

Possible therapeutic effects of berberine on bone damage in high-fat diet-induced obese rats

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Research

Keywords: osteoporosis, HFD-induced obesity, berberine

Posted Date: February 28th, 2020

DOI: <https://doi.org/10.21203/rs.2.24831/v1>

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Abstract

Background Obesity has a negative impact on bone health; thus far, no specific pharmacotherapy has been approved. Berberine has shown improvements in osteoporosis in some animal models. However, it remains unknown whether berberine ameliorates obesity-induced bone damage. This study aims to evaluate the effect of berberine on bone damage in high-fat diet (HFD)-induced obese rats.

Methods Male Sprague-Dawley rats were randomly assigned to a normal control diet (ND) group or HFD group. After the HFD induced obesity, the models were successfully established. Then, these rats were randomly divided into the HFD + berberine (HB) group or HFD group and were intraperitoneally administered berberine or an equivalent volume of DMSO, respectively, once a day for another 10 weeks. Micro-CT and three-point bending tests were conducted to evaluate bone microstructure and biomechanics. Serum was collected for the detection of P1NP, CTX-1, calcium (Ca) and phosphorus (P), tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). HE staining was performed to observe the number of trabecular and bone marrow adipocytes.

Results HFD-induced obese rats displayed decreased biomechanical properties, such as maximum load, maximum fracture load, ultimate tensile strength, stiffness, and energy absorption, compared with the ND rats ($p < 0.05$). However, berberine attenuated bone damage, including maximum load, maximum fracture load, and stiffness, in the HB group compared with the HFD group ($p < 0.05$). Trabecular bone markers were decreased in the HFD group compared with the ND group ($p < 0.05$). All parameters were improved, as shown by micro-CT and HE staining, in the HB group compared to the HFD group. Cortical bone markers were not significantly different among all groups. Moreover, CTX-1, TNF- α , IL-1 β and the number of adipocytes in bone marrow were significantly increased in HFD-induced obese rats. After treatment with berberine, TNF- α , IL-1 β and the number of adipocytes in bone marrow were significantly decreased, and P1NP levels were higher in the HB group than in the HFD group.

Conclusions Berberine might be a potential therapeutic agent for treating bone damage in HFD-induced obese rats by inhibiting inflammatory factors, reducing bone marrow adiposity and improving bone formation.

Background

Obesity has emerged as a critical public health issue related to increased prevalence rates of both mortality and morbidity, not only in Western countries but also in China [1, 2]. Its negative impact on bone damage has been recently reported. Data suggested that bone mineral density (BMD) had significant negative correlations with waist circumference, total fat area, subcutaneous fat area, appendicular fat mass and percent fat mass in premenopausal women [3]. In addition, in 20- to 49-year-old men, visceral adipose tissue was negatively associated with volumetric bone mineral density (vBMD), cortical thickness, and trabecular microstructure at the ultradistal radius and with both lumbar spine and femoral neck cortical vBMD [4]. In addition, compared to women of normal weight, obese women were more likely

to have at least one vertebral fracture and at least one mild vertebral deformity. Obese men were more likely to have a moderate and/or severe vertebral fracture compared to men of normal weight [5]. However, no specific pharmacotherapy has been approved. Hence, the search for effective therapies in the management of bone damage associated with obesity is imperative.

Berberine is the main alkaloid purified from rhizoma coptidis. It has numerous positive effects, including those on blood glucose and blood lipids, as well as antidiarrhoeal, antibacterial, anti-inflammatory, antitumour, antiarrhythmic and acute myocardial ischaemic effects [6–8]. In addition, data showed that berberine could attenuate the differentiation of osteoclasts and promote osteoblast differentiation in vitro [9]. In vivo experiments have shown that berberine can ameliorate periodontal bone loss and improve osteoporosis in some animal models [10–14]. However, it remains unknown whether berberine ameliorates obesity-induced bone damage. This study aims to evaluate the effects as well as the underlying mechanism of berberine on bone health in HFD-induced obese rats.

Material And Methods:

Animal experiments

The animal experimental protocol was approved by the Animal Ethics Committee of Shandong Provincial Hospital. The strategic abstract of the experimental process is shown in Fig. 1a. Male Sprague Dawley rats (4 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co. (Beijing, China). All experimental rats were housed in sterilized cages under conditions of 12 h light/dark cycles, 50% humidity, and controlled temperature (22-24°C) and given free access to food and water. After acclimatization for 1 week, rats were randomly assigned to one of two diets: normal control diet (ND group, n=8) (10% kcal fat) or high-fat diet (HFD group, n=30) (60% kcal fat, Beijing Keao Xieli Feed Co. Ltd., Beijing, China). Body weight was tested weekly. After 12 weeks, HFD-induced obese models were successfully established, when the body weight and fat percentage of rats in the HFD group were significantly higher than those in the ND group [15]. Then, these rats were randomly divided into the HB group (HF diet + berberine (Sigma-Aldrich, St. Louis, MO, USA), n=8) or the HFD group (HF diet, n=8) and were intraperitoneally administered berberine at a dose of 3 mg/kg or an equivalent volume of DMSO once a day for another 10 weeks. Furthermore, the ND group was intraperitoneally administered the same volume of DMSO as the HFD group.

