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High-fat/high-sucrose diet results in higher bone mass in aged rats

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ABSTRACT

Intake of high-fat/high-sucrose (HFS) diet or high fat diet influences bone metabolism in young rodents, but its effects on bone properties of aged rodents still remain unclear. This study aimed to examine the effects of HFS diet intake on trabecular bone architecture (TBA) and cortical bone geometry (CBG) in aged rats. Fifteen male Wistar rats over 1 year were randomly divided into two groups. One group was fed a standard laboratory diet (SLD) and the other group was fed a HFS diet for six months. The femur/tibia, obtained from both groups at the end of experimental period, were scanned by micro-computed tomography for TBA/CBG analyses. Serum biochemical analyses were also conducted. Body weight was significantly higher in the HFS group than in the SLD group. In both femur and tibia, the HFS group showed higher trabecular/cortical bone mass in reference to bone mineral content, volume bone mineral density and TBA/CBG parameters compared with the SLD group. In addition, serum calcium, inorganic phosphorus, total protein, triacylglycerol, HDL and TRACP-5b levels were significantly higher in the HFS group than in the SLD group. There were good correlations between body weight and bone parameters in the femur and tibia. These results suggest that HFS diet intake results in higher bone mass in aged rats. Such effects of HFS diet intake might have been induced by increased body weight.

1. Introduction

Osteoporosis and obesity are serious and prevalent health issues. Indeed, body weight positively correlated with bone mineral density (BMD), and high body mass index was associated with low fracture risk (Reid, 2008; Reid, 2010). However, there is inconsistency in the literature regarding the effects of obesity on fracture risk. For example, some studies showed that obesity reduces fracture risk and protects against osteoporosis in adults (Reid, 2010; Tang et al., 2013), while others showed that obesity does not protect against fracture in postmenopausal women (Compston et al., 2011; Tanaka et al., 2013). Interestingly, one study reported that obesity is a risk factor of fracture in children, while it is protective against fracture in adults (Dimitri et al., 2012), suggesting that the effects of obesity on bone parameters may differ with age. Thus, the effects of obesity on fracture risk were reported to be inconsistent, although BMD of obese individuals ranged from normal to high levels as compared with non-obese individuals. Therefore, BMD, which generally depends on body weight, may not be predictive of fracture risk in obesity.

Young rodents, fed with a high fat diet (HFD) or a high-fat/high-sucrose (HFS) diet, are often used to assess the effects of obesity on bone parameters. For example, bone mineral content (BMC) and mechanical properties of both the vertebral body (L6) and femoral neck were significantly decreased in rats fed with HFS diet from the age of 4 weeks until the age of 2 years, compared with rats fed with a low-fat, complex-carbohydrate diet (Zernicke et al., 1995). BMC, BMD and skeletal area, assessed by dual-energy X-ray absorptiometry (DXA), were significantly decreased in rats fed with HFD for 10 weeks from the age of 5 weeks, compared with rats fed with a standard diet (Lac et al., 2008). Furthermore, in young mice the HFD intake was reported to induce the deterioration in trabecular bone architecture (TBA) and to increase bone resorption (Cao et al., 2009; Patsch et al., 2011). In addition, bone mechanical strength and fracture toughness were decreased in mice fed with HFD for 19 weeks from the age of 4 weeks

Abbreviations: ALP, alkaline phosphatase; BMD, bone mineral density; BMC, bone mineral content; BV, bone volume; BV/TV, bone volume fraction; Ca, calcium; CBG, cortical bone geometry; Conn.D, connectivity density; Ct.Ar, cortical bone sectional area; Ct.Th, cortical bone thickness; CV, cortical bone volume; CV/(CV + MV), cortical volume fraction; DXA, dual-energy X-ray absorptiometry; Ec.Pm, endocortical perimeter; HDL, high-density lipoprotein cholesterol; HFD, high fat diet; HFS, high-fat/high-sucrose; IP, inorganic phosphorus; LDL, low-density lipoprotein cholesterol; micro-CT, x-ray micro-computed tomography; MV, medullary volume; OC, osteocalcin; Ps.Pm, periosteal perimeter; VOI, volume of interest; SLD, standard laboratory diet; TBA, trabecular bone architecture; Tb.N, trabecular number; TBPf, trabecular bone pattern factor; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tb.W, trabecular width; TC, total cholesterol; TG, triacylglycerol; TMD, tissue mineral density; TP, total protein; TRACP-5b, tartrate-resistant acid phosphatase-5b; TV, tissue volume; vBMD, volume BMD

