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Increased Nitric Oxide-Mediated Vasodilation of Bone Resistance Arteries Is Associated With Increased Trabecular Bone Volume After Endurance Training In Rats

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Abstract

Old age-associated osteoporosis is related to diminished bone blood flow and impaired nitric oxide (NO)-mediated vasodilation of the bone vasculature. Endurance exercise training restores the age-associated reduction of vasodilation in numerous vascular beds, as well as improving bone properties. The purpose of this study was to determine whether functional improvements in the bone vasculature are associated with increased bone properties after an endurance training intervention. Young adult (4–6mo) and old (24–26 mo) male Fischer-344 rats remained sedentary or were trained (15 m/min walking, 15° incline, 5 days/wk, 10–12 wk). Endothelium-dependent vasodilation of the femoral principal nutrient artery (PNA) was assessed *in vitro* using acetylcholine (ACh) and inhibitors of NO synthase (NOS) and cyclooxygenase (COX). PNA endothelium-dependent vasodilation was greater after training by 16% in young and by 24% in old animals. The NOS-mediated contribution to endothelium-dependent vasodilation was enhanced by 77% after training in old rats. Distal femur trabecular bone volume (BV/TV, %) was lower with old age in sedentary animals (young: 27±2%, old: 23±1%; P<0.05). Exercise-induced elevations in bone and marrow blood flow and the NOS signaling pathway were associated with greater BV/TV (young trained: 34±2%, old trained: 26±1%; P<0.05) relative to sedentary groups. These data demonstrate that training-induced increases in bone properties are associated with enhanced endothelium-dependent vasodilation through a NOS signaling pathway in the bone vasculature.

Keywords

Blood Flow; Nitric Oxide; Vasculature; Exercise; Aging

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INTRODUCTION

The prevailing paradigm for skeletal physiology maintains that the magnitude of the osteogenic stimulus is dictated by the magnitude of strain or deformation experienced at a particular bone region [1,2]. Since muscle forces exert the greatest strains on bone [3], strong muscles should result in stronger bones [2]. Nevertheless, low-intensity endurance-type exercise training enhances bone properties [4-6] despite eliciting relatively low strain magnitudes [1,7-9] [10], as well as reducing body and leg muscle mass [6,11]. One mechanism through which endurance training may influence bone cell activity is via the bone vasculature. For instance, systemic inhibition of blood vessel proliferation completely abolished the training-induced enhancement of tibial bone density and trabecular bone volume [12]. This inhibition also reversed the characteristic training-associated decrease in osteoclastic resorption. A similar vascular mechanism has been proposed to contribute to bone loss [13-17], such as the diminished endothelium-dependent vasodilation and nitric oxide (NO) bioavailability in the bone vasculature of old osteopenic rats [18].

The bone vasculature is proposed to directly modulate bone cell activity [19-22]. For example, an increase in vascular pressures increases regional bone properties independent of mechanical loading. This phenomenon is observable after venous occlusion [23-25], hyperhydration [26], microgravity [27], bedrest [28], hypertension [29], hindlimb unloading [22] and intramedullary fluid pumps [30]. Coincidentally, one well known effect of endurance training is a restoration of the old age-associated decline in endothelial cell function in some vascular beds [31,32]. The possibility that exercise training could induce functional alterations in the bone vasculature has not yet been investigated. Therefore, the purpose of this study was to determine whether endurance exercise training restores the old age-related decline in vascular endothelial cell function in the femoral principal nutrient artery (PNA). A secondary purpose was to determine whether putative improvements in endothelial vasodilator function are associated with positive bone adaptations.

To address these questions, a training intervention was chosen that is known to improve endothelium-dependent vasodilation in skeletal muscle arterioles of old animals [32], while eliciting relatively low strain magnitudes [1,7-9]. It was hypothesized that the old age-associated decline in NO-mediated vasodilation of the femoral PNA would be restored after endurance training. We further hypothesized that the putative restoration of NO signaling in the PNA would be associated with increased bone properties.

MATERIALS AND METHODS

The procedures used in this study were approved by the University of Florida and West Virginia University School of Medicine Institutional Animal Care and Use Committees and conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH Publication 85-23, revised 1996). Young adult (4 to 6-mo old) and aged (24 to 26-mo old) male Fischer-344 rats (National Institute of Aging colony, Harlan, Indianapolis, IN) were housed in a temperature-controlled room with a 12:12-h light:dark cycle. Water and standard rat chow were provided ad libitum. Fischer-344 males were used for this study as they are known to exhibit age-associated bone loss [13,18].

