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Effects of methionine restriction and endurance exercise on bones of ovariectomized rats: a study of histomorphometry, densitometry, and biomechanical properties

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Huang TH, Su IH, Lewis JL, Chang MS, Hsu AT, Perrone CE, Ables GP. Effects of methionine restriction and endurance exercise on bones of ovariectomized rats: a study of histomorphometry, densitometry, and biomechanical properties. *J Appl Physiol* 119: 517–526, 2015. First published July 9, 2015; doi:10.1152/japplphysiol.00395.2015.—To investigate the effects of dietary methionine restriction (MetR) and endurance exercise on bone quality under a condition of estrogen deficiency, female Sprague-Dawley rats (36-wk-old) were assigned to a sham surgery group or one of five ovariectomized groups subjected to interventions of no treatment (Ovx), endurance exercise (Exe), methionine restriction (MetR), methionine restriction plus endurance exercise (MetR + Exe), and estrogen treatment (Est). Rats in the exercise groups were subjected to a treadmill running regimen. MetR and control diets contained 0.172 and 0.86% methionine, respectively. After the 12-wk intervention, all animals were killed, and serum and bone tissues were collected for analyses. Compared with estrogen treatment, MetR diet and endurance exercise showed better or equivalent efficiency in reducing body weight gain caused by ovariectomy ($P < 0.05$). Whereas only the Est group showed evidence for reduced bone turnover compared with the Ovx group, MetR diet and/or endurance exercise demonstrated efficiencies in downregulating serum insulin, leptin, triglyceride, and thiobarbituric acid reactive substances ($P < 0.05$). Both the Exe and MetR groups showed higher femoral cortical and total volumetric bone mineral density (vBMD), but only the Exe and Est groups preserved cancellous bone volume and/or vBMD of distal femora ($P < 0.05$) compared with the Ovx group. After being normalized to body mass, femora of the MetR and MetR + Exe groups had relatively higher bending strength and dimension values followed by the Sham, Exe, and Est groups ($P < 0.05$). In conclusion, both MetR diet and endurance exercise improved cortical bone properties, but only endurance exercise preserved cancellous bone under estrogen deficiency.

estrogen deficiency; dietary restriction; amino acid; body weight

DURING MENOPAUSE, BONE METABOLISM shifts to a status characterized by high turnover and an imbalance between bone formation and bone resorption, resulting in the porosity of bone tissues. Although high body weight or obesity has been suggested to protect or preserve bone mineral content (BMC) or bone mineral density (BMD) (17, 39), body weight gain resulting from estrogen deficiency actually contributes to increased fracture risk (11, 14), and obese postmenopausal women with type 2 diabetic mellitus have increased porosity in cortical bone, despite the fact that BMD is not compromised (7, 33). Ovarian failure and the resulting loss of estrogen cause a loss of estrogen's direct ligand-receptor action in bone cells. In addition, estrogen deficiency causes a metabolic imbalance characterized by the upregulation of oxidative stress and insulin resistance, which have also been identified as contributors to impaired bone quality (12, 53). In fact, there are intercorrelations among osteoporosis, obesity, and energy metabolism (22, 33, 53, 54). Therefore, the manipulation of body weight and metabolism could have important implications in counteracting bone compromise caused by estrogen deficiency.

Over the past two decades, dietary methionine restriction (MetR) has been shown to reduce body weight gain (1, 24, 29, 31, 40) and to ameliorate energy metabolism (29), which leads to the mitigation of oxidative stress (9, 28, 52) and the delayed onset of aging-related diseases (35). This suggests the potential of MetR to mediate menopause-related estrogen deficiency effects. Previous studies demonstrated that MetR diet does not promote size-related indexes and mineral accumulation in bones (1, 24). However, measurements of serum bone markers, volumetric bone mineral density (vBMD), and bone material properties suggested that the MetR diet did not compromise bone health and that the smaller bones in MetR animals may be associated with an absolute reduction in bone development rather than an increase in bone catabolism (24). In fact, the smaller bones from rodents subjected to the MetR diet had better intrinsic biomechanical properties compared with those from rats fed a control diet (24). Therefore, it would be important to investigate the effects of the MetR diet on the pathogenesis of bone fragility under estrogen deficiency.

Aside from dietary manipulations, a lifestyle involving regular physical activity or exercise is also critical to maintaining health. Endurance exercise has been well-proven to decrease body weight gain (24) or induce body weight loss (41), benefit energy metabolism (6), and downregulate oxidative stress/inflammatory responses (30, 38). These effects are similar to those of the MetR diet. With regard to the skeletal system, endurance exercise, like the MetR diet, does not promote size-related indexes or bone mineral accumulation (24). Clinical reports and animal studies have revealed that endurance athletes (e.g., long-distance runners) or endurance-trained animals have normal or subnormal levels of linear growth, BMD, and BMC (23, 24, 44, 46, 49). However, the benefits of

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endurance exercise on intrinsic bone mechanical properties in either growing or adult rodents have been well-documented (23, 24, 49). Moreover, the downregulation of bone turnover rate (24, 56, 58) mediated by endurance exercise implies that this intervention could shift bone metabolism to a status that is more resistant to estrogen deficiency.

Because the MetR diet and endurance exercise induce similar favorable effects on body weight control, metabolism, and oxidative stress, it would be important to examine how these two interventions influence bone metabolism and overall bone health during estrogen deficiency. We hypothesized that the MetR diet and endurance exercise could benefit body weight control, energy metabolism, and bone quality in ovariectomized rats. Methods to examine energy and bone metabolic serum markers as well as bone histomorphometry, densitometry, dimension, and biomechanical properties were therefore conducted in the present study.

METHODS

Animals

Female Sprague-Dawley rats (4 wk old) were purchased from the National Cheng Kung University Animal Center and housed (2-3 rats/cage) in the same facility under conditions of 21 ± 1°C room temperature and a 12:12-h light-dark cycle. All animals had free access to food (Rodent Chow 5001, Labdiet; Purina, Richmond, IN) and water throughout the preexperimental period. During the experimental period, animal food was changed to the purified diets (Table 1) containing 0.86 or 0.172% methionine, according to the experimental design. Body weight was measured once a week. Food consumption was measured biweekly (from Thursday to Monday) and calculated based on daily food consumption per total body weight (kg) for each cage. Briefly, a given amount (500 g) of diet was put on the wire top of each cage. At the end of the food consumption measurement period, the remaining food on the wire top was weighted. To reduce measurement errors caused by diet spillage, visible pieces of food in the bottom of the cage were also collected and weighed. Food consumption per cage was then calculated as follows: (grams of food given at *time 0* − grams of food left on the cage top − collected spilled diet) ÷ total body weight in each cage (kg) ÷ time period (days). Food intake was therefore expressed as grams per kilogram body weight per day. Nevertheless, unaccountable finely shredded food and additional weight in wet pieces of spilled food still introduced some error in the measurements.

Sham and ovariectomy surgeries and death were conducted under anesthesia by intraperitoneal injection of ketamine hydrochloride (KETALAR; Pfizer) (80 mg/kg body wt) and xylazine (xylazine hydrochloride, x1251; Sigma-Aldrich, St. Louis, MO) (10 mg/kg body wt). To measure dynamic bone mineralization/formation, the fluorescent labels alizarin red (30 mg/kg body wt) (Alizarin Red S, A5533; Sigma-Aldrich) and calcein green (8 mg/kg body wt) (calcein disodium, 20130; Sigma-Aldrich) were administered intraperitoneally at 10 and 3 days, respectively, before the rats were killed. Whole blood was collected after decapitation, allowed to clot, and centrifuged at 1,500 *g* for 20 min at 4°C. Serum samples were separated into aliquots and immediately stored at −80°C for various bone turnover and energy metabolic marker analyses. All animal procedures were approved by the Committee of Animal Study in National Cheng Kung University, Tainan, Taiwan (Document No. 99104).