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(Ionova-Martine et al., 2010). Thus, the HFD/HFS diet intake appears to have negative effects on BMC, BMD, TBA and bone mechanical properties in young rodents.

In opposition to the above findings, there were some studies reporting positive effects of HFD intake on BMC, BMD, TBA, cortical bone geometry (CBG) and bone mechanical properties in young rodents. BMC, BMD and skeletal area, analyzed by DXA, were significantly increased in rats fed with HFD for 9 months from the age of 8–10 weeks, compared with rats fed with a standard diet (Malvi et al., 2014). These HFD-fed rats showed increased levels of serum alkaline phosphatase (ALP) and unchanged levels of serum tartrate-resistant acid phosphatase-5b (TRACP-5b) (Malvi et al., 2014). In young mice fed with HFD, trabecular and cortical bone structure of the tibia were also increased despite a reduction in mineral apposition and bone formation rates (Lecka-Czernik et al., 2015). Moreover, in a rat model of obesity induced by junk food intake for one month from the age of 3 months, femoral ultimate load and energy absorption capacity were significantly higher than in control rats (Brahmabhatt et al., 1998). These results suggest that the HFD intake has positive effects on bone parameters and bone mechanical properties in young rodents. Thus, in young rodents, the effects of HFD intake on bone parameters and bone mechanical properties were reported to be negative or positive, and therefore still remain controversial.

We hypothesized that the effects of HFD/HFS diet intake on bone mass/quality in aged rats may be different from those observed in young rats due to the differences in bone metabolism between growth and aging periods (Lelovas et al., 2008). In contrast to several studies on the effects of HFD/HFS diet intake on bone of young and adult rats (Zernicke et al., 1995; Brahmabhatt et al., 1998; Lac et al., 2008; Malvi et al., 2014), to the best of our knowledge, there was only one study reporting bone properties in male Wistar rats aged 11 months, which were fed with HFS diet for 4 months from 7 months of age (Gerbaix et al., 2012). This HFS diet-fed rats showed an increase in whole body bone mass and BMD compared with standard diet-fed rats, but no change in tibia TBA parameters including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), connectivity density (Conn.D) etc. (Gerbaix et al., 2012). However, we have speculated that HFS diet-feeding during the longer period would influence TBA in more aged rats. To examine this speculation, using X-ray micro-computed tomography (micro-CT), we have analyzed trabecular and cortical bone structure in rats fed with HFS diet for 6 months from 12 months of age in the present study.

2. Materials and methods

This study was approved by the Ethics Committee of Research Facilities for Laboratory Animal Sciences in the Kio University, and was performed in accordance with the Guidelines for Animal Experimentation of the University.

2.1. Animal care

Fifteen male Wistar rats over 1 year with mean body weight of 486 g (Japan SLC, Inc., Hamamatsu, Japan) were used in this study. Rats were housed in cages at 23 ± 2 °C temperature, in 50 ± 10% humidity and under a 12-hour day-night cycle. Rats were randomly divided into two groups and were allowed to feed and drink water ad libitum. One group (n = 7) was fed a standard laboratory diet (SLD) containing 4.7% crude fat (CE-2; CLEA, Inc., Hamamatsu, Japan) and the other (n = 8) was fed a HFS diet containing 13.8% crude fat and 25% sucrose (Quick Fat; CLEA, Inc., Hamamatsu, Japan), during the experimental period of 24 weeks. The Quick Fat has been generally used for diabetes mellitus/obesity studies in rodents. Nutritional details of the two diets are provided in Table 1. Body weight and food intake were measured every week throughout the experimental period.