Body composition analysis

Body composition was measured at the end of 12 weeks by dual energy X-ray absorptiometry (DEXA) using a Lunar PIXImus bone densitometer (GE, Madison, WI, USA) with small animal software (Hologic QDR 4500A). DEXA was performed under anaesthesia.

Serum sample and **bone** tissue acquisition

All rats were fasted for 12 h and anaesthetized with 1% pentobarbital sodium at the end of 12 weeks and 22 weeks. Serum was collected after centrifugation at 3000 rpm for 10 min. After removing the soft tissue, the right femurs and left tibias were removed and stored in 4% paraformaldehyde for microcomputed tomography (micro CT) analysis and bone histology analysis. Left femurs were wrapped in saline gauze and frozen at -30°C for the three-point bending test.

Biochemical analysis

Serum levels of calcium (Ca) and phosphorus (P) were measured using an Olympus AU5400 automatic biochemical analyser (Olympus Co, Ltd, Tokyo, Japan). Serum levels of procollagen type 1 N-terminal propeptide (P1NP) and C-telopeptide of type 1 collagen (CTX-1) were measured using an ELISA kit (Cusabio, Wuhan, China). Serum levels of tumour necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) were also measured using ELISA kits (ExCell Bio., Shanghai, China). All measurements were conducted according to the manufacturer's instructions.

Bone biomechanical analysis

Bone strength was assessed at the organ and tissue levels by three-point bending. The three-point bending test was performed on a universal testing machine (Bose ElectroForce® 3230, Bose Corporation, USA) by placing the bones, anterior side up on two supports that were equidistant from the ends and 16 mm apart. The load was applied at a constant deformation rate of 2 mm/min. The diaphysis of the femur was loaded until a fracture occurred to determine the yield and fracture parameters. The yield represents the point at which bone ceases to behave elastically. The data were automatically recorded in a computer interfaced with the testing machine, and a typical load-deformation curve was created. A saline solution was used to keep specimens moist during testing. The maximum load, maximum fracture load, energy absorption, stiffness, ultimate tensile strength and elastic modulus were measured.

Bone microcomputed tomography (micro CT) analysis

Bone microarchitecture was investigated by micro CT. Micro CT analyses were performed on femurs with a Skyscan 1176 micro CT system (Brook Corporation, Belgium), and the images were reconstructed to an isotropic voxel size of 12 μ m. The trabecular volume of interest was located 0.5 mm below the growth plate and extended to 2 mm. The cortical volume of interest was centred at the midshaft femur and extended out 1 mm. All 3D image manipulations and analyses were performed using system software (MicroView, v.2.1, GE Healthcare).

Histological analysis of bone

Left tibias were fixed in 4% paraformaldehyde (Sigma-Aldrich), decalcified in 10% ethylenediaminetetraacetic acid (Sigma-Aldrich) at pH 7.0, and then embedded in paraffin. Longitudinal sections (5- μ m thick) were stained with HE to observe the number of trabecular and bone marrow adipocytes. All pathological images were observed using a light microscope from several visual fields per slice (Axiovert 100 M Zeiss, Zeppelinstrasse, Germany) at 100 \times magnification. The number of trabecular

and adipocyte analyses was determined by a pathologist who was blind to the grouping situation according to the results of the images.

Statistical analysis

Data were analysed by SPSS 22.0 software and are expressed as the mean \pm standard deviation (SD). Means were compared using unpaired Student's *t*-tests for comparisons between two groups and one-way ANOVA for comparisons among multiple groups. A two-tailed *P* value <0.05 was considered significant.

Results

Rat model of HFD-induced obesity

The obese rat model was successfully established when the body weight of rats in the HFD group increased more than 20% compared with that of rats in the ND group. In our study, a HFD led to a higher body weight in rats at the end of the experiment than a ND (Fig. 1b). Body weight in the HFD group increased 26.9% compared with that in the ND group at the end of 12 weeks (Fig. 1c). The total fat percent in the HFD group increased significantly compared with that in the ND group (Fig. 1d). There was no difference in total muscle between the HFD group and the ND group (Fig. 1e).

The effect of berberine (BBR) on P, Ca, bone formation markers, bone resorption markers, and inflammatory factors IL-1 β and TNF- α in rats

There was no difference in serum P or Ca between the ND group and HFD group before treatment with BBR (12 weeks). There was also no difference in serum P or Ca among the three groups after treatment with BBR (22 weeks) (Fig. 2a-d).

In contrast, the serum levels of the bone formation marker P1NP were lower in the HFD group than in the ND group, although the difference was not significant ($p = 0.7809$ (12 weeks), $p = 0.2637$ (22 weeks)), and were significantly higher in the HB group than in the HFD group ($p = 0.0101$) (Fig. 2e-f). In contrast, the serum levels of CTX-1, a marker of bone resorption, were elevated in the HFD group compared with the ND group ($p = 0.1172$ (12 weeks), $p = 0.014$ (22 weeks)) and reduced in the HB group compared with the HFD group ($p = 0.0784$) (Fig. 2g-h).

The serum levels of IL-1 β and TNF- α were higher in the HFD group than in the ND group ($p = 0.0286$, $p = 0.0387$ at 12 weeks; $p = 0.0026$, $p = 0.0014$ at 22 weeks) and significantly lower in the HB group than in the HFD group ($p = 0.0002$, $p = 0.0139$).

