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

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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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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

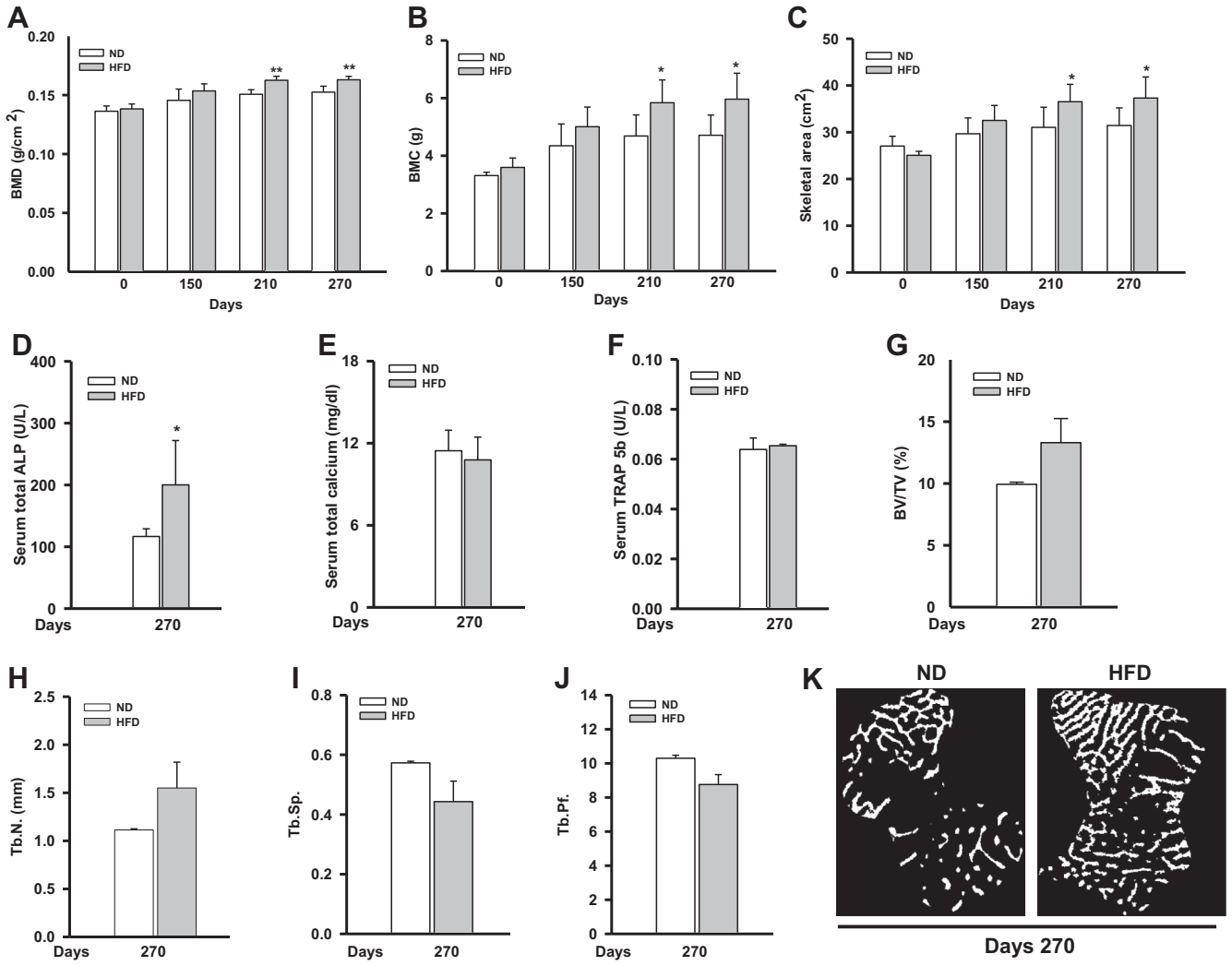

Fig. 1. Effect of high fat diet-induced obesity on bone parameters. Rats (n = 6 per group) were anaesthetized and (A) BMD, (B) BMC, and (C) skeletal area were recorded using pDEXA, Sabre at different periods. Serum biochemical parameters such as (D) total ALP, (E) total calcium, and (F) TRAP 5b were estimated using kits. Rats from normal diet and high fat diet groups (n = 3 per group) were sacrificed after 270 days. Tibias were excised and analyzed for morphological measurements of trabecular bones such as (G) BV/TV, (H) Tb.N., (I) Tb.Sp. and (J) Tb.Pf. (K) Representative μ-CT images of tibiae showing trabecular bone structures of ND and HFD rats. Results are mean ± S.D.; $^{*}p < 0.05$; $^{**}p < 0.01$.

the groups (Fig. 1F). These results suggest that increase in bone formation in HFD rats is because of increased osteoblastic activity without affecting osteoclast number.

