Voluntary running of defined distances alters bone microstructure in C57BL/6 mice fed a high-fat diet

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Complete List of Authors:	Yan, Lin; USDA ARS, Grand Forks Human Nutrition Research Center Nielsen, Forrest; USDA ARS, Grand Forks Human Nutrition Research Center Sundaram, Sneha; USDA ARS, Grand Forks Human Nutrition Research Center Cao, Jay; USDA ARS, Grand Forks Human Nutrition Research Center
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Voluntary running of defined distances alters bone microstructure in C57BL/6 mice fed a high-fat diet

Lin Yan, Forrest H Nielsen, Sneha Sundaram, and Jay Cao

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202

Corresponding author: Lin Yan, USDA, ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, Grand Forks, ND 58202, telephone: (701) 795-8499, fax: (701) 795-8220, E-mail: lin.yan@usda.gov.

Contact information for other authors:

Forrest H Nielsen: forrest.nielsen@usda.gov

Sneha Sundaram: sneha.sundaram@covance.com (current)

Jay Cao: jay.cao@usda.gov

Abstract

Obesity increases the risk for pathological conditions such as bone loss. On the other hand, physical exercise reduces body adiposity. To test the hypothesis that physical activity improves bone quality, we evaluated voluntary running of defined distances on trabecular and cortical microstructure in mice fed a high-fat diet (HFD). Sedentary mice were fed the standard AIN93G diet or the HFD. Mice fed the HFD remained sedentary or were assigned to unrestricted running or 75%, 50%, and 25% of unrestricted running with an average running activity at 8.3, 6.3, 4.2, and 2.1 km per day, respectively. The bone structural differences found in sedentary mice were that HFD, compared the AIN93G diet, resulted in a lower bone volume fraction (BV/TV) and a higher structure model index (SMI) in vertebrae. Running had a greater effect on trabecular microstructure in femurs than in vertebrae; the decrease in SMI and an increase in trabecular thickness (Tb.Th) were in a dose-dependent manner. Running was positively correlated with BV/TV and Tb.Th and inversely correlated with SMI in femurs. The HFD increased plasma concentrations of tartrate-resistant acid phosphatase 5b, a marker of bone resorption, in sedentary mice; while running decreased it in a dose-dependent manner. The findings show that voluntary running improves bone quality in young adult mice fed an HFD.

Novelty bullets

- The high-fat diet alters bone microstructure by increasing bone resorption.
- Quantitative voluntary running improves bone microstructure through its attenuation of bone resorption in mice fed a high-fat diet.

Keywords: running, trabecular, cortical, bone, high-fat diet, C57BL/6 mice

Introduction

Physical sedentariness and poor dietary habits are two major lifestyle practices that are common in the modern world. These practices result in energy imbalances that leads to obesity. The prevalence of obesity worldwide is associated with an increased risk for chronic disease, including those characterized by bone loss. An increase in body weight caused by obesity has been considered advantageous for bone health because of the reported positive correlations between body mass or fat mass and bone mass (Reid 2008; Evans et al. 2015). However, findings from both human (Zhao et al. 2007) and rodent studies (Cao et al. 2009; Patsch et al. 2011; Yan et al. 2015; Yan et al. 2017a) show that obesity may be detrimental to bone mass and structure.

Physical exercise is an effective way to reduce body adiposity. Physical activity increases energy expenditure and reduces body mass, its associated inflammatory stress, and metabolic disturbances. In humans, physical activity positively affects bone mass and microstructure in adolescents (Janz et al. 2007), adults (Falk et al. 2007) and the elderly (Nurzenski et al. 2007). Laboratory studies with rodents have found that physical activity is beneficial to bone health including increasing bone mineral density and mineral content (Umemura et al. 2002; Mori et al. 2003; Hamrick et al. 2006).