BBR improved bone biomechanical properties after bone damage in HFD-induced obese rats

The results of the three-point bending test in the femur samples showed that the biomechanical properties, such as maximum load ($p = 0.0003$), maximum fracture load ($p = 0.0024$), stiffness ($p =$

0.0028), energy absorption ($p = 0.0029$), and ultimate tensile strength ($p = 0.003$), were significantly decreased in the HFD-induced obese group compared with the ND group. The maximum load ($p = 0.0062$), maximum fracture load ($p = 0.0226$) and stiffness ($p = 0.0171$) were significantly enhanced in the HB group compared with the HFD group, but ultimate tensile strength, energy absorption, and elastic modulus showed no significant differences between the HB group and HFD group. (Fig. 3a-f).

The effect of BBR on bone microstructure in HFD-induced obese rats

Consistent with the results of the three-point bending test, the micro CT test showed that the trabecular volume bone mineral density (Tb. vBMD) ($p < 0.01$), trabecular bone volume/total volume (Tb. BV/TV) ($p < 0.01$), trabecular number (Tb. N) ($p < 0.01$), and trabecular thickness (Tb. Th) ($p < 0.01$) were decreased and that the trabecular separation (Tb. Sp) ($p < 0.01$) and structure model index (SMI) ($p < 0.01$) were increased in the HFD group compared with the ND group; furthermore, all of the above parameters (Tb. vBMD, $p = 0.1967$) (Tb. BV/TV, $p = 0.2179$) (Tb. N, $p = 0.2295$) (Tb. Th, $p = 0.3218$) (Tb. Sp, $p = 0.6898$) (SMI, $p = 0.1365$) were improved in the HB group compared to the HFD group, but the differences were not significant (Fig. 4a, 4e-j). Moreover, the cortical volume bone mineral density (Ct. vBMD), cortical bone volume/total volume (Ct. BV/TV) and cortical bone thickness (Ct. Th) showed no significant differences among the three groups (Fig. 4b-d).

The effect of BBR on the trabecular number and the number of adipocytes

The results of HE staining in the left tibia samples showed that the trabecular number was decreased in the HFD-induced obese rats compared with the ND rats ($p < 0.01$), while those in the HB group had an increased trabecular number compared with those in the HFD group ($p = 0.0546$) (Fig. 5a-d). We also found that the number of adipocytes in the HFD group was increased compared with that in the ND group ($p < 0.01$), while it was significantly decreased in the HB group compared with the HFD-induced obese group ($p < 0.01$) (Fig. 5a-c and e).

Discussion

In this study, we investigated bone health in HFD-induced obese male rats and the effect of berberine on these rats. In accordance with recent studies, our results show that obesity significantly reduces bone microstructure and bone strength, and the major finding of this study was that berberine can alleviate this damage to some extent.

Obesity is a global health issue associated with significant morbidity and mortality, which independently increases the risk for chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, hypertension, stroke, and some types of cancer [16]. In addition, its negative impact on bone damage has been recently reported. Fewer studies on osteoporosis have been conducted in males than in females. Recent studies indicated that visceral adipose tissue was also a risk factor for osteoporosis in men [4, 17] and that obese men were more likely to have vertebral fracture than men of normal weight [5]. Another study indicated that obesity induced by a HFD aggravated bone loss in the cancellous bone

compartment, with a greater loss in males than females [18]. Our current study also focused on male rats, and bone damage was observed in HFD-induced obese male rats. Trabecular bone loss and bone strength declined. Tb. vBMD, Tb. BV/TV, and Tb. N were decreased, and Tb. Th, Tb. Sp and SMI were increased in the HFD-induced obese group compared with the ND group. The maximum load, maximum fracture load, stiffness, energy absorption, and ultimate tensile strength were significantly decreased in the HFD-induced obese group compared with the ND group. However, cortical bone parameters remained unchanged in our study. Consistent with Cao et al [19], obesity induced by a high-fat diet decreases cancellous bone mass but has no effect on cortical bone mass in the tibia. The finding that cancellous rather than cortical bone is affected in HFD-induced obesity is not surprising. In general, cancellous bone is more responsive than cortical bone to diet or drug treatments, physiological status, or ageing because cancellous bone undergoes more active remodelling than cortical bone due to its larger surface-to-volume ratio [20]. In our study, bone strength decline in HFD-induced obesity was attenuated significantly by treatment with berberine, as shown by the three-point bending test. Yogesh, H. S. indicated that berberine chloride exerted protective effects on bone in an osteoporotic rat model induced by ovariectomy [10], and Xu et al demonstrated that berberine significantly increased femur load and stiffness in glucocorticoid-treated animals [11], which were consistent with our study. Xie et al showed that berberine increased femoral BMD, BV/TV, Tb. N, and Tb. Th as well as reduced Tb. Sp and SMI in rats with diabetic osteoporosis induced by streptozotocin and a high-fat diet [13]. Trabecular bone loss was also attenuated in our study, but the difference was not significant, as shown by micro CT and HE staining.