Treadmill Exercise Protocol

All rats were habituated to treadmill exercise, during which each rat walked on a motor-driven treadmill at 15 m/min (0° incline), 5 min/day for 3 days. Encouragement was provided at the rear of the treadmill by pressurized air and foot shock. After habituation, young and old rats were randomly assigned to either a sedentary control (SED) group or an exercise-trained (ET) group. For animals in the ET groups, the intensity of the exercise was increased by having the

animals walk on the treadmill (15 m/min) up a 15° incline. The time of exercise was gradually increased in the first 3-wk period until a 60 min duration was reached. The ET rats continued to exercise 5 days/wk for 60 min/day for the remainder of the 10–12 wk training period as previously described [32,33]. Vascular responses were determined at least 24-h after the last exercise bout in ET rats. To determine the efficacy of the training protocol, the soleus muscle was stored at –80 °C for determination of citrate synthase activity, a measure of muscle oxidative capacity [34].

PNA Preparation

The following experimental procedures are the same as those previously used to study PNA vasomotor responses [18]. To determine whether training influenced bone vascular reactivity, *in vitro* experiments with the femoral PNA were performed. All rats were anesthetized with isoflurane (5%):oxygen balance and euthanized by excising the heart. The femur and surrounding musculature from both hindlimbs were carefully dissected free and placed in cold (4°C) physiological saline buffer solution (PSS) contained in a dissecting dish. Using a stereomicroscope, a segment of the PNA with a length of ~1 mm was isolated from the femur and the surrounding muscle tissue where the PNA perforates the femur through the femoral foramen. One PNA from each animal was used for *in vitro* dose-response studies, while the other was frozen for protein analysis as described below. The isolated PNA for *in vitro* studies was transferred from the dissecting dish to a Lucite chamber containing PSS equilibrated to room temperature as described previously [18,32]. Each end of the PNA was cannulated with a micropipette (60–80 µm diameter tip) and secured with 11–0 nylon microfilament sutures (Alcon). The microvessel chamber was transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (Colorado Video, Boulder, CO) and data acquisition system (PowerLab) for the measurement and recording of PNA intraluminal diameter. PNAs were pressurized to 60 cm H₂O with two hydrostatic pressure reservoirs, and leaks were detected by closing the valves of the reservoirs and verifying that intraluminal diameter remained constant. PNAs that were free from leaks were warmed to 37°C and allowed to develop spontaneous baseline tone (~1-h). Vessels that leaked or did not develop at least 20% spontaneous tone were discarded.

Evaluation of Vasodilator Responses

Stock solutions of drugs were prepared in distilled water and frozen. Fresh dilutions of these stock solutions were prepared daily. All drugs were purchased from Sigma Chemical (St. Louis, MO, USA). Concentration-response relations to the cumulative addition of acetylcholine (ACh; 10⁻⁹–10⁻⁴ M) and sodium nitroprusside (SNP; 10⁻¹⁰–10⁻⁴ M) were determined in PNAs from all rats. These vasodilators were selected because they produce vasodilation through the endothelium (ACh) or directly through smooth muscle cell relaxation (SNP). After the ACh dose-responses were determined, the PNAs were rinsed with warm PSS buffer and allowed to again develop baseline tone before the determination of the SNP dose-response. After completion of the SNP response, maximal PNA diameter was determined by twice washing the PNA with a calcium-free PSS buffer and waiting 15-min after each wash to make the final diameter measure. The calcium-free PSS buffer is identical to PSS-albumin solution except that it contained 2-mM EDTA and CaCl₂ is replaced with NaCl.

Evaluation of Endothelium Signaling Pathways

PNAs from all animals were cannulated and allowed to develop spontaneous tone. Vasodilator responses to ACh were evaluated after a 20 min incubation with one of the following: 1) PSS buffer alone, 2) PSS buffer containing the NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME; 10⁻⁵ M), 3) PSS buffer containing the COX inhibitor indomethacin (Indo; 10⁻⁵ M),

or 4) PSS buffer containing L-NAME (10^{-5} M) and Indo (10^{-5} M). The concentrations of NOS and COX inhibitors used have been demonstrated successfully in previous experiments [35].

