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Original Article

Melatonin Protects Bone Microarchitecture against Deterioration due to High-Fat Diet-Induced Obesity

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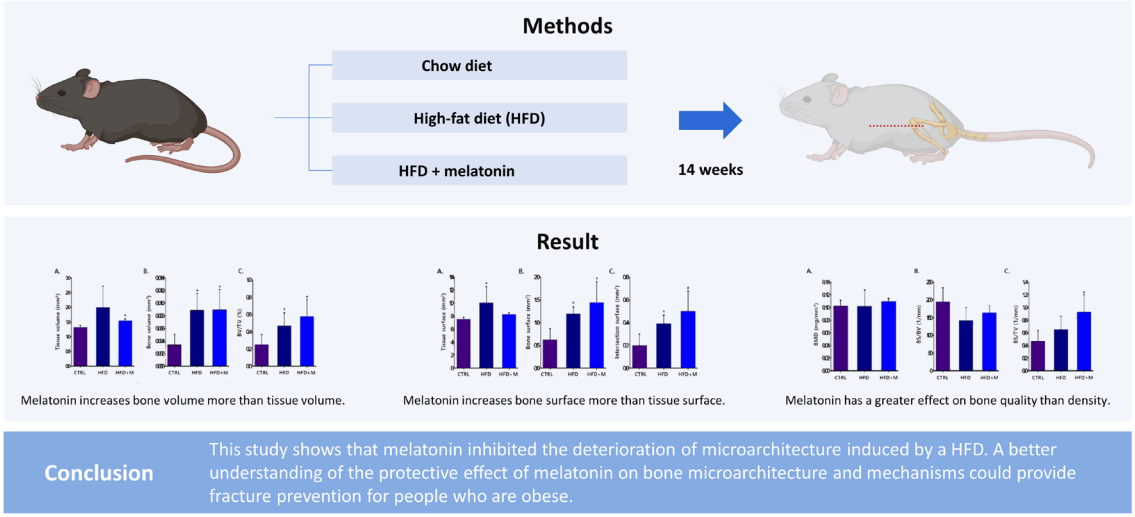
Background: We evaluated the protective effects of melatonin against high-fat diet (HFD)-induced deterioration of bone microarchitecture using high-resolution peripheral quantitative computed tomography (HR-pQCT). **Methods:** Four-week-old male C57BL/6 mice were divided into control (chow diet group), HFD, and HFD + melatonin-administered groups. Mice were sacrificed after 14 weeks, and the right femur was extracted. The microskeletal structure of the femur was analyzed using SkyScan1173 (version 1.6). A 3-dimensional image was reconstructed using the Nrecon (version 1.7.0.4) program. **Results:** Bone volume (BV) was significantly increased in the HFD group compared with that in the normal diet group, and that of the melatonin group also increased significantly compared with BV of the normal diet group ($P<0.05$). Percent BV/total volume [TV] and bone surface/BV were significantly higher in both the HFD and melatonin groups than in the normal diet group ($P<0.05$), and the melatonin group had the highest BV/total volume (TV). BMD was lower in the HFD than in the normal diet group and was the highest in the melatonin group. **Conclusions:** This study shows that melatonin inhibited the deterioration of microarchitecture induced by a HFD. A better understanding of the protective effect of melatonin on bone microarchitecture and mechanisms could provide fracture prevention for people who are obese.

Key Words: Bone and bones · High resolution QCT · Melatonin · Obesity · Quantitative computed tomography

INTRODUCTION

The prevalence of obesity is increasing worldwide. Obesity is a chronic disease that can lead to many adverse health complications including cardiovascular disease, high blood pressure, diabetes, hyperlipidemia, sleep apnea, and cancer.[1,2] The obesity rate in Korea is increasing rapidly, increasing 1.18 times in 10 years from 32.6% in 2009 to 38% in 2018.[3] Obese patients have a higher mortality rate than non-obese people due to these comorbidities.[4,5] Severe obesity is particularly serious among young people, and the prevalence of patients with class III obesity in their 20s and 30s with a body mass index greater than 35.0 kg/m^2 increased 3.8 times for men and 3.5 times for women in 2018 compared with 2009.[6] This increase in obesity among young people can lead to a sharp increase in chronic diseases and mortality as their age increases, which can subsequently result in a serious socioeconomic burden.