The results from the present study demonstrated increased inflammatory cytokine levels and bone marrow adiposity in HFD-induced obese rats, which might not be good for skeletal strength or for optimal function of the bone remodelling unit [21]. Osteoblasts and adipocytes are derived from a common multipotential mesenchymal stem cell [22]. Marrow stromal cells isolated from postmenopausal osteoporotic patients express more adipocytic differentiation markers than those with normal bone mass [23] and are more likely to enter an adipocyte differentiation programme than an osteoblast one [24]. Peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2), an essential factor in adipocyte differentiation, can inhibit osteoblast differentiation [25]. Fat in bone marrow might not only suppress osteoblastogenesis but also promote bone resorption because marrow adipocytes, much like fat cells elsewhere, secrete inflammatory cytokines [26]. Obesity is associated with a chronic inflammatory response, abnormal cytokine production, and activation of inflammatory signalling pathways, which are involved in and responsible for the development of obesity-related diseases [27]. Proinflammatory cytokines, including TNF- α , IL-1, and IL-6, are key mediators in the process of osteoclast differentiation and bone resorption. These proinflammatory cytokines are capable of stimulating osteoclast activity through the regulation of the RANKL/RANK/OPG pathway [28, 29]. Normal bone development can occur in mice lacking IL-1 and TNF genes [30]. Interestingly, overexpression of soluble TNF- α decoy receptor [31] or ovariectomy did not cause bone loss. However, blocking the action of IL-1 with an IL-1 receptor antagonist or the signalling of TNF- α with a TNF-binding protein decreased osteoclast formation and bone resorption in ovariectomized mice [32]. Furthermore, superoxide-driven oxidative stress, a common factor in obese adipose tissue, has been shown to stimulate osteoclastogenesis, bone matrix

degradation, and bone resorption [33]. In our study, the circulating bone resorption marker CTX was significantly increased in obese animals compared with the ND animals, which confirmed that bone resorption was increased. After treatment with berberine, TNF- α and IL-1 β levels and the number of adipocytes in bone marrow were significantly decreased in the HB group compared with the HFD-induced obese group. Berberine significantly increased the circulating bone formation marker P1NP. There was no significant difference in the bone resorption marker CTX. Furthermore, the number of rats in the experiment was limited, so this difference may become statistically significant if the sample size is increased. These results indicate that the inhibitory effects of berberine on inflammation and bone marrow adipocytes could promote osteoblastogenesis and inhibit bone resorption, which could be one mechanism by which berberine prevents obesity-induced osteoporosis.

There were some limitations in this study. One limitation was that the present study was conducted in a rodent model and not in humans. Another limitation was that the mechanism by which berberine alleviates bone damage was not investigated at the molecular and genetic levels. In the next step, we will conduct in vivo experiments to further elucidate the protective mechanism of berberine in alleviating bone damage induced by obesity at the molecular and genetic levels and to provide a theoretical basis for the clinical treatment of bone damage caused by obesity.

Conclusions

In conclusion, the results from the present study suggest that berberine might be a potential therapeutic agent for treating bone damage in HFD-induced obese rats by inhibiting inflammatory factors, reducing bone marrow adiposity and improving bone formation. However, whether the clinical application of berberine could be beneficial in the population needs further confirmation.

Abbreviations

CTX-1

C-telopeptide of type 1 collagen; HFD:high-fat diet; ND:normal control diet; P1NP:procollagen type I N-terminal propeptide; TNF- α :tumor necrosis factor- α ; IL-1 β :interleukin - 1 β ; Ca:calcium; P:phosphorus; micro CT:bone microcomputed tomography; BBR:berberine

Declarations

Acknowledgements

Not applicable

Funding

This work was supported by Shandong Provincial Key Research Project [Grant No. 2016GSF201025, 2016GGH3118], the National Science Foundation of China [Grant No.81370892], the Taishan Scholars Special Foundation of Shandong Province, the Medical and Health Technology Development Project of

Shandong Province [Grant No.2017WS704], Science and Technology Development Plan Project of Tai'an [Grant No 2019NS231], and the Academic Promotion Program of Shandong First Medical University [Grant No. 2019QL017].

Availability of data and materials

All data generated or analysed during this study are included in this published article or are available from the corresponding author upon reasonable request.

Authors' contributions

Design of the study was carried out by JX, YW and FC. Experiments were performed by YW, FC, YW, JL and ZH. Data analyses were performed by HW and MZ. Technical assistance was performed by SS and CY. The manuscript was written by YW and approved by JX. All authors read and approved the final manuscript.

Ethics approval

The animal experimental protocol was approved by the Animal Ethics

Committee of Shandong Provincial Hospital affiliated with Shandong University (Jinan, China).

Consent for publication

All authors support the submission to this journal.

Competing interests

The authors declare that they have no competing interests.