Measurement of Femoral Bone and Marrow Blood Flow

Young ($n = 10$) and old ($n = 8$) sedentary rats were anesthetized with pentobarbital sodium (30 mg/kg ip). A catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with heparinized (200U/ml) saline was advanced into the ascending aorta via the right carotid artery as previously described [36]. This catheter was subsequently used for the infusion of 15 μ m diameter radiolabeled (^{85}Sr , ^{113}Sn , and ^{46}Sc) microspheres (New England Nuclear) to measure tissue blood flow and recording arterial pressure. A second polyurethane catheter (Braintree Scientific, Micro-renathane; ID 0.36mm, OD 0.84 mm), used for the withdrawal of a reference blood sample, was implanted in the caudal artery of the tail and filled with heparinized saline as previously described [36]. Both catheters were externalized and secured on the dorsal cervical region.

Following 24 h of recovery from the surgical procedure, the animals were instrumented for blood flow determination. The animals were placed on a motor-driven treadmill and allowed to quietly stand for ~10 min. The first microsphere label was infused while the animals were in a normal standing position. The treadmill was then started and blood flow was measured after 5 min of exercise (15 m/min, 15° incline) with different isotopically labeled microspheres. After the final microsphere infusion, euthanasia solution (0.22 ml/kg; Euthanasia-5 Solution, Henry Schein Inc.) was infused through the carotid catheter. Bones and kidneys were then removed from the carcass. The femora from both hindlimbs were sectioned into three regions, the proximal and distal metaphyses and diaphysis; the femoral marrow was removed from the diaphysis and counted as a fourth region as previously described [18,22]. Tissue samples were weighed and placed in counting vials for flow determination. The weight of the femoral marrow was determined by weighing the shaft before and after the marrow was removed.

Radioactivity of the samples was measured with a gamma counter (Packard AutoGamma 5780), and flows were computed (PC-GERDA V2.9 Software) from counts per minute and tissue wet weights. Microsphere-blood mixing was considered adequate when right and left kidney blood flows were within 15% of each other. Based on this criterion, no bone flows were excluded from the study.

Determination of Trabecular Bone Parameters

Trabecular bone parameters of the distal femur metaphysis were assessed using microcomputed tomography (microCT; Skyscan 1172). Bones were thawed to room temperature and wrapped in parafilm to prevent drying during the scanning. Scans were obtained using an x-ray source, set at 60kV and 167 μ A over an angular range of 180 degrees (rotational steps of 0.40 degrees) with a 12- μ m pixel size. Projection images were reconstructed using standard Skyscan software. The trabecular bone compartment was segmented from the cortical shell for 50 slices in a region ~0.5-mm below the most distal portion of the growth plate for each animal. Images were binarized (threshold of 100–255) and the following parameters were assessed for the three-dimensional volume: trabecular bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). A phantom standard was scanned and reconstructed using the same parameters as above to allow conversion of gray scale values to density (mg/cm³). Two separate densities were assessed for the femora. The first was for the entire trabecular bone compartment (bone + marrow), termed bone mineral density, which represents the average density of the bone and marrow. The second was the density for only the trabecular bone, termed bone material density, which provides an index of the degree of mineralization.

Statistical Analysis

Vasodilator responses were expressed as a percentage of maximal relaxation according to the following formula: $\text{Vasodilation (\%)} = (D_s - D_b) / (D_m - D_b) \times 100$, where D_m is the maximal inner diameter recorded at 60-cm H₂O in calcium-free PSS, D_s is the steady-state inner diameter recorded after each addition of the vasodilator substance, and D_b is the initial baseline inner diameter recorded immediately before the first addition of ACh or SNP. To evaluate possible differences in the PNA sensitivity to vasodilators, IC₅₀ values were designated as the concentration of ACh or SNP producing 50% of the maximal vasodilator response. A one-way ANOVA was used to compare body mass, trabecular properties, PNA spontaneous tone, maximal diameter and IC₅₀ among groups, and a Student-Newman-Keuls method was used as a post hoc test to determine the significance of differences among means. A Two-way repeated-measures ANOVA was used to determine the effect of exercise on bone and marrow blood flow in young and old animals. Two-way repeated-measures ANOVAs with pairwise comparisons were used to determine the significance of differences between (young versus old, SED versus ET) and within (drug concentration) factors. All data are presented as mean $\pm$ SE.

RESULTS

Animal Characteristics

Body weight increased with age, whereas exercise training resulted in decreased body weight in both young and old animals (Table 1). The efficacy of the training protocol was confirmed in that citrate synthase activity in soleus muscle was higher in both young (18.3%) and old (20.1%) ET rats relative to their SED counterparts, similar to previous reports [32].

