited the highest bone density, while the Western diet group (WD) showed the lowest. Figure 1 displays a light microscope image of a cross-section of bone tissue stained with Alizarin Red.

The assumptions of normality (Shapiro–Wilk test, $p>0.05$) and homogeneity of variances (Levene's test, $p>0.05$) were satisfied. Therefore, one-way ANOVA was conducted, and as shown in Table 4, significant differences were observed among the experimental groups regarding bone density percentage ($p<0.001$), serum alkaline phosphatase levels ($p<0.01$), serum phosphorus levels ($p<0.001$), and serum calcium levels ($p<0.005$). Subsequent post hoc analysis using Fisher's LSD test revealed the following inter-group comparisons:

Bone Density: The femur bone density percentage in the WD group (24.09±2.32) was significantly lower than that in both the control (58.40±1.64) and WD/SIT (47.67±1.60) groups ($p<0.001$). Conversely, the WD/SIT group exhibited a significantly higher bone density compared to the WD group ($p<0.001$) (Fig 2A).

Serum ALP Level: The serum ALP level in the WD/SIT group (195.67±20.83) was significantly lower than that in the control (248.33±29.30) and WD (253.17±38.46) groups ($p<0.001$). However, no significant difference was observed between the WD group and the control group ($p>0.05$) (Fig 2B).

Serum Phosphorus (P) Level: The serum phosphorus level of the WD/SIT group (5.68±0.58) was significantly lower than that of the control (7.68±0.63) and WD (8.58±0.78) groups ($p<0.001$). Additionally, the WD group showed a significantly higher serum phosphorus level than the control group ($p<0.05$) (Fig 2C).

Serum Calcium (Ca) Level: The serum calcium level in the control group (10.27±0.80) was significantly higher than that in the WD/SIT (8.92±0.61) and WD (9.18± 0.28) groups ($p<0.01$). No significant difference was found between the WD and WD/SIT groups ($p>0.05$) (Fig 2D).

Discussion

The results of the present study showed that while a course of Western diet had a significant negative effect on the serum levels of ALP, phosphorus (P), calcium (Ca), and bone mineral density (BMD) of the studied rats, an eight-week course of sprint interval training significantly reduced the negative effects caused by this

