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Effects of Chronic Heavy Alcohol Consumption and Endurance Exercise on Cancellous and Cortical Bone Microarchitecture in Adult Male Rats

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Abstract

Background—Bone health is influenced by numerous lifestyle factors, including diet and exercise. Alcohol is a major non-essential constituent of diet and has dose and context-dependent effects on bone. Endurance exercise is associated with increased risk for stress fractures. The purpose of this study was to determine the long-term independent and combined effects of chronic heavy alcohol consumption and endurance exercise (treadmill running) on bone mass and microarchitecture in young adult male Sprague-Dawley rats.

Methods—Six-month-old male rats were randomized into 4 groups (9–13 rats/group): sedentary +control diet, sedentary+ethanol diet, exercise+control diet, or exercise+ethanol diet. Ethanol-fed rats consumed a liquid diet (ethanol comprised 35% of caloric intake) *ad libitum*. Control rats were pair-fed the same diet with isocaloric substitution of ethanol with maltose-dextran. Exercise was conducted on a motorized treadmill (15% grade for 30 min) 5 days/week for 16 weeks. Femur and 12th thoracic vertebra were analyzed for bone mineral content (BMC) and density (BMD) using densitometry and cortical and cancellous bone architecture using microcomputed tomography.

Results—Ethanol consumption resulted in lower femur length, BMC, and BMD, and lower midshaft femur cortical volume, cortical thickness, and polar moment of inertia. In addition, trabecular thickness was lower in vertebra of ethanol-fed rats. Endurance exercise had no independent effect on any endpoints evaluated. A significant interaction between endurance exercise and ethanol was detected for several cancellous endpoints in the distal femur metaphysis. Ethanol-consuming rats that exercised had lower distal femur metaphysis bone volume/tissue

volume, trabecular connectivity density, and trabecular thickness compared to exercising rats that consumed control diet.

Conclusions—The results obtained in this model suggest that chronic heavy alcohol consumption may reduce skeletal integrity by reducing bone size, mass, and density, and by negatively altering cancellous bone microarchitecture and may increase fracture risk associated with endurance exercise at weight bearing skeletal sites.

Keywords

Ethanol; wheel running; rat model; microcomputed tomography; densitometry

Introduction

Alcohol is a commonly consumed, non-essential constituent of diet, and is relevant to skeletal health because of its high caloric density and bioactive properties (Nielsen et al., 2012). Even modest quantities of alcohol have pronounced rapid effects on bone metabolism in humans and animal models (Turner et al., 1998, Turner et al., 2001, Marrone et al., 2012). Moderate alcohol consumption, defined as 1 standard drink (14 g ethanol)/d in women and 2 standard drinks/d in men (U.S. Department of Agriculture and U.S. Department of Health and Human Services), has been shown to reduce biomarkers of bone turnover and is associated with higher bone mineral density (BMD) in some populations (Marrone et al., 2012, Berg et al., 2008, Holbrook and Barrett-Connor, 1993). In contrast, heavy alcohol consumption, defined as > 1 standard drink/d in women and > 2 standard drinks/d in men (U.S. Department of Agriculture and U.S. Department of Health and Human Services), especially when combined with additional detrimental lifestyle factors such as smoking and poor diet, elicits pathological responses in bone, including reduced osteoblast number and function (Giuliani et al., 1999, Vignesh et al., 2006), disrupted endocrine signaling (Turner et al., 2010, Turner et al., 2012), and decreased BMD (Chappard et al., 1991, de Vernejoul et al., 1983).

Bone is influenced by the loading environment. Mechanical loads experienced by the skeleton during normal daily activities are vital for maintaining skeletal health (Wakley et al., 1992). Weight-bearing activities (exercise) over and above the activity associated with normal daily living subjects the skeleton to increased mechanical loads and can have variable effects on bone, depending on the type, intensity, and duration of exercise (Beyer et al., 1985, Frost, 1988, Wheeler et al., 1995). Under some conditions, exercise elicits a net increase in bone mass (Armas and Recker, 2012). For example, high impact skeletal loading in prepubertal boys and girls results in local increases in BMD (Fuchs et al., 2001). However, endurance exercise, including running, generally does not increase BMD. In fact, long-distance runners often exhibit osteopenia (Scofield and Hecht, 2012, Barrack et al., 2011), a well-established risk factor for fracture (Pasco et al., 2013).