Experimental Design

To examine the effects of endurance exercise and the MetR diet on bone quality in estrogen-deficient rats, 36-wk-old female rats were assigned to either a sham surgery group (Sham, $n = 9$) or one of five ovariectomized groups subjected to the following interventions: *1*) no treatment (Ovx, $n = 8$); *2*) endurance exercise (Exe, $n = 8$); *3*) MetR ($n = 9$); *4*) methionine restriction plus endurance exercise (MetR + Exe, $n = 9$); and *5*) estrogen treatment (Est, $n = 9$). Endurance exercise was introduced to the Exe and MetR + Exe groups 2 wk before ovariectomy while estrogen treatment was initiated immediately after ovariectomy surgery. Because appropriate dietary methionine content is crucial for wound healing (34), the MetR diet was given to the MetR and MetR + Exe groups 1 wk after surgery (Fig. 1). The details of the various interventions are listed below.

Table 1. *Composition of the different methionine-containing diets*

Ingredients	Diet Composition, g	
	Control diet	MetR diet
Corn starch	434.6	434.6
Sucrose	200	200
Corn oil	80	80
Dextrine	50	50
Cellulose	50	50
Glutamic acid	33.75	35.54
Mineral mix	35	35
L-Glycine	22.09	23.26
L-Lysine	13.65	14.38
L-Phenylalanine	11.00	11.58
L-Arginine	10.62	11.18
L-Leucine	10.52	11.08
Vitamin mix¥	10	10
L-Isoleucine	7.77	8.19
L-Threonine	7.77	8.19
L-Valine	7.77	8.19
L-Histidine	3.13	3.29
L-Choline bitartrate	2	2
L-Tryptophan	1.71	1.80
L-Methionine	8.60	1.72
L-Cysteine	0.00	0.00
L-Tyrosine	0.00	0.00
Total	1,000	1,000

To make the total amino acids content equivalent (138.4 g/1,000 g) between the control and methionine restriction (MetR) diets, all other amino acids were proportionally adjusted to compensate the changes in L-methionine content. In control and MetR diets, the exact L-methionine percentages were 0.86 and 0.172, respectively. In addition to L-methionine, the control and MetR diets would, respectively, need to include 138.4−8.6 = 129.8 g and 138.4−1.72 = 136.68 g of other amino acids to make the two diets both contain 138.4 g of total amino acids. Because (136.68 ÷ 129.8) × 100 @ 105.3%, all other amino acids in the MetR diet should be 5.3% higher than those in the control diet. ¥Mixtures of mineral (no. 200000; Dyets, Bethlehem, PA) and vitamin (no. 300050; Dyets) followed the compositions of the AIN-76 diet.

Diet composition. AIN-76 chemically based diets with protein replaced by amino acid mixtures containing 0.86% methionine (control diet) (519535; Dyets, Bethlehem, PA) and 0.172% methionine (MetR diet) (519540; Dyets) were purchased from Dyets. Once exercise groups were subjected to endurance training, all animals were transferred to the 0.86% methionine diet. One week after sham/ovariectomy surgery, the MetR and MetR + Exe groups were shifted to the 0.172% methionine diet until the end of the study. The total percentage of amino acids in the two diets was kept equal at 13.84%, and all diets were isocaloric. When methionine content was lowered in the MetR diet, the content of all other amino acids was changed proportionally to make the diets equal in total amino acids (Table 1) (24). The diets were devoid of cysteine, an intermediate form methionine metabolism that spares the MetR effects. In fact, cysteine supplementation reverses MetR's effects on adiposity and restores metabolism to a status equivalent to that of rats fed the control diet (15). For these reasons, cysteine was not included in diets of the current study.

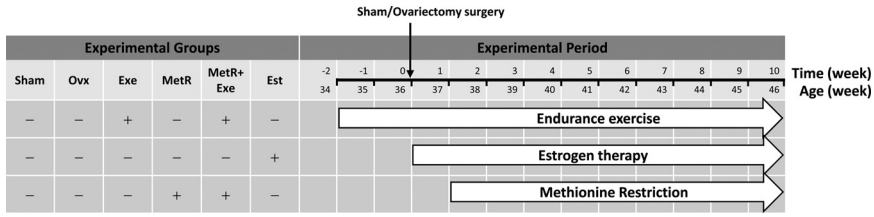

Fig. 1. Time schedule of experimental interventions and surgery. A moderate treadmill running training (from 12 m/min for 20 min/day to 16 m/min for 60 min/day) was introduced to the exercise groups 2 wk before ovariectomy. Estrogen administration and dietary methionine restriction were begun immediately and 1 wk after the ovariectomy surgery, respectively. +, Subjected to the intervention; −, not subjected to the intervention; Sham, sham surgery; Ovx, untreated ovariectomy; Exe, ovariectomy + endurance exercise; MetR, ovariectomy + methionine restriction; MetR + Exe, ovariectomy + methionine restriction and endurance exercise; Est, ovariectomy + estrogen treatment.

Endurance exercise training. The current endurance exercise protocol was modified from a previous study (26). In order for the animals to reach a level of treadmill running capable of meeting the timing of vigorous bone loss during the initial phase of estrogen deficiency, treadmill training was progressively introduced to the Exe and MetR + Exe groups 2 wk before the ovariectomy surgery. Briefly, the exercise program began at a running speed of 12 m/min on a level treadmill for 20 min and progressively increased to 16 m/min for 60 min over the 2-wk preovariectomy period. The training regimen used a progressive pattern (e.g., 12∼16 m/min and 20∼60 min) during the week right after ovariectomy surgery. Throughout the experimental period, the rats in exercise groups trained on the treadmill 5 days/wk.

Estrogen treatment. Immediately after ovariectomy, the Est group received subcutaneous injections with 17β-estradiol (E8875; Sigma-Aldrich) at a dose of 10 mg/kg body wt/day, six times a week, throughout the experimental period. In the current study, estrogen treatment was used as a positive control therapy due to its well-proven efficacy in body weight control, bone mineral preservation, and energy metabolism amelioration under the condition of estrogen deficiency (2, 19).

Serum Analyses

Serum analyses were conducted to examine the effects of the MetR diet and endurance exercise on markers of bone turnover and development, energy metabolism, and oxidative stress. Commercially available ELISA kits were used to measure serum osteocalcin (Rat-MID EIA; Immunodiagnostic Systems) [coefficient of variation (CV) = 4.46%], COOH-terminal telopeptides of type 1 collagen (CTX-1, RatLaps; Immunodiagnostic Systems) (CV <0.1%), insulin (rat insulin ELISA; Mercodia) (CV = 2.59%), insulin-like growth factor-I (IGF-I, rat/mouse IGF-I ELISA kit; Immunodiagnostic Systems) (CV = 5.39%), thiobarbituric acid reactive substances (TBARS, TBRAS assay kit; Cayman Chemical) (CV = 1.59%), and leptin (leptin mouse/rat EIA kit; Cayman Chemical) (CV = 10.5%), following the manufacturer's procedures. In addition, serum glucose and triglycerdies (TG) were measured using commercially available enzymatic kits (GLUCOSE liquicolor and TRIGLYCERIDES liquicolor; HUMAN Gesellschaft für Ciochemica und Diagnostica) (CV <2%).