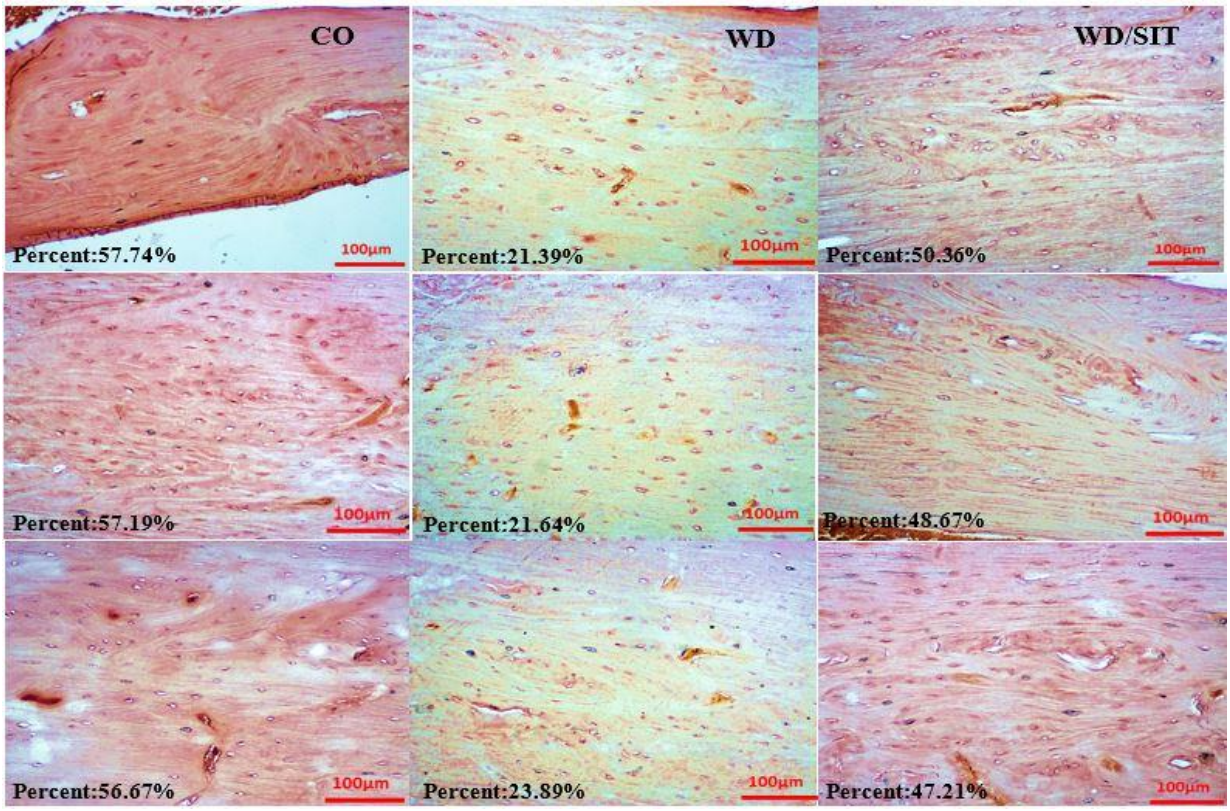

Figure 1. Alizarin red cross-sectional images of bone tissue in the normal diet (CO), Western diet (WD), and Western diet+Sprint Interval training (WD/SIT) groups. The bone density percentage was calculated using ImageJ software.

unhealthy diet.

In addition to the observed changes in bone metabolism markers, body weight was also affected during the intervention period, particularly in the Western diet group. Although high-fat diets are generally associated with weight gain, the relatively high sodium content of the present diet may have influenced fluid balance and metabolic homeostasis. High sodium intake has been reported to alter natriuresis and osmotic regulation, potentially affecting body mass dynamics (Cui et al., 2022; Robinson, Edwards, & Farquhar, 2019). Nevertheless, because daily food intake was not systematically recorded, it is not possible to determine whether reduced caloric consumption contributed to the observed weight reduction.

It is important to consider that body weight and mechanical loading are closely linked to skeletal adaptation. Mechanical forces play a central role in maintaining bone mass, and reductions in body mass may decrease skeletal loading and influence bone remodeling processes (Goolsby & Boniquit, 2017). Moreover, weight loss has been associated with endocrine adaptations, including alterations in leptin and IGF-1 signaling pathways that regulate osteoblast and osteoclast activity (Rondanelli et al., 2021). Therefore, the reduction in bone density observed in the Western diet group may reflect a combined effect of dietary composition and changes in body mass. These findings should be interpreted with caution, and future studies should monitor energy intake and body weight more closely to better isolate the independent effects of diet on bone tissue.

These findings demonstrate that prolonged consumption of a Western diet (high fat, high salt) causes an imbalance in bone remodeling processes (resorption and regeneration), leading to weakened bone structure and density. Consistent with our findings, previous studies have identified high-fat and high salt diets as contributing factors to bone mass loss (Ibrahim, Saad, Habeeb, & Abdel-raouf, 2020; Saad, Ibrahim, Habeeb, & Abdel Hafez, 2020; Suzuki et al., 2023). Specifically, Suzuki et al. (2023) demonstrated that a one-week high-fructose diet significantly elevated serum alkaline phosphatase (ALP) levels in rats (Suzuki et al., 2023). Similarly, Saad et al. (2020) observed increased serum ALP levels following seven weeks of high-fat diet consumption (Saad et al., 2020). Comparable ALP elevations have been documented in rats fed a high salt diet (Ibrahim et al., 2020). Although the precise mechanisms by which excessive consumption of fat and salt affect bone tissue remain incompletely characterized, the well-documented increase in urinary excretion of calcium and phosphorus induced by hypernatremia, along with the subsequent increase in mineral reabsorption from bone tissue, constitutes the primary mechanism responsible for the effects of excessive salt consumption (Saad et al., 2020). This pathological process has

