



The effect of multi-frequency whole-body vibration on night-shifted mouse model

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Received: 19 August 2017 / Accepted: 26 May 2018 / Published online: 14 June 2018
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Abstract

The circadian rhythm controls several biological activities; therefore, a disorganized circadian rhythm may cause fatal health problems. The aim of this study was to assess the effects of circadian rhythm disturbances induced by simulated night shift activities on the abdominal adipose tissue, bone microstructures and muscle volume in the tibiae of mice. Moreover, we evaluated the effects of multi-frequency whole-body vibration as a countermeasure against the consequences of circadian rhythm disturbances. Twenty-four 5-week-old C57BL/6J male mice were equally assigned to three groups: the normal group (Nor), night shift group (NS), and night shift with multi-frequency whole-body vibration group (NS + V). The NS and NS + V groups were exposed to circadian rhythm disturbances for 4 weeks with 3-day intervals by changing the day and night cycle based on 7 o'clock. After 4 weeks, morphological changes in the adipose tissue, bone microstructures and muscle volume in the tibiae were evaluated from three-dimensional images using *in vivo* micro-computed tomography. As a result, the volume of the abdominal adipose tissue was significantly higher in the NS than in the Nor and NS + V groups. Also, the microstructures of the tibia were more enhanced in the NS + V than the NS group. The volume of tibial muscle was increased in all groups, while there were no significant changes in muscle volume. From these results, we can conclude that circadian rhythm disturbances induced by night shift activities may reduce bone condition and increase the accumulation of abdominal adipose tissue and these negative effects may be prevented or improved through applying multi-frequency whole-body vibration.

Keywords Circadian rhythm · Whole-body vibration · Micro-CT · Adipose tissue · Muscle · Trabecular bone

Introduction

Sleep is one of the physiological functions that has 24 ± 4 h cycle within biological rhythm. It plays an important role in body by regulating hundreds of function including body temperature, blood pressure, secretion of digestive fluid and immune reaction [1]. It could be affected by zeitgebers like

LD cycle, temperature and light [2]. Therefore, irregular biological rhythm caused by jet lag, overwork, stress, disorder of endocrine system may result in sleep disorder [3].

However, there are people who do not keep their own regular biological rhythm inevitably due to their own professional or environmental factors. As the industries have been developed in today's society, double-shift and triple-shift working patterns have been increased noticeably. According to Korean working condition survey, the proportion of shift work was 7.2% in 2006 and 10.9% in 2010, which has been consistently elevated [4]. Shift work is one of the main risk factors that causes sleep disorders like insomnia and excessive daytime sleepiness. Workers with certain circumstances are more likely to develop sleep problems than those having normal working pattern [5]. Also, for athletes who receive excessive training or frequent competitions, usually suffer from sleep disorders that reduce sleep quality and time [3]. This prevents them from exercising their skills properly [6,

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7]. Also, according to National Sleep Foundation of U.S., high school students undergo lack of sleep due to considerable amount of study even though it is required for adolescents who are in important the period of growth, to maintain sleep duration at least 8 h [8].

Sleep has been emphasized for a long time since it causes various psychological problems and is strongly connected to physical problems including cardiovascular disease, musculoskeletal disease, accumulation of abdominal adipose tissue, etc [9]. In particular, shift workers are reported to have a noticeably higher incidence of musculoskeletal disease in their knee and waist than those who do not, and the spine and lower limbs have lower bone mineral density [10, 11]. Furthermore, it has been confirmed that the osteoporotic fracture risk is increased if the shift work is continued for a long time [12]. In other studies evaluating the effects of night shift on skeletal muscle using clock gene knock-out mouse, it was confirmed that the number of mitochondria in muscle tissue was significantly decreased and thus, the muscle strength was significantly diminished [13–15]. Also, obesity is generally known as the cause of complications and low quality of life, and it is influenced by irregular circadian rhythm [16]. For instance, Seo et al. reported that the volume of abdominal adipose tissue in the night shift mouse model was higher than that in the control mouse [17].

In this way, we have shown that the environment changing day and night has a negative effect on the body. However, among existing therapies, there is little research on how to treat these negative effects at once. For example, several approaches developed to treat obesity have been shown to induce loss of bones and muscles [16].

Thus, it is required to prepare an effective therapy for reducing bone loss, muscle loss, and accumulation of adipose tissue caused by night shift activities. For that reason, we would like to propose the low-magnitude high-frequency vibration (LMHFV) as a countermeasure. LMHFV is one of the mechanical stimuli that has effects of increasing blood circulation, muscle function, bone formation and muscle mass, and reducing the volume of adipose tissue without side effects [18–21]. It has been suggested that the insertion of rest period while applying LMHFV with continuous single frequency may induce bone adaptive response [22, 23]. According to a study which observed the changes in osteogenesis, it was found out that periosteal surface referent bone formation rate (pBFR/BS) of tibiae significantly improved in the group including rest insertion, relative to the group received continuous mechanical loading [24]. In addition, another study has reported that applying LMHFV with various frequency and rest period decreases body fat [25]. Also, our previous study has shown that applying a complex frequency is more effective in reducing the volume of adipose tissue than applying a single frequency [26]. Therefore, we expected that the LMHFV with multi-frequency and rest

period would be efficient for improving bone or muscle and decreasing adipose tissue. This study aimed to evaluate the effect of the LMHFV with multi-frequency and rest period as a countermeasure against negative consequences caused by circadian rhythm disturbances with some unavoidable reasons.

Materials and methods

Animals

The protocols for all procedures were approved by the Yonsei University Animal Care Committee (YWC-130729-1). Twenty-four 5-week-old male C57BL/6 mice (19 ± 1.2 g) were used in this study and randomly assigned to three groups: the normal group (Nor, $n=8$), the night shift group (NS, $n=8$), and the night shift with the LMHFV with multi-frequency and rest period (NS + V, $n=8$). All mice were maintained under a 12:12-h light–dark cycle (23 ± 3 °C, $50 \pm 10\%$ humidity) with free access to normal chow and water, ad libitum. To observe the amount of feed intake, the weight of daily chow intake was measured at 19:00 each day because the time changed from light period to dark period is 19:00. Also, the body weight of each mouse was measured every day to observe changes in weight throughout the experimental period.

Disturbance of circadian rhythm

All animals were maintained under the same conditions during a 7-day adjustment period, and inspected once a day for overall health. Subsequently, the NS and NS + V groups were induced to have circadian rhythm disturbances by rearing the animals in a room in which external light was completely blocked. The animals were only exposed to internal lighting from the mouse rack. The NS and NS + V groups were exposed to circadian rhythm disturbances for 4 weeks with 3-day intervals by changing the day and night cycle based on 7 o'clock (Fig. 1).

