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Original Article

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Bone responses to body weight and moderate treadmill exercising in growing male obese (fa/fa) and lean Zucker rats

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Abstract

Objective: To evaluate if exercise in a young growing model of obesity would be able to reduce adiposity without adversely affecting bone. **Methods:** Obese (fa/fa) and lean (Fa/Fa) male Zucker rats were randomly assigned (n=8-9 rats/group) to no exercise or to a moderate treadmill exercise regimen consisting of a speed of 20 m/min for ~1 h, 5 d/wk for 9 wks. Bone morphometry, total bone ash, and bone strength by three-point bending test were determined in the femur and the tibia. Serum alkaline phosphatase was determined using calorimetric enzyme assays. Serum osteocalcin, deoxypyridinoline (DPD), and pyridinoline (PYD) crosslinks were measured using enzyme immunoassays. Various blood chemistry measurements were determined including: serum glucose, insulin, and leptin. **Results:** Growing obese (fa/fa) Zucker rats have long bone ash comparable to lean Zucker rats, although bone size is reduced. Moderate treadmill running successfully reduced body weight without increasing muscle mass, but did not attenuate the reduction in bone growth or promote greater bone mass and strength in obese Zucker rats. **Conclusions:** Moderate treadmill running for 9 wks successfully reduced the body weight of obese Zucker rats by 7.5% without negatively affecting bone.

Keywords: Treadmill Exercise, Obesity, Bone Mineral Content, Bone Turnover, Metabolic Disorders

Introduction

The prevalence of childhood obesity continues to increase worldwide[1]. Obesity has been suggested to confer a beneficial effect of increasing bone mass[2,3]. Increased bone mass is protective against osteoporosis, a disease characterized by low bone mass that results in bone fractures with minimal trauma. Greater bone mass associated with obesity has been attributed to heavier body weight imposing a heavier mechanical load on bones[4]. The skeleton responds to mechanical loading either by stimulating osteoblast activity and/or by reducing osteoclast activity to promote bone formation[5]. Additionally, hyperinsulinemia which often accompanies obesity, has been reported to stimulate osteoblast activity[6] in both rat studies[7] and human studies[8].

The effects of obesity on growing bones have been inconclusive. Goulding et al.[9] reported that bone mineral content (BMC) was lower in obese compared to age-matched children and adolescents of normal body weight. Furthermore, risk of forearm fractures, the most common site of fracture during childhood and adolescence, was greater. The authors suggested that in overweight children bone mass was low relative to their body weight[9]. Skaggs et al.[10] reported that overweight children had smaller bone size. This combined with higher body weight places more stress on the bone resulting in increased fracture risk. In contrast, Leonard et al.[11] reported higher whole body BMC in obese (1095±502 g) compared to non-obese (732±510 g) children and adolescents. Understanding the influence of body weight on bone in children and adolescents is important because achieving a higher peak bone mass and bone size during the bone formation stage can lower the risk of bone fracture later in life.

Co-morbidities associated with adult obesity such as: dyslipidemia, insulin resistance, and type 2 diabetes also occur in children[12]. There is evidence to suggest that impaired bone formation and/or turnover is associated with metabolic

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abnormalities. The obese Zucker (fa/fa) rat is an animal model of obesity and the metabolic syndrome. In particular, the male rats display a rapid onset of obesity, insulin resistance, hyperinsulinemia, and impaired glucose tolerance at a young age[13]. A phenotype of impaired longitudinal bone growth, mineral density, and strength has been reported for Zucker obese rats[14].

Weight loss is recommended to attenuate various obesity-related co-morbidities. However, the impact of the method of weight reduction on bone, particularly during growth should be considered. Relying on caloric restriction to promote weight loss may potentially reduce the consumption of nutrients important to bone such as calcium (Ca). Studies have observed that caloric restriction resulted in bone loss[15]. Promoting physical activity for weight loss increases mechanical load, muscle mass, and improves the metabolic profile, all of which contribute to bone health. During the growth stage, exercise maximizes peak bone mass. In addition to the increased accrual of BMC, bone geometry (i.e. size) is increased, resulting in greater bone strength than provided by BMC alone[16]. However, decreased osteoblast activity has been reported during marathon running due to increased cortisol and parathyroid levels[17]. Therefore, a moderate exercise regimen is recommended.

In this study the effect of moderate exercise on bone was investigated using treadmill running. A number of studies have reported the efficacy of moderate treadmill exercise in terms of weight-bearing activity on the long bones of growing rats[18]. Mathey et al.[14] subjecting Zucker rats age 13 wks to ~13 wks treadmill running reported increased femoral BMD, Ca, and length. In our study, younger (age 6-7 wks old) rats were used based on the Wang et al.[19] study findings that in male Sprague-Dawley rats proximal tibia and femoral neck BMC showed a rapid increase until age 13 wks (3 mo). After this age, BMC accrual slowed with bone loss beginning at age 39 wks (9 mo). Exercise during the growth stage benefits PBM by increasing bone accrual and size[16]. Therefore, if physical activity is started after the rapid period of skeletal growth, the beneficial effects of exercise may be lost[20]. After 9 wks treadmill exercise, our rats (age 16 wks) are expected to have surpassed rapid skeletal growth i.e. peak bone mass. The objective of this study was to evaluate if exercise in a young growing model of obesity would be able to reduce adiposity without adversely affecting bone.

Materials and methods

Animals and moderate treadmill exercise protocol

All animal procedures used in this study conformed to the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals[21]. The protocol for this study was approved by the Institutional Animal Care and Use Committee at West Virginia University. Young (age 6 wks) male obese (fa/fa) or homogenous lean (Fa/Fa) male Zucker rats (total n=34) were purchased from Harlan Teklad (Indianapolis, IN). Animals were individually caged and housed in a facility approved by the Association for Assessment of Laboratory Animal Care. In the animal care facility, rats were kept in rooms maintained at 22±2°C with a 12:12 h light/dark cycle (6:00 am light on/6:00 pm lights off). Rats were provided free access to standard rat chow (Formlab 5008, PMI Feeds Inc, Richmond, IN) and water throughout the 9 wks study.