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Figures

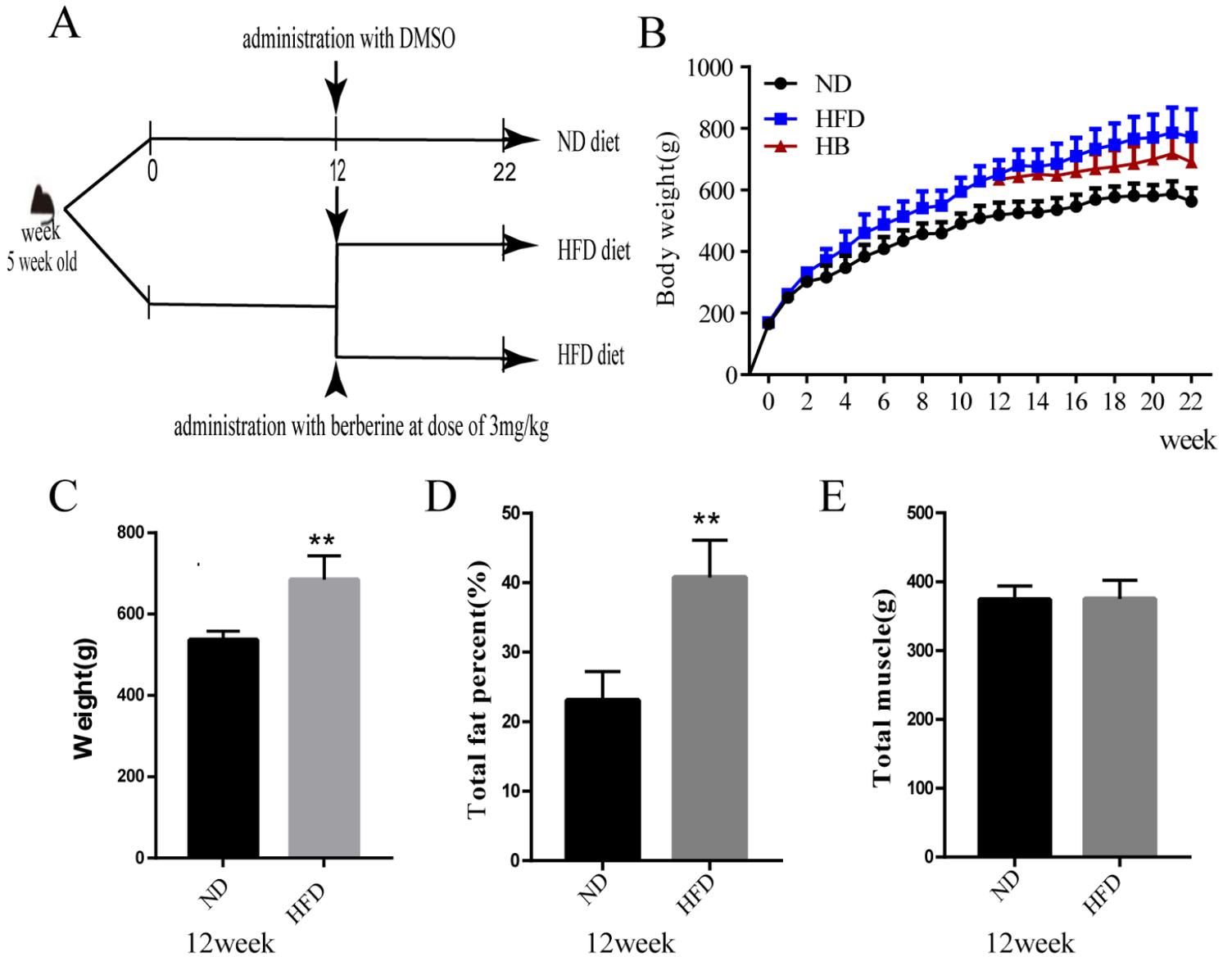


Figure 1

The effect BBR on body weight and body composition. a Strategic abstract of the experimental processes. b Body weight per rat during the experiment(n=8). c Body weight per rat in 12 week(n=8). d Total fat percent per rat in 12 week(n=6). e Total muscle per rat in 12 week(n=6). Statistical analysis were done with t test, *p<0.05, **p<0.01 versus the ND group.