Although the maximum mechanical loads experienced by the skeleton during running are generally insufficient to induce beneficial skeletal adaptation, the many repetitive cycles of skeletal loading during endurance exercise can induce fatigue damage (microdamage) to bone. Under normal circumstances microdamage is repaired when bone is remodeled;

however, an increased rate of accumulation of microdamage due to endurance exercise may outpace the ability of bone to fully repair the damage, increasing the risk for stress fractures (Bourrin et al., 1994, Burr et al., 1998, Carter and Hayes, 1977). Stress fractures of the lower extremities rarely occur in healthy young adults in the absence of endurance training but are frequently reported among athletes (Bennell et al., 1996a, Bennell et al., 1996b) and military personnel (Mattila et al., 2007, Niva et al., 2005, Kiuru et al., 2005). Lifestyle factors that independently alter bone mass, bone architecture, or rate of bone remodeling could modify the fracture risk associated with endurance exercise.

Heavy alcohol consumption is a lifestyle factor that negatively impacts bone health by reducing BMD and suppressing bone remodeling. Heavy drinking is most common among young adult males, many of whom are also physically active (Nelson and Wechsler, 2001, Yusko et al., 2008, Bracken, 2012). In particular, college student athletes and military personnel consistently report high rates of alcohol intake (Yusko et al., 2008, Bracken, 2012). Chronic heavy alcohol consumption in these populations might be expected to increase fracture risk associated with endurance exercise by inducing bone loss and/or lowering peak bone mass.

To our knowledge, there have been no human studies and only two animal studies evaluating the combined effects of heavy alcohol consumption and endurance exercise on the skeleton. We previously reported that chronic heavy alcohol consumption in young adult male rats suppressed bone formation and altered cancellous and cortical bone architecture, whereas endurance exercise (treadmill running) had no independent or interactive effect on bone (Reed et al., 2002). In contrast, Maurel et al. reported that endurance exercise (also treadmill running) attenuated alcohol-induced osteopenia in rapidly growing male rats (Maurel et al., 2013).

An improved understanding of the potential interaction between alcohol consumption and exercise on bone is important because endurance exercise and heavy alcohol consumption are common in young adult males. Thus, the purpose of this report is to extend our previously published analysis, limited to histomorphometric evaluation of tibia (Reed et al., 2002) to include analysis of additional bone specimens (archived femur and vertebra) using dual-energy X-ray absorptiometry (DXA) and microcomputed tomography (μ CT), technologies previously unavailable to us, for a more complete evaluation of the effects of endurance exercise and heavy alcohol consumption on bone mass, density, and microarchitecture in young adult male rats.

Materials and Methods

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) approaching skeletal maturity (Martin et al., 2003) were used in the experiment. The rats were housed individually in a temperature- and humidity-controlled room on a 12-hour light-dark cycle and maintained in accordance with the National Institutes of Health Guide for the Care and the Use of Laboratory Animals. All procedures were approved by the Institutional Animal Care and Use Committee.