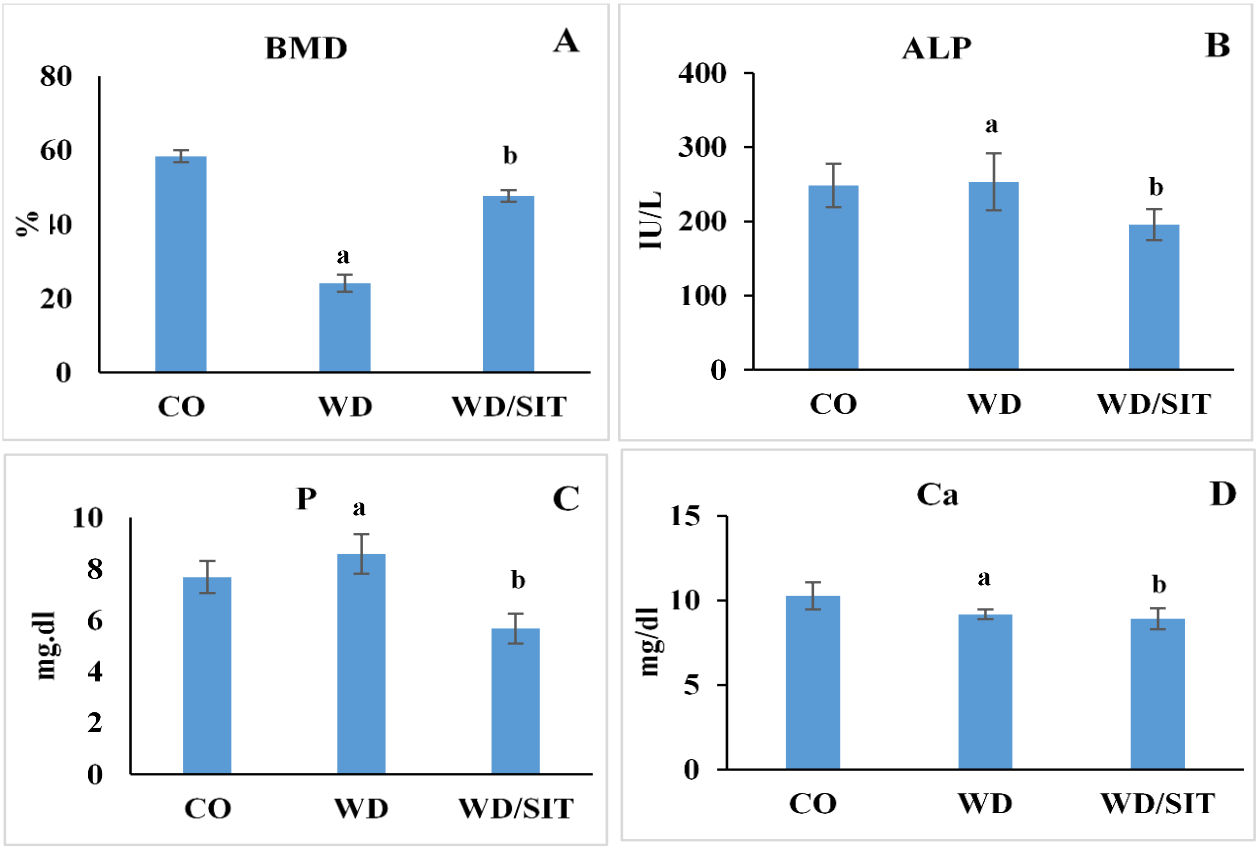

Figure 2. Comparison of bone mineral density (A), serum alkaline phosphatase (B), phosphorus (C), and calcium (D) among control (CO), Western diet (WD), and Western diet + sprint interval training (WD/SIT) groups. Bars represent mean values and error bars indicate standard deviation (SD) (n = 6 per group). Lowercase letters indicate significant differences between groups according to Fisher's LSD post hoc test ($p < 0.05$). a: Indicates a significant difference with the CO group, b: indicates a significant difference with the WD group.