Graphical Abstract



Osteoporosis, another chronic disease, is also increasing significantly as society ages.[7] Osteoporosis and osteoporotic fractures are also on the rise in Korea.[8] Osteoporosis is a severe disease with a high mortality rate following fracture incidents within one year, which is 20% to 40% and 5.4% for hip and vertebral fractures,[9,10] respectively. Interestingly, obesity is one of the protective factors against osteoporotic fractures. Osteoporotic patients of normal weight have an increased number of fractures compared to those with obesity.[11] However, obese patients have a higher mortality rate following fractures.[12] Furthermore, the rate of fracture increases in the presence of diabetes or fatty liver, which are both more prevalent in the obese population.[13] While bone density is increased in obese patients, bone quality is speculated to have deteriorated.[14] However, there are not many existing studies about the relationship between obesity and bone quality due to the lack of methods to efficiently and accurately measure bone quality. Recently, 2 studies have used high-resolution peripheral quantitative computed tomography (HR-pQCT) to measure bone volume (BV) and changes in the microscopic structure of cortical bone in high-fat diet (HFD)-fed rats.[15,16]

Melatonin inhibits bone loss and increases bone strength through multiple mechanisms.[17] Melatonin directly inhibits osteoclast differentiation and activation and promotes osteoblast differentiation.[18,19] Therefore, this study was conducted to investigate bone quality deterioration in terms of microscopic bone structure in mice with obesity induced by a HFD. Additionally, we evaluated the protective effect of melatonin on the HFD-induced deterioration of the bone microarchitecture.

METHODS

1. Animal experiments

Four-week-old male C57BL/6 mice were purchased from Orient Bio (Seongnam, Korea). The mice were housed in a humidity-controlled room maintained at 24℃ under a 12-hr light-dark cycle with free access to food and water. All animal experiments were performed according to the Animal Experimentation Ethics Committee of Kosin University (KMAP-20-8). The mice were divided into 3 groups of 5 mice each: (1) Control group (fed standard rodent chow containing 5% fat, 74% carbohydrates, and 21% protein); (2) HFD group (fed HFD that contained 60% fat, 20% protein, 20% carbohydrate); (3) Melatonin group (fed HFD and administered melatonin). Melatonin (50 mg/kg body weight) was injected into the abdominal cavity 5 afternoons per week for 14 weeks. Mice in the HFD group were injected with the same volume of saline. Body weight was measured every week in the morning (10:00-11:00). After 14 weeks, all

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experimental animals were euthanized with inhalation anesthesia, and femurs were collected.

2. Bone microarchitecture analysis

The micro-skeletal structure of the femur was analyzed using SkyScan1173 (version 1.6; Bruker-CT, Kontich, Belgium). The regions from 1.5 to 2 mm distal to the growth plate of their right femurs were analyzed. The 3-dimensional (3D) images were reconstructed using the Nrecon (version 1.7.0.4) program. The following data were collected: total volume (TV); volume of the entire region of interest, BV; volume of the region segmented as bone, total tissue surface (TS); surface of entire region of interest, bone surface (BS); surface of the region segmented as bone, and intersection surface (IS); total cross-sectional area of entire region of interest.

3. Statistical analysis

Experiments were repeated at least 3 times. Data are expressed as the mean±standard deviation (SD) from each independent experiment. Statistical analyses were per- formed using IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Student's *t*-tests were performed for continuous variables, and *P*-values lower than 0.05 were considered statistically significant.

RESULTS

The average body weight before HFD administration was 17.64 g in the normal diet group, 17.42 g in the HFD group, and 18.16 g in the melatonin administration group. After 14 weeks, the final weight gain was 31.17% (23.14 g) in the normal diet group, 109.18% (36.44 g) in the HFD group, and 78.63% (32.44 g) in the melatonin-administered group. Both the high-fat and melatonin groups gained significantly more weight than the control group ($P<0.05$). The average body weight of the melatonin-administered group was lower than that of the HFD group, but this difference was not statistically significant (Fig. 1).

