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Effects of High-Fat Diet on Mature Bone Mineral Content, Structure, and Mechanical Properties

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Abstract. Diets with a high saturated fat content can produce deleterious effects on the absorption of dietary calcium and consequently an adverse effect on bone mineralization in growing animals. Hence dietary fat may have long-term consequences for skeletal health and skeletal pathologies such as osteoporosis. Whether a diet high in saturated fat has similar negative effects on adult bone, however, remains unresolved. Thus, we investigated effects of a high-fat diet on mature bone structure and mechanics. Adult (40-week-old) roosters were maintained for 20 weeks on either a diet high in saturated fat (HF) or a low-fat (LF) diet. Cortical bone samples (tarsometatarsus) were tested mechanically in three-point bending, and cancellous bone cores from the femoral condyles and tibial plateau (four sites per knee) were tested mechanically in compression. Cortical bone cross-sectional areal data were also compared among the groups, and bone mineral content (BMC) was determined (by ashing) for both cortical bone and cancellous bone samples. There were no significant high-fat diet effects on mature cortical bone mechanical properties, geometric structure, or mineral content. Diet, however, did affect cancellous bone composition. For example, LF cancellous BMC was significantly greater than HF. Mechanical properties of the cancellous bone showed similar trends such that LF cancellous bone strength was consistently greater than HF. The potential for adverse effects of a HF diet on intestinal calcium absorption in the mature animal may be more apparent in cancellous bone, with its faster rate of turnover, than in cortical bone. Changes in cancellous bone structure and mechanical properties, related to dietary saturated fats, may have implications for understanding the role of nutrition in skeletal health and prevention of pathological bone loss (osteoporosis).

Key words: Saturated fat — Bone mechanics — Cortical bone — Cancellous bone — Osteoporosis.

The incidence of osteoporosis-related fractures in North America is increasing dramatically [1, 2]. Though this increase is partially attributable to an aging population [3], it has been suggested that the increase in fracture occurrence is disproportionately high [2]. Among other factors (i.e., genetics and hormones), nutrition—including dietary fat—may contribute to the likelihood of osteoporosis-related fractures [4, 5]. High fat diets are pervasive in North America and Europe [4, 6]. Total dietary fat levels have increased in the last half-century, and though the shift in fat content has been toward polyunsaturated fatty acids, saturated fats still constitute approximately 35% of total fat intake [6].

Zernicke et al. [4, 5, 7, 8] have shown in growing rats and Atteh et al. [9, 10] have shown in growing chicks that a diet high in saturated fat (or high in saturated fat and sucrose) adversely affects the absorption of dietary calcium and consequently deleteriously affects bone mineralization. Atteh et al. fed young roosters diets supplemented with 8% palmitic acid (a 16 chain saturated fatty acid) for 3 weeks and found a significant reduction in bone ash mass and bone calcium content [9]. Even when more calcium was added to the fat-rich diet, it was not absorbed, and the extra dietary calcium was excreted. Zernicke et al. reported a significant reduction in material and structural cortical bone properties for growing rats fed a diet high in saturated fat and sucrose (HFS) compared with rats fed a diet with low-fat and complex carbohydrates [4, 5, 7, 8]. Among the proposed mechanisms underlying the HFS diet-related changes in bone are hypercalciuria resulting from hyperinsulemia [11, 12] and reduced intestinal calcium absorption [10, 13]. In these studies, the decrease in mineralization compromised the structural and material properties of immature bone [4, 5, 7, 8]. No one, however, has demonstrated with an experimental model that the adverse effects of a high-fat diet on adult bone are comparable to those of growing animals.

Thus, the purpose of this study was to contrast the effects of high- and low-fat diets on the structure and mechanics of adult rooster bone. We hypothesized that a high-fat diet would reduce mineral content in mature bone, and would therefore result in a bone with less strength and more compliance than bone exposed to a low-fat diet. Specifically, we investigated the influence of the high- and low-fat diets on weight-bearing cortical and cancellous bones.