Voluntary running is a commonly used model to study the relation between physical activity and obesity in mice. In this model, mice have unrestricted access to in-cage activity wheels that allows them to control the frequency, duration, and intensity of their running activity (Thompson

2006). The advantage of voluntary running over forced exercise on treadmills is that it minimizes the stress associated with forced activity, a potential confounding factor for treatment outcomes. Studies have shown that voluntary running reduces body mass and its associated inflammation and improves metabolism in mice with diet-induced obesity (Bradley et al. 2008; Yan et al. 2012; Yan et al. 2013). These beneficial changes are dose-dependent with running activities ranging from 4 to 8 km per day (Yan et al. 2017b). To our knowledge, however, the quantitative assessment of voluntary running on bone turnover and microstructure has not been determined. Thus, to test the hypothesis that physical activity improves bone quality in a dose dependent manner, we performed micro-computed tomographic analysis on femurs and vertebrae collected from a study showing that quantitative voluntary running reduced body mass and improved metabolism in mice when they were fed a high-fat diet (Yan et al. 2017b).

Materials and methods

Animals and diets

Three to four-week-old male C57BL/6 mice (Harlan, Madison, WI, USA) were housed in a pathogen-free room at $22 \pm 1^\circ\text{C}$ with a 12-hour light-dark cycle. The standard AIN93G diet (Reeves et al. 1993) and a modified AIN93G diet providing 45% of energy from soybean oil (hereafter referred to as the high-fat diet, HFD) were used in this study (Table 1). Mice were weighed weekly.

Experimental design

The study was approved by the Institutional Animal Care and Use Committee of the Grand Forks Human Nutrition Research Center and conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Institute for Laboratory Animal Research 2011).

The study design was reported previously (Yan et al. 2017b). Briefly, after acclimation with the AIN93G diet for one week, mice were assigned to six groups and housed individually. Mice assigned to the two sedentary groups ($n = 20$ per group) were fed the AIN93G diet or HFD. Mice assigned to the four running groups were fed the HFD and housed in cages equipped with activity wheels with programmable brakes (Model 86120, Lafayette Instrument, Lafayette, IN, USA). These mice had free access to the wheels (unrestricted running, $n = 20$) or with running restricted to 75%, 50%, or 25% of the unrestricted running ($n = 16$ per group). Brakes were released at Zeitgeber time 12 (room light was off) for restricted runners and applied when mice met their designed daily running distances. No brakes were applied to unrestricted runners. Zeitgeber time 0 is the time when the room light is on. The average daily running distance of unrestricted runners was used to calculate running distances for the restricted runners the following day. The running regimens were initiated one week after mice were acclimated to in-cage activity wheels and lasted for 12 weeks. Food intake was measured four days a week for four consecutive weeks two weeks after starting restricted running. At the end of the study, mice were euthanized; the right femur and vertebral column (from 10th or 11th thoracic vertebra to sacrum) from each mouse were harvested and stored in 70% ethanol for micro-computed tomographic analysis of trabecular and cortical bone. Plasma was collected and stored at -80°C for quantifying osteocalcin and tartrate-resistant acid phosphatase 5b (TRAP 5b).

Bone evaluation

Femurs were cleaned with cheese cloth for physical measurements of the length along the proximal distal direction and mid-shaft widths in both medial-lateral and anterior-posterior axes (Yan et al. 2017a). Femurs and lumbar vertebrae were evaluated for trabecular and cortical bone structural properties by using high-resolution (12- μ m slice increment) micro-computed tomography (Micro CT-40; Scanco Medical, Basserdorf, Switzerland) with an x-ray source power of 55 KeV and 145 μ A and integration time of 300 ms, as described previously (Yan et al. 2015; Yan et al. 2017a). The evaluation followed guidelines for assessment of bone microstructure in rodents using micro-computed tomography (Bouxsein et al. 2010).

Trabecular bone was assessed for total volume (TV, mm^3), bone volume (BV, mm^3), bone volume fraction (BV/TV, %; ratio of the segmented bone volume to the total volume of the region evaluated), connectivity density (Conn.D, $1/\text{mm}^3$; a degree of connectivity of trabeculae normalized by TV), structure model index (SMI; an indicator of the plate- and rod-like geometry of trabecular structure where 0 represents an ideal plate), trabecular number (Tb.N, $1/\text{mm}$; the average number of trabeculae per unit length), trabecular thickness (Tb.Th, mm; mean thickness of trabeculae), trabecular separation (Tb.Sp, mm; mean distance between trabeculae), and bone mineral density (BMD, mg hydroxyapatite/ cm^3). Cortical bone was assessed for total cross-sectional area (Tt.Ar, mm^2) inside the periosteal envelope, cortical bone area (Ct.Ar, mm^2), cortical area fraction (Ct.Ar/T.Ar, %), and cortical thickness (Ct.Th, mm).