Following a 7 d acclimation period, obese Zucker rats were randomly assigned (n=8-9 rats/group) to no exercise (OB/NE) or treadmill exercise (OB/EX). Similarly, lean Zucker rats were randomly assigned (n=8-9 rats/group) to no exercise (Lean/NE) or treadmill exercise (Lean/EX). The OB/EX and Lean/EX rats were trained to run on a level motorized rodent treadmill (Columbus Instruments, Columbus, OH). The moderate treadmill exercise regimen consisted of a running speed of 10 m/min for the first 10 min and 20 m/min for 50 min with a 0% incline, at a frequency of 5 d/week for the duration of 9 wks. The duration of 9 wks was based on the Hagihara et al.[22] study finding that Wistar rats age 8 wks old subjected to treadmill running 4-5 d/wk for a duration of 8 wks had increased BMD and bone strength measured by three-point bend testing. At the end of the 9 wk study, 48 h after the last training session and an overnight fast (~16 h) rats were anesthetized with isoflurane then exsanguinated. The major leg muscles (i.e. gastrocnemius and soleus) of rats were dissected and immediately weighed.

Bone morphometry

At euthanasia, the right and left femurs and tibiae were collected from each animal. The bones were defleshed with care being taken not to damage the periosteum. Each bone was wrapped in deionized distilled water (ddH_2O)-soaked gauze and stored at -20°C until analyzed. For analysis, each bone was brought to room temperature. We measured bone morphometry because exercise of growing bones has been suggested to increase bone size and longitudinal growth[18]. Morphometry measurements of bone length, width, and depth were measured using a vernier caliper (Bel-Art Products, Pequannock, NJ). Length was measured from medial condyle to greater trochanter. Diameter was measured at mid-section of shaft from lateral to medial end i.e. diaphysis.

Bones were dried at 110°C (Oven DK-63, Baxter Scientific Products, Hayward, CA) for 48 h and dry weights determined using an analytical balance (Mettler Toledo, Columbus, OH). Morphometry measurements were averaged for bone pairs (i.e. right and left) after no bilateral differences were determined using paired t-test with significance level set at $P<0.05$.

Bone turnover markers

Rats were anesthetized with isoflurane and blood samples obtained by cardiac puncture. Serum was obtained by centrifuging blood samples at 1,500 g for 10 min at 4°C. Serum samples were stored at -80°C until assayed. Osteoblast activ-

ity was determined by measuring serum alkaline phosphatase and serum osteocalcin. Serum alkaline phosphatase was determined by Vet 16 veterinary test rotor calorimetric assay and measured using a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc, Columbia, MD). Serum osteocalcin was measured using a commercially available rat specific enzyme immunoassay (EIA) (Biomedical Technologies, Stoughton, MA). Optical densities of samples were measured at 450 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA).

Osteoclast activity was determined by measuring the bone-related degradation products of deoxypyridinoline (DPD) and pyridinoline (PYD) crosslinks. Serum DPD and PYD were determined using EIA kits (Quidel Corp, San Diego, CA). Optical densities of serum DPD and PYD samples were measured at wavelength 405 nm using a Spectramax Plus microplate reader.

Bone biomechanical strength

Bone strength indices were assessed using a TA.XT2i Texture Analyzer (Texture Technologies, Scarsdale, NY) outfitted with a three-point bending apparatus. Femora and tibiae were placed on supports. Span between the supports was 1 mm for the femora and 1.7 mm for the tibia. Diagrammed in Leppanen et al.[23], the long bone is placed on its posterior surface and force applied to the midshaft marked at a position halfway between the greater trochanter and the distal medial condyle. The bone was bent until broken by lowering a centrally placed blade (1 mm width) at a constant crosshead speed (0.1 mm/sec). The load cell was 50 kg. The load-deflection data were collected by a PC interfaced with the TA.XT2i. Sample test distance was set at 10 mm with a signal collection rate of 100 points per sec. Peak force and bending failure energy were determined according to Yuan et al.[24]. Ultimate bending stress and Young's modulus were determined according to Ortoft et al.[25].

Total bone mineral and Ca content

Total bone mineral content of the femur and of the tibia was determined by ashing the dried bones at 600°C in a muffle furnace (model CP18210, Thermolyne, Dubuque, IA) for 24 h. Total bone ash was determined by weighing the bone ash. Bone Ca content was determined by dissolved bone ash in 2 mL of 70% nitric acid. The acidified samples were neutralized in 5 ml of ddH_2O then filtered through Whatman no. 1 paper. Samples were diluted to a final volume of 50 ml with ddH_2O. The Ca concentrations in samples were determined by model P400 inductively coupled plasma optical emission spectrometry (ICP, Perkin Elmer, Shelton, CT).

Blood chemistry and hormones

Serum samples were stored at -80°C until assayed. Serum cholesterol and triglycerides were determined by the Lipid test rotor and measured using a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc, Columbia, MD). Fasting serum glucose was determined by the Vet 16 veterinary test rotor hexokinase/glucose-6-phosphate dehydrogenase assay and also measured using a Hemagen Analyst automated spectrophotometer. Serum insulin was measured using a commercially available rat specific EIA kit (Biomedical Technologies, Stoughton, MA). The optical densities of the serum insulin samples were determined at wavelength 450 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA). Biomarkers of kidney function consisting of: blood urea nitrogen (BUN), serum total protein, creatinine, Ca, and P concentrations were determined by Vet 16 veterinary test rotor enzymatic colorimetric assays and measured using a Hemagen Analyst automated spectrophotometer.