been shown to originate from dysregulation of the epithelial sodium channel expression, impaired voltage-sensitive chloride channel-3 function, downregulation of the Na-Cl transporter, and disruption of sodium-calcium exchanger activity, all triggered by excessive salt intake (Cui et al., 2022).

Elevated parathormone (PTH) levels resulting from high salt consumption represent another possible cause of bone loss. When dietary salt intake rises, calcinuria (urinary calcium excretion) increases secondary to hypernatremia-induced aldosterone elevation. This urinary calcium loss necessitates compensatory bone-derived calcium reabsorption mediated by elevated serum PTH (Robinson et al., 2019). Hypernatremia adversely affects bone health through dual mechanisms: not only by promoting urinary calcium excretion but also via direct osteoclast stimulation (Wu et al., 2017). Additionally, hypernatremia disrupts regulatory T cell (Treg) function by promoting CD-4 helper T cell (Th17) differentiation. Th17 cells play a pivotal role in osteoclastogenesis through abundant production of interleukin (IL) 17, receptor activator of nuclear factor kappa B ligand (RANKL), and tumor necrosis factor-alpha (TNF-α), coupled with reduced interferon-gamma (IFN-γ) secretion, collectively promoting bone loss. Conversely, Treg cells counteract Th17 effector functions through IL-10 and transforming growth factor-beta 1 (TGF-β1) production, while also suppressing bone resorption via inhibition of osteoclast differentiation and activity (Saad et al., 2020). High-fat diet-induced bone deterioration primarily stems from fat accumulation-triggered elevation of pro-inflammatory cytokines including TNF-α, IL-1, and IL-6. These inflammatory mediators simultaneously stimulate osteoclast differentiation/activity and suppress osteoblastogenesis through modulation of NF-kB activating receptor (RANK), RANKL, and related osteoporosis pathways, ultimately leading to decreased mineral density and microstructural bone changes (Clemente-Suárez et al., 2023). Our observed alkaline phosphatase (ALP) elevation following unhealthy Western diet consumption should be interpreted in context with this diet's ultimate effect on reduced bone density. That is ALP, phosphorus, and calcium changes must be considered collectively in relation to bone density alterations. Our observed bone density reduction, consistent with most studies examining high calorie, high salt diets' impact on bone metabolism, demonstrates diet's profound effect on bone metabolism and osteoclastic dominance over osteoblastic activity.

Despite these well-documented adverse effects, our findings reveal that eight weeks of sprint interval training significantly mitigates this diet's negative bone impacts.

While previous studies have explored the effects of high-intensity interval training in combination with high-fat diets on bone metabolism (Kang et al., 2019; Paiva, Silva, Oliveira, Souza, & Jacques, 2022), to our knowledge, this is the first study to investigate the interactive effects of a sprint interval training protocol combined with a Western diet simultaneously high in fat, sugar and salt on bone markers in an animal model.