Whole-body vibration

The vibration platform (TENSION WF-1000H, Wellfine, Korea) was used to apply multi-frequency whole-body vibration to the NS + V group. For this process, a diaphragm was used to apply the vibration in a section of $30 \times 21.5 \times 22.5$ cm in size. All the animals were allowed to move freely within the section. The LMHFV with multi-frequency was applied at an intensity of 0.3 g ($g = 9.8$ m/s²) with a frequency of 30, 40, and 50 Hz involving with rest period. A cycle is consisted of 5 repetitive times of applying 30 Hz for 35 s, 40 Hz for 20 s, and 50 Hz for

5 s of LMHFV for five times with rest period for 1 min. Then, we applied six cycles for 36 min in total [25]. Based on it, the animals in the NS + V group received 30 min of whole-body vibration each day, 5 days per week, for 4 weeks (Fig. 2).

In vivo micro-computed tomography (CT) analysis

In vivo micro-CT (Skyscan1076, Bruker, Germany) was used to observe the structural changes in the biological tissues of mice. We acquired micro-CT raw data from the right lower legs and abdomen (L2–5) at 0 and 4 weeks using high-resolution in vivo micro-CT while the mice were anesthetized by inhalation of isoflurane (Hanaph, Seoul, Korea) to minimize their movement. It is shown in Table 1 about the micro-CT scanning parameters for evaluating morphological changes in bone, muscle, and adipose tissue. To reduce the development of beam hardening, a function of beam-hardening correction was employed during the reconstruction procedure.

Structural parameters and bone mineral density (BMD) of the trabecular bone

The raw data from the right tibia acquired by micro-CT was translated into two-dimensional cross-sectional gray scale image slices using NRecon (Bruker micro-CT, Kontich, ver.1.6.9.3, Germany). From the acquired two-dimensional images, structural parameters of the tibial trabecular bone were evaluated by CT Analyzer (CT-AN ver.1.10.9.0, Bruker, Germany), including BMD (g/cm^2), reflecting the extent of bone mineral in the bone tissue, as well as the bone volume fraction (BV/TV, %), the proportion of trabecular bone in a region of interest, trabecular thickness (Tb.Th, mm), the thickness of the trabeculae in the trabecular bone, trabecular separation (Tb.Sp, mm), the distance of the space between the trabeculae, trabecular number (Tb.N, mm^{-1}), trabecular bone pattern factor (Tb.Pf, mm^{-1}), which is related to connectivity, the structure model index (SMI), which is related to the shape of trabeculae and is usually 0–3 and 3 for a rod shape, and the connectivity density of the trabeculae (Conn.dn, mm^{-3}). The analysis of structural

Fig. 1 Light–dark cycle used to disturb the circadian rhythm

	3 days						3 days					
NOR	L	D	L	D	L	D	L	D	L	D	L	D
NS	L	D	L	D	L	D	D	L	D	L	D	L
NS+V	L	D	L	D	L	D	D	L	D	L	D	L

Fig. 2 One cycle of multi-frequency whole-body vibration

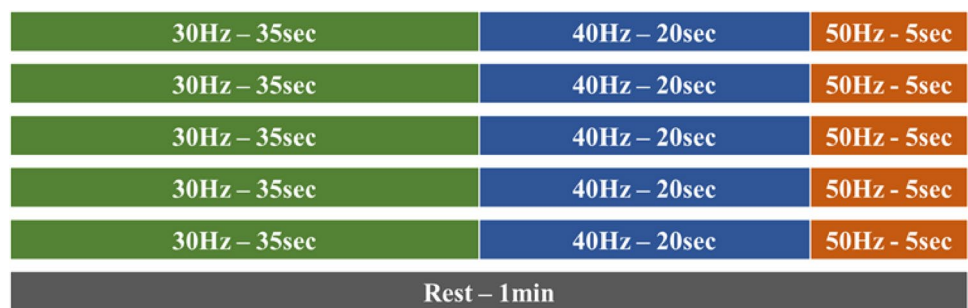


Table 1 Micro-CT scanning parameters

Parameters of micro-CT	Bone tissue	Muscle tissue	Abdominal adipose tissue
Resolution	18 μm	18 μm	35 μm
Filter	1.0 mm Al filter	1.0 mm Al filter	1.0 mm Al filter
Voltage (kV)	85	85	100
Current (μA)	118	118	100
Exposure (ms)	2065	2065	790
Rotation step (deg)	0.7	0.7	1.2
HU-calibration	8000	8000	3000
Smoothing	3	8	9
Ring artifact correction	3	3	3
Beam-hardening correction	10	10	10

parameter for relevant tibia was performed from 3.21 mm out of growth plate to 10.9 mm in distal.

Volume of abdominal adipose tissue

The raw data from the abdomen (L2–5) acquired by micro-CT was applied to NRecon (Brucker micro-CT, Kontich, ver.1.6.9.3, Germany) for analysis. The threshold method was used to extract an image of the fat among the whole image. For this process, the gray value relevant to each tissue in the abdomen was calculated. According to the gray value, the tissues were classified as bone tissue, lean tissue, adipose tissue, and skin tissue. Among these tissues, only the adipose tissue was extracted, and the total volume of abdominal adipose tissue from L2 to L5 was measured with CT Analyser (CT-AN ver.1.10.9.0, Brucker, Germany). The gray value was regulated to measure the whole volume from L2 to L5 and calculate an abdominal fat rate using the program. The volume of interest was selected as a 37.74 mm (± 0.94 mm) length from L2 to L5.

Volume of muscle

The raw data from the right tibia acquired by micro-CT was translated into two-dimensional cross-sectional gray scale image slices using NRecon (Brucker micro-CT, Kontich, ver.1.6.9.3, Germany). The threshold method was used to extract an image of the muscle among the whole-image and the total muscle volume of the right tibia was measured CT Analyser (CT-AN ver.1.10.9.0, Brucker, Germany). The volume of interest was selected from distal tibiofibular joint until it reaches 21.5 mm.

Statistical analysis

The statistical software package SPSS 18.0 (SPSS Inc., USA) was used to evaluate the effects of circadian rhythm disturbances on biological tissues and the effects of vibration on such tissues for each group. Repeated measures ANOVA

accompanied by a Bonferroni post hoc test was used and all morphological data were presented as mean \pm SEM. A p value less than 0.05 was considered to be statistically significant.

To verify the degree of morphological changes in the tibia, muscle and abdominal adipose tissue in the three groups after 4 weeks, we normalized all data at 4 weeks to that at 0 weeks and converted the data into relative variation. One-way analysis of variance accompanied by a Bonferroni post hoc test is used to compare these data and a p value less than 0.05 was considered to be statistically significant.