Experimental Protocol

The experimental protocol is described in detail elsewhere (Reed et al., 2002). In brief, 6-month-old male Sprague-Dawley rats were randomized by weight into 4 treatment groups (9 – 13 rats/group) defined by 2 binary factors (exercise and diet); 1) sedentary + control diet, 2) sedentary + ethanol diet, 3) exercise + control diet, or 4) exercise + ethanol diet. To accommodate potential noncompliance with the exercise protocol, initial group size was higher (n=13/group) in the exercise groups compared to the sedentary groups (n=10/group). The rats were adapted to a Bio-Serv Liquid Rat Diet LD82 (Bio-Serv, Frenchtown, NJ) over a one-week period during which the dietary concentration of ethanol was increased from 0% to 35%. Chronic consumption of this diet was shown to induce pathologies common in chronic heavy alcohol consumers (Lieber and DeCarli, 1982). Control diet was an isocaloric liquid diet with maltose-dextran substituted for ethanol. Exercise was performed on a motorized 20-lane treadmill. The rats were acclimated to the treadmill to reach a final intensity level of 25 m/min at 15% grade for 30 min/day 5 days/week. Sedentary rats were placed on a stationary treadmill for matching time periods. Control rats were pair-fed to their respective ethanol-fed groups for the 16-week duration of the experiment. For tissue collection, animals were subjected to CO₂ inhalation followed by cervical dislocation. Femora and 12th thoracic vertebrae were excised and stored in 70% ethanol solution and used for DXA and μ CT analysis.

Dual Energy X-ray Absorptiometry

Femoral bone mass, measured as bone mineral content (BMC) (mg), and bone area (cm²) were determined using DXA (Piximus, Lunar Corp., Madison, WI, USA). BMD was calculated as BMC/area (mg/cm²).

Microcomputed Tomography

μ CT was used for nondestructive three-dimensional evaluation of bone microarchitecture. Femora (midshaft and distal femur) and 12th thoracic vertebrae were scanned using a Scanco μ CT40 scanner (Scanco Medical AG, Basserdorf, Switzerland) at a voxel size of 16 μ m $\times$ 16 μ m $\times$ 16 μ m. The threshold value for evaluation was determined empirically and set at 245 (gray scale, 0–1000). For the midshaft femoral diaphysis, 20 slices (320 μ m) of bone were evaluated and total cross-sectional volume (cortical and marrow volume, mm³), cortical volume (mm³), marrow volume (mm³), and cortical thickness (μ m) were measured. Polar moment of inertia (I_{Polar}), a surrogate measure of bone strength in torsion, was determined. Cortical bone porosity was calculated as the difference between cortical bone volume at thresholds of 0 and 245. For the distal femur metaphysis, 75 slices (1,200 μ m) of cancellous bone were measured. Analysis of cancellous bone in the thoracic vertebra included the entire region of secondary spongiosa between the cranial and caudal growth plates (170 ± 3 slices or $2,720 \pm 48$ μ m, mean $\pm$ standard error [SE]). Direct cancellous bone measurements in the femur metaphysis and thoracic vertebra included cancellous bone volume fraction (cancellous bone volume/tissue volume or volume of total tissue occupied by cancellous bone, %), trabecular connectivity density (number of redundant connections per unit volume, mm⁻³), trabecular thickness (mean thickness of individual trabeculae, μ m),

trabecular number (number of trabecular intersects, mm^{-1}), and trabecular spacing (distance between trabeculae, μm). The regions of interest evaluated are shown in Figure 1.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to evaluate the respective effects of endurance exercise, ethanol, and their interaction on bone mass and microarchitecture. When a statistically significant interaction was identified, results from Welch's two-sample t-test were obtained separately for sedentary and exercise groups. Otherwise, the main effects of endurance exercise and ethanol were tested using F-tests from the two-way ANOVA main effects model. ANOVA model assumptions were assessed using normal quantile plots of residuals, the Anderson-Darling test of normality, and plots of residuals versus fitted values. Data analysis was conducted using R version 2.12 (R Development Core Team, 2010). Differences were considered significant at $p < 0.05$. Data are reported as mean $\pm$ SE and with 95% confidence intervals (CIs).

Results

The effects of endurance exercise and ethanol consumption on body weight, muscle mass, and cancellous and cortical bone formation in the tibia have been reported elsewhere (Reed et al., 2002). In brief, pair-feeding of control rats to ethanol-consuming rats equalized body weights between control and ethanol-fed rats in both the sedentary and the exercise groups. The average blood alcohol concentration over the 16-week study was not influenced by endurance exercise; blood alcohol level measured 90 ± 5 mg/dl in the sedentary rats and 91 ± 6 mg/dl in the exercise rats. Endurance exercise prevented weight gain but had no effect on skeletal muscle mass, bone area, or bone formation. In contrast, ethanol consumption resulted in decreased cortical (tibial diaphysis) and cancellous (proximal tibial metaphysis) bone formation. Significant interactions between endurance exercise and ethanol consumption were not detected for any tibial endpoints evaluated.