Bone Samples Preparation

Bilateral femora and tibiae were removed. The right tibiae from all animals were fixed in a 3.7% neutral paraformaldehyde solution for 24 h and decalcified with a 10% EDTA solution (pH 7.4) at 4°C for 35 days. Once decalcified, each right tibia was paraffin embedded for further histological sectioning (5 μm in thickness) and staining. The left tibiae were dehydrated in gradient alcohol, cleared with xylene, and cross sectionally cut through the midshaft. The proximal and distal segments of each tibia were embedded in methylmethacrylate (MMA) and sectioned for dynamic histomorphometry. The right femora were stored in 70% ethanol for microcomputed tomography (μCT) scanning. Finally, the left femora were cleaned of soft tissue, wrapped in gauze, immersed in PBS (pH = 7.4), and stored in aluminum foil at −80°C for biomaterial testing.

Dynamic Histomorphometry

MMA-embedded proximal tibiae were subjected to frontal sectioning (5 μm) using an automatic rotary microtome (HM 355S; Thermo Scientific). The distal segments of the tibiae were polished at the midshaft cross-sectional surface. Metaphyseal cancellous bone of the proximal tibiae (1∼3 mm below the growth plate) and the cross-sectional surfaces of midshaft tibiae were photographed under a fluorescent microscope at ×100 and 40 magnifications, respectively. Length measurements of fluorescent labeled/nonlabeled bone surfaces and the distances between two parallel fluorescent labels were performed on images using Image Pro Plus (version 6.1; Media Cybernetics). These measurements were subsequently used for the calculation of dynamic histomorphometry indexes (32), which included bone mineralization over bone surface (MS/BS), mineral apposition rate, and bone formation rate (BFR/BS). In addition, osteoclasts were stained on paraffin sections using a commercial tartrate-resistant acid phosphatase-staining kit (387A; Sigma-Aldrich). The multinucleated osteoclast number relative to the trabecular tissue perimeter of metaphyseal cancellous bone in the proximal tibiae (1∼3 mm below the growth plate) was used as a measure of bone resorption activity (32).

μCT

Right femora that were immersed in 70% ethanol were subjected to μCT scanning (Skyscan 1176; SkyScan) using the following conditions: 1 mm aluminum filter, 65 kev, 385 μA, 0.57°/picture with 1,580 minisecond exposure time and pixel size of 8.88 μm. Cross-sectional images (8-bit BMP file) of each sample were reconstructed using a NRecon (version 1.6.6.0; Skyscan) with setup as follows: dynamic range = 0∼0.06, smoothing = 2, ring artifact correction = 6, beam hardening correction (%) = 20. Densitometric and histomorphometric analyses were performed using a CT-Analyzer (version 1.12.0.0; Skyscan) with a consistent gray threshold range (66–255) selected for all sample images. vBMD and BMC measurements were conducted on the total bone, cortical bone (area selected from transverse slices 1 mm in thickness at midshaft femur), and cancellous bone (area selected from transverse slices from 0.5 to 3.5 mm under the lowest point of the growth plate at distal metaphysis) of each femur. According to the guidelines of Bouxsein et al. (5), histomorphometric indexes of bone volume ratio (BV/TV; %), trabecular thickness (Tb.Th; μm), trabecular number (Tb.N; 1/mm), trabecular separation (Tb.Sp; μm), connectivity density (Conn.Dn; 1/mm^3), and structure model index (SMI) were measured in the same area selected for cancellous bone densitometric analysis. In addition, a middle transverse-CT slice located at 50% of total length between the head of the femur and the distal condyle was acquired to assess dimensional parameters including cortical area (Ct.Ar; mm^2), cortical thickness (Ct.Th; mm), and three indexes of cross-sectional moment of inertia (CSMI; mm^4)

which were polar CSMI ($CSMI_p$), maximal CSMI ($CSMI_{max}$), and minimal CSMI ($CSMI_{min}$).

Bone Biomechanical Property Analyses

Biomechanical properties of femora were determined through a three-point bending test following details described previously (23, 51). All left femora samples were stored at −80°C for <2 mo and subjected to biomechanical testing on the same day. Briefly, an anteroposterior direction three-point bending test at a deformation rate of 1 mm/s was performed on each femur using a material testing system (MTS-858; MTS System, Minneapolis, MN). The span between the two support points was 20 mm with the middle posterior surface of each femur subjected to tensional stress. Original load (N) vs. deformation (mm) data acquired at a sampling rate of 200 Hz were used to calculate extrinsic (whole bone level) biomechanical properties of bone, including parameters of yield load (YL; N), fracture load (FL; N), yield load energy (mJ), fracture load energy (FLE; mJ), and stiffness (N/mm). Furthermore, the intrinsic (material-level) biomechanical properties were calculated based on elastic beam theory (51). Data from load-deformation curves were transformed into stress-strain data using the equations below:

$$\sigma = \frac{\mathrm{FL}_c}{4\mathrm{I}}$$

$$\varepsilon = \frac{12_c\mathrm{d}}{L^2}$$

$$\mathrm{E} = \frac{\mathrm{F}}{\mathrm{d}} \times \frac{L^3}{48\mathrm{I}}$$

where σ is longitudinal stress, ε is longitudinal strain, *c* is the maximal distance from the surface subjected to tensional forces to the line crossing the center of mass, F is the applied load (N), I is the $CSMI_{min}$ of the midshaft femora acquired by μCT, E is the elastic modulus, d is the deformation, and *L* is the span between the two support points of the bending fixture. Because beam bending theory is valid in the preyield region (51), stress-strain data were used only to determine yield stress (YS, MPa), yield toughness (mJ/mm^3), and elastic modulus (GPa) as parameters of intrinsic biomechanical properties. YL and YS were determined with the 0.002 strain-offset method described previously (23, 51).

Statistical Analysis

Results are presented as means ± SE. Because body mass plays a key role in the risk of bone fracture (11, 14), data for bone dimensional and extrinsic biomechanical properties were normalized to body mass (55). One-way ANOVA was used to compare differences among groups. *P* values <0.05 were considered significant. Fisher's least-significant difference method was used for post hoc comparisons. All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL).

RESULTS

When a statistically significant level was reached, post hoc comparisons were done pairwise between groups. For all measurements, the statistical difference between the Sham and Ovx groups was first mentioned and then followed by statements focused on the comparisons among ovariectomized groups. Although the comparisons between the Sham group and ovariectomized groups with different treatments are not described, the complete results of post hoc comparisons are shown in the Figs. 1–3 and Tables 1–6.