Materials and Methods

Animals

A total of 30 White-Leghorn roosters obtained from OK Poultry Farms, Lynden, Alberta at 20 weeks of age were used. They were fed standard vivarium chow and gang housed in two large pens at the University of Calgary farm until 40 weeks of age. At that time all 30 roosters were moved to the university vivarium where they were weighed and separated, by weight matching, into one of three groups: (1) basal control (BC), (n = 8), (2) LF diet (n = 11), and (3) HF diet (n = 11). The BC group was culled as a ''baseline''

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Table 1. LF and HF diet ingredients and nutrient content[a]

		LF (%)	HF (%)
Diet ingredients	Basal ingredients		
	Corn	27.42	27.42
	Corn gluten meal	10.00	10.00
	Corn starch	25.91	25.91
	Wheat shorts	14.20	14.20
	Fishmeal	8.00	8.00
	Dicalcium phosphate	0.60	0.60
	Iodized salt (0.011% COI_2)	0.25	0.25
	Lysine	0.45	0.45
	Tryptophan	0.05	0.05
	Mineral-vit, premix	0.75	0.75
	Total	87.63	87.63
	Palmitic acid (95.1%)	—	8.41
	Limestone	1.39	1.39
	Cellulose	10.98	2.57
	Total	100.00	100.00
Nutrient content	Crude protein	18.26	18.95
	Ash	4.07	4.29
	Moisture	8.69	9.00
	Crude fat	<1	10.00

[a] Modified from Atteh and Leeson, 1984. Palmitic acid in the HF diet (diet 8 from Atteh and Leeson) was replaced by alpha floc (wood) cellulose in the LF diet (diet 2 from Atteh and Leeson). LF = low fat, HF = high fat

control, and the remaining two groups were housed in two large pens. For 20 weeks (rooster age 41–61 weeks) half of the remaining roosters were fed a LF diet, and the other half were maintained on a HF diet rich in saturated fats (8% palmitic acid) (Table 1), modified from Atteh and Leeson's Diet 8 [9]. The HF diet fat content was 95.1% pure tri-palmitate (Emersol 143 Palmitic Acid, Henkel Canada Limited, Mississauga, ON). In the LF diet, fatty acids were substituted by alpha floc cellulose. Other ingredients of the two diets, including vitamin and mineral supplements, were the same. Diets were mixed monthly (Poultry Unit, University of Alberta, Edmonton, AB) and stored at 4 °C. The diets mixed for this study were analyzed for fatty acid (FA) content, crude protein, ash, moisture, and crude fat (Table 1). Feed consumption levels were monitored, and birds were weighed weekly.

A double fluorochrome marker (tetracycline HCL, 30 $mg \cdot kg^{-1}$, I.V.) was given to all roosters at 39 and 40 weeks just prior to culling the BC group and as the HF and LF diets were initiated. A second double label (calcein green, 15 $mg \cdot kg^{-1}$, I.V.) was given at 50 and 51 weeks, and a third double label (xylenol orange, 8 $mg \cdot kg^{-1}$, I.V.) was given at 60 and 61 weeks. Label injections were given on a 9/2 schedule (i.e., 1 week between injections and, in the cases of tetracycline and xylenol orange, 2 days prior to euthanizing the roosters). All roosters were euthanized by lethal overdose (Euthanyl, I.V.).

Bilateral limb bones (femur, tibiotarsus, tarsometatarsus–TMT) were harvested, dissected clean of soft tissue, hermetically sealed in plastic bags, and frozen (−30°C) until the day of mechanical testing.

