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Diets High in Fat or Fructose Differentially Modulate Bone Health and Lipid Metabolism

Aditi Jatkar¹, Irwin Kurland², and Stefan Judex¹

¹Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

²Department of Medicine, Albert Einstein College of Medicine, Bronx, New York, 10461 USA

Abstract

Diets high in fat or carbohydrates can lead to obesity and diabetes, two interrelated conditions that have been associated with osteoporosis. Here, we contrasted the effects of a high fat (HF) versus fructose-enriched carbohydrate (CH) versus regular chow (SC) diet on bone morphology, fat content and metabolic balance in BALB/cByJ mice over a 15 wk period. For 13 wk, there were no differences in body mass between groups with small differences in the last 2 wk. Even without the potentially confounding factor of altered body mass and levels of load bearing, HF consumption was detrimental to bone in the distal femur with lower trabecular bone volume fraction and thinner cortices than controls. These differences in bone were accompanied by 2-fold greater abdominal fat content and 4-fold greater plasma leptin concentrations. High-fat feeding caused a decrease in de-novo lipid synthesis in the liver, kidney, white adipose and brown adipose tissue. In contrast to HF, the fructose diet did not significantly impact bone quantity or architecture. Fructose consumption also did not significantly alter leptin levels or de-novo lipid synthesis but reduced epididymal adipose tissue and increased brown adipose tissue. Cortical stiffness was lower in the CH than in HF mice. There were no differences in glucose or insulin levels between groups. Together, a diet high in fat had a negative influence on bone structure, adipose tissue deposition and lipid synthesis, changes that were largely avoided with a fructose enriched diet.

Keywords

Trabecular Bone; Cortical Bone; High Fat Diet; Fructose; Lipid Synthesis

Introduction

Consumption of high-calorie diets promotes the development of obesity and related co-morbidities such as diabetes [1], conditions that have implications on other physiological systems including the skeleton. BMI, as a surrogate measure of obesity, mostly correlates

Correspondence to: Stefan Judex, Ph.D., Department of Biomedical Engineering, Bioengineering Building, Rm 213, Stony Brook University, Stony Brook, NY 11794-5281, Phone: 631-632-1549, FAX: 631-632-8577, stefan.judex@stonybrook.edu.

Conflict of Interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Standards

Human and Animal Rights and Informed Consent

All procedures involving animals were approved by the IACUC at Stony Brook University.

positively with bone density in humans [2], primarily because obese individuals have the advantage of the anabolic effect of increased mechanical loading on weight bearing bones [3].

Dietary interventions varying the relative proportions of carbohydrate, fat, and protein have been tested by their ability to improve healthy weight maintenance [4]. Eliminating non-nutritional factors such as compliance, recent clinical studies indicate that specific macronutrient content does not influence body mass and that overall caloric consumption is the primary determinant of BMI [5, 6]. Nevertheless, dietary composition can modulate other health related outcomes such as lipid synthesis, metabolic balance and bone mass independent of BMI [7].

Adipocytes and osteoblasts are derived from the same mesenchymal stem cell source [8], causing interactions between adipogenesis and osteoblastogenesis that can be influenced by dietary fat intake [9]. High-fat diet (HFD) significantly alters the bone marrow microenvironment, causing accumulation of adipose tissue in the marrow, altering the lineage potential of stem cells and reducing the number of B-cells [9–11]. In male mice and despite of increased body mass, HFD may deteriorate trabecular bone volume and architecture without significantly impacting the cortical compartment [12–14]. Other investigations showed that HFD initially increases bone deposition due to increased mechanical demand but consequently impairs bone formation and turnover due to metabolic dysregulation [15]. HFD induced adiposity is also a source of the hormone leptin which modulates bone deposition and is an important regulator of energy intake [16]. The effect of dietary fat on bone health is thus complex, depends on multiple factors besides body mass, and may be compartment-specific [13, 17].