Body Weight and Food Consumption

Table 2 shows body weight data from 2 wk before (pre-2 wk), the week immediately after (0 week), and 10 wk after ovariectomy. At the end of the experimental interventions, marginal differences were observed between the Sham and Ovx groups ($P = 0.094$). Among the five ovariectomized groups, the Exe and MetR + Exe groups showed lower body weight than the Ovx, Est, and/or MetR groups ($P < 0.05$) at 0 week. By the end of the experimental period, the rats in the Exe, MetR, MetR + Exe, and Est groups had lower body weight than the Ovx group ($P < 0.05$). Furthermore, the MetR and MetR + Exe groups showed lower body weight than the Est group by the end of the intervention period ($P < 0.05$) (Table 2). With respect to body weight gain, the Ovx group showed higher body weight gain compared with the Sham group ($P < 0.05$) from *week 1* to *week 8* after ovariectomy. The

Table 2. *Body weight changes and body weight gain during the experimental period*

	Sham	Ovx	Exe	MetR	MetR + Exe	Est	*P* Value
Body weight, g							
Pre-2 wk	368.8 ± 11.2	381.4 ± 9.2	374.1 ± 17.1	385.1 ± 10.8	370.1 ± 10.0	412.8 ± 20.3	0.221
0 wk	410.7 ± 13.5^{a,b,c}	428.1 ± 8.5^{a}	369.4 ± 8.6^{c}	415.6 ± 14.7^{a,b}	383.6 ± 8.8^{b,c}	440.8 ± 26.2^{a}	0.017
10 wk	469.4 ± 21.9^{a,b}	514.9 ± 8.6^{a}	427.3 ± 9.1^{b,c}	379.9 ± 15.3^{c,d}	364.6 ± 10.4^{d}	453.1 ± 31.1^{b}	<0.001
Body weight gain, g							
Pre-2 wk	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	NA
Pre-1 wk	30.0 ± 3.3^{a,b}	36.0 ± 3.7^{a}	11.8 ± 8.1^{c}	33.8 ± 4.3^{a}	18.9 ± 3.5^{b,c}	38.1 ± 5.2^{a}	0.002
0 wk	41.9 ± 5.4^{a}	46.8 ± 5.2^{a}	−4.8 ± 10.9^{c}	30.4 ± 6.0^{a,b}	13.4 ± 5.0^{b,c}	28.0 ± 7.3^{a,b}	<0.001
1 wk	36.0 ± 5.6^{b,c}	69.8 ± 6.7^{a}	5.5 ± 9.9^{d}	47.4 ± 6.6^{b}	18.4 ± 8.5^{c,d}	28.9 ± 7.9^{b,c}	<0.001
2 wk	57.2 ± 7.6^{b}	96.8 ± 5.7^{a}	31.8 ± 9.4^{c}	43.2 ± 7.7^{b,c}	−1.7 ± 9.1^{d}	41.3 ± 7.8^{b,c}	<0.001
3 wk	63.8 ± 9.6^{b}	109.9 ± 6.3^{a}	39.6 ± 9.1^{b,c}	19.2 ± 9.3^{c}	−7.6 ± 8.6^{d}	30.9 ± 9.8^{c}	<0.001
4 wk	76.7 ± 11.5^{b}	118.4 ± 7.1^{a}	47.4 ± 10.1^{c}	−8.6 ± 10.3^{d}	−11.1 ± 7.9^{d}	33.8 ± 10.6^{c}	<0.001
5 wk	88.6 ± 13.1^{b}	125.6 ± 7.5^{a}	56.0 ± 11.0^{c}	−15.6 ± 12.0^{d}	−4.1 ± 6.9^{d}	44.6 ± 12.1^{c}	<0.001
6 wk	98.2 ± 13.4^{b}	140.1 ± 7.9^{a}	62.8 ± 11.2^{c}	−12.9 ± 14.3^{d}	4.7 ± 6.3^{d}	46.1 ± 11.3^{c}	<0.001
7 wk	109.2 ± 13.7^{b}	148.9 ± 7.6^{a}	67.4 ± 15.6^{c}	−5.0 ± 15.4^{d}	14.1 ± 6.0^{d}	49.0 ± 12.2^{c}	<0.001
8 wk	119.1 ± 14.9^{b}	160.3 ± 7.6^{a}	75.4 ± 17.5^{c}	9.4 ± 15.4^{e}	23.3 ± 5.5^{d,e}	53.9 ± 12.5^{c,d}	<0.001
9 wk	113.2 ± 14.8^{a,b}	142.8 ± 8.2^{a}	59.4 ± 16.9^{c}	2.0 ± 13.2^{d}	6.2 ± 6.8^{d}	50.6 ± 13.5^{c}	<0.001
10 wk	100.6 ± 13.4^{a,b}	133.5 ± 7.7^{a}	53.1 ± 17.1^{c}	−5.2 ± 13.4^{d}	−5.6 ± 6.0^{d}	40.3 ± 13.9^{c}	<0.001

Data are means ± SE; $n = 8$ or 9 rats/group. Sham, sham surgery; Ovx, untreated ovariectomy; Exe, ovariectomy plus endurance exercise; MetR, ovariectomy plus methionine restriction; MetR + Exe, ovariectomy plus methionine restriction and endurance exercise; Est, ovariectomy plus estrogen treatment; NA, not available. Mean values within a parameter not sharing a common superscript were considered significantly different ($P < 0.05$). For example, data with the superscript "a" were significantly different from those without the superscript a.

Ovx group also had a marginally (25–33%) higher body weight gain than the Sham group during the last 2 wk of the experimental period. Among the five ovariectomized groups, the MetR and MetR + Exe showed the lowest body weight gain followed by the Exe and Est groups, and all treatments demonstrated lower body weight gain compared with the Ovx group after the intervention period ($P < 0.05$) (Table 2).

Food consumption data were recorded biweekly and normalized to total body weight (kg) for each cage. The Ovx group was found to have a lower food consumption than the Sham group at the end of the experimental period. A decrease in food consumption was observed at the beginning of the interventions in the Exe and MetR + Exe groups compared with the Ovx and Est groups ($P < 0.05$) (Table 3). However, by the end of the experimental period, food consumption in the Exe, MetR, MetR + Exe, and Est groups was greater than that in the Ovx group ($P < 0.05$) (Table 3). Caution should be taken regarding the dominance hierarchy occurring within the group housing system, which could cause variations in endocrine status and food intake. In general, dominant animals have lower levels of stress hormones but higher food intake (50), which would limit both the accuracy and the precision of the data shown in Table 3 and would also increase the variation in body weight. To determine the possible effects of dominance hierarchy, pairwise correlations were done among the data of original body weight and body weight gain of all time points. Briefly, the correlation coefficients (Pearson's r) between the baseline (pre-2 wk) body weight and those of subsequent time points were significant but progressively reduced from the initial phase (pre-2 wk vs. pre-1 wk, $r = 0.936$, $P < 0.001$) to the later phase of the experiment (pre-2 wk vs. 10 week, $r = 0.533$, $P < 0.001$). However, the baseline body weight showed no significant correlation to body weight gain throughout the experimental period ($r = -0.088 \sim 0.137$, $P > 0.05$). Taken together, the correlation between initial body weight and those of other time points might imply the existence of dominance hierarchy-related body weight variation among animals. However, the impact of various interventions on body weight gain seemed to be independent of the actual baseline body weight as well as the possible dominance hierarchy.

Serum Markers

A significant upregulation in serum bone markers (e.g., osteocalcin and CTX-1) and energy metabolism markers (e.g., insulin and leptin) was observed in the Ovx group compared with the Sham group. The level of serum osteocalcin was lower in the Est group compared with the other four ovariectomized groups ($P < 0.05$). CTX-1 levels were significantly lower in the Est group compared with the Ovx group and the two MetR groups ($P < 0.05$). With regard to energy metabolism markers, the MetR and MetR + Exe groups had lower serum insulin compared with the other ovariectomized groups. The Est group also had lower insulin levels than the Ovx group ($P < 0.05$). Serum leptin was lower in the Exe, MetR, MetR + Exe, and Est groups with the lowest levels found in the two MetR groups compared with the Ovx group ($P < 0.05$). Although no difference in serum TG was found between the Ovx and Est groups, the Ovx group had higher serum TG and TBARS than other ovariectomized groups ($P < 0.05$) (Table 4).