Bone Mechanical Testing

On the day of testing, right-side limb bones were thawed (21°C). To assess cortical bone mechanical properties, TMT bones were tested in three-point bending. Bone lengths were measured (±30 μm) with a precision caliper (Brown and Sharpe, North Kingstown, RI) and midlengths were marked on the diaphyses with ink. Bones were temperature equilibrated (37°C) in physiological buffer solution (PBS, pH 7.4) for at least 1 hour prior to testing. A servo-controlled electromechanical testing system (Model 1122, Instron Corporation, Canton, MA) loaded each bone in three-point bending (42.3 $mm \cdot s^{-1}$) until failure. The three-point intersupport distance for the TMTs was 45 mm, and they were loaded anterior (compression surface) to posterior (tensile surface). A small preload was applied to each bone to ensure that the bones rested securely on the support points and did not shift upon full load application.

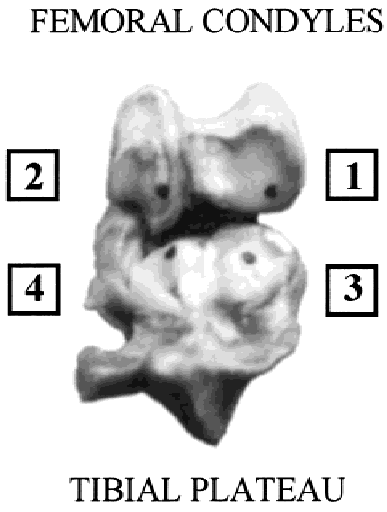

Fig. 1. Subchondral bone cores. Bone cores were taken from the (1) medial femoral condyle, (2) lateral femoral condyle, (3) medial tibial condyle, and (4) lateral tibial condyle.

To assess subchondral bone mechanical properties, we used a stainless-steel coring bit to extract four cylindrical dowels of subchondral/cancellous bone (2-mm diameter) from the joint surface of the right distal femur and proximal tibiotarsus (tibia). One core was removed from each of the medial and lateral condyles of the femur and tibia (Fig. 1). PBS (pH 7.4, 21°C) was forced through the core of the drill bit to moisten and cool the specimen during drilling. The uneven ends of the core were cut to produce a 4-mm long dowel of cancellous bone. While immersed in PBS, each dowel of cancellous bone was compressed (Instron 1122, Canton, MA) to 50% of its original height at a rate of 50% $strain \cdot s^{-1}$ [14].

Analog load-time data from the Instron (from three-point bending and subchondral bone compression tests) were converted to digital data at a sample rate of 2 kHz (IBM-AT, Boca Raton, FL, and Computerscope ISC-16, RC Electronics, Santa Barbara, CA). Custom-designed programs (Run Technologies, Laguna, CA) were used to calculate structural and material properties. For three-point bending, we calculated maximal load, tensile linear stress (stress on the tensile surface of the bone at the point where the load-deformation curve becomes nonlinear), and flexural rigidity for each TMT. For the cancellous bone, we calculated maximal load, stiffness, and compressional stress of each bone core.

Cross-sectional Areal Analysis

Using a low-speed (130 $rev \cdot min^{-1}$) slotting saw (Unimat 3, EMCO, Colombus, OH), a 1-mm cross-section wafer was cut from the middiaphysis of each left (contralateral) TMT. Radiographs were taken of each wafer (Kodak SR-5, Eastman Kodak, Rochester, NY, cabinet X-ray system—faxitron, Hewlett-Packard Co., McMinnville, OR) and then scanned into digital TIFF image files (PC/P5-60, Gateway 2000, N. Sioux City, SD, MasterScan Interpretive Densitometer/Camscan, Scanalytics, Billerica, MA). Digital images were then converted and custom-designed pixel counting routines were used to quantify areal properties (PV-WAVE, Visual Numerics Inc., Houston, TX). Total bone cross-sectional