Trabecular microstructure analysis by μ-CT showed that the HFD group had an increasing trend in BV/TV (Fig. 1G), and Tb.N. was found to be increased (Fig. 1H), but a decreasing trend in Tb.Sp. was observed (Fig. 1I). Lower Tb.Pf. signifies well connected trabecular lattices while its higher value is indicative of a more disconnected trabecular structure. HFD group showed a decreasing trend in Tb.pf. (Fig. 1J). The representative images of trabecular structures of the rats are shown in Fig. 1K. The pattern shown by these parameters suggests that increase in BMD and BMC by high fat diet is associated with acquisition of better trabecular microarchitecture at an early stage.

3.2. Dietary intervention by switching rats from high fat diet to normal diet normalizes body weight and obesity-related parameters but retains bone mass

To further investigate the effect of dietary intervention on skeletal parameters, HFD rats were shifted from high fat diet to normal diet for an additional period of 6 months. ND rats were continued on normal diet during this period. There was a significant reduction ($p < 0.05$) in body weight of HFD rats while body weight of ND rats remained unchanged (Fig. 2A). In addition, there was a significant decrease in fat mass, serum TGs, serum total cholesterol and serum LDLc ($p < 0.05$) in HFD rats corresponding to decrease in body weight (Fig. 2B and D–F). Lean mass was significantly increased in HFD rats at day 180 after shifting the diet (Fig. 2C). There was no change in all these parameters in ND rats during this period. These results suggest that dietary intervention by shifting HFD rats from high fat diet to normal diet contributes towards normalization of obesity-related parameters in these rats.

After shifting HFD rats from high fat diet to normal diet, bone-related parameters were measured at different periods. Interestingly, there was no alteration in skeletal parameters including BMD, BMC, skeletal area and serum TRAP 5b in HFD rats (Fig. 3A–D) indicating that the positive bone phenotype was maintained even after withdrawal of high fat diet. Various trabecular parameters including BV/TV, Tb.N., Tb.Sp. and Tb.Pf. remained unaltered in HFD rats after diet shifting (Fig. 4A–D). However, no significant changes were observed in all these parameters of ND