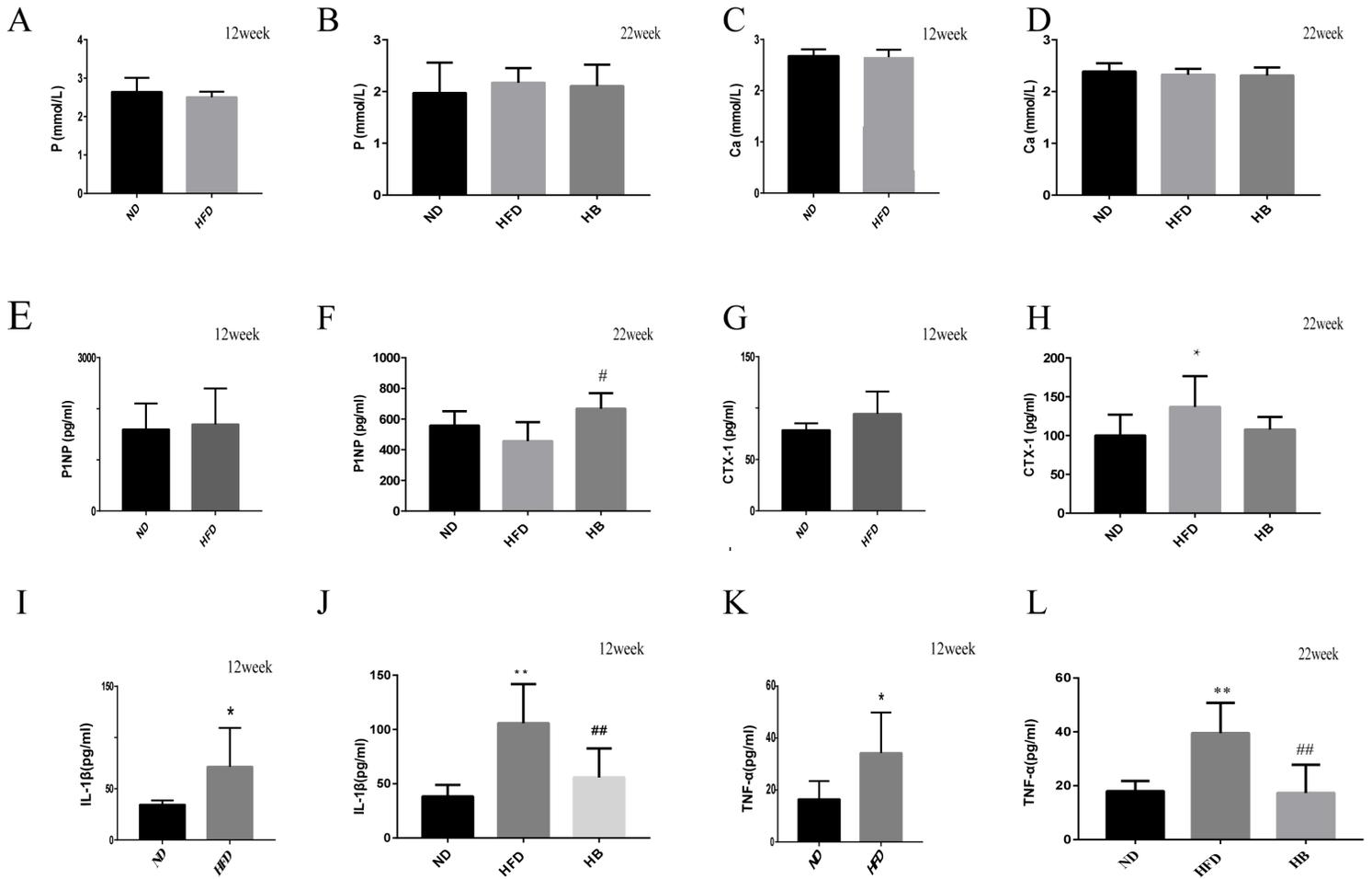


Figure 2

The effect of BBR on serum phosphorus(P), calcium(Ca), bone formation markers, bone resorption marker, inflammatory cytokine tumor necrosis factors- α (TNF- α) and inflammatory factor-1 β (IL-1 β). a b serum P in 12 week and 22 week. c d serum Ca in 12week and 22week e f bone formation markers: P1NP, procollagen type I N-terminal propeptide in 12week and 22week. g h bone resorption marker: CTX-1, C-telopeptide of type 1 collagen in 12week and 22 week. i j TNF- α in 12 week and 22 week. k l IL-1 β in 12 week and 22 week. Data are expressed as the mean \pm SD (n=6). *p<0.05, **p<0.01 versus the ND group and # p<0.05, ## p< 0.01 versus the HFD group by t test or one-way ANOVA.