Quantification of osteocalcin and TRAP 5b in plasma

Sandwich enzyme-linked immunosorbent assay kits were used to quantify osteocalcin (Alpco Diagnostics, Salem, NH, USA) and TRAP 5b (Immunodiagnostic Systems, Scottsdale, AZ, USA) in plasma, following manufacturers' protocols. Samples values were read within the linear range of the assay. The accuracy of the analysis was confirmed by the controls provided in each kit.

Statistical analyses

One-way analysis of variance (ANOVA) with Tukey contrasts was performed to compare differences among the six groups. Weekly body weight differences among the groups were analyzed by using a repeated measures ANOVA followed by Tukey contrasts at each week. Pearson correlation analysis was performed to determine body weight, body fat mass, and daily running distance in association with microstructural changes in trabecular and cortical bone. Data are presented as means $\pm$ standard error of the mean (SEM). Differences with p values of 0.05 or less are considered significant. All analyses were performed by using SAS software (version 9.4, SAS Institute, Cary, NC, USA).

Results

Body weight, body fat mass, and caloric intake

Mice assigned to unrestricted running, 75%, 50%, and 25% of unrestricted running ran an average of 8.3, 6.3, 4.2, and 2.1 km/day, respectively (Yan et al. 2017b). Sedentary mice fed the HFD were heavier than their AIN93G-fed counterparts (Figure 1). The difference was

significant two weeks after starting the HFD ($p < 0.05$); the increase in body weight was maintained throughout the remainder of the study (Figure 1). There were no differences in body weight between unrestricted runners (8.3 km/day) and sedentary mice fed the AIN93G diet for the duration of the study (Figure 1). The HFD-fed runners at 6.3 km/day were lighter than their HFD-fed sedentary counterparts (Figure 1A). The difference was significant 7 weeks after the running; the decrease was maintained throughout the remainder of the study ($p < 0.05$). The HFD-fed runners allowed at 4.2 km/day tended to be lighter in body weight compared to HFD-fed sedentary mice; the difference was not significant until the last two weeks of running ($p < 0.05$) (Figure 1B). There was no difference in body weight between HFD-fed runners at 2.1 km/day and sedentary mice fed the HFD (Figure 1C). There were no differences in caloric intakes among HFD-fed runners. Caloric intakes were 18.94 ± 0.43 , 18.93 ± 0.40 , 18.39 ± 0.42 , and 18.79 ± 0.51 kcal/day/mouse for runners at 8.3, 6.3, 4.2, and 2.1 km/day, respectively (Yan et al. 2017b). Caloric intakes of HFD-fed runners, regardless of running protocol, were not significantly different from that of HFD-fed sedentary mice (16.70 ± 0.69 kcal/day/mouse)(Yan et al. 2017b). However, they were significantly higher than that of AIN93G-fed sedentary mice (15.2 ± 0.15 kcal/day/mouse)(Yan et al. 2017b).

Physical bone measurement

The lengths of right femurs from HFD-fed unrestricted runners were slightly but significantly shorter than that of their sedentary counterparts (Table 2). There were no differences in medial-lateral axis width and anterior-posterior axis width among the six groups (Table 2).

Micro-computed tomographic assessment of femoral trabecular bone

In femurs, there were no differences in BV/TV, Conn.D, and BMD among the six groups nor differences in SMI, Tb.N, Tb.Th, and Tb.Sp between the sedentary mice fed the AIN93G diet and their HFD-fed counterparts (Table 3). Mice running from 8.3 to 2.1 km/day exhibited increased SMI and Tb.N and decreased Tb.Th and Tb.Sp in a dose-dependent manner (Table 3). Pearson correlation analysis showed that body weight was positively correlated with SMI and Tb.N and inversely correlated with Tb.Sp (Table 4). Similar results were observed when body fat weight was correlated with SMI, Tb.N, and Tb.Sp (Table 4). Running was positively correlated with BV/TV, Tb.Th, and Tb.Sp and inversely correlated with SMI and Tb.N in femurs (Table 4).