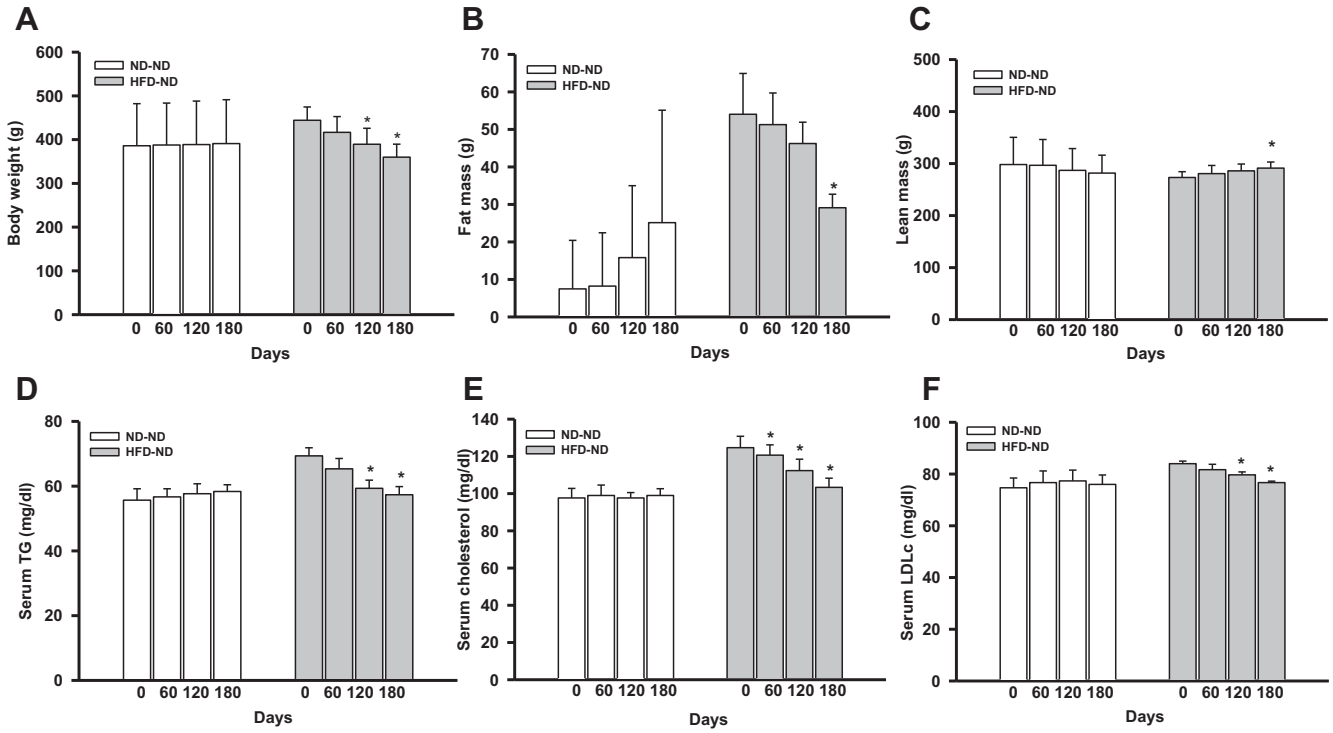

Fig. 2. Effect of changing HFD to ND on obesity in rats. After feeding HFD for 270 days, rats (n = 3 per group) were shifted to ND (HFD–ND) and the remaining 3 rats from ND group were used as control (ND–ND). (A) Body weight, (B) fat mass, (C) lean mass, (D) serum TGs, (E) serum cholesterol, and (F) serum LDLc were measured at the indicated periods. Results are mean ± S.D.; $^{*}p < 0.05$.