PNA Characteristics

Maximal PNA inner diameter was greater in old SED, old ET and young ET compared to young SED (Table 1). However, the percentage of spontaneous tone was not different among groups (Table 1).

Endothelium-Dependent and -Independent Vasodilation in SED Rats

ACh-induced vasodilation of the PNA was lower in old SED rats relative to young SED rats (Fig 1A). NOS inhibition abolished this age-related difference in ACh-mediated vasodilation (Fig 1B). COX inhibition lowered the ACh-induced vasodilator response in both young and old SED rat PNAs. However, the old age-related difference in endothelium-dependent vasodilation persisted (data not shown). Relative to NOS inhibition alone, the combination of the NOS and COX inhibitors together had little or no further effect on the ACh-mediated vasodilation of old and young SED rat PNAs (data not shown). SNP-induced vasodilation was not different between young and old SED rat PNAs (Fig 2).

Endothelium-Dependent and -Independent Vasodilation in ET Rats

Exercise training was associated with increased endothelium-dependent vasodilation of both young and old rat PNAs compared to SED (Fig 1A). These values correspond to ~25% increase in endothelium-dependent vasodilation after training in the old, and ~17% increase after training in the young. In old ET rats, the NOS-mediated contribution to endothelium-dependent vasodilation was enhanced by 77% after training (Fig 1A and 1B). COX inhibition lowered the ACh-induced vasodilator response of both young and old ET rat PNAs to a similar extent (data not shown). Relative to NOS inhibition alone, the combination of the NOS and COX inhibitors together had little or no further effect on the ACh-mediated vasodilation of old and young rat PNAs. SNP-mediated vasodilation was similar between young and old ET rats (Fig 2).

Exercise and Bone Blood Flow

In young rats (Fig 3A) exercise elevated blood flow in the diaphyseal marrow and distal metaphysis, and tended to increase perfusion in the proximal metaphysis. There was no change in perfusion of the diaphysis. Likewise, in the old animals, treadmill walking increased the perfusion of the diaphyseal marrow and distal metaphysis (Fig 3B). Blood flows to the proximal and distal metaphyses and the diaphyseal marrow were higher in the young rats during standing and exercise relative to that in the old animals.

Bone Properties with Aging and Exercise Training

Advancing age was associated with lower distal femur trabecular bone volume (BV/TV, %) (Figs 4 and 5). Exercise training resulted in higher BV/TV in both young and old compared to SED (Figs 4 and 5). Trabecular thickness was not different among any of the groups. However, trabecular number was lower with advancing age and higher in both groups with training (Fig 6). Trabecular bone mineral density was not different with advancing age, but was increased in both groups after training (Fig 7). Bone material density, an index of trabecular mineralization, was increased with age, but did not change in either group with training (data not shown).

DISCUSSION

The principal finding from this study is that exercise training induced an increase in endothelium-dependent, NO-mediated vasodilation (Figs 1A and 1B) of the femoral vasculature, which was associated with enhanced indicators of trabecular bone quality (Figs 4-7). To our knowledge, this study is the first to demonstrate that old age-associated reductions in NO-mediated vasodilation of the bone vasculature [18] are restored with endurance exercise training, and that a relation exists between enhanced bone properties and a functional improvement of the endothelial NO signaling pathway in the bone vasculature.

These findings are significant in that they support at least two potential mechanisms whereby the vasculature may influence bone structure, independent of mechanical loading (Fig 8). The first mechanism is through the coupling of paracrine signals between vascular cells and bone cells [18-21]. A second potential mechanism for vascular control of bone cell activity is through direct control of bone blood flow and the consequent increase in intramedullary pressure and bone interstitial fluid flow [22,30,37].

Paracrine Signaling

Previous work from Villanueva and Nimni [38] determined that endothelial cells, when seeded along with calvarial cells, promote a 70-fold increase in calcium deposition, compared to calvarial cells seeded alone. Indeed, endothelial cells synthesize and secrete many molecules that influence bone cell activity [19-21], including various growth factors, macrophage colony-stimulating factor, prostanoids, cytokines, and NO. Data from the present study demonstrate that endurance training enhances NO signaling in the bone vasculature of old rats (Figs 1A and 1B) without altering the vascular smooth muscle cell sensitivity to NO (Fig 2). Moreover, the COX signaling pathway did not contribute to either the old age-related impairment or the training-induced restoration of endothelium-dependent vasodilation. NO stimulates osteoblastic bone formation [39,40] and inhibits osteoclastic resorption [41,42]. Therefore, the effect of endurance training to promote endothelial NO signaling, in contrast to advancing age, may augment an osteogenic effect from the vascular endothelium to nearby bone cells.