Results

Changes in BMD and structural parameters of the bone tissue

Figure 3 shows the results of BMD. BMD decreased in all groups at 4 weeks, and the decreases in the NS group were significant ($p < 0.001$). BV/TV also decreased in all groups at 4 weeks, and the decrease in the NS group was significant (Fig. 4 (left), $p < 0.01$). As it is shown in Fig. 4 (right), 20.2% for the Nor group, 5.9% for the NS + V group, and 44.1% for the NS group decreased after 4 weeks. In particular, a significant difference was found between the NS and NS + V groups ($p < 0.01$). The relative value for decreases in each group was shown in Fig. 4 (right). Tb.Th increased by 7.2% in the Nor group and by 12.2% in the NS + V group, but decreased by 6.6% in the NS group, with a significant difference between the relative variance of the NS and NS + V groups ($p < 0.01$) similar to the observations of BV/TV and a significant difference between the relative variance of the Nor and NS groups ($p < 0.05$) (Fig. 5). Tb. Sp increased by 40.1% in the Nor group ($p < 0.05$), 30.4% in the NS group, and 41.6% in the NS + V group ($p < 0.01$); however, there were no significant changes in the relative variance for all groups (Fig. 6). Tb. N decreased in all groups at 4 weeks, and the decrease in the NS group was significant ($p < 0.001$)

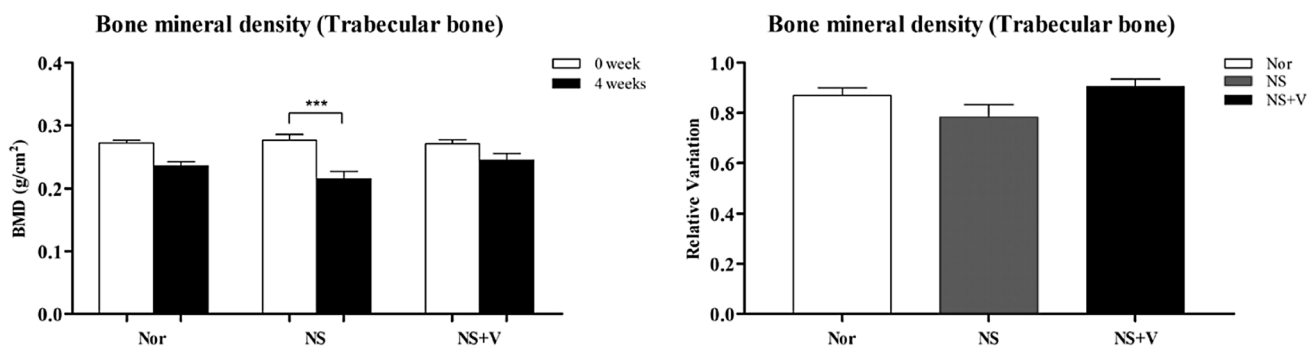


Fig. 3 Bone mineral density of the trabecular bone (left) and relative variations based on the values at day 0 (right)

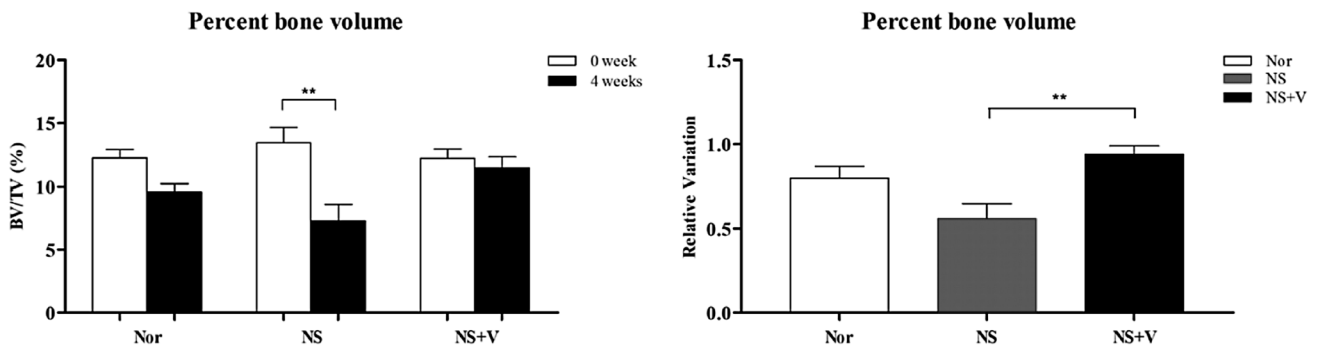


Fig. 4 Percent bone volume (BV/TV) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

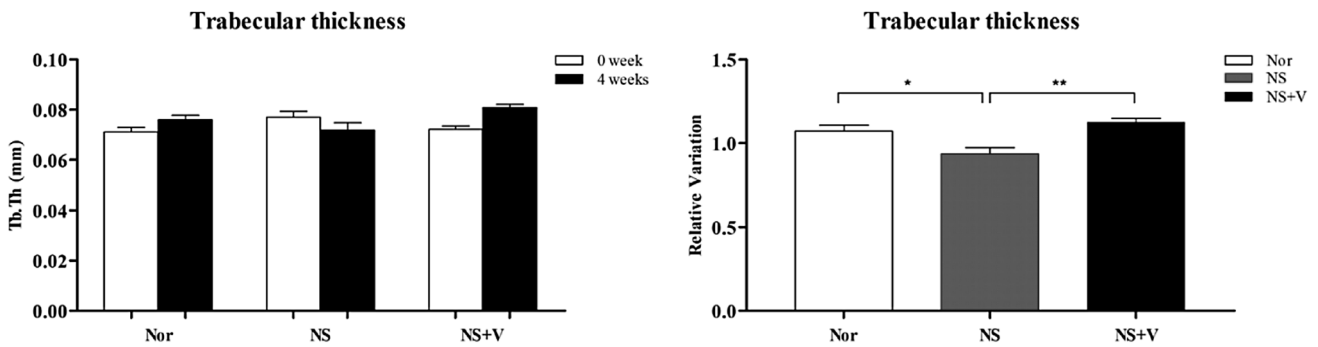


Fig. 5 Trabecular thickness (Tb. Th) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