Serum leptin was measured by a rat specific EIA (Assay Designs, Ann Arbor, MI). The optical densities of the samples were determined at wavelength 450 nm by Spectramax Plus microplate reader. Serum total testosterone was determined using a Coat-a-Count radioimmunoassay kit (Siemens Healthcare Diagnostics, Los Angeles, CA). Radioisotope counting was done using a Wallac 1470 Wizard Gamma Counter (Perkin Elmer, Waltham, MA). The intra-assay coefficient of variation was 8%.

Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). Upper and lower 95% confidence intervals were analyzed by Microsoft Office Excel 2007. The relationships between femoral or tibial total ash and body weight were determined by a simple regression of individual animals from all treatment groups. Two-way ANOVA was used to determine the effect of body weight, exercise, and body weight x exercise on measured bone and serum variables. Fisher's t-test was used to evaluate differences among the means of the treatment groups. An alpha level of 0.05 or $P<0.05$ was used to determine statistically significant differences. All statistical analyses were performed using Sigma Stat (Abacus Concepts, Berkeley, CA).

Results

Body weight and muscle mass

At the start of the study, obese Zucker rats were already heavier ($P<0.05$) than lean Zucker rats. According to Table 1, weight gain was greater ($P<0.05$) and final body weight was heavier ($P<0.05$) in OB/NE compared to Lean/NE rats. The absolute and relative weights of the major leg muscles: gastrocnemius ($P<0.05$) and soleus ($P=0.002$) were lower in OB/NE compared to Lean/NE rats. Moderate treadmill running lowered weight gain ($P=0.01$) final body weight ($P=0.004$) of OB/EX rats compared to OB/NE, but had no significant effect on the absolute or relative gastrocnemius

	Treatment[1]			
Measurements	**LEAN/NE**	**OB/NE**	**LEAN/EX**	**OB/EX**
Initial Body Weights (g)	170±5 (158-182)	220±3[a] (212-228)	172±6[b] (158-186)	229±5[a] (216-242)
Body Weight Gain (g)	199±11 (174-224)	363±13[a] (331-395)	172±6[b] (153-191)	310 ±14[ab] (287-333)
Final Body Weights (g)	369±7 (352-386)	583±14[a] (551-615)	344±8[b] (329-359)	539±10[ab] (508-570)
Gastrocnemius (g)	1.7±0.03 (1.6-1.8)	1.3±0.04[a] (1.2-1.4)	1.7±0.03[b] (1.6-1.8)	1.2±0.04[a] (1.1-1.3)
Relative Gastrocnemius (mg/g bwt)	4.55±0.07 (4.4-4.7)	2.17±0.05[a] (2.0-2.3)	4.82±0.08[ab] (4.6-5.0)	2.29±0.07[a] (2.2-2.4)
Soleus (g)	0.17±0.01 (0.16-0.18)	0.15±0.01[a] (0.14-0.16)	0.17±0.01[b] (0.16-0.18)	0.14±0.01[a] (0.13-0.15)
Relative Soleus (mg/g bwt)	0.47±0.01 (0.45-0.49)	0.25±0.01[a] (0.24-0.26)	0.49±0.01[b] (0.46-0.52)	0.26±0.01[a] (0.24-0.28)
Femur total BMC (mg)	302±8 (284-320)	298±6 (285-311)	310±9 (288-332)	294±7 (278-310)
Femur calcium (mg/g bone)	43.3±0.7 (42-45)	45.1±0.7 (43-47)	43.5±0.9 (41-46)	44.7±0.6 (43-46)
Tibia total BMC (mg)	293±26 (234-352)	287±26 (226-348)	283±21 (234-332)	320±40 (278-362)
Tibia calcium (mg/g bone)	47.3±3.0 (41-54)	47.9±1.4 (44-52)	45.4±0.9 (43-48)	48.7±1.8 (47-50)

[1] Values are expressed as the mean ±SEM of n=8-9 rats/group. In parentheses are upper and lower 95% confidence intervals. Superscript a=$P<0.05$ vs Lean/NE, b=$P<0.05$ vs OB/NE. Fisher's t-test was used to evaluate differences among the means of the treatment groups. An alpha level of 0.05 or $P<0.05$ was used to determine statistically significant differences. Abbreviations: OB obese, NE non-exercise, EX exercise, BMC bone mineral content, bwt body weight.

Table 1. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on body weight, muscle mass, and bone mineral content.

and soleus muscle mass. Body mass remained heavier and muscle mass reduced in OB/EX rats compared to lean Zucker rats. In Lean/EX rats, moderate treadmill running had no significant effect on body weight compared to Lean/NE rats. In Lean/EX rats relative gastrocnemius weight was increased ($P<0.05$), but moderate treadmill running had no effect on the weight of the soleus muscle. Two-way ANOVA showed an effect of body weight ($P<0.001$), but no effect of exercise or body x exercise on absolute or relative soleus weight. Two-way ANOVA showed an effect of body weight ($P<0.001$) and body x exercise ($P=0.008$) on relative gastrocnemius weight.

Total Bone Ash and Ca content

As shown in Table 1, there were no significant differences in femoral or tibial Ca content or total bone ash between OB/NE and Lean/NE rats. Linear regression analysis showed no relationship between body weight and femoral total ash ($r^2=0.05$, $P=0.20$) or tibial total ash ($r^2=0.01$, $P=0.50$). A moderate treadmill running exercise regimen showed no significant differences in femoral or tibial Ca content or total bone ash in OB/EX compared to OB/NE or lean rats. In Lean/EX rats, moderate treadmill running showed no significant differences in femoral or tibial Ca content or total bone ash compared Lean/NE rats. Two-way ANOVA showed no effect of body weight ($P=0.19$), exercise ($P=0.84$) or body x exercise ($P=0.45$) on femoral total ash. Two-way ANOVA showed no effect of body weight ($P=0.61$), exercise ($P=0.71$) or body x exercise ($P=0.46$) on tibial total ash.