The effects of endurance exercise and ethanol consumption on femur length, bone area, BMC, and BMD are shown in Table 1. Sixteen weeks of endurance exercise had no independent effect on femur length, bone area, BMC, or BMD. In contrast, ethanol consumption resulted in lower femur length and lower femoral BMC and BMD. Controlling for exercise, the estimated mean femur length in the ethanol group was 0.6 mm (95% CI: 0.03 mm, 1.2 mm) lower than the mean for the control group, while BMC and BMD were 0.05 mg (95% CI: 0.02 mg, 0.08 mg) and 0.01 mg/cm^2 (95% CI: 0.006 mg/cm^2 , 0.02 mg/cm^2) lower than the means for the control groups, respectively. Significant interactions between endurance exercise and ethanol consumption were not detected for any endpoints evaluated.

The effects of endurance exercise and ethanol consumption on cortical bone microarchitecture in femur mid-diaphysis are shown in Table 2. An independent effect of endurance exercise was not detected for any of the cortical endpoints measured. In contrast, ethanol consumption resulted in lower midshaft femur cortical volume, cortical thickness, and I_{polar} . Controlling for exercise, the estimated mean cortical volume was 0.3 mm^3 (95% CI: 0.2 mm^3 , 0.4 mm^3) lower, cortical thickness was 81 μm (95% CI: 46 μm , 117 μm)

lower, and I_{Polar} was 3.2 mm^4 (95% CI: 0.9 mm^4 , 5.4 mm^4) lower in the ethanol group than in the control group. Significant interactions between endurance exercise and ethanol consumption were not detected for any of the cortical endpoints evaluated.

The effects of endurance exercise and ethanol consumption on cancellous bone microarchitecture in distal femur metaphysis are shown in Figure 2. There was an interaction between endurance exercise and ethanol in the distal femur metaphysis for bone volume/tissue volume, trabecular connectivity density, and trabecular thickness. In the exercise group, ethanol-consuming rats had lower bone volume/tissue volume (Welch's t-test $p=0.002$), trabecular connectivity density (Welch's t-test $p=0.03$), and trabecular thickness (Welch's t-test $p<0.0001$) compared to rats consuming control diet. More specifically, average bone volume/tissue volume was 2.9% (95% CI: 1.2%, 4.6%) lower, average trabecular connectivity density was 5.8 mm^{-3} (95% CI: 0.6 mm^{-3} , 10.9 mm^{-3}) lower, and average trabecular thickness was $11 \mu\text{m}$ (95% CI: $7 \mu\text{m}$, $16 \mu\text{m}$) lower in the ethanol group than in the control group. In contrast, significant differences in cancellous bone volume/tissue volume, trabecular connectivity density, and trabecular thickness were not detected between control and ethanol-consuming rats in the sedentary group.

The effects of endurance exercise and ethanol consumption on cancellous bone microarchitecture in thoracic vertebra are shown in Table 3. Endurance exercise had no independent effect on cancellous bone volume/tissue volume, trabecular connectivity density, trabecular number, trabecular thickness, or trabecular spacing in the thoracic vertebra. In contrast, ethanol consumption resulted in higher trabecular connectivity density and lower trabecular thickness. The estimated mean trabecular connectivity density in the ethanol group was 5.6 mm^{-3} (95% CI: 1.0 mm^{-3} , 10.1 mm^{-3}) higher than the mean for the control group, while the estimated mean for trabecular thickness was $8 \mu\text{m}$ (95% CI: $4 \mu\text{m}$, $11 \mu\text{m}$) lower in the ethanol group than the control group. There was no significant interaction between endurance exercise and ethanol consumption for cancellous bone in thoracic vertebra.