Consumption of diets high in saccharides, such as aerated beverages and processed foods, causes glucose intolerance and changes in de-novo lipid synthesis and has been associated with poor bone health [7, 18]. In rats, administration of sucrose reduced calcium and phosphorous content in the tibia and compromised its breaking strength [19]. A combined high-fat sucrose diet increased body mass and fat while deteriorating bone mechanical and structural properties but no comparisons were performed between the two individual dietary components [20]. When contrasting the effects of fructose versus glucose administration, bone's microarchitecture and strength were enhanced in fructose-fed rats [21], however, normal control animals were not included in this study. Regardless, fructose does not appear to exacerbate bone loss caused by consumption of a high-fat diet [22]. Together, these studies suggest that saccharide consumption may influence bone mass, morphology and mineralization and that altering dietary composition may provide an opportunity to impact an individual's bone health and metabolic state even in the absence of changes in body mass [6].

Previous skeletal studies mostly focused on high-fat diet induced deterioration of bone architecture or used high-fat diets supplemented with carbohydrates. No direct contrasts between longer-term fructose and high-fat consumption on bone architecture and mechanical properties are currently available. Further, some carbohydrate- and high-fat diets used in pre-clinical studies are difficult to translate to human physiology because of use of supra-

physiological doses of sugars or because of secondary influences including increased body mass. In an effort to provide critical data on the comparative effects of high-fructose and high-fat consumption on fat and bone, we fed mice a high-fructose, high-fat, or control diet and quantified visceral and subcutaneous fat deposition, de-novo lipid synthesis, plasma concentrations of glucose/insulin/leptin, bone architecture and stiffness. We hypothesized that across our outcome measures, a high-fat diet will be more detrimental than a fructose-enriched diet.

Materials and Methods

Experimental Design

Thirty BALB/cByJ (BALB) male mice (Jackson Laboratories) were assigned to either a high-fat (HF), fructose-enriched (CH) or a standard chow (SC) diet group at 7 wk of age (n=10/group). The high-fat diet provided 45% kcal from fat (VanHeek 58V8, TestDiet). The high-fructose regimen consisted of a standard chow diet (Purina Rodent Chow 5001, LabDiet) and 10% w/v powdered fructose mixed into the drinking water. 10% w/v fructose administration is physiologically relevant and consistent with the average consumption of fructose in the U.S [23]. The control group was fed standard lab-chow. To minimize variability in cage activities (mechanical loading), all mice were housed in individual cages with a 12 hr light/dark cycle and given free access to food and water. All mice were on their respective regimes for 15 wk (until 22 wk of age). Body mass was recorded at the end of each week. Food intake was recorded at the end of each week except for the last week. In the 8th wk of the 15 wk experiment, one mouse of the fructose group had to be euthanized because of excessive weight loss.

Lipid Synthesis

Mice in all groups received deuterium as a stable isotope tracer for 2 wk prior to sacrifice. At baseline, mice were administered an intraperitoneal injection of deuterated water equal to 4% saline. Mice were then maintained on drinking water containing 6% deuterated water for 2 wk before sacrifice. This procedure is designed to maintain deuterium enrichment in body water of at least 3%. After sacrifice, mouse livers were frozen and powdered. Hepatic lipids were extracted by homogenizing 50 mg of liver in 1 ml chloroform/methanol (2:1) [24]. The lipid extract was dried under a stream of liquid nitrogen followed by saponification (90% MeOH/0.3M KOH at 80°C for 1 h). Non-saponified lipids were extracted into petroleum ether, leaving fatty acids. Palmitate was analyzed as its trimethylsilyl derivative using gas chromatography-electron impact ionization mass spectrometry using a single quadrupole Agilent 7890A/5975C GC/MS [25, 26]. The palmitate 2H enrichment was determined by using selective ion monitoring under electron impact ionization of m/z 313, 314, 315 and 316 (M+0, M+1, M+2 and M+3), with a dwell time of 10 ms per ion. Rate of lipid synthesis was determined as the percent contribution of denovo synthesized lipid. The total 2H-labeling of palmitate was calculated as (1 x M+1 + 2 x M+2 + 3 x M+3):

$$\text{Fraction newly made palmitate} = \left[\frac{\text{total 2H-labeling palmitate}}{\text{2H-labeling body water} \times n} \right];$$

where M+1, M+2, M+3 are the palmitate enrichments, the 2H-labeling of body water is the fraction water labeled with 2H, and n is the number of exchangeable hydrogens on palmitate [25, 26].