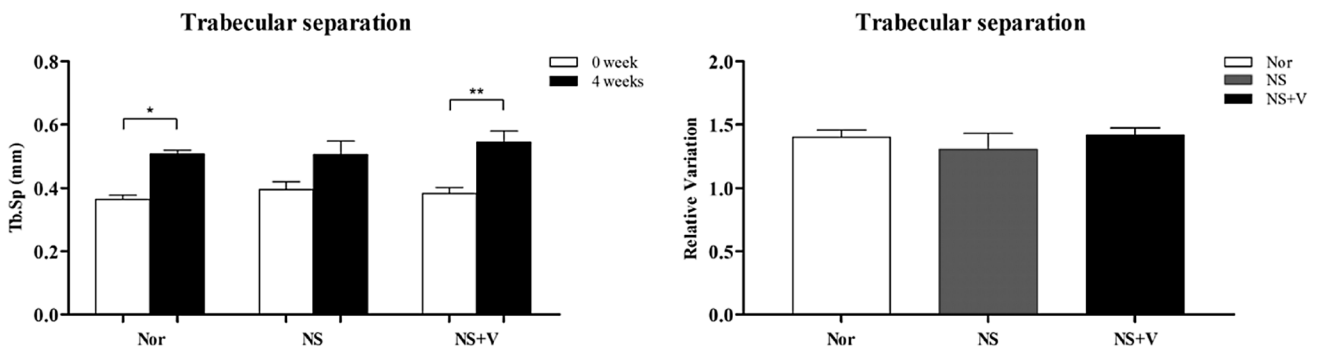


Fig. 6 Trabecular separation (Tb. Sp) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

and significant difference was found between the NS and NS + V groups ($p < 0.05$) (Fig. 7). Tb. Pf increased by 19.2% in the Nor group, 33.7% in the NS group ($p < 0.05$), and 1.7% in the NS + V group, with a significant difference between the relative variance of the NS and NS + V groups ($p < 0.05$) (Fig. 8). SMI increased in all groups by 8.6–12.1%, but significant differences were found in the Nor ($p < 0.01$) and NS + V groups ($p < 0.05$). (Fig. 9). Conn. Dn increased by 24.6% in the Nor group and by 28.8% in the NS + V group, but decreased by 65.8% in the NS group ($p < 0.001$), with a significant difference between the relative variation of the

NS and other groups ($p < 0.001$) (Fig. 10). Also, representative 3D reconstructed images of the trabecular bone for each group at day 0 and 4 weeks after showed in Fig. 11.

Changes in the volume of abdominal adipose tissue (ADT)

The volumes of the abdominal adipose tissue (ADT) in each group are shown in Figs. 12, 13. Throughout the experimental period, the ADT volume of the NS and NS + V groups increased and significant difference was

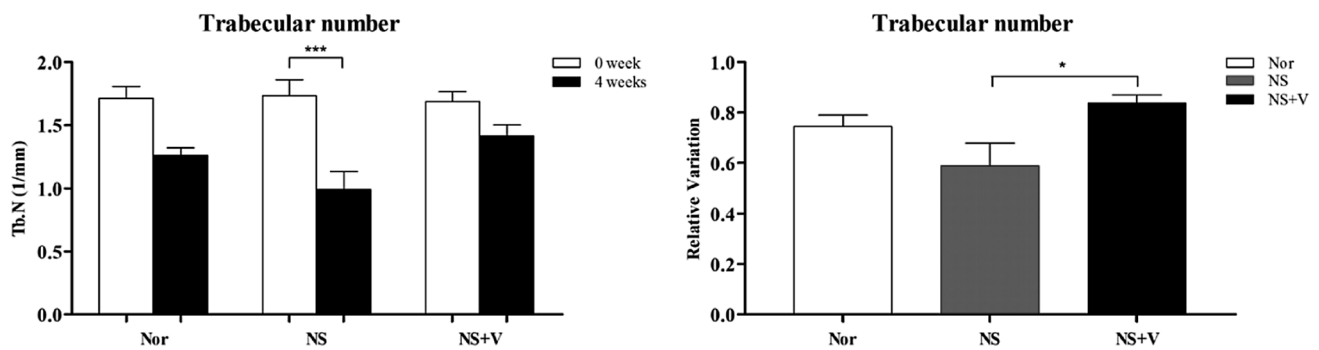


Fig. 7 Trabecular number (Tb. N) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

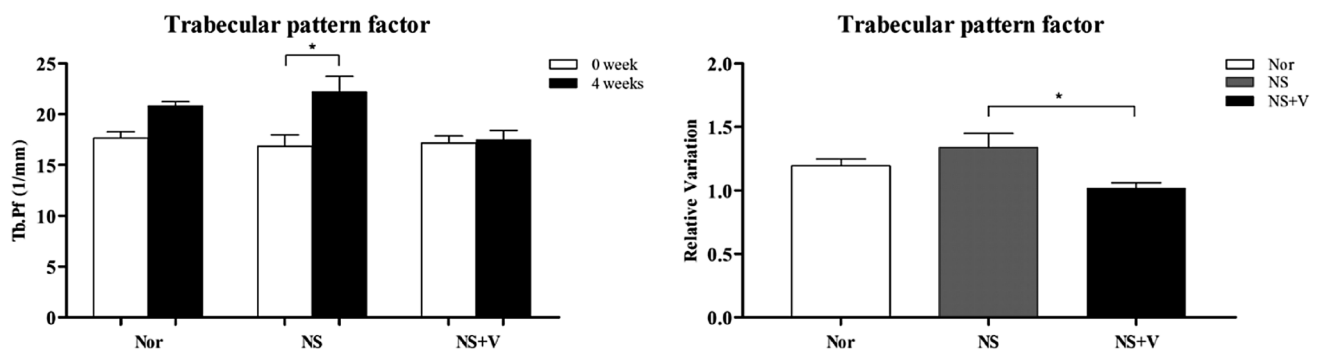


Fig. 8 Trabecular pattern factor (Tb. Pf) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

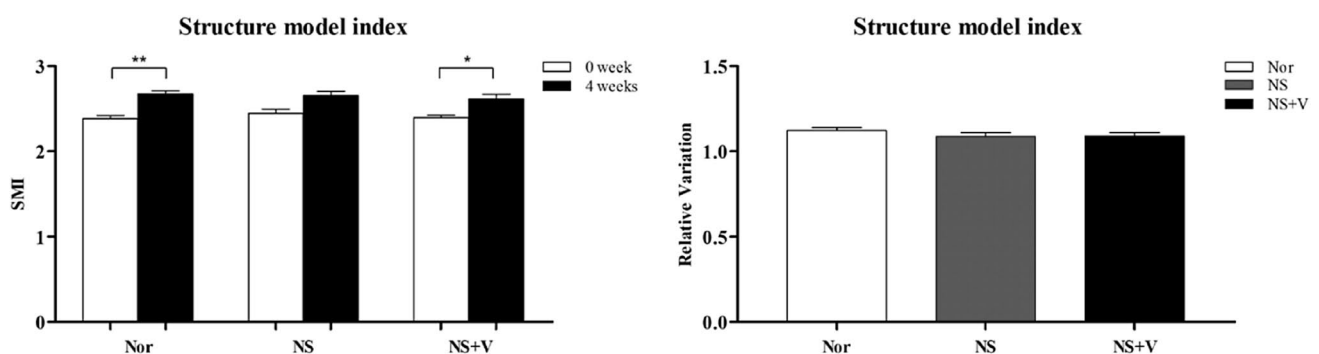


Fig. 9 Structure model index (SMI) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

found only in the NS group ($p < 0.001$). The amount of increase in the NS group was relatively higher than the Nor group ($p < 0.05$). Figure 13 shows the ratio of ADT volume in the total volume (L2–L5) at 0 and 4 weeks. The ratio of ADT volume was increased from 5.55 to 6.65% in the NS group, from 4.73 to 15.55% in the NS group ($p < 0.001$), and from 7.14 to 11.49% in the NS + V group. Representative 3D reconstructed images of the ADT for each group at day 0 and 4 weeks are shown in Fig. 14.