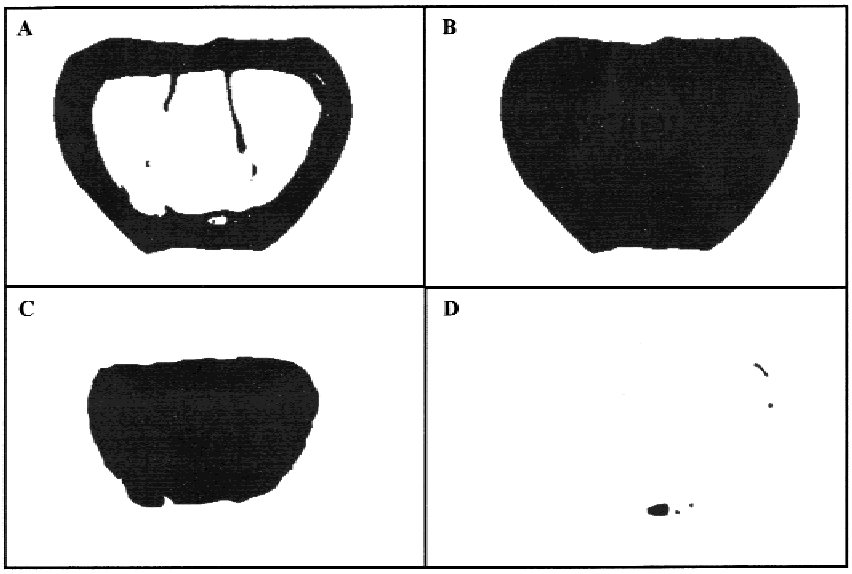

Fig. 2. Cross-sectional area measures for morphometry and bending calculations; the area of consideration in each case is black. **(A)** Total bone XSA; **(B)** area within the periosteal envelope; **(C)** area within the endosteal envelope (marrow space); **(D)** area of intracortical porosities.

area (XSA) (Fig. 2), endosteal and periosteal envelopes, area of intracortical porosities, second moment of area about the medial-lateral axis, and *c*-distance from the neutral (medial-lateral) axis to the tensile surface were determined for each section.

A 250 μm middiaphyseal cross-section was also cut (Isomet low speed saw, Buehler, Lake Bluff, IL) from each left TMT. The sections were then hand ground to 100 μm and viewed under fluorescent light (Leitz Wetzlar, Germany). Each section was assessed qualitatively for the presence of label at the periosteal and endosteal surfaces, and the number and size of labeled intracortical osteons.

Ash Content

Dry and ash masses of the 1-mm middiaphyseal cross-sections and cancellous bone cores were determined with the procedures described by Atteh et al. [10]. The samples were defatted (100% ethanol) for two weeks. Each bone section was then dried at 100°C for 24 hours and later ashed at 600°C for 24 hours (Model 62700, Barnstead/Thermodyne, Dubuque, IA). Bone mineral content was calculated as the ratio of ash mass to dry mass.

Data Analysis and Statistics

For each animal, cancellous bone data from the four test sites were averaged to produce an aggregate cancellous bone value for each of maximal load, stiffness, and ash content. Only these aggregate data were used in the statistical analyses for the cancellous bone results. Multivariate analysis of variance (ANOVA) (SPSS Inc., Chicago IL) was used to detect significant main effects and interactions in the morphological and biomechanical dependent variables. Posthoc comparisons with Kruskal-Wallis nonparametric one-way ANOVA revealed locations of significant differences in the main-effect means. A significance level of $p < 0.05$ was used for all statistical tests.

Results

At 62 week of age, HF rooster body mass (2.6 ± 0.20 kg) was significantly greater than the 41 week old BC roosters (2.1 ± 0.23 kg), and LF rooster mass (2.3 ± 0.23 kg) was not significantly different from BC. During dissections, we also observed greater fatty deposits throughout the body cavities of the HF roosters than the other two groups.

There was no significant difference in the maximal bending load required to break the HF and LF TMT (Table 2). Both HF and LF maximal bending loads, however, were significantly greater than BC. Similarly, HF flexural rigidity was not significantly different from LF, but both HF and LF TMT flexural rigidities were significantly greater than BC. Tensile linear stress was not significantly different among the three groups.