Abdominal Adiposity

To regionally determine fat volumes, the abdominal region (between L1 to L5) of all mice was scanned by in-vivo micro-computed tomography (μ CT) at the end of the 15 wk experimental phase (VivaCT 75, Scanco Medical, SUI). This method has previously been validated via correlations with fat pad weights [27]. The scans were generated at a voxel size of 82 μ m (45 KV, 133 μ A, 300 ms integration time) and reconstructed into 3D images of visceral and subcutaneous fat [28]. An automated script individually quantified the volume of both subcutaneous and visceral fat [29].

Tissue Collection and Plasma Assays

All animals were sacrificed at 22 wk of age. After overnight fasting, blood was collected via cardiac puncture with the mouse under anesthesia. Mice were then euthanized via cervical dislocation. Right femurs were preserved in 70% ethanol at 4°C. Weights of the epididymal and brown fat pad were recorded. Plasma was isolated via centrifugation. Kits were used to determine plasma concentrations of glucose (Diagnostic Chemicals Limited) as well as insulin, triglycerides and leptin (EMD Millipore).

Bone Phenotype

Bone geometry, architecture, and tissue mineral density of the distal femur were examined via ex vivo μ CT (Micro-CT 40, Scanco Medical) using a voxel size of 12 μ m. Over a distance of 1.5 mm of the distal metaphysis, total cortical area (Tt.Ar), cortical bone volume fraction (BV/TV), cortical thickness (Ct.Th), trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular connectivity density (Conn.D), trabecular separation (Tb.Sp), trabecular structural model index (SMI), and cortical & trabecular tissue mineral density (Ct.TMD & Tb.TMD) were determined with an automated separation algorithm [30].

Nanoindentation

Mechanical properties of femoral cortical bone samples were tested using nanoindentation (Hysitron Triboindenter). Cortical sections from the distal diaphysis of the femur were cut using a diamond wheel blade saw and dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, 100% for 48 h each). Dehydrated sections were embedded in epoxy resin using disposable molds [31]. Surfaces of sample blocks were ground and polished using a Buehler grinder- polisher using sand-paper of decreasing grit size (1200 μ m and 800 μ m) and diamond suspensions of decreasing particle size (3 μ m, 1 μ m, 0.25 μ m, 0.05 μ m). Each mouse bone sample was indented at three points spaced 3 μ m apart on the anterior cortex. A maximum load of 1000 μ N with a hold time of 10 sec. and a loading rate of 100 μ N/sec was used. Hardness and the reduced modulus were calculated from the unloading portion of the force-displacement curve [31].

Statistics

The three diet groups (SC vs CH vs HF) were compared using one-way ANOVA followed by a (protected) Fischer's least significant difference post-hoc test. Statistical analyses were performed with SPSS and GraphPad Prism software. $P < 0.05$ was considered significant.

Results

Food Intake and Body Mass

Starting at the end of the first week compared to SC and end of second week compared to CH, the amount of food (mass) eaten by the HF group was significantly less and this difference persisted to the end of the study (with HF diet providing 4.6 kCal/gram energy and the SC diet providing 4.1 kCal/gram, caloric intake of the HF group was also less). Compared to SC, the CH group had a slightly lower (but statistically significant) food intake during the first, third, fifth and thirteenth week but not at any other time-point (Figure 1A). Body mass was not significantly different between groups throughout the study, except at the end of week 14 and week 15 when the HF group had approximately 10% greater body mass than SC (Figure 1B).