Discussion

We investigated the independent and combined effects of chronic heavy alcohol consumption and endurance exercise (treadmill running) on cortical and cancellous bone mass, density, and microarchitecture. Alcohol provided 35% of total energy intake, analogous to the distribution observed with chronic heavy alcohol consumption in humans (Maurel et al., 2012). The running protocol used in this animal study modeled levels of endurance exercise that could reasonably be achieved by healthy young adults. Whereas endurance exercise had no independent effects on bone parameters, alcohol consumption led to osteopenia and abnormalities in cortical and cancellous bone microarchitecture. Endurance exercise, in the setting of chronic heavy alcohol consumption, exacerbated detrimental effects of alcohol on cancellous bone microarchitecture in distal femur.

Chronic heavy alcohol consumption is a lifestyle factor known to have detrimental effects on bone health (de Vernejoul et al., 1983) and, as a consequence, may increase fracture risk associated with endurance exercise. However, the combined effects of chronic heavy alcohol

consumption and endurance exercise on bone health have not been studied in humans. Long-duration (16 week) heavy alcohol consumption (35% calories) was previously shown to result in reductions in periosteal bone formation, cortical area, and cross sectional area in tibia in young adult male rats (Reed et al., 2002, Sibonga et al., 2007). DXA and μ CT used in the present analysis provide a more complete assessment of the effects of alcohol on bone in this animal model. Our results indicate that chronic heavy alcohol consumption, by lowering BMC, BMD, cortical thickness, and I_{Polar} , has detrimental skeletal effects that would contribute to the reduction in bone mechanical and material properties previously reported in alcohol-fed rats (Hogan et al., 1999, Nyquist et al., 2002).

The present analysis also extends earlier findings by evaluating the respective effects of endurance exercise and heavy alcohol consumption on cancellous bone microarchitecture in distal femur (high weight bearing site) and thoracic vertebra (low weight bearing site). Chronic heavy alcohol consumption resulted in thinner trabeculae in both the distal femur metaphysis and vertebral body, which corroborates earlier findings of decreased trabecular thickness in the proximal tibia metaphysis of alcohol-fed rats (Reed et al., 2002). Since decreased thickness confers reduced strength, it would be anticipated to preferentially increase fracture risk at weight-bearing skeletal sites (McColl et al., 2006).

Alcohol reduces acquisition of bone in growing rats, at least in part, by antagonizing growth hormone signaling (Turner et al., 2010, Turner et al., 2012) and, as a consequence, can result in reduced peak bone mass (Maddalozzo et al., 2009). There have been relatively few studies investigating the effects of heavy alcohol consumption in skeletally mature rats and, to our knowledge, no prior studies utilizing μ CT to evaluate bone microarchitecture. However, histomorphometry studies performed in male and female skeletally mature rats have consistently reported cancellous bone loss following long duration (>3 months) heavy alcohol consumption (35% caloric intake) (Sibonga et al., 2007, Reed et al., 2002, Turner et al., 2001, Sampson, 1998).

Maurel and colleagues (Maurel et al., 2013) reported that treadmill running reduced the detrimental effect of alcohol administered in drinking water on cortical and cancellous bone microarchitecture in rapidly growing male rats (Maurel et al., 2013). The exercise protocol used in their study had detrimental effects on several important structural and biomechanical parameters, including femur BMC, cross sectional bone area, energy to failure, and I_{Polar} (Maurel et al., 2013). The findings suggest that endurance exercise reduces the magnitude of the detrimental skeletal response to alcohol in growing rats. However, the findings do not support endurance exercise as a viable countermeasure to prevent alcohol-induced bone loss since endurance exercise is strongly associated with increased stress fracture risk.

