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Effects of Different Intensity Exercises on Bone Structure of Young Male Wistar Rats

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Abstract

Many adolescents have insufficient physical activity. Regular physical activity, from young age, can improve health and fitness. Getting regular exercise and eating healthy diet should be encouraged among kids and teenagers to prevent various diseases in adulthood and old age, such as osteoporosis and fractures. When exercising, a prescription tailored to each individual abilities is needed. The intensity of exercise will be different for each individual in accordance with their age, physiological functions, genetics, economic status, and previous comorbid diseases. There is no standardised exercise prescription proven to increase bone mineral density in adolescents. Based on the above description, a study was conducted on the provision of different intensity exercise and its effect on bone morphology and density. In this study, male Wistar rats, aged 15–16 weeks (body weight ± 250-350 grams; n = 20), were split into 4 groups, namely control group, low intensity exercise group, moderate intensity exercise group, and high intensity exercise group. All groups were receiving interventions for 8 weeks. Then, the rats were sacrificed and the femoral bones were isolated. Femur weight and femur length were checked. Bone density was examined using X-Ray and calculated by statistical tests. Research was conducted in the Central Laboratory, Universitas Padjadjaran. There were significant differences of femur weight between high intensity exercise group and control and low intensity exercise groups ($p < 0.05$). Moderate and high intensity exercises significantly increased femur length as opposed to control and low intensity exercise groups ($p < 0.05$). Bone density was higher in moderate and high intensity exercise groups ($p < 0.05$). Moderate to high intensity exercise can increase bone health, seen from significances of bone morphology analysis and a higher density compared to low intensity exercise.

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INTRODUCTION

According to World Health Organisation in 2016, around 86.4% of Indonesian adolescents aged 11-17 years lack of physical activity (Guthold et al., 2020). Regular physical activity from young age can improve health and fitness. Based on American Academy of Paediatrics data, the incidence of noncommunicable diseases, such as hypertension and type 2 diabetes, is now increasing even at a young age (May et al., 2012). To reduce these risk factors, physical activity is needed to improve cardiorespiratory function, strengthen muscles and bones, reduce body fat, maintain body weight, and even reduce symptoms of anxiety and depression. Physical activity will enhance cardiorespiratory fitness which can also boost anti-inflammatory factors that have an effect on increasing bone mineralization (Gil-Cosano et al., 2023; Wainstein et al., 2016). Parents rarely realise the importance of being aware of their child bone health. Getting regular exercise and eating healthy diet should be encouraged among children and adolescents to prevent various diseases in adulthood and old age, such as osteoporosis and fractures (Dorn et al., 2012; IQ Solutions, 2012).

Any movement of the body resulting from the contraction of skeletal muscles that uses more energy than resting energy is called physical activity. If performed regularly, physical activity can improve the body physiological functions, including repairing bone damage and accelerating bone healing (Konopka et al., 2014; Konopka & Harber, 2014). Exercise is an organized physical activity that involves repeated bodily motions and serves to increase fitness and good health. Aerobic exercise is a type of exercise that uses oxygen-burning energy, where oxygen is needed but there is no oxygen debt. Jogging, cycling, and swimming are a few examples of aerobic exercise. Aerobic-type exercise also improves capillary function, decreases the amount of blood fat, and improves bone and muscle functions (Kluwer, 2018; Powers & Howley, 2018). When exercising, a prescription tailored to each individual abilities is needed. The prescription is called FITT (frequency, intensity, time, type). The quantity of workouts is known as frequency, while intensity includes low, moderate, and high. Time is the length of exercise and type is the form of exercise, either aerobic or anaerobic exercise. A good physical activity recommendation is 150-300 minutes per week (duration) divided into 3-5 times per week (frequency) for moderate-high intensity or 75 - 150 minutes per week for high intensity. The intensity of exercise will be different for each individual according to their age, physiological functions, genetics, economic status, and previous comorbid diseases (Junjie, 2020; Kluwer, 2018).

Many studies have shown that physical activity can help increase bone density. Aerobic activities and resistance trainings are believed to increase the activation of osteoblasts. There is no standardised exercise prescription proven to increase bone mineral density in adolescents. Based on the above description, this study was conducted to examine the effect of the provision of different intensity exercises on bone morphology and density.