Micro-computed tomographic assessment of vertebral trabecular bone

In vertebrae, there were no differences in Conn.D, Tb.N, and Tb.Sp among the six groups (Table 3). Compared to the AIN93G diet, the HFD decreased BV/TV by 11% and elevated SMI by 16% in sedentary mice (Table 3). Compared to sedentary mice fed the HFD, unrestricted runners (8.3 km/day) increased Tb.Th by 6%; mice running 6.3 km/day increased BV/TV by 16% and Tb.Th by 9% and decreased SMI by 12% (Table 3). Running 4.2 km/day compared to sedentariness increased both BV/TV and BMD by 12%; running 2.1 km/day increased Tb.Th by 8% (Table 3). Pearson correlation analysis showed that neither body weight nor body fat weight was correlated with vertebral trabecular measurements (Table 4). Running was positively correlated with Tb.Th in vertebrae (Table 4).

Micro-computed tomographic assessment of cortical bone

There were no differences in Ct.Ar/Tt.Ar and Ct.Th in both femurs and vertebrae Between AIN93G-fed and HFD-fed sedentary mice (Table 5). There were no differences in Ct.Ar/Tt.Ar and Ct.Th in both femurs and vertebrae among the runners, regardless of running protocol. However, running 4.2 km/day increased Ct.Th by 7% compared to that found in sedentary mice fed the HFD (Table 5). Body weight and body adiposity were positively correlated with femoral Ct.Th; running was positively correlated with femoral Ct.Ar/Tt.Ar and vertebral Ct.Th (Table 4).

Concentrations of osteocalcin and TRAP 5b in plasma

There were no differences in osteocalcin between AIN93G-fed and HFD-fed sedentary mice nor differences in HFD-fed runners regardless of running protocol (Figure 2A). Plasma concentration of TRAP 5b was 44% higher in HFD-fed sedentary mice than in their AIN93G-fed counterparts (Figure 2B). Plasma concentration of TRAP 5b was 35% lower in unrestricted runners (8.3 km/day) than in sedentary mice fed the HFD. A dose dependent increase in TRAP 5b was observed with decreases in daily running distance from 8.3 to 2.1 km/day (Figure 2B).

Discussion

Obesity was considered beneficial to bone health because mechanical loading through increased body weight was associated with increased bone mass (Reid 2008; Evans et al. 2015). However, the association has not been consistently supported by laboratory studies. While there have been studies showing increased bone mineral density and content (Ma et al. 2010) and bone strength (Iwaniec et al. 2009) in rodent models of obesity; others have found bone loss and attenuated

bone strength in different bone sites in rodents fed a high-fat diet (Cao et al. 2009; Patsch et al. 2011; Shu et al. 2015). In the present study, the HFD decreased BV/TV and increased SMI in vertebrae of sedentary mice. In addition, the only correlations between body weight and measured bone variables were a positive correlation with SMI and an inverse correlation with Tb.Sp in femoral trabecular bone, and a positive correlation with Ct.Th in femoral cortical bone. Thus, findings from our study do not support the notion that diet-induced obesity, that increases the body mass, is beneficial to bone health. Rather, they suggest that the HFD may alter trabecular structure in a detrimental manner.

The present study showed that quantitative voluntary running altered both trabecular and cortical microstructure in femurs and vertebrae from mice fed the HFD. Running, ranging from 4.2 to 8.3 km per day, resulted in decreases in SMI and Tb.N and increases in Tb.Th and Tb.Sp in femurs in a dose dependent manner. The positive correlations between running and BV/TV and Tb.Th and the inverses correlation between the running and SMI in femurs indicate that running changes microstructure such that it increases bone strength in runners fed the HFD. However, the positive correlation between running and Tb.Sp and the inverse correlation between running and Tb.N contradict this indication. A possible explanation for these findings is that our study was conducted on growing mice. It has been reported that trabecular number continuously decreases from the age of six weeks old and trabecular spacing increases up to 24 weeks of age in C57BL/6 mice (Halloran et al. 2002; Somerville et al. 2004). Our findings were likely affected by physiological changes in the growing mice. Thus, the present study supports the notion that voluntary running improves bone microstructure in young adult mice with diet-

induced obesity and that physical activity during the growth period is beneficial to bone health (Daly 2007).

