Biochemical and Biophysical Research Communications 455 (2014) 133–138

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

High fat diet promotes achievement of peak bone mass in young rats

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ARTICLE INFO

Article history:
Received 24 October 2014
Available online 1 November 2014

Keywords:
High fat diet
Obesity
Peak bone mass
Osteoporosis

ABSTRACT

The relationship between obesity and bone is complex. Epidemiological studies demonstrate positive as well as negative correlation between obesity and bone health. In the present study, we investigated the impact of high fat diet-induced obesity on peak bone mass. After 9 months of feeding young rats with high fat diet, we observed obesity phenotype in rats with increased body weight, fat mass, serum triglycerides and cholesterol. There were significant increases in serum total alkaline phosphatase, bone mineral density and bone mineral content. By micro-computed tomography (μ-CT), we observed a trend of better trabecular bones with respect to their microarchitecture and geometry. This indicated that high fat diet helps in achieving peak bone mass and microstructure at younger age. We subsequently shifted rats from high fat diet to normal diet for 6 months and evaluated bone/obesity parameters. It was observed that after shifting rats from high fat diet to normal diet, fat mass, serum triglycerides and cholesterol were significantly decreased. Interestingly, the gain in bone mineral density, bone mineral content and trabecular bone parameters by HFD was retained even after body weight and obesity were normalized. These results suggest that fat rich diet during growth could accelerate achievement of peak bone mass that is sustainable even after withdrawal of high fat diet.

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1. Introduction

Osteoporosis, a systemic skeletal disorder, is a common condition affecting millions of individuals worldwide including both men and women [1]. It is characterized by low bone mass leading to fragility fractures occurring mainly at hip, spine and vertebra [2,3]. Osteoporosis occurs due to normal ageing process and because of deficiency of sex hormones. It can also result from failure to achieve peak bone mass during adolescence or excessive bone loss during adulthood [4]. At cellular level, osteoporosis arises as a consequence of imbalanced activities of osteoblasts and osteoclasts. In osteoporosis, osteoclast number and activity are increased while osteoblast number and function are decreased leading to low bone mass [5]. Life style and diet may play a crucial role in bone health, but the contribution of these factors towards bone homeostasis is not well understood.

Obesity is a metabolic disorder, which is characterized by excessive accumulation of adipose tissue in the body resulting in alterations in serum levels of lipids, cytokines and hormonal factors, which are attributed to excessive accumulation of white adipose tissue especially in visceral parts of the body [6]. It is prevalent in both developed and developing countries, and about 2.1 billion people are overweight worldwide [7]. The major causes of obesity are excessive intake of food, decreased energy expenditure and lack of physical activity [8]. Life style changes, physical exercise and pharmacological approaches remain the main strategies towards prevention and management of obesity.

Both osteoporosis and obesity are complex disorders and are related to each other pathophysiologically. Body weight has a profound impact on bone density and bone turnover [9,10]. The link between obesity/overweight and bone-related parameters has been explored in human subjects, animal models and through *in vitro* studies, however the reports are controversial. A few reports highlight the negative correlation between diet-induced obesity and bone mass [11,12], whereas some studies demonstrate

Abbreviations: HFD, high fat diet; ND, normal diet; BMD, bone mineral density; BMC, bone mineral content; ALP, alkaline phosphatase; TRAP 5b, tartrate-resistant acid phosphatase; μ-CT, micro-computed tomography.

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http://dx.doi.org/10.1016/j.bbrc.2014.10.131
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the positive association of diet-induced obesity with bone mass [13–15]. Studies also suggest that overweight or moderate obesity is associated with increased bone mineral density (BMD) and reduced risk of fractures [16], whereas extreme obesity adversely affects the bone health [17]. Thus, the pathophysiological association of obesity with bone health is still not clearly understood. In the present study, we evaluated the impact of diet-induced obesity on achieving peak bone mass in young male Wistar rats.

2. Materials and methods

2.1. Animals

Male Wistar rats of 8–10 weeks age were obtained from Experimental Animal Facility of National Centre for Cell Science, Pune, India. Water and food were provided *ad libitum* to all the rats. All experiments involving animals were carried out after approval from the Institutional Animal Ethics Committee.

2.2. Diet and feeding

Wistar rats were divided into two groups: normal diet (ND) rats ($n = 6$) fed with normal chow diet (Amrut Laboratory Animal Feed, Pune, India), and high fat diet (HFD) rats ($n = 6$) fed with diet containing 24% fat (Provimi Animal Nutrition Pvt. Ltd., Bangalore, India) along with ground nut (100 g/kg body weight/day) and dried coconut (50 g/kg body weight/day). The detailed composition of the two diets is shown in Supplementary Table 1. Rats of both the groups were fed with their respective diet for 9 months. Body weight, serum triglycerides (TGs), serum cholesterol and serum LDL-cholesterol (LDLc), and bone related parameters were measured at the indicated periods.