In the most comparable study, Kang et al. (2019) demonstrated that eight weeks of swimming training in high-fat diet-fed rats significantly increased femoral FNDC5 and PGC-1α expression, while bone resorption markers (CTX-1 and IL-1) decreased and osteogenic factors (osteocalcin and β-catenin) increased. They attributed these benefits to elevated serum irisin, enhanced PGC-1α/FNDC5 bone expression, and improved bone metabolism markers (Kang et al., 2019). Similarly, Paiva et al. (2022) reported improved bone mineral density and cortical thickness after six weeks of 49-minute HIIT sessions in high-fat diet rats (Paiva et al., 2022). Supporting these findings, Yang et al. (2024) recently demonstrated that HIIT positively influenced bone health in a rat model of postmenopausal osteoporosis through mechanisms involving the irisin/PGC-1α pathway, further confirming the osteogenic potential of high-intensity exercise (Yang et al., 2024). Similarly, another study reported enhanced bone thickness and strength using a four-minute interval HIIT protocol at 85-90% VO_2max (Choobineh & Ghardashi Afousi, 2020).

Current evidence confirms that exercise intensity critically determines its osteogenic impact, with weight-bearing, high-intensity activities providing maximal bone-building effects (Goolsby & Boniquit, 2017). Mechanistically, high-intensity exercise generates mechanical forces that deform the bone matrix and alter interstitial fluid flow, thereby activating osteocytes and osteoblasts. Beyond these direct mechanical effects, emerging evidence suggests that exercise-induced myokines, particularly interleukin-6 (IL-6), may mediate the osteogenic response to high-intensity interval training. Sasimontonkul et al. (2024) demonstrated that a single session of HIIT acutely increased bone formation markers, likely through IL-6 mediated pathways and adiponectin activation, highlighting the important role of muscle-bone crosstalk in exercise-induced bone health benefits (Sasimontonkul & Sirivarasai, 2024).

Conclusion

In conclusion, these results indicate that sprint interval training mitigated, but did not necessarily fully normalize, the adverse effects of the Western diet on bone metabolism. However, because a normal diet plus exercise group was not included in the study design, it remains unclear whether SIT fully restores bone parameters to physiological levels or primarily attenuates diet-induced impairments. Future studies incorporating appropriate control groups are warranted to address this question.

Limitations

This study has several limitations that should be acknowledged. First, daily food intake was not systematically recorded, which limits interpretation of the unexpected body weight reduction observed in the Western diet group and its potential contribution to bone outcomes. Second, the absence of a normal diet plus exercise control group prevents determination of whether sprint interval training fully normalizes bone parameters or only partially attenuates the effects of the Western diet. Third, bone mechanical strength was not directly assessed, and conclusions are based on biochemical markers and histological evaluation. Finally, although the study provides insight into short-term adaptations, longer intervention periods may be required to fully characterize the chronic effects of diet and high-intensity exercise on bone metabolism. Future studies addressing these factors would further clarify the independent and interactive effects of diet composition, body mass, and exercise on skeletal health.

What is already known on this subject?

Western dietary patterns, characterized by high levels of saturated fat, refined sugar, and sodium, are known to negatively affect bone health through mechanisms including chronic low-grade inflammation, oxidative stress, impaired intestinal calcium absorption, and increased urinary calcium excretion (hypercalciuria).

High-intensity, weight-bearing physical activity is a well-established non-pharmacological intervention for promoting bone formation and maintaining bone mineral density, primarily through direct mechanical signals and the indirect action of muscle-derived cytokines (myokines).

Previous animal studies have demonstrated that high-fat or high-salt diets individually can elevate bone resorption markers and reduce bone density, while various forms of exercise (e.g., swimming, continuous running) can mitigate some of these negative effects.

What this study adds?

This is the first study to investigate the interactive effects of a sprint interval training (SIT) protocol specifically combined with a Western diet simultaneously high in fat, sugar, and salt (rather than a single component) on bone tissue in an animal model.

It provides novel evidence that a time-efficient SIT protocol (only 8 weeks, with three 10-second sprints per session, three days per

week) significantly attenuates the severe negative impact of a multi-component unhealthy diet on bone mineral density, even when diet-induced weight loss occurred.

The study uniquely reports that SIT led to a significant reduction in serum alkaline phosphatase (ALP) and phosphorus in the Western diet group, which, in the context of the histological bone density findings, suggests a shift away from the high-turnover, catabolic state induced by the diet. This contrasts with the diet-only group, which exhibited high bone turnover markers alongside low bone density.