Abdominal Fat Volume

In-vivo μ CT scans showed that after 15 wk, the HF group had 112% greater ($p < 0.001$) total abdominal fat than the SC group and 72% total greater ($p = 0.002$) abdominal fat than the CH group. This increase was the result of increased fat accumulation both in the visceral (+119%, $p < 0.001$ HF vs. SC and +81%, $p = 0.003$ HF vs. CH) and subcutaneous (+100%, $p < 0.001$ HF vs. SC and +60%, $p = 0.006$ HF vs. CH) depots (Table 1). There were no significant differences between the abdominal fat volumes of the CH and SC groups, as measured by μ CT.

Fat Pad Weights

Similar to the in-vivo μ CT data, the epididymal fat pad weights of animals in the high-fat group were markedly elevated after 15 wk when compared to SC (+128%, $p < 0.001$) and CH (+430%, $p < 0.001$). In fructose-fed mice, epididymal fat pad weight was 57% lower compared to SC ($p = 0.02$). Further, in fructose-fed mice, brown fat pad weight was 156% greater ($p < 0.001$) compared to SC and 65% greater ($p = 0.02$) compared to HF (Table 1).

Plasma Insulin, Glucose, Leptin

After 15 wk, fasting plasma insulin levels were 91% greater in HF than SC mice, approaching statistical significance ($p = 0.06$). Plasma glucose was not significantly different between groups. Concurrent with the markedly increased fat volume, plasma leptin levels were approximately 4-fold greater ($p < 0.001$) in HF than SC and 2-fold greater in HF compared to CH ($p = 0.003$). The fructose-enriched diet did not significantly alter plasma leptin levels compared to SC (Table 1).

De Novo Lipid Synthesis

Deuterium enrichment in body water reached a plateau of 2–3% in fatty acids of all tissues. As measured by the incorporation of stable deuterium label into fatty acid synthesis, palmitate FNS was significantly lower ($p<0.05$ for all) in the high-fat group compared to standard chow in the liver (–52%), kidney (–46%), white adipose tissue (–40%) and brown adipose tissue (–34%). Palmitate FNS was also significantly lower ($p<0.05$ for all) in the liver (–60%), kidney (–56%), white adipose tissue (–46%) and brown adipose tissue (–33%) of the high-fat group compared to the fructose group. There were no significant differences in the fractional new lipid synthesis between CH and SC mice (Figure 2).

Bone Morphology and Mechanical Properties

High fat diet consumption significantly impacted cortical bone in the distal femur (Table 2). In the absence of differences in total cortical area, cortical bone volume fraction (–5%, $p<0.001$ HF vs. SC and –4%, $p=0.007$ HF vs. CH), cortical thickness (–8%, $p<0.001$ HF vs. SC and –8% $p=0.006$ HF vs. CH), and tissue mineral density (–2%, $p<0.001$ HF vs. SC and –2%, $p<0.001$ HF vs. CH) were altered. In contrast, there were no cortical differences between CH and SC.

Similar to cortical bone, HF diet detrimentally impacted trabecular bone, yielding a structure characterized by a smaller trabecular bone volume fraction compared to mice fed a standard chow (–18%, $p=0.03$). Differences in outcome variables describing the trabecular architecture of HF mice compared to SC also indicated a compromised structure but reached statistical significance only for the structural model index (+13%, $p=0.005$). Differences in connectivity density (–27%, $p=0.07$), trabecular number (–7%, $p=0.09$), and trabecular separation (+10%, $p=0.09$) approached significance for HF vs. SC. Compared to the CH group, the HF group had significantly lower trabecular bone volume fraction (–9%, $p=0.04$) and a greater structural model index (+13%, $p=0.008$) (Table 2).

We used nanoindentation on cortical bone to test for differences in two mechanical properties – bone's elastic modulus and bone hardness. Neither the fructose nor the high-fat group displayed significant differences in these two mechanical properties when compared to controls (Table 2). When comparing the two dietary regimes to each other, bone's elastic modulus was 22% smaller ($p=0.01$) in CH than in HF and the 16% difference in hardness between the two groups approached significance ($p=0.07$).