Bone Interstitial Fluid Flow

Endurance training elicited a ~17–25% increase in maximal diameter induced through an endothelium-dependent mechanism in both ages (Figs 1A). This increase in arterial diameter is biologically significant when considering Poiseuille's law for fluid flow, $Q = (\pi \Delta P r^4) / 8 \eta l$, where blood flow (Q) is proportional to the arterio-venous pressure difference (ΔP) and the radius of the vessel to the fourth power (r^4), and inversely proportional to the viscosity of the blood (η) and the length of the vessel (l). Therefore, this 17–25% increase in the radius of resistance blood vessels through an endothelium-dependent mechanism could result in a more than doubling of blood flow to the bone.

This exercise-induced increases in bone blood flow and local arterial pressure would elevate medullary pressure [37] and correspondingly elevate bone interstitial fluid flow [43]. In fact, *in vivo* increases in bone interstitial fluid flow increase bone properties independent of mechanical loading [23,30] through the putative release of NO and prostaglandins from osteoblastic bone lining cells [44]. NO and prostaglandin E_2 stimulate osteoblastic bone formation [39,40,45] and inhibit osteoclastic bone resorption [41,42]. Thus, enhancements in bone blood flow during acute bouts of exercise (Figs 3A and 3B) over a prolonged period of time may represent an important mechanism for modulating bone cell activity and shifting the focal balance toward bone accumulation.

It should be noted, however, that the literature is divided regarding the effects of acute exercise on bone blood flow, with studies showing both increases [46,47] and no change [48,49]. Results from the present study may provide some insight into these disparate findings by revealing a regional variability of bone and marrow blood flow response to acute exercise. The results demonstrate increased perfusion of the distal metaphysis and diaphyseal marrow in both old and young animals during treadmill walking, with no change in blood flow to the diaphysis (Figs 3A and 3B). These data indicate that low-intensity, low-impact exercise has no effect on cortical bone perfusion, but increases blood flow to the marrow and regions of cancellous bone in the femur, which corresponds with regions of training-induced enhancement in cancellous bone quality (Figs 4-7).

Mechanical loading is well known to enhance the structural properties of bone [50-52]. The prevailing paradigm for skeletal physiology asserts that bone modeling and remodeling manipulate bone properties to effectively adjust strains to within some pre-determined threshold range of strain, regulated in classic negative-feedback fashion (C.F. [1]). Implicitly, mechanical events that do not exceed a minimum strain threshold will fail to promote positive structural acclimation and may even result in permanent removal of bone tissue [1]. This minimum effective strain is thought to be in the range of 800 – 2,000 μ strain [1,7,8] across most species, although this may be site-specific, with locations more proximal to the appendicular skeleton having a lower threshold [53]. Strains in the rat femur during treadmill locomotion were not assessed in the present study, and therefore, measures of strain reported in the literature are presented to provide insight into the strains animals in our study likely experienced. We are aware of only two studies that have directly assessed strains in rats during treadmill exercise. Indrekvam et al. [9] determined strain magnitudes in rat femurs during acute treadmill exercise at 10 m/min to be 329 μ strain in 3 mo old rats and 230 μ strain in 12 mo old rats, well below the proposed minimum effective strain. In 5–6 mo old rats, Rabkin et al. [10] measured slightly higher strains (727 ± 190 μ strain in tension and 163 ± 60 μ strain in compression) in the tibia during treadmill walking at 16 m/min. Although our treadmill exercise protocol had the additional factor of an incline, work in humans has shown that strains are not different in the tibia during horizontal running versus that on an incline [54]. Based on these data, we propose that the levels of strain specifically on the trabecular bone, which is likely lower than that on the periosteal surface [8,53], is below the threshold for bone adaptation. However, we acknowledge that it is unclear what the osteogenic threshold is for trabecular

bone in the rat femur and what the actual strain induced by low-intensity exercise is at this bone site. Therefore, although we suggest that improvements in the bone properties observed in the present study are associated with the enhanced endothelium-dependent vasodilator properties of the bone vasculature (Fig 8), we cannot definitively exclude the influence of direct mechanical effects at this time.