Organ Cross-Talk Tips:

- SIT partially attenuated the diet-induced bone loss, likely through mechanisms involving muscle-bone signaling, where mechanical loading and exercise-induced myokines (e.g., IL-6, irisin) may counteract the pro-inflammatory and metabolic disturbances caused by the diet.

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None.

Data availability

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

Compliance with ethical standards

Conflict of interest the authors declare that there is no conflict of interest in the present research.

Ethical approval This study was performed in line with the principles of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, as well as the National Research Council's Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Research Ethics Committee of Shahed University, Tehran, Iran (Approval Code: IR.SHAHED.REC.1400.005).

Informed consent Animal study.

Author contributions

Conceptualization: A.T., Methodology: M.R., Software: M.K., Validation: A.T.,; Formal analysis: M.R.,; Investigation: M.K.,; Resources: A.T.,; Data curation: M.R.,; Writing - original draft: M.K.,; Writing–review & editing A.T.,; Visualization: M.R.,; Supervision: M.R.; Project administration: A.T.,.; Funding acquisition: M.R.

References

Asadi, M., Rahmani, M., & Samadi, A. (2021). Effect of short-term sprint interval training (SIT) on sperm parameters and spermatogenesis indexes in adult male wistar rats. Daneshvar Medicine, 28(6), 12-23. https://doi.org/10.22070/daneshmed.2021.12933.0

Choobineh, S., & Ghardashi Afousi, A. (2020). Variability in Bone Histomorphometric Parameters of Male Rats Induced by High Intensity Interval Training. Sport Physiology & Management Investigations, 12(1), 21-32.

Chung, S. I., Ryu, S. N., & Kang, M. Y. (2021). Changes in bone metabolism and antioxidant defense systems in menopause-induced rats fed bran extract from dark purple rice (Oryza sativa L. Cv. Superjami). Nutrients, 13(9), 2926. https://doi.org/10.3390/nu13092926.

Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global impacts of western diet and its effects on metabolism and health: A narrative review. Nutrients, 15(12), 2749. https://doi.org/10.3390/nu15122749.

Cui, Y., Sun, K., Xiao, Y., Li, X., Mo, S., Yuan, Y., . . . Zhu, X. (2022). High-salt diet accelerates bone loss accompanied by activation of ion channels related to kidney and bone tissue in ovariectomized rats. Ecotoxicology and Environmental Safety, 244, 114024. https://doi.org/10.1016/j.ecoenv.2022.114024.

Dias-da-Silva, G., Panissa, V. L., Derchain, S. F., Ferreira, M. L., Telles, G. D., Buzaglo, G. B., . . . Conceição, M. S. (2024). High-Intensity Interval Training for Cancer Patients: A Review of Key Considerations for Exercise Prescription. Sports Medicine, 1-21. https://doi.org/10.1007/s40279-024-02145-7

Gibala, M. J. (2007). High-intensity interval training: a time-efficient strategy for health promotion? Current sports medicine reports, 6(4), 211-213. https://doi.org/10.1097/01.CSMR.0000306472.95337.e9

Goolsby, M. A., & Boniquit, N. (2017). Bone health in athletes: the role of exercise, nutrition, and hormones. Sports health, 9(2), 108-117. https://doi.org/10.1177/1941738116677732

Grant, W. B. (2024). A brief history of the progress in our understanding of genetics and lifestyle, especially diet, in the risk of Alzheimer's disease. Journal of Alzheimer's Disease, 100(s1), S165-S178. https://doi.org/10.3233/JAD-240658

Ibrahim, H. M., Saad, A. H., Habeeb, W. N., & Abdel-raouf, S. M. (2020). Effect of high salt diet on bone in adult male albino rats. Minia Journal of Medical Research, 31(4), 136-140. https://doi.org/10.21608/mjmr.2022.217549

Kang, Y.-S., Kim, J.-C., Kim, J.-S., & Kim, S. H. (2019). Effects of swimming exercise on serum irisin and bone FNDC5 in rat models of high-fat diet-induced osteoporosis. Journal of sports science & medicine, 18(4), 596.