The tissue volume increased significantly in the HFD group compared to the control group, but the difference was not statistically significant between the melatonin group and the HFD group. In the melatonin group, tissue volume was lower than that of the HFD group and higher than the control group with statistical significance ($P<0.05$) (Fig. 2A). BV was significantly increased in the HFD group compared with the control group, and it was also significantly increased in the melatonin group compared with the control group

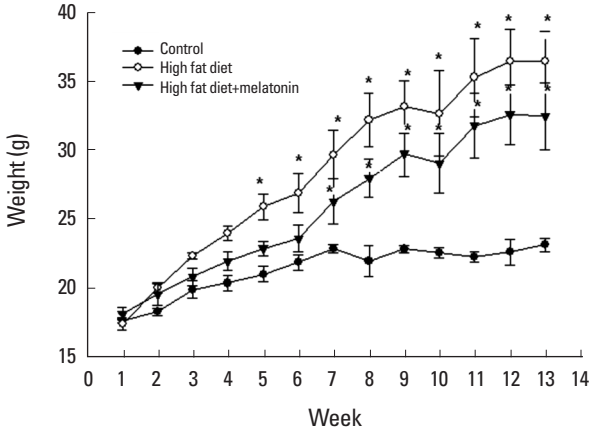

Fig. 1. Weight change of each group over 14 weeks. *$P<0.05$ compared with the control.

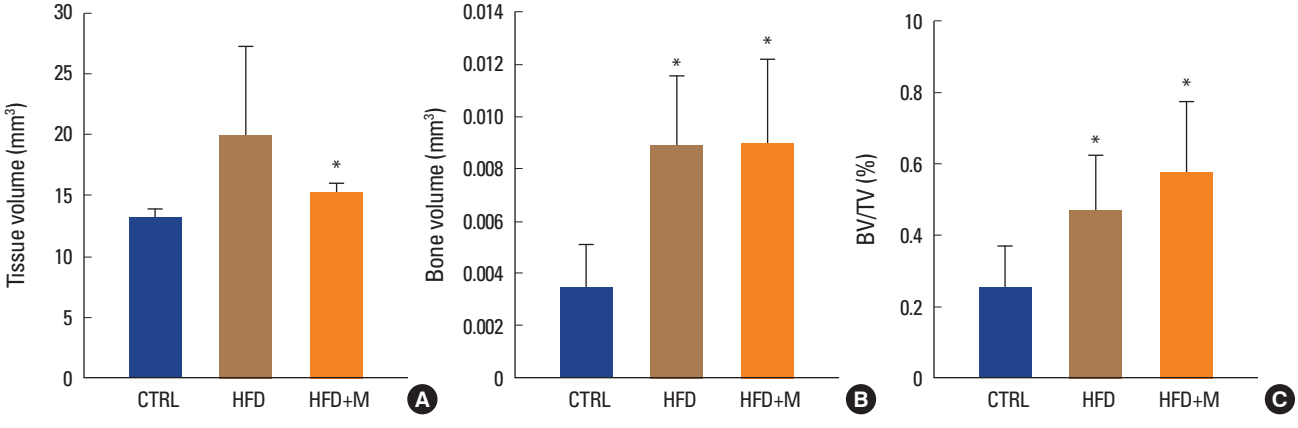

Fig. 2. Melatonin increases bone volume (BV) more than tissue volume (A), BV (B), and percent BV (C) at the 14th week. *$P<0.05$ compared with the control. TV, total volume; CTRL, control; HFD, high-fat diet; HFD+M, high-fat diet with melatonin injection.