METHODS

Subject

Male Wistar rats, aged 15–16 weeks-old (body weight ± 250-350 grams; n = 20), were obtained from PT Bio Farma, Bandung, Indonesia. Rats were housed at 24°C, 55% relative humidity, a 12-hr light-dark cycle, and given food ad libitum and water ad libitum. The calculation of the number of rats was based on the Mead's equation formula. Male rats were used to avoid the effects of oestrogen which could affect bone formation (Sample et al., 2012). Rats (n=20) were split into four groups, namely the control group, low intensity exercise group, moderate intensity exercise group, and high intensity exercise group. The control group (n=5) was not given any exercise intervention. The other groups were given exercises with low intensity (treadmill speed 10 metres/minute/30 minutes, n=5), moderate intensity (treadmill speed 20 metres/minute/30 minutes, n=5), and high intensity (treadmill speed 30 metres/minute/30 minutes, n=5). The speed of each intensity was determined based on previous research referring to the level of serum lactate accumulation, called sub-lactate threshold, where the treadmill speed needs to reach 30m/min to gain the supra-lactate threshold stage (Gunadi et al., 2019; Lesmana et al., 2016). All groups were receiving intervention for 8 weeks. After the completion of experiment, the rats were sacrificed and femoral bones were isolated. Femoral bones were weighed using electronic pocket scale (Camry®). Length measurement used digital calliper

(TriiCles®). Femoral bones were kept in formalin 10% solution until used. Research was conducted in Central Laboratory, Universitas Padjadjaran.

Animal treatments were carried out by observing the 3R principles, which refers to refinement, reduction, and replacement, according to the guide for use and care of laboratory animals (Council NR, 2011) after obtaining approval from Animal Ethics Committee of Universitas Padjadjaran Bandung, Indonesia (No. 1398/ UN6.KEP/EC/2022).

Bone Xray

The femoral bones were subjected to X-Ray imaging using commercial imaging system (Cube X series, Portable X-Ray, JPI Healthcare Co., Ltd, South Korea), 50 kVp, 300 ma, exposure time 0.16 ms, 6.60 mAs, Xray beam 90 cm. Bone density was analyzed using ImageJ software.

Data Analysis

All data were presented as mean ± standard deviation. Statistical analysis was conducted using IBM SPSS Statistics version 21 (SPSS IBM, Armonk, NY, USA).

Data were analysed using Shapiro-Wilk test to see if the data were normally distributed and Levene's test to check if the data were homogenous. One-Way Analysis of Variance was administered to see if the data distribution was normal and homogenous, then retrieved with post hoc analysis of LSD test. If the distribution of data was not normal and/or homogenous, Kruskal-Wallis test was run, continued with Mann-Whitney test. Statistical significance was designated at $p < 0.05$.

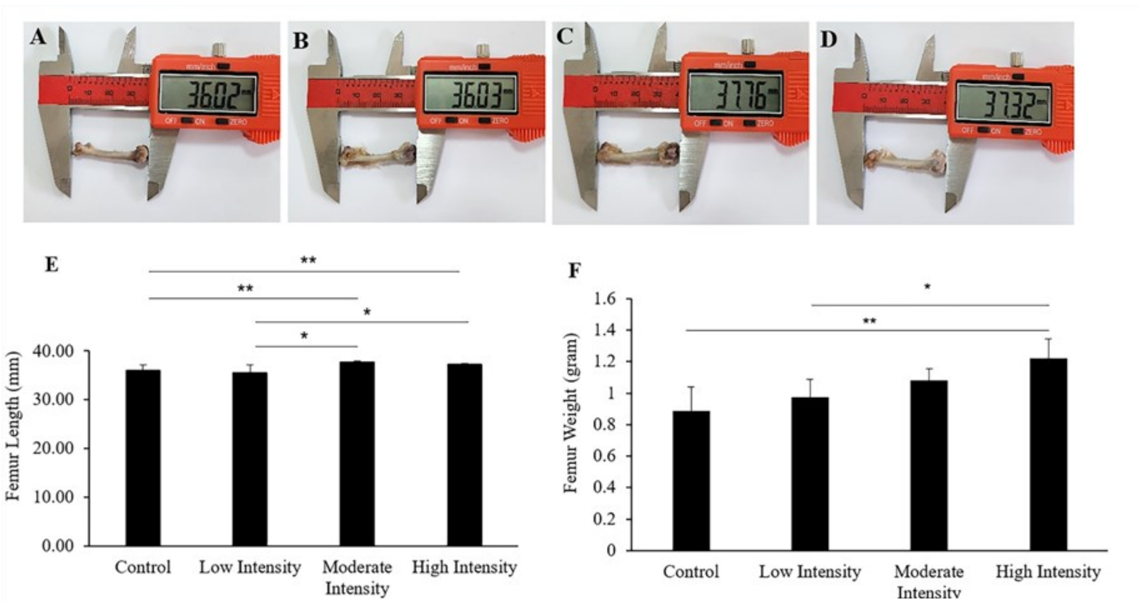

Figure 1. Macroscopic of Femur Rat

RESULT

We found that femur length of the moderate and high intensity group was significantly longer than the control and low intensity groups (Figure 1A, 1B, 1C, 1D, 1E). Analysis of femur length using Kruskal-Wallis test showed significant differences among rats in control group, low intensity exercise group, moderate intensity exercise group, and high intensity exercise group ($p < 0.05$). Control group had average length of 36.05 ± 1.14 mm, showing significant difference from moderate intensity group (37.66 ± 0.33 mm), as well as from the high intensity group (37.29 ± 0.22 mm). The low intensity group mean length was 35.47 ± 1.68 mm, differed considerably from the moderate and high intensity groups, as shown in the Figure 1E.