Both HF and LF TMT had significantly larger XSAs than BC (Table 2), but, as with the load data, LF and HF XSA were not statistically different. The same was true for the second moment of area about the medial-lateral axis—HF and LF were significantly greater than BC. The LF periosteal envelope was significantly larger than BC, but there was no statistical difference between HF and the other two groups. There were no significant differences among the groups for area within the endosteal envelope (marrow space). The cross-sectional area displaced by intracortical porosities in the BC TMT was significantly greater than in LF and HF TMT, but the area of intracortical porosities was not significantly different between LF and HF. TMT bone mineral content was consistent with the trend in mechanical properties; there was no significant difference between HF and LF.

Fluorochrome Labels

Xylenol orange, the final label given to the HF and LF birds at weeks 61 and 62, did not show in the bone cross-sections because the dose was too low ($8\ mg \cdot kg^{-1}$). The earlier tetracycline and calcein green labels, however, were effective. The BC TMT had some periosteal tetracycline label, primarily on the medial and lateral surfaces, but no endosteal label. There were many large tetracycline-labeled osteons in the BC TMT that were positioned mostly toward the endosteal surface, although a few were also seen around the posteromedial ridge.

Tetracycline label was visible in both HF and LF sections and had a pattern similar to the BC labeling, although

Table 2. Cortical bone (TMT) mechanical properties, geometric measures, and mineral content

Measure	BC	LF	HF
Maximal load (N)	419 ± 82.8	530 ± 85.4[a]	555 ± 67.7[a]
Flexural rigidity ($N \cdot m^2$)	0.71 ± 0.13	0.93 ± 0.14[a]	0.96 ± 0.14[a]
Tensile linear stress (MPa)	96.4 ± 22.17	99.2 ± 12.87	112.9 ± 16.99
XSA (mm^2)	20.6 ± 2.65	23.9 ± 1.59[a]	23.1 ± 1.55[a]
Periosteal envelope (mm^2)	40.7 ± 2.51	46.3 ± 4.01[a]	44.2 ± 3.62
Endosteal envelope (mm^2)	19.4 ± 1.82	22.4 ± 2.90	21.0 ± 3.28
Porosity (mm^2)	0.60 ± 0.48	0.05 ± 0.05[a]	0.09 ± 0.10[a]
2nd Moment of area (mm^4)	76.7 ± 11.11	99.4 ± 14.19[a]	91.8 ± 12.15[a]
BMC (%)	65.0 ± 0.93	65.7 ± 0.46	66.3 ± 0.49[a]

There were no significant differences between HF and LF measures
BC = basal control, LF = low fat, HF = high fat
[a] $P < 0.05$, HF and LF measures that are significantly different from BC

some of the osteons were removed by subsequent remodeling between weeks 41 and 61. Noteably, the tetracycline-labeled osteons appeared partially resorbed at the endosteal surface. This apparent resorption was followed by formation of primary lamellar bone on the endosteal surface, as indicated by the calcein green label at the endosteum of every HF and LF TMT. Though all TMTs had endosteal calcein green label, there was rarely any double-calcein green label on the endosteum. The LF and HF TMT had larger XSA than their BC counterparts, but there was little periosteal calcein green label to indicate that the LF and HF groups had any significant periosteal apposition at the 10-week time point (age 50 weeks). The primary periosteal formation may have occurred early in the diet protocol (*between* the tetracycline and calcein green labels).

Cancellous Bone Cores

Mechanical properties and mineral content of the bone cores varied among the four test sites (medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau). LF maximal load tended to be greater than HF, though the difference (with the small sample size) was not statistically significant (Table 3). Both LF and HF maximal loads were significantly greater than BC. Similarly, LF core stiffness was generally greater than HF, but the difference was not significant. Both HF and LF stiffness were significantly greater than BC. A statistical power analysis indicated the sample size was too small ($\beta > 0.2$), and a larger sample size would have revealed the differences between the diet groups.