The dose-dependent alteration in femoral trabecular structure in runners (e.g., SMI, Tb.N, Tb.Th, and Tb.Sp) is not related to changes in caloric intake. There were no differences in caloric intake among the runners, regardless of running protocol, and runners consumed more calories than sedentary mice (Yan et al. 2017b). Rather, the alteration may be related to the improved metabolism in these HFD-fed runners. This is evidenced by an improvement in insulin sensitivity and a decrease in hepatic triacylglycerides in dose-dependent manners in running mice (Yan et al. 2017b).

Adipose tissue is no longer considered an inert storage of excess energy. It now has been shown to be an endocrine organ. Adipose tissue produces cytokines that play roles in both physiological and pathological processes in various organs including the skeleton (Farr and Dimitri 2017; Walsh and Vilaca 2017). For example, monocyte chemotactic protein-1 (MCP-1), which is found in adipose tissue, has pro-osteoclastogenic properties. High expression of *Mcp-1* has been found in osteoporotic bone in humans (Hopwood et al. 2009). *In vitro* studies have shown that MCP-1 stimulates osteoclast formation (Kim et al. 2005). *Mcp-1* knockout improves trabecular microstructure in both femurs and vertebrae in C57BL/6 mice bearing Lewis lung carcinoma (Yan et al. 2017a). We have shown that quantitative voluntary running results in dose-dependent decreases in body fat mass and MCP-1 in both adipose tissue and plasma in mice fed the HFD (Yan et al. 2017b). Thus, decreases in body adiposity as well as its associated

inflammatory cytokines including MCP-1 may have contributed, at least partly, to the improvement in bone quality in runners fed the HFD.

Bone formation and bone resorption are highly coordinated in bone remodeling. Osteocalcin, produced by osteoblasts, is considered a marker of bone formation and involved in bone mineralization (Gundberg et al. 2002). TRAP 5b is secreted by osteoclasts during bone resorption; the secretion correlates with resorptive behavior (Halleen et al. 2000; Kirstein et al. 2006). The lack of differences in osteocalcin between AIN93G-fed and HFD-fed sedentary mice and among the HFD-fed runners regardless of running protocol suggests that either the HFD or voluntary running may have less impact on bone formation. Consistent with previous reports (Cao et al. 2010; Cao et al. 2017), the elevation in TRAP 5b in sedentary mice fed the HFD suggests that the diet-induced obesity may have promoted bone resorption in this study. The dose-dependent decrease in TRAP 5b in HFD-fed runners indicates that running may attenuate HFD-mediated bone resorption. Thus, changes in bone microstructure in runners observed in this study is likely a result from a decrease in bone resorption rather than an increase in bone formation.

In this study, there were no differences in most of the measurements analyzed between running at 8.3 and 6.3 km per day. This suggests that running beyond a certain amount, in the present case 6 km per day for C57BL/6 mice, would be of no further benefit to bone health. On the other end of the spectrum, we found that running at 2.1 km per day did not affect body weight and body adiposity (Yan et al. 2017b), but it significantly increased trabecular thickness in both femurs and vertebrae compared to sedentary mice fed the HFD. Furthermore, running at 2.1 km per day decreases MCP-1 in both plasma and adipose tissue (Yan et al. 2017b). Elevated MCP-1

expression has been found at sites of osteoporotic bone in humans (Hopwood et al. 2009). Depletion of MCP-1 improves trabecular microstructure (Yan et al. 2017a) and increases bone mass in mice (Sul et al. 2012). Findings from the present study indicate that the benefit of running at a minimum of 2 km per day is not related to changes in body weight or body adiposity but to attenuation in adipose-mediated inflammation, and through which it improves bone quality.

In summary, the HFD resulted in undesirable alteration in trabecular microstructure in sedentary mice. The elevation of plasma TRAP 5b in these mice indicate that the HFD may have promoted bone resorption. Voluntary running was beneficial to bone microstructure in both femurs and vertebrae, particularly femoral trabecular bone, and decreased plasma TRAP 5b in a dose-dependent manner in mice fed the HFD. Findings from this study indicate that voluntary running may improve bone quality in young adult mice fed the HFD by attenuating bone resorption.

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Competing interests

The authors declare there are no competing interests.

Author contributions

LY and SS designed the study, conducted experiments, and collected and analyzed data. All authors contributed to interpret data, manuscript preparation and revision, and agreed to be accountable for the content of the work.

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Data availability

Datasets generated for this study are available on request to the corresponding author.