Liu, L., Guo, J., Chen, X., Tong, X., Xu, J., & Zou, J. (2021). The role of irisin in exercise-mediated bone health. Frontiers in cell and developmental biology, 9, 668759. https://doi.org/10.3389/fcell.2021.668759

Liu, X., Wu, Y., Bennett, S., Zou, J., Xu, J., & Zhang, L. (2024). The effects of different dietary patterns on bone health. Nutrients, 16(14), 2289. https://doi.org/10.3390/nu16142289

Liu, Y., Zhang, L., Wang, Q., Liu, H., Zhu, X., Li, H., & Zhang, H. (2024). The effects of high-intensity interval training/moderate-intensity continuous training on the inhibition of fat accumulation in rats fed a high-fat diet during training and detraining. Lipids in Health and Disease, 23(1), 221. https://doi.org/10.1186/s12944-024-02209-7

Metcalfe, R. S., Atef, H., Mackintosh, K., McNarry, M., Ryde, G., Hill, D. M., & Vollaard, N. B. (2020). Time-efficient and computer-guided sprint interval exercise training for improving health in the workplace: a randomised mixed-methods feasibility study in office-based employees. BMC Public Health, 20, 1-13. https://doi.org/10.1186/s12889-020-8444-z

Muñoz-Garach, A., García-Fontana, B., & Muñoz-Torres, M. (2020). Nutrients and dietary patterns related to osteoporosis. Nutrients, 12(7), 1986. https://doi.org/10.3390/nu12071986

Naeimi, S., Sazvar, A., & Feyzi, A. (2022). Investigating the relationship between serum calcium and alkaline phosphatase with bone mineral density in active and inactive men. New Approaches in Exercise Physiology, 4(8), 1-13. https://doi.org/10.22054/nass.2023.72534.1125

Nazari, M., Azarbayjani, M. A., Rahmati-Ahmadabad, S., & Guerra, M. (2022). A Review of the Effects of Physical Activity (PA) on Bone Density: Relying on Iranian Studies. Thrita, 11(1), 1-7. https://doi.org/10.5812/thrita-128483

Paine, A., Woeller, C. F., Zhang, H., de la Luz Garcia-Hernandez, M., Huertas, N., Xing, L., . . . Ritchlin, C. T. (2018). Thy1 is a positive regulator of osteoblast differentiation and modulates bone homeostasis in obese mice. The FASEB Journal, 32(6), 3174. https://doi.org/10.1096/fj.201701379R

Paiva, L. A., Silva, I. S., Oliveira, S. A. d., Souza, A. S. d., & Jacques, C. O. B. (2022). Analysis of high-intensity interval training on bone mineral density in an experimental model of type 2 diabetes. Acta Cirúrgica Brasileira, 37(2), e370207. https://doi.org/10.1590/acb370207

Robinson, A. T., Edwards, D. G., & Farquhar, W. B. (2019). The influence of dietary salt beyond blood pressure. Current hypertension reports, 21, 1-11. https://doi.org/10.1007/s11906-019-0948-5

Rondanelli, M., Faliva, M. A., Barrile, G. C., Cavioni, A., Mansueto, F., Mazzola, G., . . . Tartara, A. (2021). Nutrition, physical activity, and dietary supplementation to prevent bone mineral density loss: a food pyramid. Nutrients, 14(1), 74. https://doi.org/10.3390/nu14010074

Saad, A. H., Ibrahim, H. M., Habeeb, W. N., & Abdel Hafez, S. M. (2020). Expolring how sex difference impacts bone response to high salt diet in adult albino rats. Minia Journal of Medical Research, 31(3), 193-205. https://doi.org/10.21608/mjmr.2022.220260