BMC differences paralleled the mechanical properties (Table 3). Both HF and LF cancellous BMCs were significantly greater than BC. For ash content, LF cancellous BMC was significantly greater than HF.

Discussion

After 20 weeks on their respective diets, HF roosters were significantly heavier (20%) than BC, but the difference (10%) between the average mass of the LF and HF roosters was not statistically different. Maximal load and flexural rigidity of the 62-week (HF and LF) TMT were significantly greater than the 42 week (BC) TMT, likely due to significantly larger cross-sectional area and reduced intracortical porosities at 62 weeks. There were, however, no significant differences in TMT material properties among the groups, and TMT measures were similar to those reported for other mature-rooster TMT cortical bone [15] (maximal load of 500–600 N compared with 545–660 N for this study) and tensile linear stress (72–78 MPa compared with 78–93 MPa for this study).

In contrast to cortical bone (TMT), significant diet effects were found in the weight-bearing cancellous bone of the rooster knee joint. We found that mechanical properties and mineral content of cancellous bone varied from location to location within the joint, a result similar to reports for cancellous bone in the joints of other species [16–20]. Despite these location-related variations, cancellous bone mechanical properties (maximal load and stiffness) and BMC were consistently and reliably greater in LF than in HF or BC. Both mechanical properties (maximal load and stiffness) and mineral ash content of the 62-week birds (HF and LF) were significantly greater than BC. More noteworthy, however, was the trend between HF and LF cancellous bone properties which was not apparent in the assessment of the cortical bone. All LF cancellous bone measures were greater than HF, and LF mineral content was significantly greater than HF. Thus, cancellous bone was apparently more sensitive to the high-fat diet than the cortical bone.

Our results highlighted the fact that adult cortical bone was not as sensitive to a high-fat diet as are growing animals. Atteh and Leeson [9] fed male broiler chicks diets containing 8% palmitic acid, oleic acid (18:1), or a 50:50 combination of both for a period of 3 weeks. Both oleic and palmitic acids formed intestinal soaps with calcium, but the calcium palmitate soap was used less effectively than calcium oleate. Fecal fatty acid content from the chicks fed the palmitic acid diet was 71% fatty acid soap compared with 8% in the oleic acid group and 18% in the control group. Consequently, there was a significant reduction in the tibiotarsus ash content of chicks fed diets supplemented with palmitic acid [9]. Atteh and Leeson did not assess bone mechanical properties, nor did they consider cancellous and cortical bone separately, thus, other direct comparisons between the studies are not possible.

Zernicke et al. [4, 5, 7, 8] reported a series of studies comparing rats fed diets high in saturated fat and sucrose (HFS) with those fed low-fat complex-carbohydrate diets (LFCC). In their studies with young, rapidly growing rats (8–18 weeks old), they found that HFS tibial mechanical properties were reduced, even though HFS and LFCC tibiae had similar geometry [7], indicating that the deleterious effects of the HFS diet were related to changes in material

Table 3. Cancellous bone mechanical properties and mineral content

Measure	BC	LF	HF
Maximal load (N)	32.9 ± 7.86	51.7 ± 6.03[a]	47.4 ± 4.80[a]
Stiffness (N/mm)	213 ± 52.1	347 ± 50.8[a]	313 ± 37.5[a]
BM Content (%)	46.6 ± 3.45	56.0 ± 2.04[a,b]	53.3 ± 1.56[a]

For each animal, cancellous bone data from each of the four test locations in the knee (medial and lateral femoral condyles and tibial plateau condyles) were averaged to produce an aggregate value for each measure (maximal load, stiffness, and BMC).
[a] $P < 0.05$, all cancellous bone measures, both LF and HF were significantly greater than BC.
[b] $P < 0.05$, significantly different between LF and HF mineral content (cancellous bone measures were greater than HF)

properties of the cortical bone as well as the femoral neck (FN) [4] and lumbar vertebra (L6) [5]. In a subsequent long-term study (2 years), Zernicke et al. [8] reported that, although the HFS rats had significantly greater mass than the LFCC rats, the HFS L6 and FN had significantly smaller cross-sectional areas. When normalized to body mass, mechanical properties of the L6 and FN were also significantly lower in the HFS compared with the LFCC group.