To study the effect of dietary intervention on bone-related parameters, HFD rats were shifted from high fat diet to normal diet for additional period of 6 months. The ND rats were continuously fed with normal chow diet. Obesity and bone-related parameters were measured at indicated periods. The tibiae were preserved in 10% formalin at room temperature before subjecting them to micro-computed tomography (μ-CT) analysis.

2.3. Biochemical analysis of serum samples

Blood was withdrawn from rats by orbital sinus puncture using sterile bleeding capillaries. The serum was collected and stored in sterile tubes at -80 °C until further use. The obesity-related parameters such as serum TGs, cholesterol and LDLc were estimated using kits obtained from Spinreact (Girona, Spain) as per the manufacturer's instructions. Other biochemical parameters including total alkaline phosphatase (ALP) and calcium were estimated in the serum by pNPP (p-nitro-phenylphosphate) kinetic method and Ortho–Cresolphthalein Complexone respectively at Golwilkar Metropolis Health Service Pvt. Ltd., Pune, India. Serum tartrate resistant acid phosphatase 5b (TRAP 5b) activity was measured using RatTRAP™ Assay (KRISHGEN Biosystems, USA).

2.4. Dual-energy X-ray absorptiometry (DEXA) measurements

For dual-energy X-ray absorptiometry (DEXA) measurements, rats were anesthetized by intraperitoneal injections of xylazine (10 mg/kg) and ketamine (100 mg/kg). The whole body was scanned using pDEXA® SABRE™ X-ray Bone Densitometer (Orthometrix Inc., USA) for evaluation of BMD, BMC, skeletal area, fat mass and lean mass.

2.5. Micro-computed tomography (μ-CT) analysis of bones

μ-CT of excised tibiae was carried out using the Sky Scan 1076 μ-CT scanner (Sky Scan, Ltd., Kartuizersweg, Kontich, Belgium). Animals were sacrificed and tibiae were dissected and cleaned of soft tissues. Bones were preserved in 10% formalin until subjected to μ-CT analysis. The X-ray source was set at 70 kV and 100 mA, with a pixel size of 18 μm. 3D reconstruction of tibiae was done using NReconn software. Proximal tibial metaphysis lying approximately 120 slices below the growth plate was selected and about 100 slices of the trabecular bone were extracted by drawing ellipsoid contours and analyzed with the CT Analyzer software (CTAn, Skyscan). Various trabecular bone parameters such as trabecular bone volume fraction (bone volume/tissue volume, BV/TV, %), trabecular number (Tb.N.), and trabecular separation (Tb.Sp., mm) and trabecular pattern factor (Tb.Pf.) values were calculated by Batmann software (Skyscan).

2.6. Statistical analysis

All data were represented as the mean ± standard deviation (SD). Statistical analysis was performed using Sigma Plot 12.0 (Systat Software Inc., CA, USA). In case of two different groups of rats, two-tailed unpaired Student's *t*-test was used, whereas two-tailed paired Student's *t*-test was employed to compare the same groups. The values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered as statistically significant (∗), very significant (∗∗) and highly significant (∗∗∗) respectively, unless otherwise mentioned.

3. Results

3.1. High fat diet induces obesity and increases bone mass in rats

Consumption of fat rich diet leads to development of obesity. To generate diet-induced obese phenotype, male Wistar rats of 8–10 weeks old were fed either with normal diet or high fat diet for 9 months and various obesity/skeletal parameters were measured. We observed significant increase in body mass and weight at days 210 and 270 in HFD rats as compared to ND rats. The average body weight of HFD rats at day 270 was 460.50 ± 49.02 g as compared to 388.66 ± 64.54 g in ND rats ($p < 0.05$) (Supplementary Fig. 1A and B). Accumulation of visceral adipose tissue was profoundly increased in HFD rats (Supplementary Fig. 1C). Analysis of DEXA measurements indicated that fat mass of HFD rats was significantly increased at days 150, 210 and 270 (Supplementary Fig. 1D). However, there was no difference in lean mass between HFD and ND rats (Supplementary Fig. 1E). Increased body weight was associated with significant increase in serum TGs, total cholesterol and LDLc at days 150, 210 and 270 (Supplementary Fig. 1F–H). These factors are well-accepted parameters of an obese phenotype, thus indicative of obesity in HFD rats.

To address whether diet-induced obesity has any impact on bone mass, we measured skeletal parameters such as BMD, BMC and skeletal areas in both ND and HFD rats. For this, we regularly monitored skeletal parameters in these rats at different time points in both ND and HFD rats. When there was ~70–80 g difference in body weight between ND and HFD rats, corresponding to increased body weight at days 210 and 270, there was significant increase in BMD and BMC in HFD rats as measured by DEXA (Fig. 1A and B). Also, there was significant increase in skeletal area and serum total ALP in HFD rats in comparison to ND rats (Fig. 1C and D). However, there was no difference in levels of serum total calcium between ND and HFD rats (Fig. 1E). We also measured serum TRAP 5b to check whether the increase in bone mass is because of decrease in osteoclast number. There was no change in TRAP 5b between

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