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Table 1. Composition of diets

	AIN93G Diet	High-Fat Diet
Ingredient	g/kg	g/kg
Corn Starch	397.5	42.5
Casein	200	239.4
Dextrin	132	239.4
Sucrose	100	119.7
Soybean oil	70	239.4
Cellulose	50	59.8
AIN93 mineral mix ¹	35	41.9
AIN93 vitamin mix ²	10	12
L-Cystine	3	3.6
Choline bitartrate	2.5	3
<i>t</i> -Butylhydroquinone	0.014	0.017
Total	1000	1000
Energy	%	%
Protein	20	20
Fat	16	45
Carbohydrate	64	35
Analyzed gross energy		
Kcal/g ^a	4.3 0.1	5.2 0.1

^a Values are means ± SEM of three samples analyzed from each diet.

¹ AIN93G mineral mix

Ingredient	g/kg
Calcium carbonate	357
Potassium phosphate monobasic	196
Potassium citrate, tri-potassium, monohydrate	70.78
Sodium chloride	74
Potassium sulfate	46.6
Magnesium oxide	24
Ferric citrate	6.06
Zinc carbonate	1.65
Sodium meta-silicate	1.45
Manganous carbonate	0.63
Cupric carbonate	0.3
Chromium potassium sulfate	0.275
Boric acid	0.0815
Sodium fluoride	0.0635
Nickel carbonate	0.0318
Lithium chloride	0.0174
Sodium selenate, anhydrous	0.01025
Potassium iodate	0.01
Ammonium paramolybdate	0.00795
Ammonium vanadate	0.0066
Powdered sucrose	221.026
Total	1000

² AIN93G vitamin mix

Ingredient	g/kg
Nicotinic acid	3
Calcium Pantothenate	1.6
Pyridoxine-HCl	0.7
Thiamine-HCl	0.6
Riboflavin	0.6
Folic Acid	0.2
Biotin	0.02
Vitamin B-12 (Cyanocobalamine) (0.1% in mannitol)	2.5
Vitamin E (all-rac- α -tocopheryl acetate) (500 IU/g)	15
Vitamin A (all-trans-retinyl palmitate) (500,000 IU/g)	0.8
Vitamin D-3 (cholecalciferol) (400,000 IU/g)	0.25
Vitamin K-1 (phylloquinone)	0.075
Powdered sucrose	974.655
Total	1000

Table 2. Physical measurement of right femurs from sedentary mice fed the AIN93G or a high-fat diet and mice fed the high-fat diet with different running protocols.

	AIN93G Diet	High-Fat Diet				
	Sedentary (19)	Sedentary (20)	8.3 km/day (20)	6.3 km/day (16)	4.2 km/day (16)	2.1 km/day (16)
Length, mm	14.90 ± 0.05 ^a	14.62 ± 0.09 ^a	14.28 ± 0.15 ^b	14.60 ± 0.04 ^{ab}	14.64 ± 0.05 ^a	14.56 ± 0.03 ^{ab}
WML, mm	1.38 ± 0.05	1.29 ± 0.05	1.27 ± 0.02	1.24 ± 0.01	1.24 ± 0.02	1.28 ± 0.02
WAP, mm	1.92 ± 0.08	1.98 ± 0.05	1.98 ± 0.02	2.03 ± 0.01	1.94 ± 0.02	2.02 ± 0.03

WML: medial-lateral axis width; WAP: anterior-posterior axis width. Values (means ± SEM) in a row with different letters are different at $p \leq 0.05$. Numbers in parentheses are number of mice per group.

Table 3. Trabecular structure and bone mineral density in femurs and vertebrae from sedentary mice fed the AIN93G or a high-fat diet and mice fed the high-fat diet with different running protocols.