Feed intake and weight changes

Figure 15 shows the weight of weekly chow intake and the average total amount of feed intake for each group throughout the experimental period, indicating no significant differences among all groups. As shown in Fig. 16, body weight significantly increased in all groups at 4 weeks ($p < 0.001$) and the amount of weight changes in the NS group was significantly higher than those of

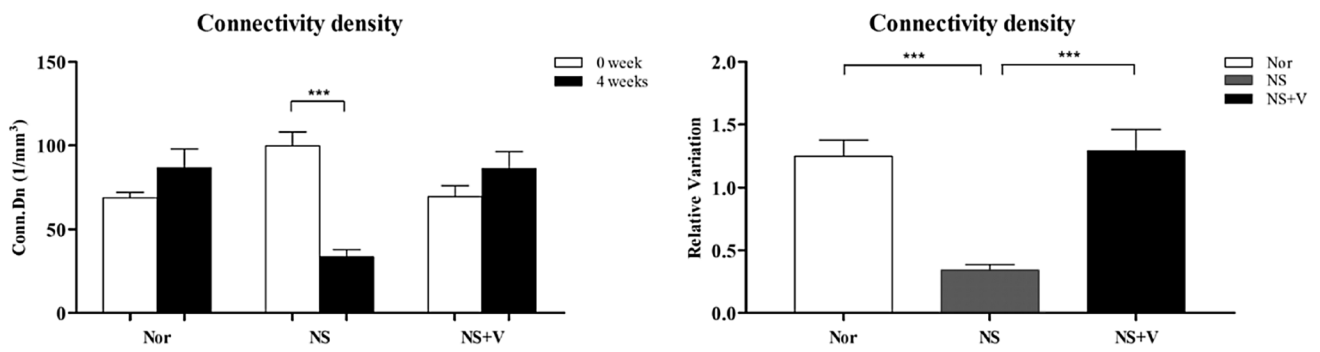
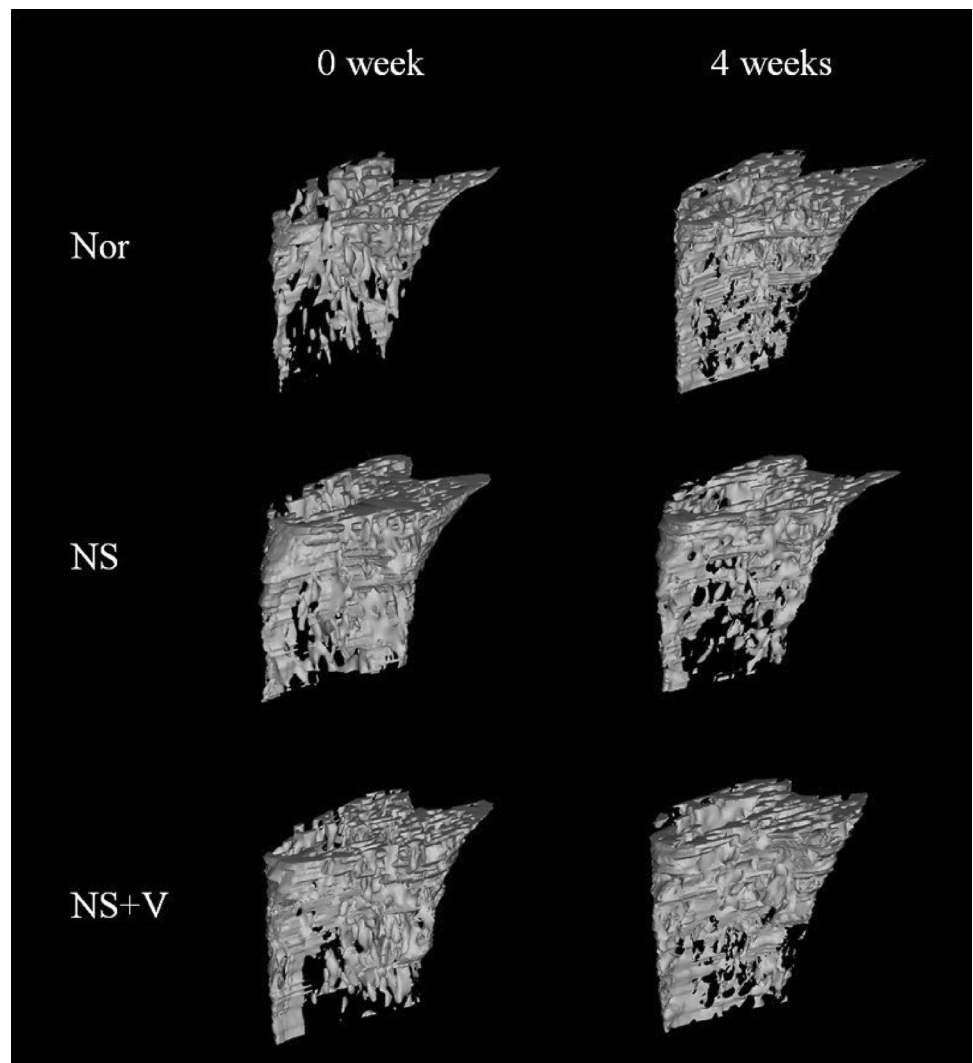


Fig. 10 Connectivity density (Conn. Dn) of the trabecular bone (left) and relative variations based on the values at day 0 (right)

Fig. 11 Representative 3D reconstructed images of the trabecular bone for each group at 4 weeks compared to 0 week



the other two groups. Moreover, the weight changes in the NS group were significantly higher than those of the Nor group ($p < 0.001$) and those of the NS + V group ($p < 0.01$).