In the present study, endurance exercise had no independent effect on femur mass, density, or cortical architecture and did not reduce the detrimental effects of heavy alcohol consumption on these parameters. In fact, endurance exercise exacerbated the detrimental effects of chronic heavy alcohol consumption on cancellous bone microarchitecture in distal femur, a common site for stress fractures in military personnel, as evidenced by decreased cancellous bone volume fraction, trabecular connectivity density, and trabecular thickness. Cancellous bone volume fraction represents the volume of bone tissue per volume of total

tissue and is an important index of cancellous bone mass. Trabecular connectivity density (a measure of the interconnectedness of the trabecular network) and trabecular thickness are important indices of bone microarchitecture integrity (McColl et al., 2006). Whereas greater trabecular connectivity indicates greater stability and fracture-resistance, lower connectivity suggests flaws in the quality of cancellous bone microarchitecture (Trevisiol et al., 2007, Tai et al., 2008). The present results suggest that chronic heavy alcohol consumption could increase fracture risk by reducing bone mass and bone quality while endurance exercise may potentiate this risk by increasing fatigue damage. Our prior analysis suggests that chronic heavy alcohol consumption has the potential to further increase fracture risk associated with endurance exercise by antagonizing bone remodeling (Reed et al., 2002), the process that repairs bone microdamage.

There were several important differences between Maurel et al. (2013) and the present study. Maurel et al. used younger animals and delivered alcohol in drinking water. Delivery of alcohol in drinking water resulted in 1) reduced water intake and decreased micronutrient intake in the alcohol-fed rats, as well as 2) large differences in body weight among treatment groups. Thus, it is difficult to differentiate the specific effects of alcohol on bone from those associated with dehydration, micronutrient availability, and/or large body weight differences (Maddalozzo et al., 2009, Piano et al., 2001, Leichter and Lee, 1982, Turner, 2012). In contrast, in the present study alcohol was administered as a component of a liquid diet. Pair-feeding control rats using an isocaloric liquid diet equalized water and micronutrient intake among groups. Exercised animals were lighter than sedentary animals at the end of the 16-week-long study, likely due to increased energy expenditure associated with physical activity, but there were no differences in weight between alcohol-consuming and control rats within the sedentary or exercise groups.

The present study has several limitations. The lack of an independent effect of endurance exercise on bone parameters observed in our young adult rats may not be applicable to growing rats or to forms of exercise that impose greater mechanical loads on the skeleton. Furthermore, the present study was performed in male rats. There is evidence suggesting gender differences in the skeletal response to alcohol consumption (Malik et al., 2009). Although previously a large gender gap existed in alcohol consumption among young adults in the US (with males drinking earlier, heavier, and at higher rates than females), evidence suggests drinking rates and consumption patterns are changing among females, closing this gap (Keyes et al., 2008). It would therefore be valuable to investigate the possibility of interactions between alcohol and endurance exercise in females in an animal model. Finally, the endurance exercise protocol used in this study does not induce stress fractures in rats. In spite of this notable limitation, bone metabolism in rats shares many similarities with bone metabolism in humans. In this regard, the rat has been invaluable as a preclinical model to evaluate the skeletal responses to exercise and alcohol consumption (Iwaniec and Turner, 2008).

In summary, this study reports the independent and combined effects of endurance exercise and chronic heavy alcohol consumption in male rats approaching skeletal maturity. Whereas endurance exercise had no independent effect on parameters of bone health, alcohol induced osteopenia and other pathological perturbations, including decreased cortical bone size,

mass, and density, and altered cancellous bone microarchitecture. A significant interaction between endurance exercise and alcohol was detected in distal femur: in particular, endurance exercise exacerbated the negative effects of alcohol on cancellous bone microarchitecture at this site. Taken together, these data suggest that fracture risk associated with endurance exercise may be increased by chronic heavy alcohol consumption.

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Figure 1. Regions of interest evaluated in femur and thoracic vertebra using microcomputed tomography.

Figure 2.

Effects of long-duration (16 weeks) endurance exercise (treadmill running; 25 m/min at 15% grade for 30 min/day 5 days/week) and ethanol consumption (35% caloric intake) on distal femur metaphysis cancellous bone volume/tissue volume (A), connectivity density (B), trabecular number (C), trabecular thickness (D), and trabecular spacing (E). Data are mean $\pm$ SE. ^aDifferent from control within treatment, $P < 0.05$.