Static/Dynamic Histomorphometry

Regarding the static histomorphometry, the Ovx group showed a significant loss of bone volume (e.g., BV/TV) and an increase in architectural degradation of cancellous bone (e.g., Tb.Sp, Tb.N, Conn.Dn, and SMI) compared with the Sham group ($P < 0.05$). In addition to demonstrating the beneficial effects of estrogen as a treatment for preserving the cancellous bone histomorphometry, the Exe group showed higher BV/TV, Tb.Th, Tb.N, and Conn.Dn and lowered Tb.Sp and SMI compared with the Ovx, MetR, or MetR + Exe groups ($P < 0.05$) (Fig. 2). The two MetR-treated groups showed no preservation of trabecular bone and revealed similar histomorphometric features to those of the Ovx group.

Dynamic histomorphometry was conducted in cancellous bone and cortical bone (Table 5). In cancellous bone, the Ovx group showed higher bone mineralization activity (e.g., MS/BS and BRF/BS) and osteoclast density compared with the Sham group ($P < 0.05$). Although only the Est group had lower MS/BS, all four treatment groups revealed lower BFR/BS compared with the Ovx group ($P < 0.05$). The Est group showed lower osteoclast density than the Ovx group and two MetR groups ($P < 0.05$). Conversely, the MetR group showed higher osteoclast density than the Ovx group ($P < 0.05$). In cortical bone, the Ovx group showed higher endosteal MS/BS compared with the Sham group ($P < 0.05$). The Exe group had marginally lower ($P = 0.085$) while the other three treatment groups had significantly lower ($P < 0.05$) endosteal MS/BS than the Ovx group. Periosteal MS/BS was higher in the Exe group compared with the MetR + Exe and Est groups ($P < 0.05$).

Densitometry and Dimensional Measurements

No difference in vBMD was shown between the Sham and Ovx groups. The rats in both the Exe and MetR groups had higher total and cortical vBMD than the other ovariectomized

Table 3. *Diet consumption during the experimental period*

	Sham	Ovx	Exe	MetR	MetR + Exe	Est	*P* Value
0 wk	48.9 ± 1.6^{a}	$45.8 \pm 2.1^{a,b}$	27.4 ± 4.4^{c}	$34.0 \pm 5.0^{b,c}$	23.4 ± 4.5^{c}	$43.8 \pm 5.2^{a,b}$	0.003
2 wk	$46.9 \pm 0.3^{a,b}$	$45.7 \pm 0.8^{a,b}$	46.6 ± 1.1^{a}	38.7 ± 3.3^{b}	30.9 ± 4.5^{c}	$45.8 \pm 0.8^{a,b}$	0.003
4 wk	42.5 ± 0.3^{a}	40.0 ± 2.2^{a}	42.8 ± 1.9^{a}	27.9 ± 1.9^{b}	41.2 ± 1.3^{a}	40.6 ± 2.6^{a}	<0.001
6 wk	41.1 ± 3.2^{b}	38.4 ± 1.1^{b}	41.4 ± 2.0^{b}	41.2 ± 0.5^{b}	50.3 ± 1.1^{a}	40.7 ± 3.4^{b}	0.010
8 wk	$36.7 \pm 0.8^{c,d}$	33.7 ± 0.2^{d}	$36.5 \pm 1.8^{c,d}$	$43.3 \pm 1.7^{a,b}$	46.7 ± 1.9^{a}	$40.5 \pm 3.5^{b,c}$	0.002
10 wk	24.3 ± 0.5^{c}	18.0 ± 1.9^{d}	$26.8 \pm 2.7^{b,c}$	$32.5 \pm 1.6^{a,b}$	$32.3 \pm 2.2^{a,b}$	34.4 ± 2.0^{a}	0.001

Data are means ± SE; $n = 3$ or 4 rats/group. Units are $g \cdot kg^{-1} \cdot day^{-1}$. Diet consumption was measured biweekly within a 4-day period (Thursday to Monday). Mean values within a parameter not sharing a common superscript were considered significantly different ($P < 0.05$). For example, data with the superscript a were significantly different from those without the superscript a.

Table 4. *Serum markers of bone metabolism, energy metabolism, and oxidative stress*

	Sham	Ovx	Exe	MetR	MetR + Exe	Est	*P* Value
Osteocalcin, ng/ml	115.2 ± 6.3[b]	221.3 ± 21.9[a]	180.1 ± 14.5[a]	175.8 ± 17.1[a]	196.1 ± 22.1[a]	81.6 ± 15.1[b]	<0.001
CTX-1, ng/ml	11.0 ± 1.9[b,c]	15.2 ± 0.6[a]	12.3 ± 1.5[a,b,c]	13.5 ± 1.1[a,b]	12.7 ± 1.0[a,b]	8.7 ± 1.4[c]	0.031
Insulin, μg/l	0.53 ± 0.09[b]	0.79 ± 0.13[a]	0.60 ± 0.05[a,b]	0.36 ± 0.05[c]	0.38 ± 0.04[c]	0.49 ± 0.05[b]	0.002
IGF-I, ng/ml	1178 ± 99	1,563 ± 104	1,160 ± 136	1,152 ± 188	1,277 ± 105	1,101 ± 167	0.248
Leptin, ng/ml	25.8 ± 5.4[b]	49.3 ± 4.6[a]	20.3 ± 4.9[b]	3.9 ± 0.7[c]	3.9 ± 1.1[c]	15.8 ± 3.7[b]	<0.001
Glucose, mmol/l	13.6 ± 1.0	13.3 ± 0.9	13.5 ± 1.2	13.2 ± 0.7	12.1 ± 0.6	10.8 ± 0.6	0.142
TG, mmol/l	2.38 ± 0.19[a]	2.34 ± 0.190.18[a]	1.67 ± 0.11[b]	1.54 ± 0.06[b]	1.42 ± 0.04[b]	2.12 ± 0.11[a]	0.001
TBARS, μmol/l	30.9 ± 3.0[a]	30.7 ± 3.3[a]	22.4 ± 1.0[b]	23.6 ± 2.0[b]	23.1 ± 1.0[b]	24.3 ± 1.5[b]	0.013

Data are means ± SE; $n = 8$ or 9 rats/group. CTX-1, COOH-terminal telopeptides of type 1 collagen; IGF-I, insulin-like growth factor-I; TG, triglyceride; TBARS, thiobarbituric acid reactive substances. Mean values within a parameter not sharing a common superscripted letter were considered significantly different ($P < 0.05$). For example, data with the superscript a were significantly different from those without the superscript a.

groups ($P < 0.05$); however, no difference in total vBMD was found between the MetR and MetR + Exe groups. Additionally, the Exe group had higher cancellous vBMD than the Ovx and MetR + Exe groups ($P < 0.05$). The Ovx group had lower total and cancellous BMC compared with the Sham group ($P < 0.05$). The Exe and Est groups had higher total BMC compared with the Ovx group ($P < 0.05$), whereas the Est group had higher total BMC than the MetR + Exe group ($P < 0.05$).