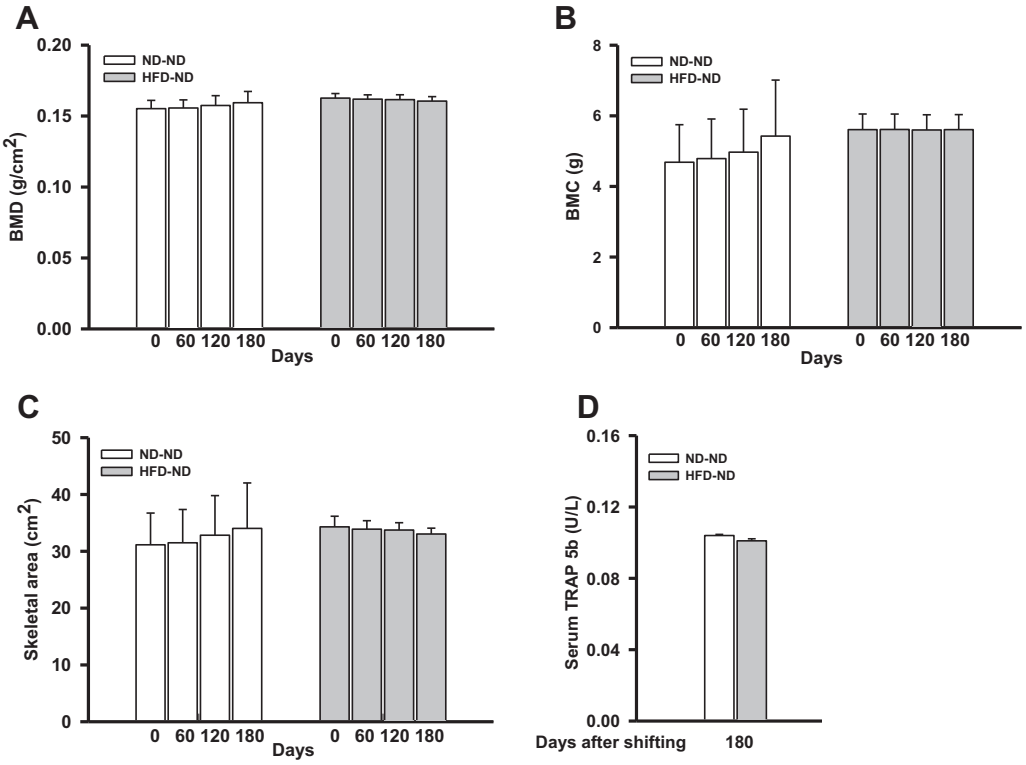

Fig. 3. Effect of changing HFD to ND on skeleton. After 270 days of feeding with HFD, rats (n = 3 per group) were shifted to ND (HFD–ND). The remaining 3 rats from ND group were used as control (ND–ND). (A) BMD, (B) BMC, (C) skeletal area, and (D) serum TRAP 5b were measured at the indicated periods. Results are mean ± S.D.

rats during this period. The representative images of trabecular structures of the ND–ND and HFD–ND rat bones are shown in Fig. 4E. This finding clearly suggests that once higher peak bone mass is achieved due to obesity, the gain is maintained even after reduction in body weight due to withdrawal of high fat diet.

4. Discussion

The link between obesity and bone is complex and multifactorial. Previous reports suggest either positive or negative correlation between obesity and the skeletal system. Diet rich in

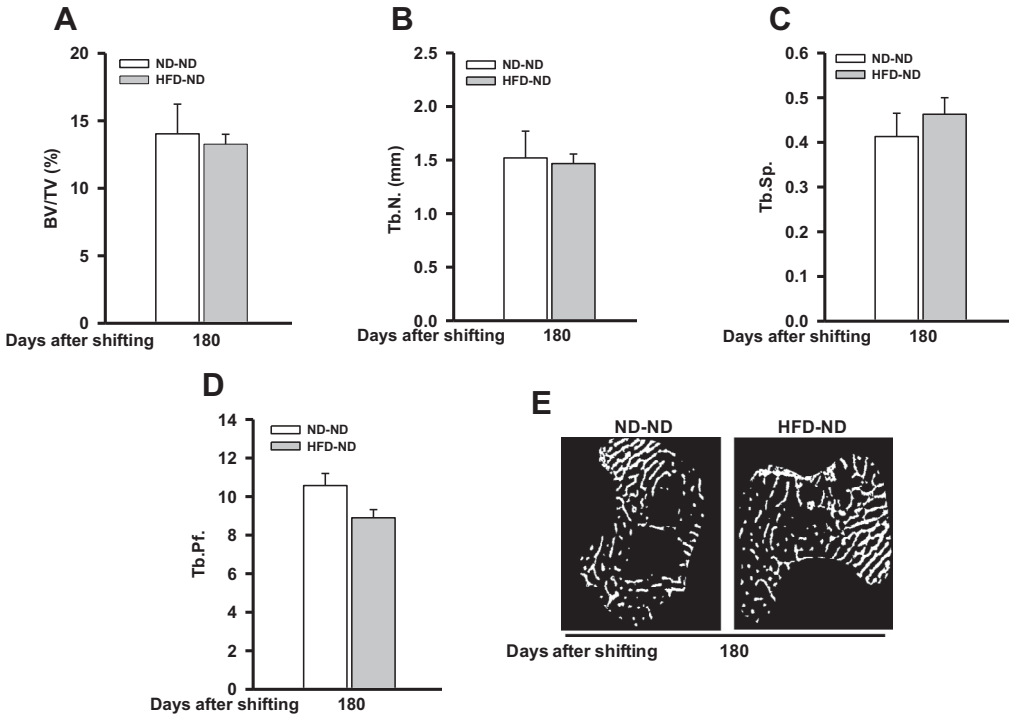

Fig. 4. Effects of changing HFD to ND on trabecular bone parameters. Three rats each from ND and HFD group were sacrificed after 270 days. Remaining 3 rats of HFD group were shifted to ND (HFD–ND) for 6 months and then killed. The remaining 3 rats from ND group were used as control (ND–ND). Tibias were analyzed and data presented for (A) BV/TV, (B) Tb.N., (C) Tb.Sp., and (D) Tb.Pf. (E) Representative μ-CT images of tibias showing trabecular bone structures of ND–ND and HFD–ND rats. Results are mean ± S.D.