Bone morphometry

As shown in Table 2, femur length was shorter ($P=0.002$) in OB/NE compared to the Lean/NE rats. There were no significant differences in femur width, depth, and weight between OB/NE and Lean/NE rats. In the femur, adjusting for length resulted in no significant differences in total bone ash between

	Treatment[1]			
Morphometry Measurements	**LEAN/NE**	**OB/NE**	**LEAN/EX**	**OB/EX**
Femur				
Length (mm)	31.23±0.23 (30.7-31.8)	29.72±0.27[a] (29.1-30.3)	31.67±0.27[b] (31.0-32.3)	29.37±0.30[a] (28.6-30.1)
Width (mm)	4.18±0.06 (4.0-4.4)	4.02±0.08 (3.8-4.2)	4.09±0.09 (3.9-4.3)	4.13±0.06 (4.0-4.3)
Depth (mm)	3.17±0.04 (3.0-3.3)	3.28±0.06 (3.1-3.5)	3.13±0.05 (3.0-3.3)	3.23±0.05 (3.1-3.4)
Weight (mg)	648±28 (602-694)	625±25 (571-679)	651±27 (593-709)	616±23 (571-661)
Tibia				
Length (mm)	37.16±0.27 (36.6-37.7)	35.43±0.25[a] (34.8-36.1)	37.13±0.27[b] (36.5-37.8)	35.03±0.22[a] (34.6-35.5)
Width (mm)	3.14±0.05 (3.0-3.3)	2.88±0.04[a] (2.8-3.0)	3.28±0.08[b] (3.1-3.5)	2.88±0.05[a] (2.8-3.0)
Depth (mm)	2.44±0.05 (2.3-2.6)	2.43±0.05 (2.4-2.5)	2.44±0.04 (2.4-2.5)	2.45±0.04 (2.4-2.5)
Weight (mg)	518±26 (455-581)	469±19[a] (427-511)	521±22[b] (467-575)	467±17[a] (427-507)

[1] Values are expressed as the mean ±SEM of n=8-9 rats/group. In parentheses are upper and lower 95% confidence intervals. Superscript a=$P<0.05$ vs Lean/NE, b=$P<0.05$ vs OB/NE. Fisher's t-test was used to evaluate differences among the means of the treatment groups. An alpha level of 0.05 or $P<0.05$ was used to determine statistically significant differences. Abbreviations: OB obese, NE non-exercise, EX exercise.

Table 2. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on femur and tibia morphometry.

OB/NE (10.0±0.1 mg/mm) and Lean/NE (9.7±0.2 mg/mm). The moderate treadmill running exercise regimen had no significant effect on femoral morphometry in either OB/EX or Lean/EX Zucker male rats. The femur expressed as g total ash/g bone showed no significant differences among the treatment groups Lean/NE (0.47±0.01), OB/NE (0.48±0.01), Lean/EX (0.48±0.01), OB/EX (0.48±0.01). Two-way ANOVA showed an effect of body weight ($P<0.01$), but no effect of exercise ($P=0.81$) or body x exercise ($P=0.15$) on femur length.

In the tibia, width ($P=0.003$), weight ($P<0.05$), and length ($P=0.01$) were lower in OB/NE compared to Lean/NE rats. Adjusting for length resulted in no significant differences in total ash between OB/NE (8.1±0.7 mg/mm) and Lean/NE (7.9±0.6 mg/mm). Adjusting for width resulted in no significant differences in total bone ash between OB/NE (99±8 mg/mm) and Lean/NE (94±9 mg/mm). The tibia expressed as g total ash/g bone showed no significant differences among the treatment groups Lean/NE (0.57±0.05), OB/NE (0.61±0.08), Lean/EX (0.54±0.04) or OB/EX (0.69±0.09). The moderate treadmill running exercise regimen had no significant effect on tibia morphometry in either OB/EX or Lean/EX Zucker male rats. Two-way ANOVA showed an effect of body weight ($P<0.05$), but no effect of exercise or body x exercise ($P>0.1$) on tibia length, width or weight.

Bone Turnover Markers

Indicators of osteoblast activity was increased indicated by a tendency ($P=0.049$) for higher serum alkaline phosphatase (Figure 1A) and higher ($P<0.05$) serum osteocalcin concentration in OB/NE compared to the Lean/NE rats (Figure 1B). There was no difference in serum PYD or DPD concentration between OB/NE compared to the Lean/NE rats (Figure 2A,B). The moderate treadmill running exercise regimen had no significant effect on bone turnover biomarkers of serum alkaline phosphatase, osteocalcin, PYD or DPD concentration in either OB/EX or Lean/EX rats. Two-way ANOVA showed an effect of body weight ($P<0.01$), but no effect of exercise ($P=0.10$) or body x exercise ($P=0.65$) on serum osteocalcin.

Bone biomechanical strength

As shown in Table 3, the femur and tibia of OB/NE showed no significant differences in biomechanical strength measurements of: peak force, bending failure energy, ultimate bending stress or Young's modulus compared Lean/NE rats. The moderate treadmill running exercise regimen had no significant effect on any of the biochemical strength parameters in OB/EX or Lean/EX rats.