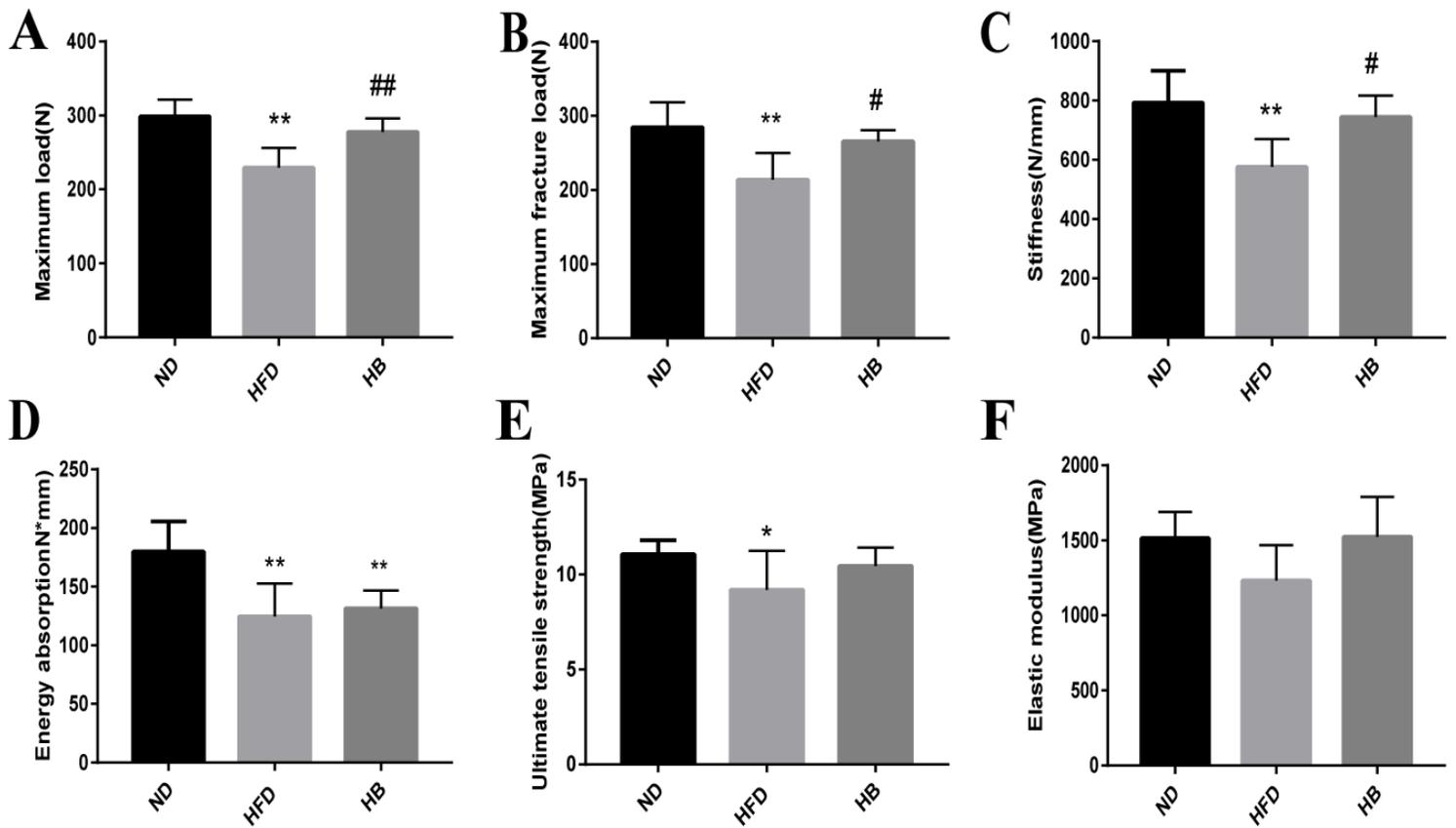


Figure 3

The effect of BBR on bone biomechanical properties. a The maximum load of the bone before crushing. b Maximum fracture load of the bone crushing. c Stiffness, the slope of the linear region. d Energy absorption, AUC of the load multiplied by the displacement. e Ultimate tensile strength of the bone before brittle fracture. f Elastic modulus, maximum slope of the stress-strain curve. Data represent the mean \pm SD (n=6). *p<0.05, **p<0.01 versus the ND group and # p<0.05, ## p< 0.01 versus the HFD group by one-way ANOVA.