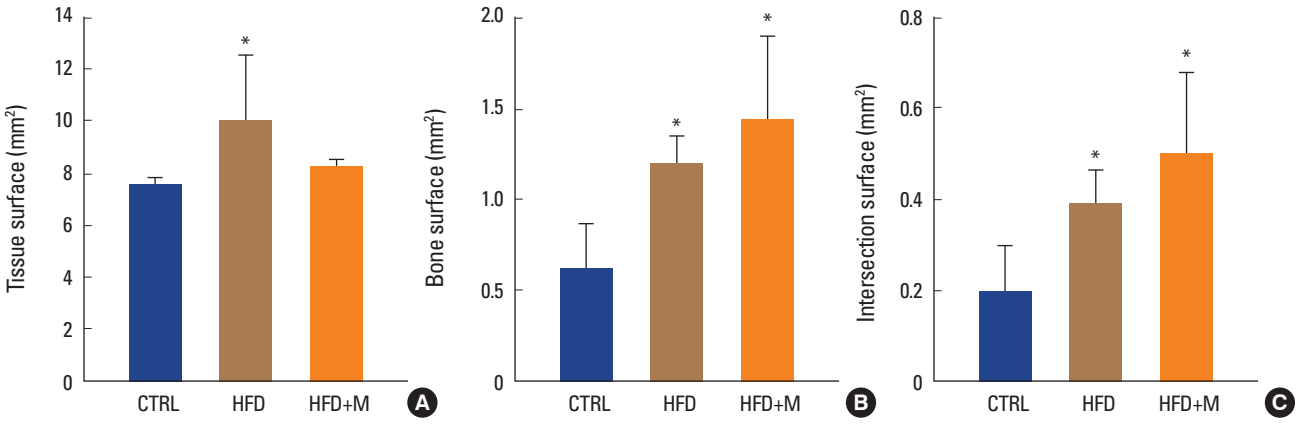

Fig. 3. Melatonin increases bone surface more than tissue surface (A), bone surface (B), and intersection surface (C) at week 14. **P*<0.05 compared with the control. CTRL, control; HFD, high-fat diet; HFD+M, high-fat diet with melatonin injection.

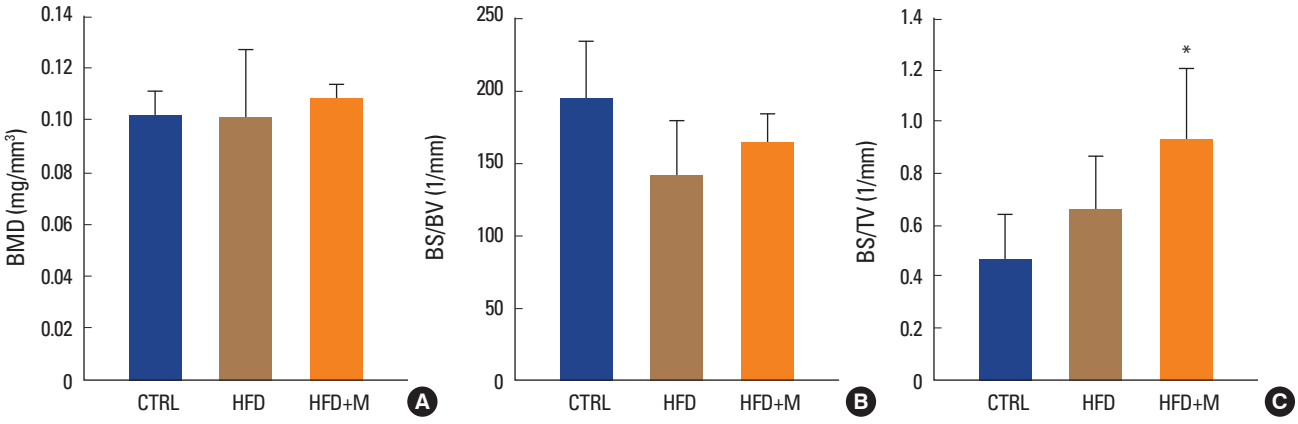

Fig. 4. Melatonin has a greater effect on bone quality than density (A), bone surface/bone volume (B), and bone surface/tissue volume (C) at week 14. **P*<0.05 compared with the control. CTRL, control; HFD, high-fat diet; HFD+M, high-fat diet with melatonin injection.

(*P*<0.05) (Fig. 2B). Percent BV (BV/TV) was significantly higher in both the HFD group and the melatonin group compared with the control group (*P*<0.05), and the melatonin group had the highest BV/TV (*P*<0.05) (Fig. 2C).

The total TS increased significantly in the HFD group compared to the normal diet group, but the difference between the HFD group and the melatonin-administered group was not significant. In the melatonin-administered group, the total TS was lower than that of the HFD group and was higher than that of the normal diet group (Fig. 3A). The BS and IS were significantly increased in both the HFD group and the melatonin group compared with the normal diet group (*P*<0.05), and the values were highest in the melatonin group (Fig. 3B, C).

Bone mineral density (BMD) was lower in the HFD group than in the control group and highest in the melatonin-administered group, but the differences were not significant (Fig. 4A). BS per BV was lower in the HFD group than in the control group and highest in the melatonin group, but these differences were not significant (Fig. 4B). BS per TV (BS/TV) was highest in the melatonin group, followed by the HFD group and then the control group, with significantly higher BS/TV in the melatonin group than in the control group (*P*<0.05) (Fig. 4C).

There were no gross differences in the images of the femurs reconstructed in 3D among the 3 groups.