Sasimontonkul, S., & Sirivarasai, J. (2024). The 40-min HIIT acutely induced bone formation which was likely through the increases in muscle derived interleukin 6 and adiponectin activation: The 16 weeks of HIIT intervention, longitudinal randomized controlled trial. Bone, 184, 117105. https://doi.org/10.1016/j.bone.2024.117105

Sequeira, S., Cruz, C., Pinto, D., Santos, L., & Marques, A. (2011). Prevalence of barriers for physical activity in adults according to gender and socioeconomic status. British Journal of Sports Medicine, 45(15), A18-A19. https://doi.org/10.1136/bjsports-2011-090606.59

Suzuki, Y., Yokoyama, D., Matsuura, C., Kondo, K., Shimazaki, T., Ryoke, K., . . . Sakakibara, H. (2023). Active-phase Plasma Alkaline Phosphatase Isozyme Activity Is a Sensitive Biomarker for Excessive Fructose Intake. in vivo, 37(5), 1967-1974. https://doi.org/10.21873/invivo.13293

Ticinesi, A., Lauretani, F., Tana, C., Nouvenne, A., Ridolo, E., & Meschi, T. (2019). Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis. Exercise Immunology Review, 25, 84-95. URL: http://www.eir-isei.de/2019/eir-2019-084-article.pdf

Wahlström, A., Sayin, S. I., Marschall, H. U., & Bäckhed, F. (2016). Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metabolism, 24(1), 41-50.

Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology, 14(2), 88-98. doi: https://doi.org/10.1038/nrendo.2017.151

Vannucci, L., Fossi, C., Quattrini, S., Guasti, L., Pampaloni, B., Gronchi, G., . . . Marcucci, G. (2018). Calcium intake in bone health: a focus on calcium-rich mineral waters. Nutrients, 10(12), 1930. https://doi.org/10.3390/nu10121930

Varley, I., James, L. J., Willis, S. A., King, J. A., & Clayton, D. J. (2022). One week of high-fat overfeeding alters bone metabolism in healthy males: a pilot study. Nutrition, 96, 111589. https://doi.org/10.1016/j.nut.2022.111589

Wilson-Barnes, S. L., Lanham-New, S. A., & Lambert, H. (2022). Modifiable risk factors for bone health & fragility fractures. Best Practice & Research Clinical Rheumatology, 36(3), 101758. https://doi.org/10.1016/j.berh.2022.101758

Wong, S. K., Chin, K.-Y., Suhaimi, F. H., Ahmad, F., Jamil, N. A., & Ima-Nirwana, S. (2018). Osteoporosis is associated with metabolic syndrome induced by high-carbohydrate high-fat diet in a rat model. Biomedicine & pharmacotherapy, 98, 191-200. https://doi.org/10.1016/j.biopha.2017.12.042

Wu, L., Luthringer, B., Feyerabend, F., Zhang, Z., Machens, H., Maeda, M., . . . Schilling, A. (2017). Increased levels of sodium chloride directly increase osteoclastic differentiation and resorption in mice and men. Osteoporosis International, 28, 3215-3228. https://doi.org/10.1007/s00198-017-4163-4

Xu, Z., Ma, Z., Zhao, X., & Zhang, B. (2024). Aerobic exercise mitigates high-fat diet-induced cardiac dysfunction, pyroptosis, and inflammation by inhibiting STING-NLRP3 signaling pathway. Molecular and Cellular Biochemistry, 1-12. https://doi.org/10.1007/s11010-024-04950-0

Yang, R., Cao, K., Zhao, W., Wang, Q., Lu, C., & Zhang, Y. (2024). Mechanisms by which high-intensity interval training influences bone health in a rat model of postmenopausal osteoporosis. Chinese Journal of Tissue Engineering Research, 28(32), 5141. https://doi.org/10.12307/2024.498