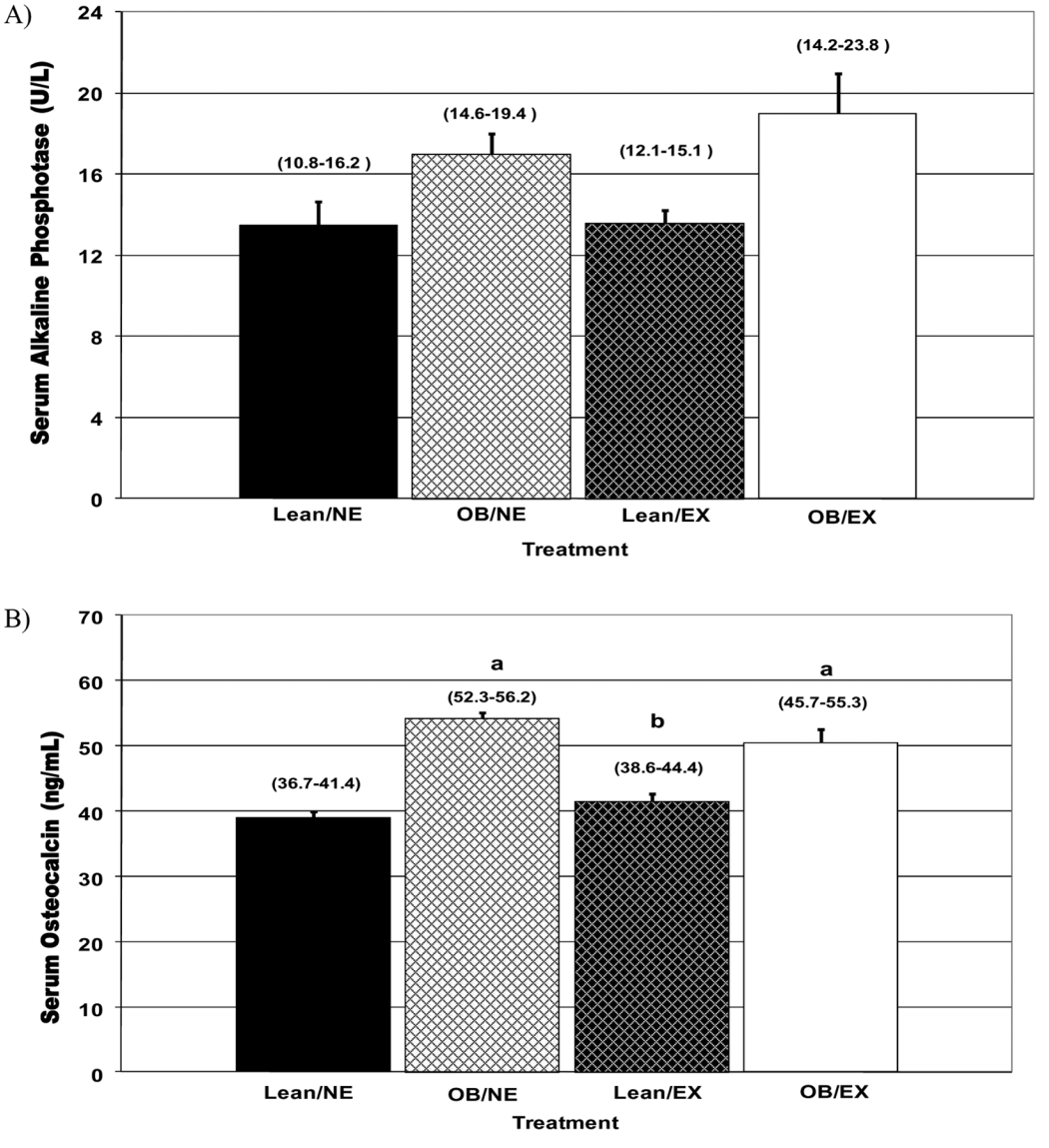

Figure 1. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on indicators of osteoblast activity: A) serum alkaline phosphotase and B) serum osteocalcin. Values are the mean ±SEM of the absolute values of n=6-7 rats/group. In parentheses are upper and lower 95% confidence intervals. Superscripts a=$P<0.05$ vs Lean/NE, b=$P<0.05$ vs OB/NE. Fisher's t-test was used to evaluate differences among the mean of treatment groups. An alpha level of 0.05 or $P<0.05$ was used to determine statistically significant differences.

Blood chemistry and hormones

According to Table 4, serum cholesterol ($P<0.05$) and triglyceride ($P<0.05$) concentrations were higher for OB/NE compared to the Lean/NE rats. Fasting glucose ($P<0.05$) and serum insulin ($P<0.05$) concentrations were higher for OB/NE compared to Lean/NE rats. Hormone measurements showed higher ($P<0.05$) serum leptin concentrations, but no differences for serum testosterone concentration between OB/NE and Lean/NE rats. There were no significant differences in biomarkers of kidney function consisting of: serum BUN, creatinine, total protein, albumin, Ca or P between OB/NE and Lean/NE rats.

The moderate treadmill running exercise regimen had no significant effect on serum cholesterol, triglyceride or biomarkers of kidney function in either OB/EX or Lean/EX rats. The treadmill exercise regimen had no significant effect on serum insulin, but lower ($P<0.05$) fasting glucose in OB/EX compared to OB/NE. The moderate treadmill running exercise regimen had no significant effect on serum leptin or testosterone concentrations in either OB/EX or Lean/EX rats. Two-way ANOVA showed an effect of body weight ($P<0.001$), but no effect on exercise or body x exercise on serum cholesterol, triglycerides, insulin or leptin. However, there was an effect of body weight ($P<0.001$) and body x exercise ($P=0.04$) on fasting glucose.