Changes in the volume of muscle volume

The volumes of muscle tissue in each group are shown in Fig. 17. It was increased by 23.1% in the Nor group, 12.3%

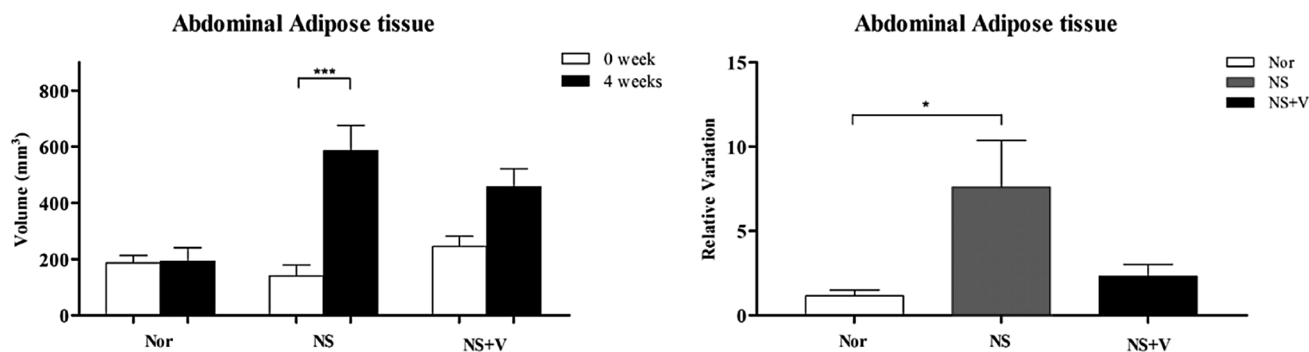


Fig. 12 Abdominal adipose tissue (ADT) volume at 0 and 4 weeks (left) and relative variations of ATV volume based on day 0 (right)

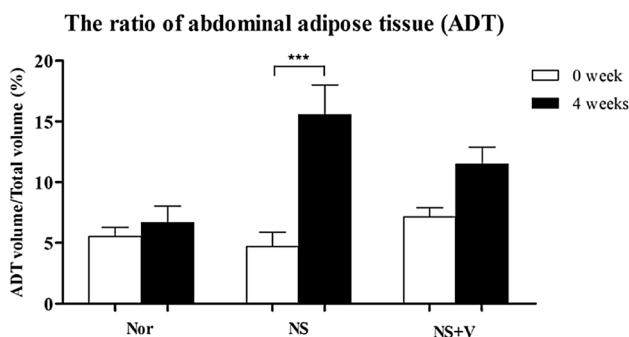


Fig. 13 The ratio of abdominal adipose tissue (ADT) volume in the total volume (L2–L5) at 0 and 4 weeks

in the NS group, 35.9% in the NS + V group after 4 weeks. However, there were no significant changes for the amount of increase in all groups.

Discussion

This study aimed to investigate the effects of circadian rhythm disturbances according to night shift activities on the abdominal adipose tissue, bone microstructures and muscle volume in the tibiae of mice. Moreover, the effects of LMHFV with multi-frequency and rest period as a countermeasure against the negative changes caused by circadian rhythm disturbances were evaluated.

Biological rhythms, where sleep and wake cycles are included, are an indispensable part of practically all aspects of life. These rhythms are mostly controlled by circadian clocks providing internal daily periodicity and allowing various organisms to anticipate daily changes in its environment [27]. To be more specific, it optimizes wide range of biological activities involving cell, tissue, and organism with a proper response at an appropriate time. Therefore, any disturbances in circadian clocks or circadian rhythms may cause various disease states in humans. In the present study,

we mainly focused on the changes in the abdominal adipose tissue, bone microstructures and muscle volume when the circadian rhythm is disturbed by abnormal sleep/wake patterns, which are night shift activities.

For the effects of circadian rhythm disturbances on bone condition, Seo et al. [28] reported that circadian rhythm disturbances weaken both cortical bones and trabecular bones at the tibia. This was verified by significant decreases in both BMD ($p < 0.001$) and structural parameters including BV/TV ($p < 0.01$) and Tb. N ($p < 0.001$) of the trabecular bone at the tibia in the NS group at 4 weeks which is shown in Figs. 3, 4, 5, 6, 7, 8, 9, 10 and 11 at the present study. For the mechanism of this phenomenon, previous studies have suggested that night shift activities might negatively affect bone health owing to reduce melatonin levels [29]. As melatonin controls the secretion of estrogen, which maintains bone mass and stimulates bone formation, a decrease in melatonin due to night shift activities has a direct influence on the bone through accelerating estrogen suppression which results in bone resorption and repression of bone formation. In addition, a decline in the secretion of melatonin due to night shift activities has been reported to be associated with the development of osteoporosis [30].

For the effects of circadian rhythm disturbances on abdominal adipose tissue, a number of studies have clarified that disruptions in sleep patterns increase the accumulation of abdominal adipose tissue and is likely to be the cause of obesity through inducing changes in both systemic and peripheral physiology [17, 28]. This observation is in accordance with the results in the present study, which is shown in Figs. 12, 13 and 14 indicating that the abdominal adipose tissue volume is significantly higher in the NS group ($p < 0.05$) at 4 weeks. Also, for the body weight of each group, presented in Fig. 16, the weight changes in the NS group were significantly higher than those of the Nor group ($p < 0.001$) and those of the NS + V group ($p < 0.01$). Although the mechanism for the association between disrupted sleep/wake patterns and adiposity has not been clearly established, a previous study mentioned that mechanism