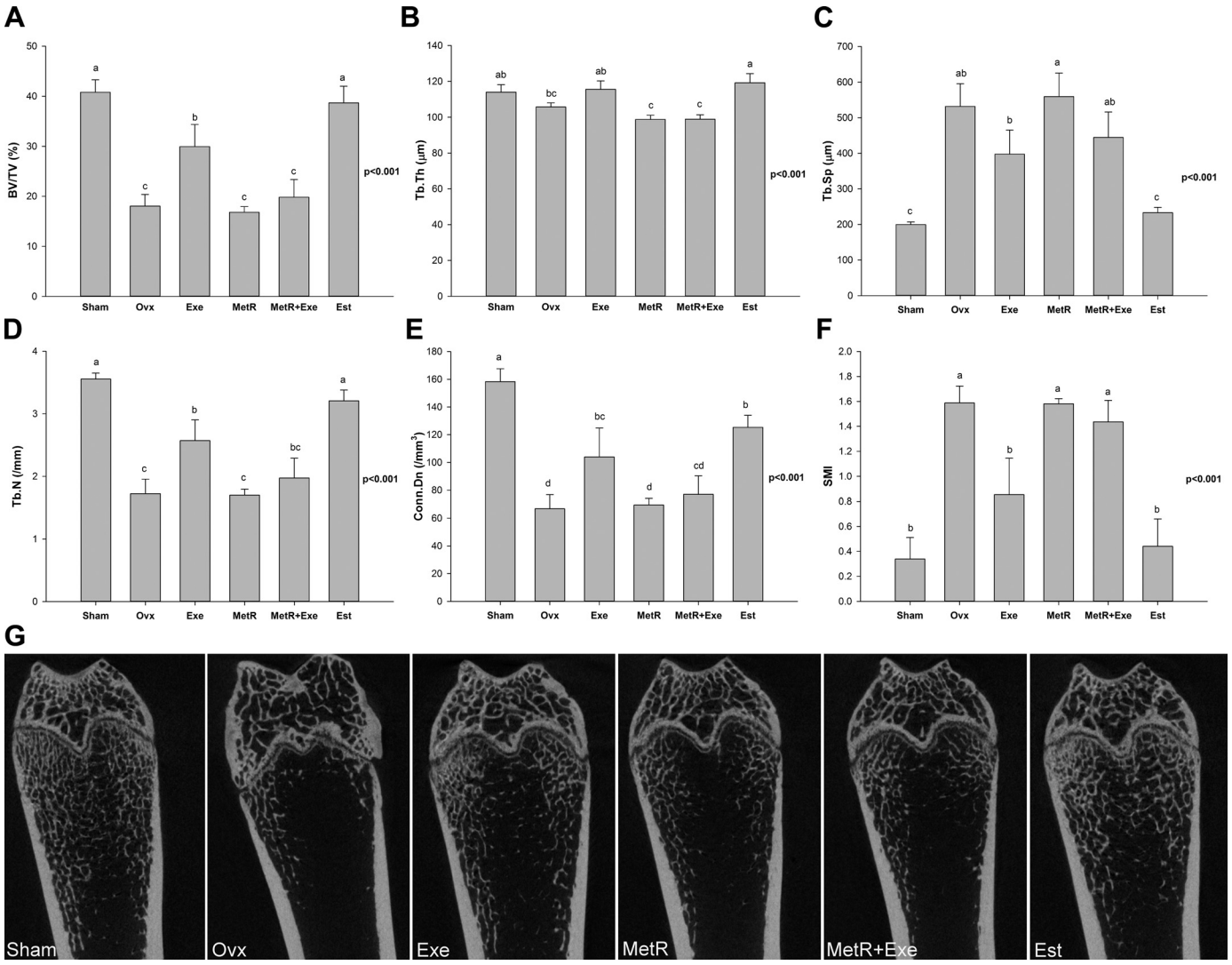

Fig. 2. Histomorphometry of metaphyseal cancellous bone from distal femora. Architectural indexes including bone volume over total volume ratio (BV/TV; *A*), trabecular thickness (Tb.Th; *B*), trabecular separation (Tb.Sp; *C*), trabecular number (Tb.N; *D*), connectivity density (Conn.Dn; *E*), structure model index (SMI; *F*), and frontral microcomputed tomography (μCT) slices of distal femora (*G*) were acquired from μCT analyses. Data are means ± SE; $n = 8$–9 experiments. Mean values within a parameter not sharing a common superscript (a–d) were considered significantly different ($P < 0.05$). For example, data with the superscript "a" were significantly different from those without the superscript a.

Table 5. *Dynamic histomorphometry of cancellous bone and cortical bone in tibiae*

	Sham	Ovx	Exe	MetR	MetR + Exe	Est	*P* Value
Metaphysis							
MS/BS, %	8.82 ± 1.63[b,c]	20.39 ± 2.50[a]	14.81 ± 2.06[a,b]	16.56 ± 2.92[a]	14.11 ± 2.59[a,b]	6.66 ± 0.86[c]	0.001
MAR, μm/day	9.22 ± 2.53	15.36 ± 2.42	10.50 ± 3.03	10.10 ± 3.26	6.76 ± 1.93	5.95 ± 1.26	0.142
BFR/BS, $\mu m^3 \cdot \mu m^{-2} \cdot day^{-1}$	0.99 ± 0.30[b]	3.34 ± 0.60[a]	1.70 ± 0.61[b]	1.30 ± 0.49[b]	1.25 ± 0.49[b]	0.44 ± 0.14[b]	0.002
N.Oc/BS, 1/mm	1.23 ± 0.17[c]	3.17 ± 0.41[b]	2.59 ± 0.34[b,c]	4.32 ± 0.58[a]	3.29 ± 0.40[a,b]	1.70 ± 0.26[c]	<0.001
Endosteum							
MS/BS, %	4.84 ± 0.81[c]	13.09 ± 1.71[a]	10.14 ± 2.80[a,b]	6.49 ± 1.23[b,c]	6.36 ± 1.21[b,c]	6.40 ± 1.04[b,c]	0.005
Periosteum							
MS/BS, %	5.46 ± 2.30[b]	9.94 ± 3.04[a,b]	15.86 ± 4.81[a]	8.12 ± 2.93[a,b]	7.38 ± 2.64[b]	1.83 ± 1.15[b]	0.044

Data are means ± SE; $n = 8$ or 9 rats/group. BFR/BS, bone formation rate; MAR, mineral apposition rate; MS/BS, bone mineralization over bone surface; N.Oc/BS, no. of osteoclasts relative to the trabecular tissue perimeter. Mean values within a parameter not sharing a common superscript were considered significantly different ($P < 0.05$). For example, data with the superscript a were significantly different from those without the superscript a. Dynamic histomorphometic analyses were done in metaphyseal cancellous bone of proximal tibia and the cross section of midshaft tibia.

Cancellous BMC was higher in the Exe and Est groups compared with the Ovx group and the two MetR groups ($P < 0.05$) (Table 6).

In dimensional measurements, the Ovx group had lower Ct.Ar, $CSMI_{min}$, and Ct.Th compared with the Sham group when normalized to body mass. The MetR and MetR + Exe groups had significantly higher dimensional parameters ($P < 0.05$) than most of the other ovariectomized groups. Finally, the Exe and Est groups had similar dimensional parameter values, the majority of which were higher than those from the Ovx group ($P < 0.05$) (Table 6).

Bone Biomechanical Properties

Indexes of extrinsic bone strength were also normalized to body mass. The Ovx group showed significantly lower YL, FL, and FLE and marginally lower stiffness ($P = 0.056$) compared with the Sham group (Fig. 3). The four treatment groups had higher values for YL, FL, FLE, and stiffness compared with the OVX group ($P < 0.05$), with the exception of numerical differences between the Ovx and Exe groups' FLE ($P = 0.1$) and stiffness ($P = 0.061$). Moreover, the two MetR groups were found to have higher YL and FL, respectively, compared with the Est and Exe groups ($P < 0.05$). The MetR group had higher stiffness ($P < 0.05$) than the EXE groups. Intrinsic biomechanical properties were not normalized to body mass, and no differences were observed among groups.