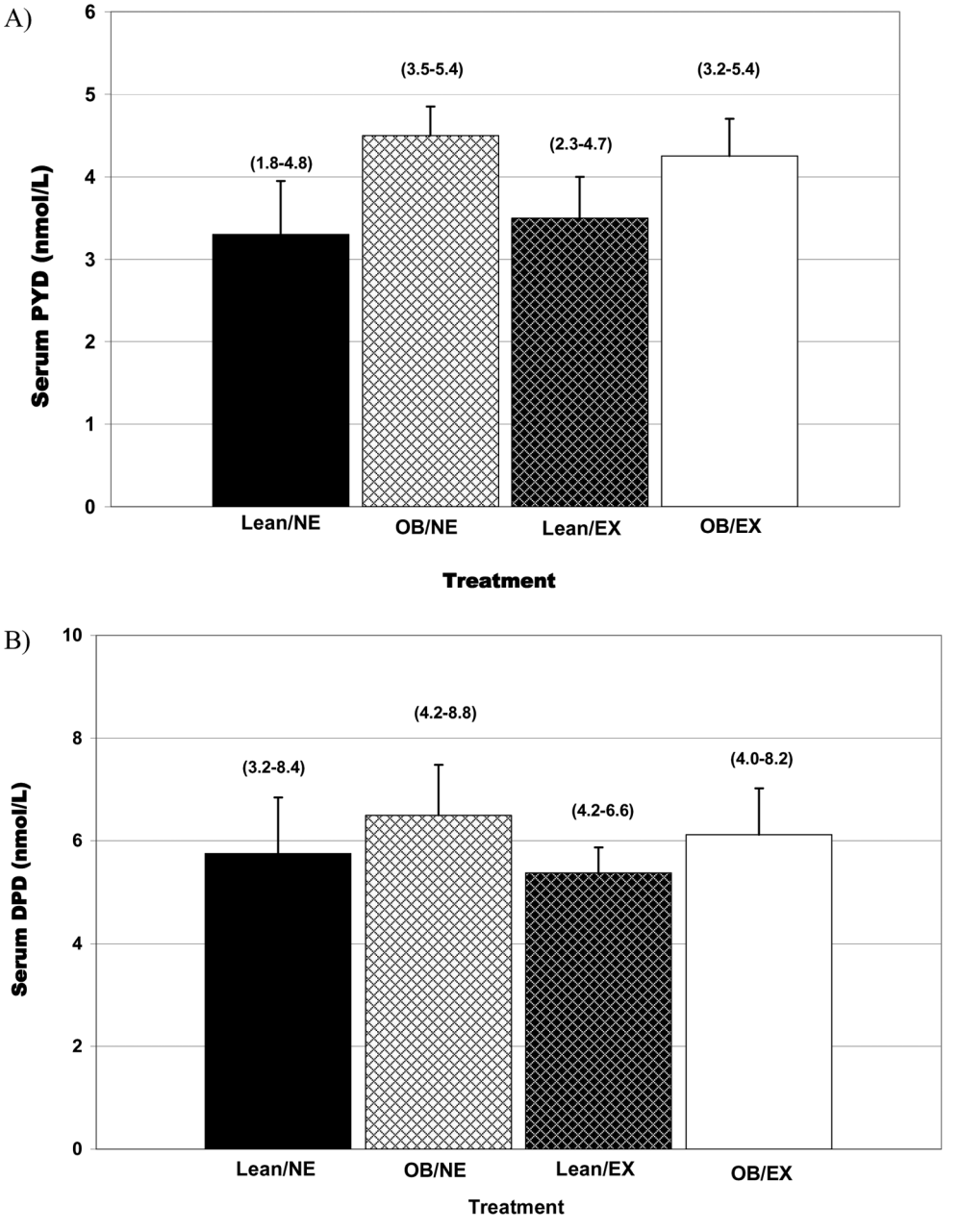

Figure 2. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on indicators of osteoclast activity A) serum pyridinoline (PYD) crosslinks and B) serum deoxypyridinoline (DPD). Values are the mean ±SEM of the absolute values of n=6-7 rats/group. In parentheses are upper and lower 95% confidence intervals.

Discussion

At the end of the 9 wk study, obese male Zucker rats age 16 wks (3.7 mo) were ~37% heavier than lean male Zucker rats. There were no significant differences in femoral or tibial total ash between obese and lean male Zucker rats despite the suggestion that higher mechanical loading provided by heavier body weights increase bone mass. Similarly, Picherit et al.[26] reported no difference in femoral mass in obese compared to lean Zucker rats at age 13 wks (3 mo). In an animal model of diet-induced obesity, rats achieving a fat mass of 19.1% greater than controls, showed no increase in femoral mass[27]. Heavier body weights associated with greater muscle rather than fat mass may be necessary to promote bone mass in young obese rats. A close association has been reported between skeletal muscle and bone development in children and adolescents[28]. Obesity has been suggested to promote higher lean body mass due to the greater muscle force

	Treatment[1]			
Strength Measurements	**LEAN/NE**	**OB/NE**	**LEAN/EX**	**OB/EX**
Femur				
Peak Force (N)	145±30 (76-214)	138±28 (73-203)	129±27 (66-192)	133±25 (77-189)
Bending Failure Energy (N s)	34+4 (25-43)	33+3 (26-40)	34±3 (26-42)	35±4 (25-45)
Ultimate Bending Stress (N/mm^2)	145±27 (83-207)	142±27 (77-207)	140±27 (75-205)	133±22 (82-184)
Young's Modulus (N/mm^2)	1289±199 (829-1749)	1270±252 (674-1866)	1379±260 (765-1993)	1159±182 (738-1580)
Tibia				
Peak Force (N)	66±7 (49-83)	65±9 (45-85)	76±11 (50-102)	56±7 (39-73)
Bending Failure Energy (N s)	24±3 (16-32)	24±3 (16-32)	31±6 (17-45)	23±3 (15-31)
Ultimate Bending Stress (N/mm^2)	183±25 (126-240)	193±30 (123-263)	191±29 (162-220)	164±23 (111-217)
Young's Modulus (N/mm^2)	10345±1846 (6088-14602)	11695±2235 (6411-16979)	10065±1174 (5720-14410)	9700±1838 (5609-13791)

[1] Values are expressed as the mean ±SEM of n=8-9 rats/group. Abbreviations: OB obese, NE non-exercise, EX exercise.