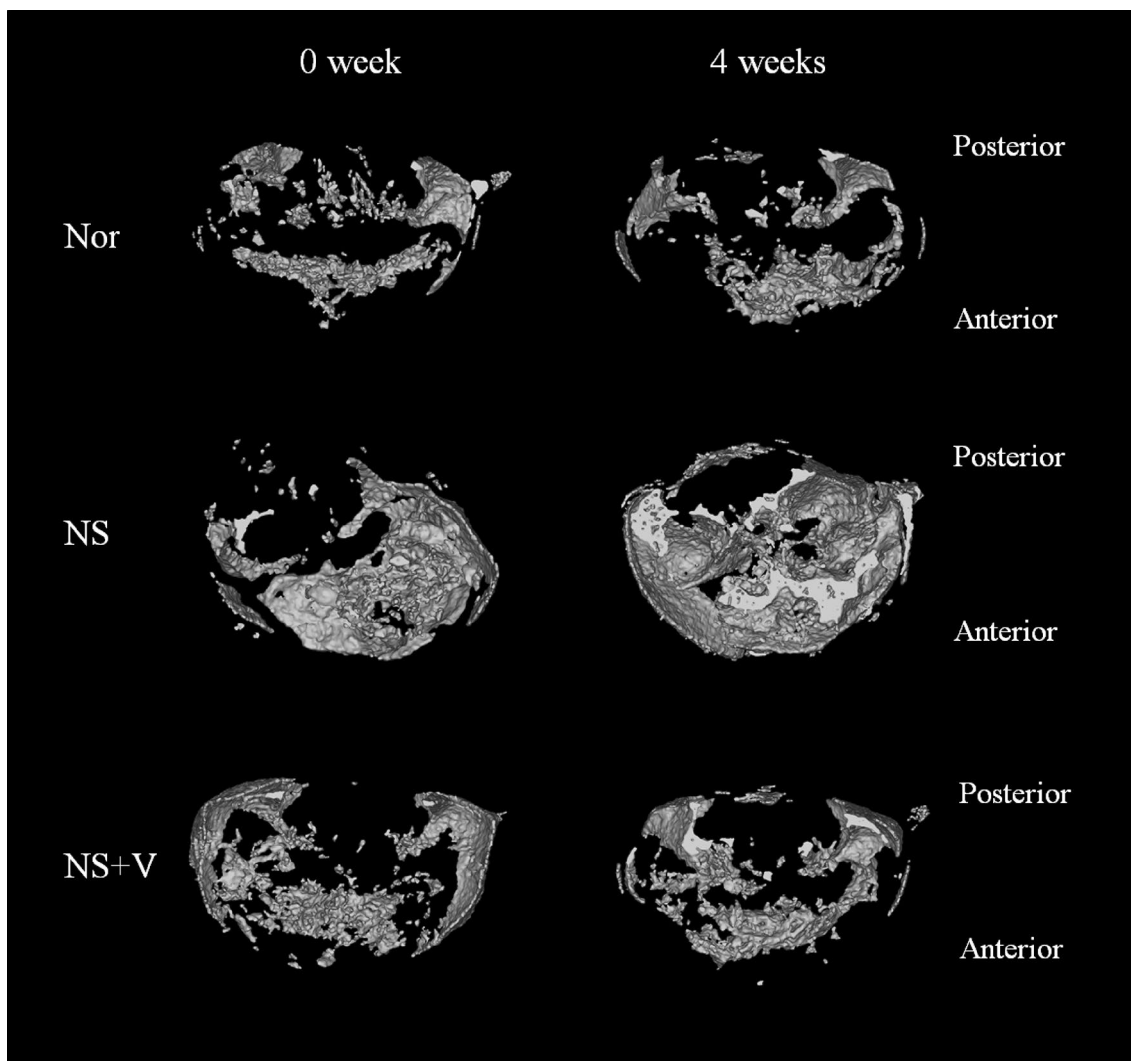


Fig. 14 Representative 3D reconstructed images of the abdominal adipose tissue for each group at 4 weeks compared to 0 week

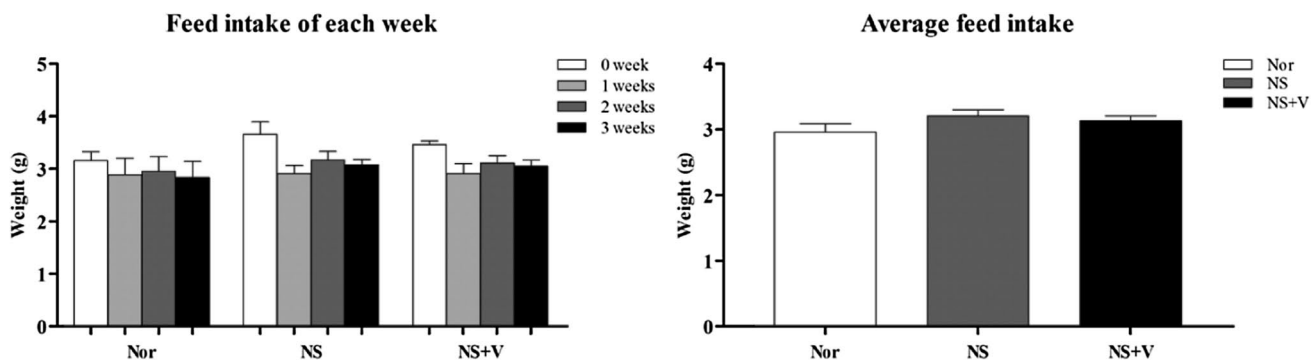


Fig. 15 The weight of feed intake of each week (left) and average feed intake of each group during experiment period (right)

between two factors is related to impaired circadian clock intrinsic to the adipocyte. Such defective circadian clock may take place via asynchrony between sleeping and eating

patterns and/or through changes in the DNA sequence of genes containing or controlled by the circadian clock mechanism [31]. Since there was no significant difference in food

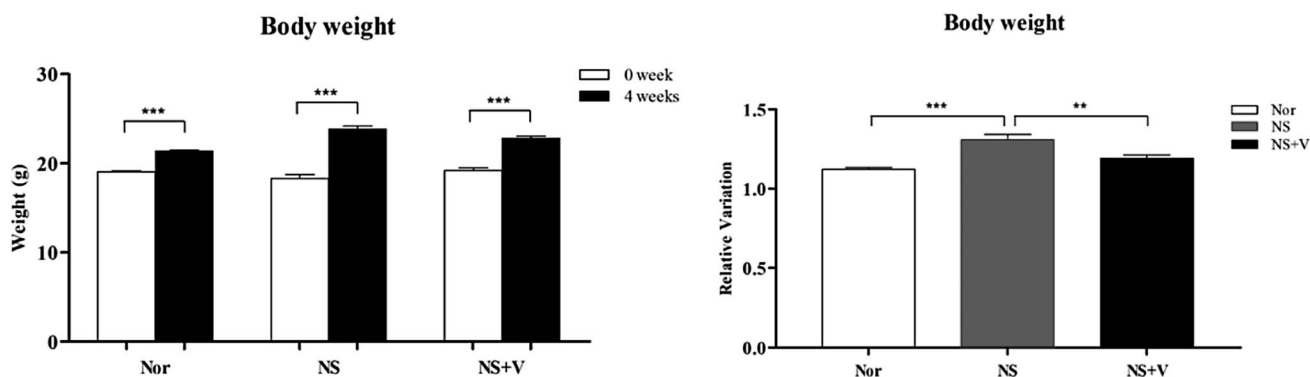


Fig. 16 Body weight of each group at day 0 and 4 weeks after (left) and the relative variation of weight based on values at day 0 (right)