	AIN93G Diet	High-Fat Diet				
	Sedentary (19)	Sedentary (20)	8.3 km/day (20)	6.3 km/day (16)	4.2 km/day (16)	2.1 km/day (16)
Femurs						
BV/TV, %	5.67 ± 0.33	5.45 ± 0.17	6.42 ± 0.28	6.42 ± 0.24	5.90 ± 0.43	6.14 ± 0.34
Conn.D, 1/mm ³	29.04 ± 4.09	24.45 ± 1.95	25.12 ± 1.98	26.04 ± 2.30	22.66 ± 2.87	25.31 ± 2.63
SMI	2.96 ± 0.04 ^{ab}	3.04 ± 0.03 ^a	2.69 ± 0.03 ^c	2.70 ± 0.05 ^c	2.84 ± 0.07 ^{bc}	2.87 ± 0.05 ^{abc}
Tb.N, 1/mm	3.30 ± 0.12 ^a	3.21 ± 0.06 ^a	2.57 ± 0.08 ^c	2.74 ± 0.08 ^{bc}	2.73 ± 0.11 ^{bc}	2.98 ± 0.06 ^{ab}
Tb.Th, µm	44.60 ± 0.55 ^b	46.51 ± 0.57 ^b	54.10 ± 0.94 ^a	53.25 ± 0.83 ^a	52.07 ± 1.08 ^a	51.84 ± 1.27 ^a
Tb.Sp, µm	308.73 ± 11.98 ^c	311.55 ± 6.15 ^c	397.11 ± 14.76 ^a	368.73 ± 11.12 ^{ab}	374.16 ± 15.99 ^{ab}	336.69 ± 6.48 ^{bc}
BMD, mg HA/cm ³	69.39 ± 5.62	66.02 ± 3.98	55.85 ± 6.37	60.16 ± 3.75	60.42 ± 6.40	65.49 ± 7.07
Vertebrae						
BV/TV, %	17.53 ± 0.55 ^a	15.60 ± 0.28 ^b	17.08 ± 0.45 ^{ab}	18.05 ± 0.36 ^a	17.50 ± 0.63 ^a	17.11 ± 0.41 ^{ab}
Conn.D, 1/mm ³	216.69 ± 6.68	202.18 ± 5.26	197.55 ± 5.75	199.96 ± 7.15	200.34 ± 6.29	196.71 ± 5.61
SMI	1.48 ± 0.06 ^c	1.72 ± 0.03 ^a	1.63 ± 0.04 ^{abc}	1.52 ± 0.03 ^{bc}	1.62 ± 0.07 ^{abc}	1.68 ± 0.05 ^{ab}
Tb.N, 1/mm	5.34 ± 0.05	5.27 ± 0.06	5.21 ± 0.05	5.25 ± 0.06	5.25 ± 0.07	5.22 ± 0.06
Tb.Th, µm	41.24 ± 0.49 ^{bc}	40.26 ± 0.34 ^c	42.66 ± 0.45 ^{ab}	43.73 ± 0.45 ^a	43.31 ± 0.79 ^{ab}	43.46 ± 0.68 ^a
Tb.Sp, µm	182.05 ± 2.07	184.98 ± 2.10	184.86 ± 1.86	183.86 ± 2.36	184.15 ± 2.66	184.66 ± 2.18
BMD, mg HA/cm ³	181.42 ± 4.28 ^{ab}	166.24 ± 3.69 ^b	184.72 ± 4.32 ^{ab}	173.16 ± 12.45 ^{ab}	186.82 ± 6.18 ^a	177.56 ± 5.14 ^{ab}

BV/TV: bone volume fraction; Conn.D: connectivity density; SMI: structure model index; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; BMD: bone mineral density. Values (means $\pm$ SEM) in a row with different letters are different at $p \leq 0.05$. Numbers in parentheses are number of mice per group.

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Table 4. Pearson correlations of body weight, fat weight, and daily running distance with trabecular and cortical structure in femurs and vertebrae.