Table 1

Effects of long-duration (16 weeks) endurance exercise (treadmill running; 25 m/min at 15% grade for 30 min/day 5 days/week) and ethanol consumption (35% caloric intake) on femur length, bone area, bone mineral content (BMC) and bone mineral density (BMD).

	Sedentary		Exercise		ANOVA, P =		
	Control	Ethanol	Control	Ethanol	Exercise	Ethanol	Interaction
Total Femur							
Length (mm)	41.3 ± 0.2	40.7 ± 0.4	41.0 ± 0.3	40.3 ± 0.3	0.34	0.04	0.87
Bone area (cm ²)	3.0 ± 0.0	2.9 ± 0.0	3.0 ± 0.0	2.9 ± 0.1	0.59	0.10	0.28
Bone mineral content (mg)	0.584 ± 0.008	0.546 ± 0.017	0.589 ± 0.015	0.528 ± 0.013	0.97	0.001	0.42
Bone mineral density (mg/cm ²)	0.197 ± 0.004	0.185 ± 0.004	0.197 ± 0.003	0.184 ± 0.002	0.78	0.0002	0.86

Data are mean ± SE

Effects of long-duration (16 weeks) endurance exercise (treadmill running; 25 m/min at 15% grade for 30 min/day 5 days/week) and ethanol consumption (35% caloric intake) on cortical bone microarchitecture in femur midshaft diaphysis.

Table 2

	Sedentary		Exercise		ANOVA, P =		
	Control	Ethanol	Control	Ethanol	Exercise	Ethanol	Interaction
Femur Midshaft Diaphysis (cortical bone)							
Cross-sectional volume (mm ³)	4.63 ± 0.07	4.46 ± 0.09	4.65 ± 0.10	4.46 ± 0.12	0.75	0.09	0.92
Cortical volume (mm ³)	2.71 ± 0.06	2.46 ± 0.08	2.76 ± 0.08	2.40 ± 0.06	0.73	0.0001	0.46
Marrow volume (mm ³)	1.92 ± 0.06	2.00 ± 0.06	1.90 ± 0.07	2.06 ± 0.09	0.92	0.08	0.59
Cortical thickness (µm)	735 ± 17	669 ± 19	748 ± 19	653 ± 11	0.67	0.0001	0.42
Polar moment of inertia (mm ⁴)	27.7 ± 0.8	24.8 ± 1.1	28.1 ± 1.2	24.7 ± 1.0	0.66	0.007	0.81
Cortical porosity (%)	2.3 ± 0.3	2.1 ± 0.2	2.3 ± 0.2	2.1 ± 0.1	0.71	0.36	0.97

Data are mean ± SE

Table 3

Effects of long-duration (16 weeks) endurance exercise (treadmill running; 25 m/min at 15% grade for 30 min/day 5 days/week) and ethanol consumption (35% caloric intake) on cancellous bone microarchitecture in thoracic vertebra.

	Sedentary		Exercise		ANOVA, P =		
	Control	Ethanol	Control	Ethanol	Exercise	Ethanol	Interaction
Thoracic Vertebra (cancellous bone)							
Bone volume/Tissue volume (%)	26.5 ± 1.7	26.3 ± 1.4	26.9 ± 1.4	25.3 ± 1.1	0.91	0.49	0.61
Connectivity density (mm ⁻³)	33.1 ± 1.9	39.7 ± 3.1	35.6 ± 1.4	40.3 ± 2.7	0.59	0.02	0.68
Trabecular number (mm ⁻¹)	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	0.75	0.76	0.88
Trabecular thickness (µm)	93 ± 2	85 ± 2	91 ± 1	85 ± 2	0.73	0.0002	0.64
Trabecular spacing (µm)	315 ± 14	308 ± 14	317 ± 13	313 ± 12	0.78	0.69	0.93

Data are mean ± SE