DISCUSSION

Melatonin is a sleep-regulating hormone secreted by the pineal gland.[20,21] Circadian rhythms play a very important role in metabolic diseases, and it is well-known that

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various metabolic diseases upon disruption of this rhythm.[22-24] Interestingly, melatonin also affects bone density. After pinealectomy in female sheep, bone density decreased even more than when their ovaries were removed.[22] In addition, melatonin administration decreased the expression of receptor activator of nuclear factor-κB ligand, which activates osteoclasts.[19] Moreover, there are reports that melatonin induces the differentiation of mesenchymal stem cells into osteoblasts.[25,26]

Conversely, in obese rats induced by a HFD, adipose tissue increases in the bone marrow, and the microarchitecture is destroyed.[27] Various inflammatory cytokines derived from adipocytes negatively affect the bone,[28] which was supported by our current results. The tissue volume was the smallest in the control group with the smallest body weight, and the tissue volume was the largest in the HFD group with the largest body weight. Similarly, the tissue volume in the melatonin group was larger than that in the normal diet group and smaller than that in the HFD group. However, there was no significant difference in BV between the HFD group and the melatonin group. As a result, BV/TV, the volume occupied by bone among the TV of the femur, was highest in the melatonin group. This shows that melatonin is effective in preventing bone loss caused by a HFD.

Another indicator of bone microstructure is the BS. We determined that the TS showed the same pattern as total body weight and volume. The TS was smallest in the control group with the smallest body weight and largest in the HFD group with the largest body weight. The TS was larger in the melatonin group than in the normal diet group and smaller than in the HFD group. However, as shown in Figure 3, the BS was higher in the melatonin group than in the HFD group. These results, we can predict the structure of the trabecular bone. Increased bone erosion by osteoclasts can increase the BS. On the contrary, if the trabecular number increases, the surface could be increased. In this study the BV increased, and it can be thought that the trabecular number also increased. Therefore, it is necessary to check the bending and breaking test and a more precise 3D image of the bone through further studies.

The larger the BS is, the more complicated the structure of the trabecular bone is, and there is no disconnection and perforation, suggesting that melatonin strengthens the microstructure of the bone. As in Figure 3B, the BS was highest in the melatonin group.

BMD in this study was lower in the HFD group than in the control group and highest in the melatonin group (Fig. 4A). According to a previous study by Masanova et al.[28], a HFD interferes with mineral deposition in bone. Even if a bone structure is eventually formed by osteoblasts, bone strength cannot be maintained if the mineralization process is not performed well and will result in a condition similar to that of osteomalacia or rickets. In this study, the HFD group showed a lower BMD than the control, but it was not significant. The melatonin group has the highest BMD, but it was also not significant, it would be difficult to explain the relationship between melatonin and bone density using only the results of this study.

Contrary to the general laboratory experimental results showing that fatty or HFDs result in poor consequences for bones, clinical epidemiology shows that a higher body weight leads to higher bone density and fewer fractures.[29] In our experiments, TV was proportional to body weight. Bone strength is closely related to the microstructure of the bone, but it is also closely related to the overall outer bone diameter. Therefore, larger absolute tissue volume imparts higher bone strength.[30] In obesity, bone density must compensate for the fragility of the microscopic bone structure, and the muscle mass or adipose tissue around the bone acts as a cushion, protecting against fracture.

There are several limitations of this study. Due to the small number of experimental subjects, several factors did not reach statistical significance. In addition, the absolute size of the mouse femur was small, and the bone structure of the cancellous bone could not be visually confirmed in the 3D reconstructed image. Nevertheless, we were able to show the microstructure of bone by excluding all the effects of soft tissues seen in human clinical practice.

In this study, we confirmed that melatonin inhibited high-fat-diet-induced deterioration of bone microarchitecture over a 14-week period in mice. Melatonin increased percent BV, BS per BV, and BMD. To our knowledge, this is the first HR-pQCT study to examine the protective effect of melatonin on bone microarchitecture changes due to a HFD. Conventionally, obesity has been considered a protective factor against fractures, but we confirmed that the microstructure deteriorated and was vulnerable to fracture and that melatonin inhibited it. We propose a better understanding of the protective effect of melatonin on bone microar-

chitecture and its mechanism in the future could provide fracture prevention for obese people.

DECLARATIONS

Funding

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Ethics approval and consent to participate

All animals experiments were performed according to the Animal Experimentation Ethics Committee of Kosin University (KMAP-20-8).

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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