Discussion

The goal of this study was to test for differential effects of chronic high fat vs. fructose consumption on bone, fat, and metabolic markers. Similar to clinical studies, both dietary modifications did not lead to changes in body mass (except for the last 2 wk of the protocol). Even in the absence of substantial differences in body mass, 15 weeks of HF consumption compromised trabecular and cortical bone volume and architecture in the femur. Mice in the HF group also had larger subcutaneous and visceral fat deposits together with increased plasma leptin concentrations. Synthesis of new lipids in the major organs capable of de-novo lipogenesis (kidney, liver, adipose tissue) was significantly lower in the HF group compared

to SC. In contrast, elevated fructose consumption for 15 weeks did not significantly alter bone structure and density but gave rise to compromised cortical bone stiffness when compared to high-fat diet bones. Fructose treatment did not significantly alter de-novo lipogenesis or plasma metabolites but increased interscapular brown adipose tissue mass. Together, these data suggest that in the BALB mouse, a long-term diet high in fat has greater negative health consequences on bone and adiposity than a diet high in fructose.

Our study has limitations. We used a moderate dose of fructose via daily water consumption and it is possible that much higher doses of saccharides are required to elicit alterations in lipid synthesis or bone morphology. Also, we did not quantify water intake, and it is therefore difficult to compare the caloric consumption of CH mice with the other two groups. Finally, observational data such as ours provide essential information of dietary effects on bone and fat but inherently, mechanistic studies with the inclusion of a greater number of outcome variables will be required to unravel the underlying physiologic and molecular basis.

Although obesity induced increases in body mass can act as growth factor on bone through augmented mechanical loading, high-fat diets can increase osteoclastogenesis, marrow adipogenesis, and decrease bone mass [12, 14]. Consumption of high-fat diets also increases circulating levels of the hormone leptin, a principal regulator of energy homeostasis that can impact bone growth [16]. HF diet causes preferential attrition of trabecular bone volume and architecture, with some investigations not observing negative effects on cortical bone [12–14, 22]. In addition to altered trabecular bone properties with HF, here, we also observed a negative impact of HF on the cortical femur including decreased cortical thickness and TMD. The effects of high-fat feeding on trabecular bone observed in our study were modest compared to changes reported by others [12, 13, 32]. We speculate that some of the differences between studies may be accounted for by specific aspects of the diet (e.g., 45% fat content vs. 60%), differences in species (e.g., rat vs. mouse), or differences in genetic makeup within a species (e.g., BALB/c vs C57BL/6 mice). Compared to more commonly used B6 mice, our BALB mice experienced less bone loss, particularly in the trabecular compartment [32]. While baseline as well as skeletal developmental patterns differ between the two strains of mice [33], the (genetic) basis for the differential response it is yet to be determined.

In spite of the continued pervasiveness of fructose in processed foods and drinks, its impact on bone structure and mechanical properties has received little attention. We administered fructose as a 10% w/v solution via the drinking water to approximate human fructose consumption that approaches 10% of total daily energy intake [23]. In contrast to diets high in sucrose or fat [12–14, 21, 34], our data from BALB mice demonstrate that fructose consumption at physiologically relevant levels does not significantly alter trabecular and cortical bone volume and architecture over controls. Considering emerging data indicating a positive relation between brown adipose tissue and bone mass [35], it is conceivable that the larger brown adipose tissue mass in fructose supplemented mice exerted an osteoprotective effect.

We also tested for the presence of altered mechanical material properties in the cortical region of the femur. The lack of differences in bone stiffness or hardness between fructose fed animals and controls matched previous data [36]. When compared to high-fat mice, however, fructose-fed mice revealed reduced stiffness. Fructose can alter the absorption of phosphorus and magnesium [37] and clinical studies have demonstrated an association between carbohydrate consumption and whole body macro-mineral homeostasis [36, 38]. We observed small but significant differences in bone calcification (tissue mineral density) between the fructose and high fat group which, together with other potential chemical and nano-structural changes [39–41], may have caused the difference in elastic modulus between fructose and high-fat mice.