The mean of femur weight of the control group, low intensity exercise group, moderate intensity exercise group, and high intensity exercise group, retrieved with ANOVA test, displayed significant differences ($p < 0.05$). The femur weight mean of control group rats was 0.886 ± 0.15 grams, significantly different from the mean of the high intensity exercise group (1.22 ± 1.126 grams). The femur weight mean of low intensity exercise rats (0.972 ± 0.117 grams) was also significantly different from the weight mean of high intensity exercise rats as depicted in the Figure 2F.

Figure 1A-D is a representative figure of the macroscopic rat femur bone (A: control, B: low intensity exercise group, C: moderate intensity exercise group, D: high intensity exercise group). Figure 1E displays the average femur bone length. Figure 1F showed the average of femur weight between groups. All data were presented as mean ± standard deviation. Asterisk indicates $p<0.05$, indicates $p<0.01$.

The anteroposterior X-ray of the rat femur showed that the higher the exercise intensity, the thicker the bone cortex area and the more opaque the trabecular area. The X-ray images (Figure 2A) of the control group were similar to the X-ray images of the low intensity group. In the moderate and high intensity groups, the bone cortex area looked thicker and the trabeculae area looked more filled or more opaque as seen in the Figure 2A. This is consistent with the calculation using ImageJ software, where the statistical test using Kruskal-Wallis test of the control group (35.707 ± 5.613 AU) did not show a significant difference in density with the low intensity group rats (35.296 ± 7.752 AU). The bone density of the moderate intensity group (54.964 ± 1.587 AU) and the high intensity group (64.135 ± 1.547 AU) had significant differences. The control and low intensity groups had significantly different densities to the moderate exercise group and the high exercise group as shown in the Figure 2B.

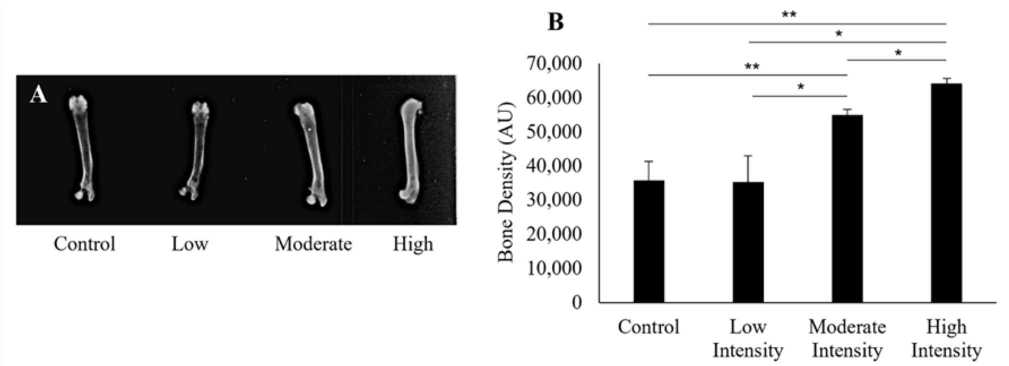

Figure 2. Bone density measurement using X-Ray

Figure 2A shows X-rays of rat femurs (from left to right): control group, low intensity exercise group, moderate intensity exercise group, and high intensity exercise group. Figure 2B shows the quantification of femur density. All data were presented as mean ± standard deviation. Asterisk indicates $p<0.05$, indicates $p<0.01$.

DISCUSSION

Bone development is affected by genetics and environmental factors, such as nutrition and exercise (Salazar et al., 2016). Human bone growth stops at the age of 16-18 years. Rat bone growth reaches its peaks at age 4-6 months. After age 6 months, bone starts to become porous as extra bone mineralisation, such as blood vessels, begins to occur (Nguyen-Yamamoto et al., 2019). Before the growth stops, it is necessary to intervene to maximise the bone growth. Many studies have shown that physical activity can help increase bone density. This intervention depends on many variables, namely FITT (frequency, intensity, time, type), training principles, such as progressivity, periodisation, and training conditions, whether or not supervision is provided, and whether or not equipment is used (Faienza et al., 2020).