In rapidly growing animals, cortical bone modeling drifts quickly form new bone at the periosteal surface and resorb bone at the endosteal surface causing the cortical cross-section to become larger [21]. Bone formation rates for human children (1–9 years) and adolescent monkeys are in the range of 35–40% volume · year^{-1} [22]. During a 2-week period of rapid growth (6–8 weeks old), Zernicke et al. [23] reported that the female rat tibial XSA increased by approximately 23%, and the male tibial XSA by 48%. With the rapid new bone formation in the skeletally immature animal, nutritional deficiencies are more likely to affect the material content of bone.

Although modeling drifts can occur in mature bone as a response to changes in mechanical stimuli [22, 24], remodeling is the primary mechanism of mature bone turnover [25]. Remodeling does not increase bone mass but only maintains it [22, 25, 26], and the new bone formation rate is of an order of magnitude less for mature bone than for immature bone [22]. Thus, mature cortical bone is less likely to be affected as dramatically as immature cortical bone. Rather, in the adult, cancellous bone is more vulnerable to mineral fluctuations. Through its intimate contact with marrow vascularity and endosteal lining cells that initiate the remodeling cycle [22, 26], cancellous bone turnover (both formation and loss) occurs more rapidly than in cortical bone. The predilection of cancellous bone to a greater rate of bone loss than cortical bone has been demonstrated repeatedly in animal models and clinical studies of immobilization [27–29], weightlessness [29], nutritional deficiencies [30], hormone-mediated osteopenia [31, 32], and postmenopausal and senile osteoporosis [3, 33].

The roosters at 20 weeks old were assumed to be skeletally mature [34]. Both the LF and HF TMT XSA, however, were significantly greater than BC. The TMT periosteal and endosteal envelopes were greater for HF and LF than for BC suggesting that some periosteal formation and endosteal resorption (i.e., modeling) happened after 42 weeks. This modeling may have been a continuation of cortical bone growth, or it may have been a response to the dietary changes. Regardless, periosteal calcein green label was rare in either of the HF or LF bones, therefore the periosteal growth likely occurred early in the diet, before the calcein green label at 50 weeks. In contrast, all 60-week (both HF and LF) TMT had calcein green label around the entire endosteal surface, after which there was further endosteal bone apposition. Some of the tetracycline-labeled osteons near the endosteum in the HF and LF TMT were partially resorbed (endosteal resorption), and subsequent endosteal formation replaced the resorbed bone. Hence, the post-BC modeling was followed by endosteal formation (a reduction in the endosteal envelope). There were numerous calcein green-labeled secondary osteons in both HF and LF TMT. There was variability in the label of the cross-sections, and no marked differences were apparent between the HF and LF groups for the bone labeling.

This study investigated the effects of high- and low-fat diets on the structure and mechanical properties of adult bone. The adverse effects of a high-fat diet on intestinal calcium absorption have been demonstrated to reduce mineral content and structural and material properties of bone in growing animals. We found that the mature cortical bone was less sensitive to the high-fat diet than was immature bone. This disparity may have been related to the different nature of skeletal adaptation in young (modeling) versus adult (remodeling) bone. Significant differences did exist, however, between high- and low-fat cancellous BMC. The potential adverse effects of a HF diet on intestinal calcium absorption in the mature animal may be more apparent in cancellous bone, with its faster rate of turnover, than in cortical bone.

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