Diet is an environmental determinant of metabolic indicators including circulating levels of glucose, insulin, and leptin. After 15 weeks, insulin and glucose levels were not statistically different between groups. Leptin is secreted by adipocytes and its levels are proportional to the amount of adipose tissue, acting as a sensor of energy stores and regulator of food intake via centrally mediated appetite control [42]. Not surprisingly, we measured a large increase in circulating leptin after 15 wk of HF treatment. The HF group also had reduced food intake as early as the end of the first week. We measured leptin levels only at the end of 15 weeks but it is likely that the high intake of exogenous fat caused an early induction of the leptin response and decreased food intake. Food consumption is responsive to the central action of leptin for up to 11–12 weeks of high-fat diet feeding, after which both peripheral and central leptin resistance develops and body mass starts to increase [42]. We observed a similar effect in our study, with a slight increase in body mass after 12 weeks of HFD despite lower caloric intake in the HF group compared to SC. Changes in body mass with HF feeding depend on several factors other than caloric intake including genetic background, energy expenditure, duration of diet, or leptin signaling [43]. Long-term exposure to HFD can decrease energy expenditure by down regulating genes involved in oxidation-reduction and metabolism [44], enabling rodents to differentially gain weight despite similar caloric intake.

Leptin signaling protects against obesity by increasing energy expenditure and reducing appetite in acute settings [45]. The hypophagia we observed is considered an intermediate stage in the development of DIO during which the oncoming peripheral resistance to leptin results in fat deposition while central leptin sensitivity attempts to prevent obesity by reducing food intake [46]. The lower food intake we observed in HF mice was paralleled by similar body mass between HF and SC for the first 13 weeks, consistent with an 8wk study in which BALB mice on HFD resisted increases in body mass and had increased energy expenditure [43]. During the last two weeks of the study, however, the increase in body mass and the supernormal leptin plasma concentrations in HFD over SC at the completion of our 15-week study were possibly related to leptin resistance and decreased energy expenditure in HF mice, resulting in weight gain. Nevertheless, the HF group had comparable body mass to the other two group for 13 weeks, facilitating the contrast between the two diets largely independent of body mass, an often-confounding variable in skeletal research.

Lipid accumulation can occur due to increased exogenous intake of fat or due to endogenous synthesis of lipids [7]. The de-novo lipogenesis (DNL) pathway is the primary means by which cells maintain energy stores, converting excess carbohydrate substrate into fatty acids

and triacylglycerol [7]. Conversely, it would be expected that the consumption of excess exogenous fatty acids via a HF diet leads to a down-regulation of DNL. However, studies quantifying DNL during high-fat diet exposure have been equivocal in rodent models [47, 48]. Clinical studies have examined dietary effects on whole body DNL but these studies cannot yield direct information on lipid synthesis in individual organs. In our study, DNL in kidney, liver, white adipose tissue and brown adipose tissue was significantly lower in HF than in SC mice, consistent with the physiological function of the de-novo lipogenesis pathway [7] and animal studies showing decreases in liver specific DNL with HF consumption [47, 48]. High-fat diet consumption reduced DNL in the kidney and white adipose tissue to levels observed in the liver, perhaps indicating that these tissues re-esterify liver exports of DNL. The greater DNL levels in brown adipose tissue may suggest de novo lipogenesis in excess of that seen in liver under high-fat conditions.

Dietary fructose intake is known to be a strong modulator of DNL since it is delivered to the liver directly at high concentrations and it increases the expression of enzymes involved in lipogenesis [7]. For example, fructose is a direct stimulator of the transcription factor SREBP1-c which increases the expression of lipogenic genes [7]. In our study, consumption of the fructose regimen did not alter new palmitate synthesis in any of the organs examined. This discrepancy may be attributed to the supra-physiological doses of fructose used in previous studies.