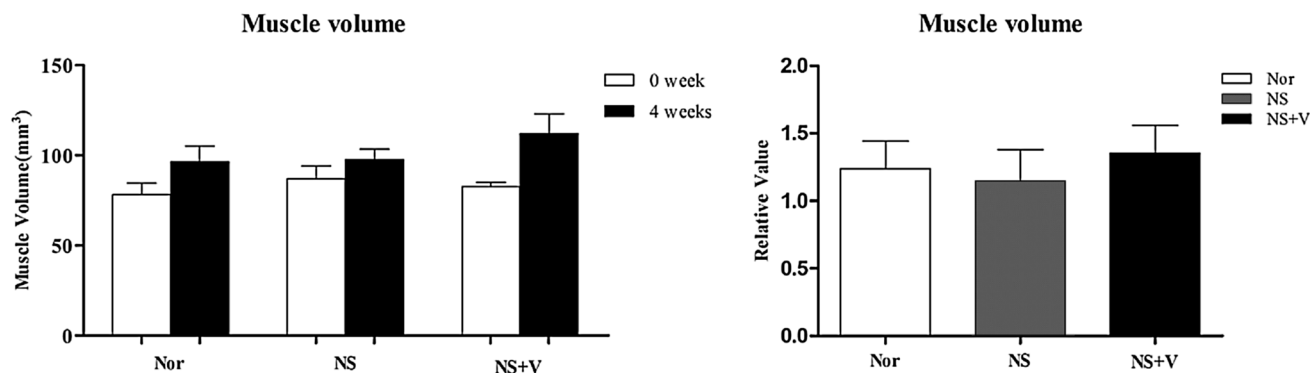


Fig. 17 Muscle volume at day 0 and after 4 weeks (left) and the relative variation of muscle volume based on values at day 0 (right)

intake between two groups in the present study, shown in Fig. 15, an increase in abdominal adipose tissue volume in the NS group seems to be related to the latter.

For the effects of circadian rhythm disturbances on skeletal muscle, related researches are insufficient although muscle also play an important role as much as bone and adipose tissue. In the present study, we investigated the changes in muscle volume by night shift activities and there were no significant changes between the Nor and NS groups. It might be inferred that night shift activity is not a major factor influencing muscle condition.

Likewise, circadian disturbances caused by night shift activities or changes in sleep/wake patterns affect human health states through increasing abdominal adipose tissue volume and reducing both BMD and structural parameters for bone condition.

As aforementioned, we introduced LMHFV with multi-frequency as a countermeasure for these negative changes. It has been confirmed in previous studies that whole-body vibration is more effective than other kinds of exercise in reducing prediction of bone fracture risk and accumulation of visceral fat, and increasing muscle force. The application of whole-body vibration with high frequency and low

magnitude has been suggested to combat the negative effects driven by circadian rhythms disturbances on bone condition by promoting the regeneration of lost bone tissue, and could protect and augment cellular indices, tissue quantity, and trabecular morphology [32].

The application of LMHFV has been discovered to restore the thickness and connectivity of the trabeculae of bones that had been weakened by the circadian rhythm disturbance [33, 34]. These results also support previous findings which demonstrated that bone is a responsive tissue to mechanical stimulation, and that multi-frequency vibration could accelerate osteogenesis by artificially generating mechanical loading to the bone [24, 25, 35, 36]. In addition, for the use of multi-frequency, it has been reported that dynamic loading is more efficient for bone adaptation than static loading when bone is given with repetitive stimulation [37]. Based on it, in our study, we found that a decrease in BMD of the NS + V group is relatively smaller than it of NS group after 4 weeks of disturbing circadian rhythm. Furthermore, increases were shown in Tb. Th ($p < 0.01$), Tb. Sp, and Conn. Dn ($p < 0.001$) and smaller decreases were shown in BV/TV ($p < 0.01$), Tb. N ($p < 0.05$) in the NS + V group compared to those of the NS group. These results represent the positive

effect of LMHFV with multi-frequency on bone condition after being exposed to circadian rhythm disturbances.

Several studies have also shown that the LMHFV with multi-frequency with the insertion of rest periods has clinically beneficial effects on the reduction of accumulated fat by decreasing the concentration of serum leptin, which is strongly related to the peripheral tissue and adiposity [25]. As aforementioned, circadian rhythm disturbances caused by night shift activities may increase the accumulation of abdominal adipose tissue and is likely to be the cause of obesity. Additional research suggests that obese humans have marked increases in leptin, which are closely related to energy regulation and play a major role in controlling the body mass through reducing appetite and weight by interacting with receptors in the hypothalamus. Despite of its weight-reducing effects, obese humans have marked increases in leptin levels, generally in proportion to body fat content due to their resistance to the effects of leptin [38, 39]. Therefore, we expected that LMHFV with multi-frequency can be an effective countermeasure against the accumulation of abdominal adipose tissue caused by circadian rhythm disturbances through reducing the concentration of serum leptin. In addition, this result accords with the results reported by Maddalozzo et al. which stated that the LMHFV with multi-frequency slowed down the acquisition of fat in mature female rats [25]. Indeed, it is generally believed that physical activity increases energy metabolism, and can, therefore, be beneficial to decrease fat mass. Accordingly, whole-body vibration has been proposed as a potential alternative to exercise [25]. In our study, in Figs. 12 and 13, the changes in the volume of abdominal adipose tissue tended to be lower in the Nor and NS + V groups than that in the NS group, which increased about 7.6 times. Thus, collectively, the application of LMHFV with multi-frequency appears to be an effective way to reduce the accumulation of abdominal adipose tissue.

LMHFV with multi-frequency might be effective on increasing muscle volume. Several systematic reviews have already clearly indicated that whole-body vibration (WBV) exercise enhances muscle strength. In addition, some previous studies have evaluated that WBV is a time-saving, safe and effective intervention for improving functional capacity for muscle [40–42]. Despite there is no significant difference in muscle volume among all the groups, an increase in muscle volume in NS + V group was higher than that of other groups between 0 and 4 weeks as it is shown in Fig. 17.

According to the results of this study, we observed weakened bone tissue and an increase in accumulated adipose tissue due to circadian rhythm disturbances. However, for the muscle volume, no significant changes were observed. After applying the LMHFV with multi-frequency, we confirmed a reduction in both bone tissue weakening and abdominal adipose tissue accumulation, while there was no significant

increase in muscle tissue for the group with whole-body vibration. In conclusion, the LMHFV with multi-frequency is expected to be a potential method to prevent negative changes in biological tissue caused by abnormal sleep/wake patterns. Therefore, it might be an effective method for shift workers, athletes, students, and others to prevent or improve negative effects on their biological tissues caused by inevitable irregular biological rhythm with abnormal sleep/wake patterns.

Furthermore, it is expected to be clearer to understand detailed mechanisms after observing the changes in hormone, such as melatonin and leptin, or proteins before and after applying circadian rhythm disturbances and LMHFV with multi-frequency.

Acknowledgements This work was supported by the Yonsei University Future-leading Research Initiative of 2015 (2017-22-0143).

Compliance with ethical standards

Ethical approval Ethical Committee Permission and Animals/the protocols for all procedures were approved by the Yonsei University Animal Care Committee (YWC-130729-1) and twenty-four 5-week-old male C57BL/6 mice (19 ± 1.2 g) were used in this study.

Conflict of interest All of the authors have nothing to disclose.

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