An additional consideration for the stimulus to bone remodeling with exercise is that low-intensity endurance type exercise training does not build leg muscle mass and decreases loading on bones (i.e., diminishes total body mass) in Fischer-344 rats [6,11,32], which is directly oppositional to the idea that stronger muscles are requisite for stronger bones [2]. Nevertheless, endurance training commonly results in increased bone properties [4-6]. This further supports our hypothesis that the aforementioned vascular mechanisms are responsible, at least in part, for the improved bone properties during low-strain endurance activities.

We expect that the training-induced enhancements demonstrated at the femoral PNA are representative of those occurring in arterioles downstream of the PNA as well. Such downstream effects are characteristic of functional changes within skeletal muscle vasculature due to aging and training. For example, aging diminishes endothelium-dependent NO-mediated vasodilation of feed arteries leading to the soleus muscle [55], as well as in the arterial branch orders within the muscle [35]. Likewise, training enhances the NO signaling pathway along the resistance vasculature tree in skeletal muscle [32]. Therefore, aging and training are likely to impact the PNA and its downstream arterioles in a similar fashion.

In summary, the present data indicate that a program of endurance exercise training, sufficient to restore endothelial function in the femoral PNA, yet shown previously to elicit relatively low strains, results in significant increases in trabecular bone volume and mineral density in the distal femur of old rats. These findings provide support for at least two potential mechanisms through which the bone vasculature could modulate bone structural properties (Fig 8), specifically, 1) vascular endothelial cell release of paracrine substances, such as NO, and 2) enhanced endothelium-dependent vasodilation and bone blood flow and the consequent increase in bone interstitial fluid flow and shear stress on bone cells.

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Figure 1.

The effects of old age and exercise training on vasodilator responses of the PNA to increasing doses of the endothelium-dependent vasodilator acetylcholine in the absence (A) and presence (B) of the nitric oxide inhibitor, L-NAME. SED = sedentary, ET = exercise trained. n = number of animals per group. *Different vs. SED ($P < 0.05$).

Figure 2.

The effects of old age and exercise training on vasodilator responses of the PNA to increasing doses of the NO donor sodium nitroprusside. SED = sedentary, ET = exercise trained. n = number of animals per group.

Figure 3.

Regional blood flow rates in the femur of young (A) and old (B) sedentary rats during standing and treadmill exercise. *Different vs. standing ($P < 0.05$); ‡different vs. standing ($P < 0.10$).

†Blood flow lower in old (B) vs. young (A) in the same region and under the same condition.

Figure 4. Representative microCT scan images of trabecular bone in the distal femoral metaphysis from young sedentary (A), old sedentary (B), young exercise trained (C) and old exercise trained (D) rats. SED = sedentary, ET = exercise trained.

Figure 5.

Effects of old age and exercise training on trabecular bone volume (BV/TV) of the distal femur. SED = sedentary, ET = exercise trained. $n = 12$, 1 femur per animal. *Different vs. Young SED ($P < 0.05$), †different vs. Old SED ($P < 0.05$).

Figure 6.

Effects of old age and exercise training on trabecular number in the distal femur. SED = sedentary, ET = exercise trained. $n = 12$, 1 femur per animal. *Different vs. Young SED ($P < 0.05$), ‡different vs. Old SED ($P < 0.10$).

Figure 7.

Effects of old age and exercise training on cancellous bone mineral density of the distal femur. SED = sedentary, ET = exercise trained. $n = 12$, 1 femur per animal. *Different vs. Young SED ($P < 0.05$), ‡different vs. Old SED ($P < 0.10$).

Figure 8.

Scattergram showing relation between the peak percent endothelium-dependent vasodilation and the percent trabecular bone volume of the distal femur ($n = 53$).

Table 1

Animal and PNA characteristics of young and old, sedentary and exercise-trained rats

	Young SED	Old SED	Young ET	Old ET
<i>n</i>	17	19	15	13
Body weight, g	344 ± 6	400 ± 10 [*]	352 ± 4	372 ± 6 ^{†‡}
Maximal diameter, μm	146 ± 8	175 ± 9 [*]	173 ± 5 [*]	185 ± 8 [*]
PNA Spontaneous Tone, %	32 ± 3	36 ± 3	34 ± 2	40 ± 4

Values are means ± SE.

^{*} Significant difference from young SED ($P \leq 0.05$).[†] Significant difference from young ET ($P \leq 0.05$).[‡] Significant difference from old SED ($P \leq 0.05$).