DISCUSSION

As in previous studies (1, 24, 36), MetR diet in the current study showed significant efficiency in body weight control of estrogen-deficient rats, which was stronger than that mediated by endurance exercise and hormone replacement therapy. In addition, the downregulation of serum insulin, TG, leptin, and TBARS by the MetR diet was in agreement with its effects on energy metabolism, as reported in previous studies (1, 20, 24, 29, 36). Whereas endurance exercise kept the estrogen-deficient female rats in a physiological status comparable to that of the Sham group, the effects of the MetR diet on energy metabolism were more pronounced. Despite such strong MetR diet and endurance exercise effects on metabolic parameters, the properties of bones in ovariectomized rats subjected to a MetR diet and endurance exercise improved relative to body mass (Table 6 and Fig. 3, *A–E*). Reports stating that a higher body weight (e.g., obesity) maintains BMD and BMC and protects from bone fracture risk (17, 39) are controversial, since metabolic disturbances accompanied with obesity (e.g., diabetes, inflammatory responses) can impair bone quality (8). Although obese postmenopausal women show a lower inci-

Table 6. *Densitometric and dimensional measurements in femora*

	Sham	Ovx	Exe	MetR	MetR + Exe	Est	*P* Value
Densitometry							
Tt.BMD, g/cm^3	0.997 ± 0.006[b]	0.994 ± 0.012[b]	1.035 ± 0.014[a]	1.034 ± 0.006[a]	1.010 ± 0.011[a,b]	0.994 ± 0.008[b]	0.004
Tt.BMC, mg	472.1 ± 10.6[a]	396.4 ± 11.0[d]	457.4 ± 11.7[a,b,c]	425.7 ± 12.2[b,c,d]	419.5 ± 14.5[c,d]	460.3 ± 17.8[a,b]	0.002
Ct.BMD, g/cm^3	1.208 ± 0.008[b]	1.190 ± 0.011[b]	1.255 ± 0.013[a]	1.241 ± 0.007[a]	1.210 ± 0.010[b]	1.198 ± 0.010[b]	<0.001
Ct.BMC, mg	9.7 ± 0.2	9.1 ± 0.3	10.1 ± 0.2	9.7 ± 0.3	9.4 ± 0.3	9.8 ± 0.3	0.168
Cn.BMD, g/cm^3	0.656 ± 0.006[a,b]	0.641 ± 0.011[b]	0.686 ± 0.017[a]	0.655 ± 0.007[a,b]	0.636 ± 0.005[b]	0.664 ± 0.014[a,b]	0.046
Cn.BMC, mg	11.7 ± 0.8[a]	4.1 ± 0.4[b]	9.0 ± 1.3[a]	4.6 ± 0.3[b]	5.1 ± 0.9[b]	10.8 ± 1.4[a]	<0.001
Dimension							
Ct.Ar, mm^2/body wt	17.2 ± 0.7[b,c]	14.9 ± 0.3[d]	18.7 ± 0.5[b]	20.8 ± 0.8[a]	21.5 ± 0.7[a]	18.4 ± 0.9[b,c]	<0.001
$CSMI_p$, mm^4/body wt	47.0 ± 2.2[c,d]	40.8 ± 2.5[d]	51.1 ± 2.1[b,c]	55.9 ± 2.3[a,b]	58.8 ± 3.3[a]	49.2 ± 2.4[b,c]	<0.001
$CSMI_{max}$, mm^4/body wt	28.9 ± 1.3[c,d]	25.9 ± 1.6[d]	31.9 ± 1.2[b,c]	34.9 ± 1.5[a,b]	37.4 ± 2.2[a]	31.5 ± 1.6[b,c]	<0.001
$CSMI_{min}$, mm^4/ body wt	18.1 ± 1.0[b]	14.9 ± 1.0[c]	19.2 ± 1.1[a,b]	21.0 ± 0.9[a]	21.4 ± 1.1[a]	17.6 ± 1.0[b,c]	<0.001
Ct. Th, mm/body wt	1.52 ± 0.07[b]	1.30 ± 0.03[c]	1.68 ± 0.05[b]	1.87 ± 0.08[a]	1.92 ± 0.04[a]	1.67 ± 0.09[b]	<0.001

Data are means ± SE; $n = 8$ or 9 rats/group. Denstitometric measurements were conducted in total, cortical, and cancellous bone of femora. Dimensional measurements were done at midshaft femur. Ar, area; BMC, bone mineral content; BMD, bone mineral density; Cn, cancellous; $CSMI_{max}$, $CSMI_{min}$, and $CSMI_p$, maximal, minimal, and polar cross-sectional moment of inertia, respectively; Ct, cortical; Tt, total; Ct.Th, cortical thickness. Mean values within a parameter not sharing a common superscript were considered significantly different ($P < 0.05$). For example, data with the superscript a were significantly different from those without the superscript a.