Table 3. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on femur and tibia biomechanical strength measurements.

required to move a heavier body weight[11]. In contrast, our study showed absolute and relative gastrocnemius and soleus muscle weights were significantly lower in obese compared to lean male Zucker rats. In agreement, Mathey et al.[14] also reported lower lean body mass in obese compared to lean male Zucker rats. In the present study, heavier body weight without greater muscle mass in obese male Zucker rats may explain the absence of greater femoral or tibial total ash compared to lean male Zucker rats. In our study, bone size was also reduced in obese compared to lean Zucker rats. Similarly, Foldes et al.[29] reported shorter and lighter femurs. Histomorphometric evaluation showed increase bone resorption in obese Zucker rats age 14 wks compared to their normal littermates. In our study, there was no difference in bone resorption in obese compared to lean male Zucker rats age 16 wks. However, osteoclast activity was determined by measuring serum PYD and DPD rather than by histomorphometry. Adjusting for the differences in bones size in our study showed that despite smaller bone size in obese Zucker male rats, there was no difference in femoral or tibial total ash compared to lean male Zucker rats.

Obesity-related metabolic abnormalities have the potential to affect bone growth through multiple pathways. The phenotype of the obese Zucker rat consists of juvenile onset of extreme obesity accompanied by various obesity-related metabolic abnormalities[30]. In our study, obese male Zucker rats were dyslipidemic, hyperinsulinemic, and hyperglycemic. According to Reid[31], hyperinsulinemia reduces liver production of sex hormone binding globulins which increases circulating sex steroids. In turn, elevated sex steroids promote bone formation by decreased osteoclast activity. In the present study, insulin was elevated, but failed to increase serum testosterone concentrations or to decrease osteoclast activity (i.e. serum PYD and DPD) in obese compared to lean male Zucker rats. High circulating insulin and normal glucose concentrations has been reported to enhance bone; whereas, conditions associated with fasting hyperglycemia negatively impact bone[7]. In our study, hyperinsulinemia was accompanied by hyperglycemia. Osteoblast activity was increased rather than decreased indicated by higher ($P<0.05$) serum osteocalcin concentrations and a tendency ($P=0.049$) for increased serum alkaline phosphatase concentrations in obese compared to lean male Zucker rats. Leptin, a hormone mainly secreted by white adipose tissue has been reported to stimulate osteoblast activity by binding to receptors present on osteoblasts[32]. In our study, elevated serum leptin concentrations may explain the increased osteoblast activity in obese Zucker rats. Despite this increase in osteoblast activity, total bone ash was not increased in obese compared to lean Zucker rats.

Another adverse effect of hyperglycemia is impaired renal injury associated with glycosuria and in turn, hypercalciuria

	Treatment[1]			
Blood Chemistry	**LEAN/NE**	**OB/NE**	**LEAN/EX**	**OB/EX**
Cholesterol (mg/dL)	87±6 (74-100)	154±10[a] (121-187)	99±5[b] (88-110)	142±12[a] (118-166)
Triglycerides (mg/dL)	103±5 (90-116)	260±24[a] (217-303)	92±4[b] (80-104)	230±16[a] (196-264)
Fasting glucose (mg/dL)	110±7 (94-126)	185±9[a] (163-207)	115±7[b] (103-127)	158±9[ab] (118-198)
Insulin (ng/mL)	1.1±0.1 (0.7-1.5)	7.7±0.9[a] (6.0-9.4)	1.3±0.2[b] (0.9-1.7)	7.6±0.7[a] (6.6-8.6)
Leptin (ng/mL)	2.1±0.9 (1.3-2.9)	27.9±7.4[a] (20.1-35.7)	2.4±0.6[b] (1.9-2.9)	25.2±6.2[a] (18.7-31.7)
Testosterone (ng/dL)	137±8 (117-157)	121±6 (113-129)	139±8 (121-157)	122±4 (112-132)
BUN (mg/dL)	13.2±1.8 (8.4-18)	15.1±1.4 (11-19)	12.9±1.0 (11-15)	15.4±1.6 (11-20)
Creatinine (mg/dL)	0.48±0.03 (0.41-0.55)	0.45±0.04 (0.34-0.56)	0.50±0.03 (0.44-0.56)	0.46±0.03 (0.40-0.52)
Total Protein (g/dL)	3.8±0.4 (2.9-4.7)	3.6±0.6 (2.1-5.1)	4.3±0.4 (3.4-5.2)	4.8±0.5 (3.5-6.1)
Albumin (g/dL)	2.8±0.4 (1.8-3.8)	3.0±0.3 (2.3-3.7)	2.9±0.4 (2.0-3.8)	3.0±0.4 (1.9-4.1)
Calcium (mg/dL)	5.0±0.01 (4.5-5.5)	5.0±0.01 (4.5-5.5)	5.8±0.02 (3.9-7.7)	5.6±0.01 (3.9-7.3)
Phosphorus (mg/dL)	7.2±1.0 (4.7-9.7)	7.3±1.4 (6.3-8.3)	7.4±1.3 (4.3-10.5)	8.2±1.0 (5.4-11)

[1] Values are expressed as the mean ±SEM of n=6-7 rats/group. In parentheses are upper and lower 95% confidence intervals. Superscript a=$P<0.05$ vs Lean/NE, b=$P<0.05$ vs OB/NE. Fisher's t-test was used to evaluate differences among the means of the treatment groups. An alpha level of 0.05 or $P<0.05$ was used to determine statistically significant differences. Abbreviations: OB obese, NE non-exercise, EX exercise. BUN blood urea nitrogen.

Table 4. The effect of body weight and moderate treadmill running of young obese and lean male Zucker rats on blood chemistry and hormones.

leading to bone mineral loss. In the present study, there was no significant difference in serum biomarkers of kidney function consisting of: BUN, creatinine, total protein, albumin or Ca concentrations between obese and lean male Zucker rats. No significant difference were observed in the total bone ash or Ca content of the long bones of obese compared to lean male Zucker rats. Even after adjusting for shorter femur length and tibia width, and weight, we found no differences in femoral or tibial total ash between obese and lean male Zucker rats. The results indicated that bone ash was not affected although bones size was decreased in obese male Zucker rats. Reduced bone size accompanied by equivalent bone ash may be due to leptin affecting endocrine systems such as the growth hormone /insulin-like growth factor pathway[33]. However, growth hormone and insulin-like factors were not measured in this study. Tamasi et al.[34] reported decreased structural parameters, but no differences in BMD between obese and the lean female Zucker rats. Histomorphometric evaluation showed no effect on osteoblast activity, but osteoclast surface was increased in obese compared to lean Zucker rats age 15 wks. In our study, there was no effect on osteoclast activity (serum DPD, PYD) and osteoblast activity (serum osteocalcin) was increased. Whether obese female Zucker rats in the Tamasi et al study[34] showed similar metabolic disorders to obese male Zucker rats in our study could not be determined since leptin and serum glucose concentrations were not measured.