	Body Weight, g		Fat Weight, g		Running, km	
	r	p	r	p	r	p
Femoral Trabecular						
BV/TV, %	0.09	0.92	0.033	0.74	0.221	< 0.05
Conn.D, 1/mm ³	0.039	0.69	0.018	0.86	-0.090	0.36
SMI	0.300	< 0.01	0.221	< 0.05	-0.534	< 0.01
Tb.N, 1/mm	0.282	< 0.01	0.195	< 0.05	-0.629	< 0.01
Tb.Th, µm	-0.008	0.94	0.110	0.26	0.642	< 0.01
Tb.Sp, µm	-0.303	< 0.01	-0.231	< 0.05	0.610	< 0.01
BMD, mg HA/cm ³	0.102	0.29	0.065	0.50	-0.184	0.06
Vertebral Trabecular						
BV/TV, %	0.006	0.95	-0.013	0.89	0.136	0.16
Conn.D, 1/mm ³	0.032	0.75	-0.049	0.62	-0.176	0.07
SMI	0.142	0.14	0.172	0.08	-0.011	0.91
Tb.N, 1/mm	0.096	0.33	0.010	0.92	-0.160	0.10
Tb.Th, µm	0.130	0.18	0.173	0.07	0.316	< 0.01
Tb.Sp, µm	-0.101	0.30	-0.020	0.83	0.073	0.46
BMD, mg HA/cm ³	0.056	0.56	0.023	0.81	0.095	0.33
Femoral Cortical						
Ct.Ar/Tt.Ar, %	0.12	0.23	0.22	0.02	0.32	< 0.01
Ct.Th, µm	0.39	< 0.01	0.43	< 0.01	0.17	0.08
Vertebral Cortical						
Ct.Ar/Tt.Ar, %	-0.05	0.65	-0.11	0.27	0.17	0.08
Ct.Th, µm	0.07	0.48	0.05	0.59	0.24	0.01

BV/TV: bone volume fraction; Conn.D: connectivity density; SMI: structure model index; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; BMD: bone mineral density; Ct.Ar/Tt.Ar: cortical area fraction; Ct.Th: cortical thickness.

Table 5. Cortical structure of femurs and vertebrae from sedentary mice fed the AIN93G or a high-fat diet and mice fed the high-fat diet with different running protocols.

	AIN93G Diet	High-Fat Diet				
	Sedentary (19)	Sedentary (20)	8.3 km/day (20)	6.3 km/day (16)	4.2 km/day (16)	2.1 km/day (16)
Femurs						
Ct.Ar/Tt.Ar, %	43.66 ± 0.32 ^b	44.14 ± 0.25 ^{ab}	44.99 ± 0.29 ^a	45.16 ± 0.36 ^a	45.15 ± 0.34 ^a	44.84 ± 0.35 ^{ab}
Ct.Th, µm	144.53 ± 1.11 ^b	146.85 ± 0.97 ^{ab}	147.60 ± 1.34 ^{ab}	149.75 ± 1.11 ^a	150.25 ± 1.41 ^a	150.25 ± 1.51 ^a
Vertebrae						
Ct.Ar/Tt.Ar, %	68.91 ± 0.66	67.26 ± 0.68	69.67 ± 1.01	69.32 ± 1.04	69.43 ± 1.11	68.80 ± 0.97
Ct.Th, µm	39.53 ± 0.42 ^{ab}	38.55 ± 0.41 ^b	40.50 ± 0.78 ^{ab}	40.31 ± 0.60 ^{ab}	41.3 ± 0.61 ^a	40.06 ± 0.54 ^{ab}

Ct.Ar/Tt.Ar: cortical area fraction; Ct.Th: cortical thickness. Values (means ± SEM) in a row with different letters are different at $p \leq 0.05$. Numbers in parentheses are number of mice per group.

Figure legends

Figure 1. Body weight of sedentary mice fed the AIN93G or a high-fat diet (HFD) and HFD-fed mice allowed to have unrestricted running (8.3 km/day) in comparison to HFD-fed runners at 6.3 km/day (A), 4.2 km/day (B), and 2.1 km/day (C). The HFD, compared to the AIN93G diet, significantly increased body weight in sedentary mice two weeks after starting the HFD (1A-C, a). Running at 6.3 km/day (1A, b) and 4.2 km/day (1B, b) significantly decreased body weight, compared to HFD-fed sedentary mice, seven and 10 weeks after the running, respectively.

Values are means $\pm$ SEM ($n = 19$ for sedentary AIN93G, $n = 20$ each for sedentary HFD and 8.3 km/day HFD, and $n = 16$ each for 6.3 km/day HFD, 4.2 km/day HFD, and 2.1 km/day HFD, respectively). Data were analyzed by a repeated measures ANOVA followed by Tukey contrasts at each week.

Figure 2. Plasma concentrations of osteocalcin (A) and TRAP 5b (B) from sedentary mice fed the AIN93G or a high-fat diet and high-fat diet-fed mice with different running protocols.

Values (means $\pm$ SEM) with different letters are different at $p \leq 0.05$ ($n = 12$ per group). Data were analyzed by one-way ANOVA followed by Tukey contrasts.

Figure 1

Figure 2

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