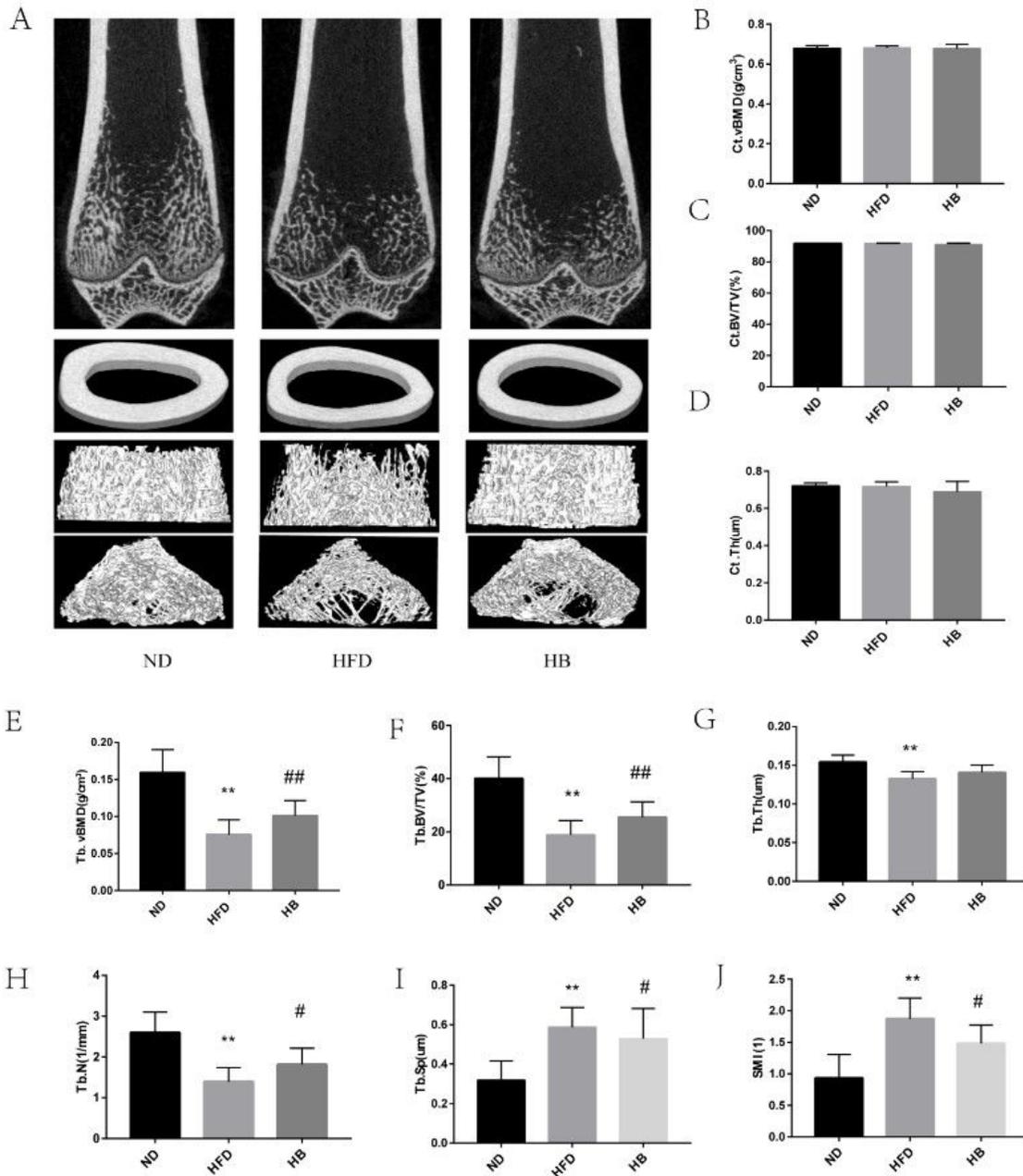


Figure 4

The effect of BBR on bone structural characteristics by micro CT. a Representative 2D and 3D images of micro CT reconstruction of distal femurs. b-d Micro CT analysis of cortical bone parameters: Ct. vBMD, cortical volumetric bone mineral density; Ct. BV/TV, cortical bone volume/total volume; Ct.Th, cortical bone thickness. e-j Micro CT analysis of trabecular bone parameters: Tb. vBMD, trabecular volumetric bone mineral density; Tb. BV/TV, trabecular bone volume/total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; SMI, structure model index. The results are shown as the mean \pm SD (n=6). *p<0.05, **p<0.01 versus the ND group and # p<0.05, ## p<0.01 versus the HFD group by one-way ANOVA.

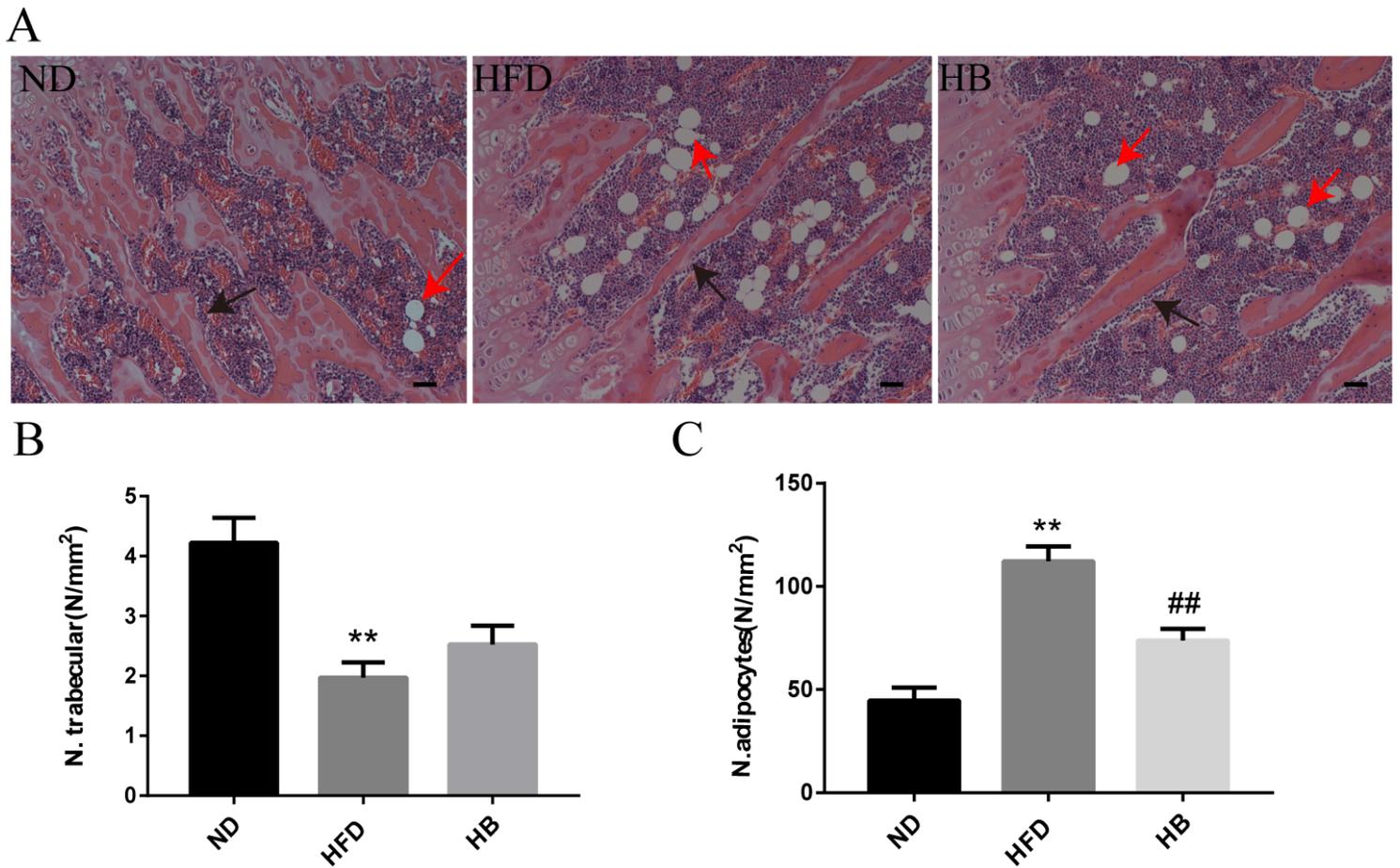


Figure 5

H&E staining to observe the trabecular number and number of adipocytes from rat tibial sections. a Representative images are shown. Scale bar, 100 μ m. The red arrows represent adipocytes. The black arrows indicate the trabecular bone structure. The red arrows represent adipocytes. b The trabecular number (N. trabecular) per square millimetre was evaluated (n=5). c The number of adipocytes (N. adipocytes) per square millimetre was evaluated (n=5). *p<0.05, **p<0.01 versus the ND group and ## p<0.01 versus the HFD group by one-way ANOVA.