In addition to investigating the effect of body weight on bone, another study objective was to investigate the effect of exercise on bone. Dynamic rather than static (i.e. body mass) loads have been suggested to provide the appropriate mechanical loading required to promote bone formation[35,36]. Treadmill running was used because this type of exercise has been demonstrated to promote fat loss and to provide mechanical load on the long bones[18]. An exercise regimen of moderate intensity and frequency was selected because most

adolescents fail to achieve 60 min/d of daily vigorous intensity physical activity[37]. According to Hagihara et al.[22] higher bone mass in rats are obtainable by a moderate treadmill running at an intensity of 15 m/min for 1 h, frequency of 5 d/wk, and a duration of 8 wks. In our study, treadmill running consisting of an intensity of 20 m/min for ~1 h, frequency of 5 d/week, and duration of 9 wks successfully reduced the body weight of obese Zucker rats by 7.5%. The reduction of weight-bearing load associated with weight loss did not negatively affect total bone ash or Ca content in the long bones. In fact, Shiga et al.[38] reported that exercise not only induced weight loss, but increased femoral and tibial weight and Ca content compared to non-exercised animals.

Mathey et al.[14] reported that using a treadmill exercise regimen that reduced fat mass while increasing lean mass without significant changes in total body weight resulted in higher BMD determined by dual energy X-ray absorptiometry in obese as well as lean Zucker male rats. The results suggested that an exercise regimen associated with muscle gain during the growth stage is necessary for bone protective effects. In our study, moderate treadmill running of lean male Zucker rats increased relative gastrocnemius muscle mass. However, there was no increase in soleus muscle mass or bone mass. On the other hand, moderate treadmill running of obese male Zucker rats successfully reduced body weight without increasing muscle mass. Lack of significant increases in lean body mass may explain the absence of increased total bone ash in our treadmill exercised rats.

In addition to increasing bone mass, treadmill exercise of growing rats has also been reported to enhance bone growth[18,39]. Mathey et al.[14] reported that treadmill running of obese male Zucker rats increased femoral length and decreased urinary DPD excretion. However, in our study, moderate treadmill exercise of obese male Zucker rats failed to stimulate femur longitudinal growth or to affect bone turnover indicated by absence of changes in serum DPD, PYD or osteocalcin. An exercise regimen of longer duration and greater intensity may be necessary to attenuate decreased bone size in obese male Zucker rats. In the Mathey et al.[14] study, improvements in bone size occurred with treadmill running frequency of 7 d/week for the duration of ~13 wks compared to our study with a frequency of 5 d/wk for the duration of 9 wks. Exercise also benefits bone health by improving obesity-related metabolic abnormalities. In our study, moderate treadmill running improved fasting serum glucose concentration in obese Zucker rats, although circulating serum glucose did not reach normal values. At the end of our study, rats were age 16 wks compared to the Mathey et al. study[14] where rats were age 26 wks. Age difference is important to consider because obese Zucker rats exhibit a range of metabolic aberrations that progressively worsens with age[40]. For example at ~18 wks and older, obese Zucker rats spontaneously develop renal damage and this in turn, may affect bone health[41].

In our study, moderate treadmill exercise of young obese male Zucker rats had no effect on bone size or total bone ash. However, Huang et al.[42] reported that moderate endurance training benefited bone by strengthening tissue properties without increasing size and/or bone mineral accumulation. Therefore, we measured bone strength. Moderate treadmill exercise had no effect on femoral strength measurements of stiffness, strength or ability to absorb energy elastically indicated by the absence of significant differences in strength measures of: peak force, bending failure energy, ultimate bending stress or Young's modulus in either obese or lean male Zucker rats. However, there was large variability in the bone strength measurements. Biomechanical tests produce large variability due to limited precision, relatively small responses, and slow response times. According to Leppanen et al.[23], studies measuring bone strength measurements, particularly stiffness and energy absorption, are typically underpowered. Therefore, increasing the sample size may have enabled detection of small bone responses to exercise or body weight. In human studies, Leonard et al.[11] reported that higher weight in adolescents resulted in higher BMD but did not examine bone fracture risk. Goulding et al.[9] reported high body weight decreased BMC and increased risk of forearm fractures during childhood and adolescence. Differences in study results may be due to body composition. Petit et al.[43] reported that overweight children had wider and stronger bones at the proximal femur appropriate for their higher lean mass and height. However, body weight in the form of fat mass did not contribute to bone strength. Therefore, in our study, failure of exercise to promote greater bone mass and strength in obese Zucker rats may be due to the absence of significant muscle gain during moderate treadmill running.

In summary, obesity during the bone growth stage resulted in equivalent bone ash, but bone size was reduced compared to their lean counterparts. Moderate treadmill running improved hyperglycemia and successfully reduced body weight without negative effects on bone. However, moderate treadmill running failed to attenuate decreased bone growth in obese Zucker rats or to promote greater bone mass and strength. During the bone formation stage, an exercise regimen resolves obesity-related metabolic abnormalities and/or promotes muscle gain appears necessary in order to stimulate bone growth and mass. This suggests that including some other resistance training with treadmill running may be required. The study results contribute knowledge towards defining physical activity for obese individuals that will reduce body weight, improve health while maximizing bone mass during the bone formation stage.

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