In summary, we showed that the BALB/cByJ mouse may be a suitable model to investigate dietary modulation of bone and fat over a longer term because of the lack of substantial differences in body mass, a potentially confounding factor in many other rodent studies. In absence of the protective effect of increased load bearing, chronic high-fat consumption was detrimental to bone mass and its architecture as well as tissue density. The high fat diet also increased deposition of subcutaneous and visceral fat stores and reduced fractional new synthesis of lipids. In contrast, chronic consumption of fructose at moderate levels did not impact bone and fat mass, new lipid synthesis or other indicators of metabolic health but elevated interscapular brown adipose tissue mass. It also compromised bone's internal stiffness when compared to the high-fat group. While increased sugar consumption has been clinically linked to poor bone health, these studies are usually association based [18]. Here we provide direct evidence that in the BALB/cByJ mouse, chronic fructose feeding has mostly insignificant effects on lipid storage, synthesis, or bone health.

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Figure 1.

Changes in (A) food intake and (B) body mass of mice consuming a standard chow (SC), fructose (CH), or high-fat (HF) group for 15 weeks. Food intake was not measured during the last week. Values are mean $\pm$ SD. #: $p < 0.05$ HF vs. SC, *: $p < 0.05$ CH vs. SC, †: $p < 0.05$ HF vs. CH.

Figure 2.

De-novo lipogenesis was measured as the fraction of newly synthesized palmitate (fractional net synthesis or FNS) present in lipogenic tissues of mice in the standard chow (SC), fructose (CH), or high-fat (HF) group at end of 15 weeks of diet treatment. Theoretical FNS maximum value =1. Values are mean $\pm$ SD. #: $p < 0.05$ HF vs. SC, *: $p < 0.05$ CH vs SC, †: $p < 0.05$ HF vs. CH.

Table 1

Abdominal fat volumes, fat pad weights and plasma metabolites.

	Standard Chow	Fructose	High-Fat
Total fat [mm ³]	647±292	797±374	1369±377 ^{#†}
Visceral [mm ³]	373±217	454±257	819±238 ^{#†}
Subcutaneous [mm ³]	274±88	343±143	550±183 ^{#†}
Insulin [ng/ml]	0.9±0.6	1.3±1.0	1.8±1.3
Glucose [mg/dl]	159±47	184±37	181±28
Leptin [ng/ml]	1.6±1.2	2.9±2.5	6.1±2.4 ^{#†}
Epididymal fat pad [mg]	368±144	157±57 [*]	840±258 ^{#†}
Brown fat [mg]	157±35	403±210 [*]	246±99 [†]

Values are mean±SD.

[#]
p< 0.05 HF vs. SC,^{*}
p< 0.05 CH vs SC,[†]
p< 0.05 HF vs. CH.

Table 2

Femoral structural and mechanical properties.

		Standard Chow	Fructose	High-Fat
<i>Cortical</i>	Total cortical area [mm ²]	0.95±0.04	0.95±0.05	0.95±0.05
	Cortical bone volume fraction [-]	0.85±0.01	0.84±0.02	0.81±0.02 ^{#†}
	Cortical thickness [mm]	0.14±0.01	0.14±0.01	0.13±0.01 ^{#†}
	Tissue mineral density [mg HA/ccm]	1152±8	1146±12	1126±11 ^{#†}
	Elastic Modulus [GPa]	23.5±4.3	20.9±6.0	26.7±2.9 [†]
	Hardness [GPa]	1.1±0.2	1.0±0.2	1.2±0.2
<i>Trabecular</i>	Trabecular bone volume fraction [-]	0.13±0.03	0.12±0.02	0.11±0.02 ^{#†}
	Connectivity Density [1/mm ³]	120±35	108±29	95±30
	SMI [-]	2.2±0.3	2.2±0.3	2.5±0.2 ^{#†}
	Trabecular Number [1/mm]	4.6±0.4	4.4±0.3	4.3±0.4
	Trabecular thickness [mm]	0.044±0.002	0.045±0.002	0.044±0.002
	Trabecular separation [mm]	0.21±0.02	0.22±0.02	0.23±0.02
	Tissue mineral density [mg HA/ccm]	881±12.8	877±15.3	882±12.5

Values are mean±SD.

[#]
p< 0.05 HF vs. SC,^{*}
p< 0.05 CH vs SC,[†]
p< 0.05 HF vs. CH.