Table 1
Nutritional content of two experimental diets.

	CE-2	Quick Fat
Moisture (%)	8.9	6.9
Crude protein (%)	24.2	23.7
Crude fat (%)	4.7	13.8
Crude fiber (%)	4.1	2.6
Crude ash (%)	6.9	5.3
Supplemented sucrose (%)	0	25
Ca (%)	1.1	1.0
P (%)	1.1	0.8
Vitamin D (IU/g)	2.15	2.90
Energy (kcal/100 g)	345.5	412.5
Fat energy (%)	12.2	30.1

2.2. Analyses of TBA and CBG

The procedures for analyses of TBA and CBG were performed according to the micro-CT guidelines for assessment of rodent bone microstructure (Bouxsein et al., 2010), as previously reported by our group (Minematsu et al., 2016a; Minematsu et al., 2016b; Minematsu et al., 2017). The bilateral femurs and tibias were dissected out, and then soft tissues were removed. The bones were put into the vials filled with 70% ethanol solution (25 °C), and the vials were tightly sealed. In an attempt to prevent the generation of air bubbles/micro-air bubbles in bones, the vials were stored under occasional light vibration in a refrigerator (4 °C) until analyzed. To analyze TBA and CBG using micro-CT (Hitachi Medical Corporation. Tokyo, Japan), the distal femur, proximal tibia and mid-shaft of the tibia were scanned at 65 kVp and 90 μA, with a voxel size of 19.1 μm in the high-definition mode. A BMD phantom was also scanned using micro-CT under the same conditions to calculate tissue mineral density (TMD), BMC and volume BMD (vBMD, i.e., BMC/tissue volume). All micro-CT images were inspected visually to identify possible scanning artifacts, like ring artifact (Bouxsein et al., 2010). Scanned data of images without scanning artifacts were transmitted to a personal computer. After the removal of random image noise was accomplished by median-filtering, bone images were reconstructed by binary coded processing using a discriminative analysis method for global segmentation. TBA and CBG of the volume of interest (VOI) were analyzed using a bone analysis software (TRI BON 3D; Ratoc System Engineering Co., Ltd., Tokyo, Japan). Tissue volume (TV), bone volume (BV), BV/TV, Tb.Th, trabecular width (Tb.W), Tb.N, Tb.Sp, Conn.D and trabecular bone pattern factor (TBPf, i.e., ratio of changed bone surface area to changed bone surface volume in trabecular bone) were assessed as TBA parameters in the distal femur and proximal tibia. Cortical bone volume (CV), medullary volume (MV), cortical volume fraction (CV/(CV + MV)), cortical bone thickness (Ct.Th), cortical bone sectional area (Ct.Ar), periosteal perimeter (Ps.Pm), and endocortical perimeter (Ec.Pm) were assessed as CBG parameters in the tibia. The VOI of TBA in the femur was an area of 2 mm in the femoral metaphysis, with the first slice starting 1 mm away from the distal femoral growth plate physeal-metaphyseal demarcation in the proximal direction. The VOI of TBA in the tibia was an area of 2 mm in the tibial metaphysis, with the first slice starting 1 mm away from the proximal tibial growth plate physeal-metaphyseal demarcation in the distal direction. The VOI of CBG in the tibia was an area of 2 mm in the tibial shaft, with the first slice starting 1 mm away from the inferior tibiofibular junction in the proximal direction.

2.3. Dry bone weight and ash weight measurements

After TBA and CBG analyses, the femur and tibia were dehydrated in 100% ethanol for 48 h and dried at 100 °C for 24 h with a drying machine (Yamato Scientific Co., Ltd. Tokyo, Japan) to measure dry weight of the whole bone. Subsequently, the bones were burned to ash at 600 °C for 24 h with an electric furnace (Nitto Kagaku Co., Ltd.,

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