polyunsaturated fatty acids, calcium, vitamin D and phosphorus are associated with good bone health [18]. However, diet with high levels of saturated fatty acids and reduced levels of calcium, vitamin D have deleterious effect on bones [19]. In diet-induced obesity, the levels of proinflammatory factors have been reported to be elevated [20]. Extreme obesity has been shown to have negative impact on bone health, which is associated with profound increase in the levels of obesity-related factors like TNF-α and IL-6 [17]. On the contrary, overweight or moderate obesity supports bone development and health [21]. Moreover, dietary restriction has been shown to reduce BMD and BMC [22,23]. A critical balance between adipocyte and osteoblast differentiation is an important regulator of bone homeostasis. A shift in the balance towards adipogenesis has been shown to be associated with bone loss. In various bone disorders, particularly in osteoporosis, increased adipocytes have been reported in the bone marrow [24]. On the contrary, reports suggest that individuals with increased body weight also have enhanced BMD [25]. So, the relationship between osteoblasts and adipocytes is still controversial. This contradiction could mainly be due to difference in the extent of obese state, dietary pattern and the age at which obesity is attained or the age at which obesity onsets.

In the present study, to evaluate the impact of diet-induced obesity on bones, we developed obese phenotype in young Wistar rats by feeding them with high fat diet. As anticipated, HFD rats exhibited increase in body weight, visceral fat accumulation (fat mass), serum TGs, cholesterol, and LDLc levels. However, lean mass was not altered markedly in these rats suggesting development of obese phenotype. Diet-induced obesity caused an increase in bone-related parameters such as BMD, BMC and skeletal area in HFD rats. Our findings suggest that high fat diet-induced obese phenotype supports bone health. Diet-induced obesity caused no significant change in serum calcium levels as both normal chow and high fat diets used in the study contained approximately same quantity of calcium (ND = 2.15% and HFD = 1.89%). Bone health is dependent on the balance between the activity of osteoblasts and osteoclasts. One way to evaluate function of osteoblast is to measure ALP levels in serum. In the present study, we found a significant increase in serum total ALP in HFD rats as compared to ND rats suggesting an increase in bone formation [26]. We observed no change in serum TRAP 5b activity between ND and HFD groups thereby implying that the increase in bone mass was not because of decreased osteoclast number. μ-CT data showed a trend of better connectivity and geometry of trabecular bones of HFD group that had achieved higher bone mass over the ND group. The status of trabecular microarchitecture significantly contributes to bone strength and may reduce fracture risk beyond BMD [27]. From our data showing increased BMD accompanied by a trend of better microarchitecture in the HFD over ND group, it appears that HFD group may have stronger resistance to fractures than the ND group. Since HFD was supplemented with ground nut and coconut it is likely that lipid components (unsaturated fatty acids and others) present in dried ground nut and coconut could be helpful in enhancing bone-related parameters. Additionally, the possibility of obesity-related serum factors (nutritional or hormonal) in HFD rats being associated with improved bone health cannot be ruled out.

Dietary intervention or manipulation plays an important role in bone health and strength. Therefore, we speculated that shifting HFD rats to normal chow diet could also have an impact on bone mass. Interestingly, HFD rats shifted to normal diet have had normalization in body weight, fat mass, serum TGs, cholesterol and LDLc. However, the gain in skeletal parameters (BMD, BMC and skeletal area) were maintained even after six months of shifting the HFD rats to ND. Similar observations were found in μ-CT analysis, where the parameters remained unchanged after diet shifting. These observations imply that high fat diet promotes peak bone mass achievement at younger age. ND rats required 450 days to achieve the BMD that was achieved by HFD fed rats in 270 days. Thus, high fat diet helps in achieving peak bone mass during early

growth. Since individuals with high peak bone mass are less likely to suffer from osteoporosis, high fat diet during growing age may mitigate bone loss later in life.

Conflict of interest

None declared.

Note

This work was partly presented in "12th International Congress on Obesity (ICO) 2014" organized by World Obesity Federation [formerly International Association for Studies on Obesity (IASO)] at Kuala Lumpur, Malaysia during March, 17–20, 2014. The abstract was published in Volume 15, Issue Supplement S2 of Obesity Reviews, March 2014. PM received the International Travel Award from Science and Engineering Research Board, Department of Science and Technology (DST), Government of India to attend this conference under International Travel Support (ITS) scheme.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.131.

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