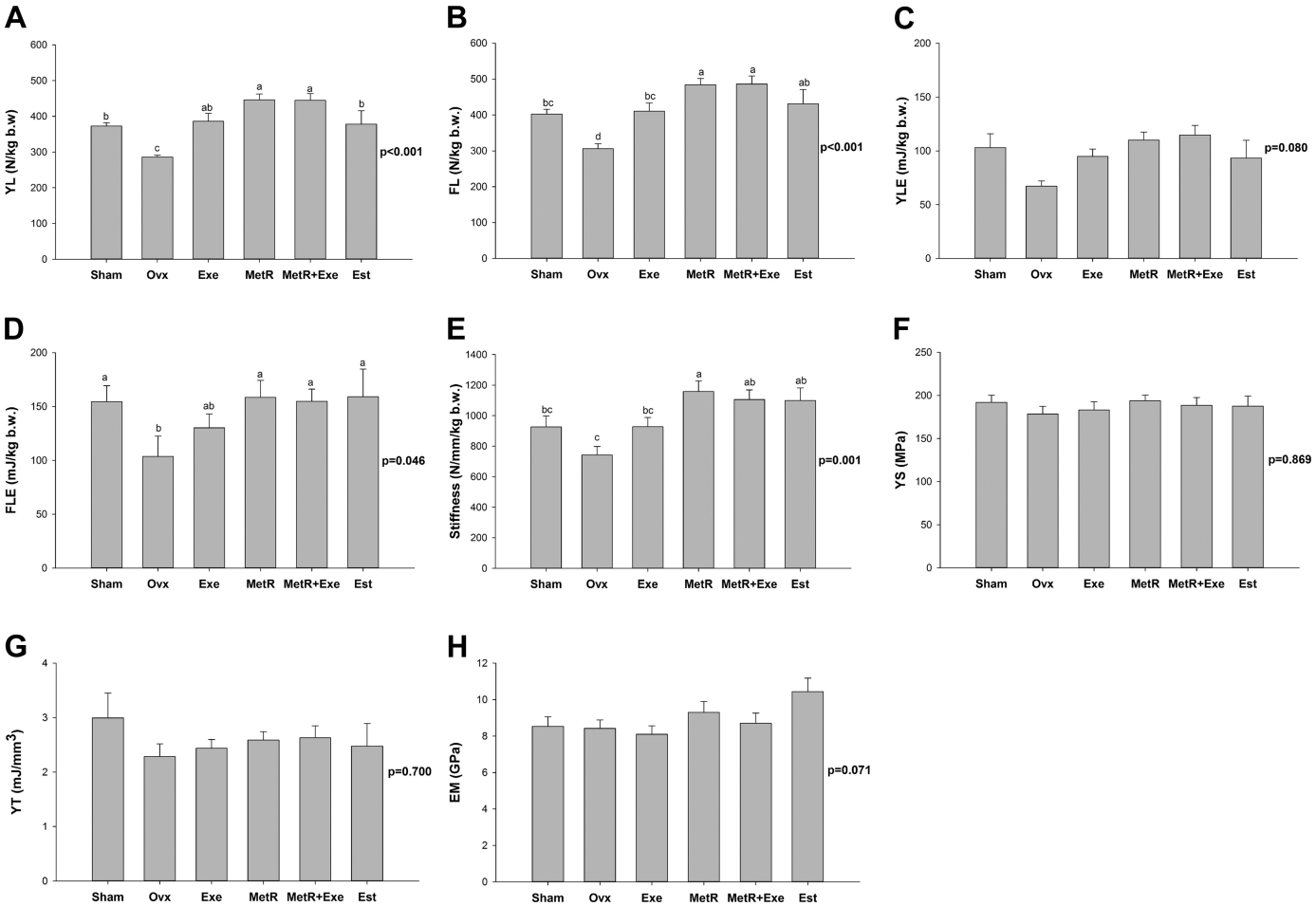

Fig. 3. Bone biomechanical properties in femora. Extrinsic biomechanical properties normalized to body mass included yield load (YL; *A*), fracture load (FL; *B*), yield load energy (YLE; *C*), fracture load energy (FLE; *D*), and stiffness (*E*), and indexes of intrinsic biomechanical properties included yield stress (YS; *F*), yield toughness (YT; *G*), and elastic modulus (EM; *H*). Data are means ± SE; $n = 8$–9. Mean values within a parameter not sharing a common superscript (a–c) were considered significantly different ($P < 0.05$). For example, data with the superscript a were significantly different from those without the superscript a.

dence of wrist fractures than nonobese women, major fractures in the ankle and upper leg were reported to be more common in obese postmenopausal women (14). Therefore, body weight has been used to normalize the mechanical properties and dimensional indexes of bones (48, 55). In this study, bone dimensions and biomechanical properties of MetR-fed and exercise-trained rats were relatively larger and stronger, respectively, when normalized to body weight (Table 5 and Fig. 3, *A*–*E*). Taken together, the ameliorated metabolic status and lower body weight induced by the MetR diet and endurance exercise could, respectively, benefit bone quality and reduce obesity-associated fracture risk.

The entire metabolic status should be taken into consideration when estimating bone health. Although the MetR diet caused a slight, yet significant, increase in local osteoclast density in cancellous bone, both the MetR diet and endurance exercise mitigated the effects of ovariectomy on local cancellous bone formation rate to a level comparable to that of Sham and Est groups. Systemically, the MetR diet and endurance exercise appeared to lower bone resorption activity, since *1*) there were no differences in serum CTX-1 between the Exe and MetR groups compared with the Sham group, and *2*) the Exe and MetR groups had 12–19% lower serum CTX-1 compared with the Ovx group (Table 4).

Energy metabolism and inflammation also play roles on bone mineral homeostasis (8, 53). In the current study, the MetR diet and endurance exercise lowered levels of insulin, leptin, TG, and TBARS, suggesting a downregulation of signaling pathways that control energy metabolism and oxidative stress. It is well known that the upregulation of insulin levels is associated with metabolic syndrome (25, 37). Although obese or type 2 diabetic individuals show normal or higher bone accumulation (17, 39), obesity in postmenopausal women with type 2 diabetes is correlated with serious cortical bone porosity and loss of bone strength (7, 33). Although the evaluation of bone collagen cross linking was not conducted in this study, increased advanced glycation end products following the onset of menopause and type 2 diabetes has been suggested to impair bone remodeling and bone material properties (42, 57). Therefore, dietary restriction or endurance exercise may not dominantly favor bone mineral accumulation (13, 24, 44). However, according to published reports, the downregulation of metabolic tones (e.g., lower insulin, IGF-I, and inflammatory proteins) (6, 24, 29, 30, 38, 47) could make the organism more resistant to estrogen deficiency-caused hormone imbalances, inflammation, and detrimental effects on the skeleton (27, 53). Further studies are needed to investigate whether the lower body weight and changes in metabolic parameters observed in

MetR-fed and endurance exercise-trained rats can help maintain better matrix organization and, ultimately, better bone material properties.

The downregulation of leptin and insulin through endurance exercise was not as significant as that observed in MetR-fed rats. However, endurance exercise shifted the physiological profile to a level relatively close to or slightly better than that of the Sham and Est groups. Because the Exe group was fed a control diet ad libitum, it is suggested that endurance exercise alone is capable of counteracting estrogen deficiency-related disturbances on metabolism and bone morphology. In previous studies, endurance exercise has been reported to be inefficient (26, 45) or beneficial (4, 43) in the preservation of bone mineral of ovariectomized rodents. Reasons for the inconsistency could be the inherent defect of densitometry and/or the design of exercise training protocols. Conventionally, clinical densitometry, such as dual energy X-ray absorptiometry, provides areal BMD (aBMD) measurements, which ignore tissue thickness. Thus, the tissue density of bones with bigger size, which have greater tissue thickness, would be overestimated when measured by an aBMD densitometer. It has also been suggested that higher body weight can partially contribute to higher aBMD in postmenopausal women (18). To minimize the overestimation of aBMD due to size-related factors and obesity, we used a volumetric densitometer in this study and demonstrated that endurance exercise and the MetR diet independently benefitted vBMD indexes in ovariectomized rats. However, one should also be aware of overestimation of dimensional indexes (e.g., Ct.Th) or histomorphometric measurements (e.g., BV/TV, Tb.Th, etc.) coinciding with higher tissue vBMD, which could occur in μCT analysis when using a consistent global threshold (gray scale: 66~255) (5). With regard to exercise training effects, there is the possibility that the training regimen (e.g., intensity, volume, or timing) used in some studies may not have been efficient enough to neutralize the vigorous metabolic impact produced during the initial phase of estrogen deficiency (26, 45). Therefore, in the current study, we introduced treadmill running training 2 wk before ovariectomy surgery. With this modification, we were able to demonstrate that endurance exercise alone can benefit both cancellous bone and cortical bone in measurements of histomorphometry or densitometry.

Endurance exercise combined with the MetR diet had no additive effects. In fact, the responses observed in the MetR + Exe group were comparable to those observed in the MetR group. As shown in published studies, the MetR diet and endurance exercise have similar effects on energy metabolism (1, 6, 20, 21, 29), inflammation and oxidative status (9, 28, 30, 38, 47, 52), aging-related diseases (3, 10, 16, 35), and bone metabolism (1, 24, 56, 58). Also, the MetR diet appears to have a relatively stronger impact on metabolism according to the current data on body weight gain and serum markers (e.g., insulin and leptin). Similar phenomena have also been shown in young growing male rats subjected to interventions of endurance exercise and the MetR diet (24).

In conclusion, the MetR diet and endurance exercise displayed different beneficial effects on metabolism and body weight control in ovariectomized rats. Both the MetR diet and endurance exercise ameliorated estrogen deficiency effects on cortical bone, but only endurance exercise preserved cancellous bone. The combination of the MetR diet plus endurance exercise showed similar efficiencies as those in the MetR diet alone group. Further studies would be valuable to optimize the combination of dietary MetR and endurance exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: T.H.H. conception and design of research; T.H.H. and I.-H.S. performed experiments; T.H.H., I.-H.S., and G.P.A. analyzed data; T.H.H., I.-H.S., J.L.L., M.-S.C., A.-T.H., C.E.P., and G.P.A. interpreted results of experiments; T.H.H. prepared figures; T.H.H. and C.E.P. drafted manuscript; T.H.H. and C.E.P. edited and revised manuscript; T.H.H., I.-H.S., J.L.L., M.-S.C., A.-T.H., C.E.P., and G.P.A. approved final version of manuscript.

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