In our study, measurement of femur length at the end of the intervention showed that there were differences in the average in each group. The moderate and high intensity groups had a significant mean femur length compared to the control group and the low intensity group. The moderate intensity group had the longest mean femur length compared to the other groups. The average femur weight of the high intensity group was the highest among the other groups.

There was a mean difference of femur weight among each treatment group, where the high intensity exercise group was significantly different from the control group, low intensity group, and moderate intensity group. Running on a treadmill increases mechanical stress on the femur, tibia, and humerus. Seventy percent of bone strength comes from density, while 30% depends on bone quality assessed by the degree of mineralisation, microstructural damage, bone size, collagen amount, and bone alteration processes. Mice given exercise interventions tend to eat less, resulting in lighter body weight and femur weight compared to non-exercised mice (Takeda et al., 2012).

X-Ray examination showed that the high intensity group had the highest density, which was substantially different from the control, low intensity, and moderate intensity groups. There was no discernible difference between the control group and the low intensity group. The low intensity group and the control group did not compare favourably with the moderate intensity group. Rats given high intensity exercise had thicker bone subchondral plates, higher density, thicker trabeculae, and higher carbonate-phosphate ratios than other intensity groups. The carbonate-phosphate ratio indicates remodelling process. It is suspected that low and moderate intensity exercises can maintain cartilage homeostasis, while high intensity exercises can cause cartilage degradation and lead to bone remodelling (Li et al., 2017). The treadmill exercise in rats is an aerobic exercise because it is considered not to provide oxygen debt. In addition, the treadmill exercise provides a biomechanical effect for weight-bearing activities, thus triggering new bone formation, cartilage remodelling, and decreased bone resorption in the parts exposed to the greatest mechanical load. Low and moderate intensity exercises can maintain cartilage integrity, but high intensity exercise can stimulate deformity formation due to the greater stress, resulting in a more extensive bone formation (S. Y. Liu et al., 2018).

The high intensity exercise has a greater impact for increasing bone density than low and moderate exercises, because the greater the load, the greater the deformation that occurs and triggers new bone formation. In humans, training that is considered influential is an above 6 month training (Kemmler, Shojaa, Kohl, & von Stengel, 2020; Kemmler, Shojaa, Kohl, Schoene, et al., 2020).

Osteocytes will respond to mechanical stress. Mechanical load will affect the differentiation of bone marrow mesenchymal cells (BMSC) into osteoblasts, resulting in increased osteogenesis and decreased adipogenesis. Liu et al argue that moderate intensity exercise stimulates the potentiation of BMSC changes into osteoblasts compared to low and high intensity exercises. Bone trabeculae of rats given moderate intensity exercise was denser. In the administration of high intensity exercise, there was a decrease in mRNA expression of Runx2 as a transcription factor for the differentiation of pre-osteoblasts into osteoblasts and type 1 collagen as a marker of bone development in each phase, but there was an increase in mRNA expression in the administration of moderate intensity exercise. Low intensity exercise cannot put enough pressure on the bone, while moderate intensity is enough to exceed the threshold of mechanical stress. Mechanical load on the bone will activate the Wnt/β-catenin signal which will stimulate the formation of osteoblasts (S. Y. Liu et al., 2018). The bone can adapt to adjust its function according to the surrounding conditions. After 5 weeks of treadmill running in rats, the number of mesenchymal stem cells and serum ALP activity increased. After 8 weeks of running intervention, there was an increase in osteogenesis and inhibition of bone resorption (Z. Liu et al., 2019). There was an increase in the expression of alkaline phosphatase and osteocalcin in the femur of rats given treadmill intervention, where these two indicators have association with the formation and mineralization of bone matrix. Treadmill exercise can increase the maturation and differentiation of bone tissue (Yang et al., 2021).

Running affects several osteogenesis pathways, including the Wnt/β-catenin pathway and the BMP/SMAD pathway. Runx2, an osteoblast-forming transcription factor, will be activated by the Wnt/β-catenin pathway. In another pathway, running will increase the expression of Bone Morphogenetic Protein-2 (BMP2) which will bind to receptor SMAD 1, 5, 8. Along with SMAD 4, it creates a complex that activates Runx2. Those pathways trigger osteoblast differentiation and increase bone formation (Chen et al., 2012; Katagiri & Watabe, 2016; Luo, 2017; Yang et al., 2021). Bone strength is influenced by internal conditions, such as structure and geometry, and external conditions, such as load and nutrient intake that affect the bone. Age, gender, nutritional status, dietary intake, and the external environment, such as mechanical stress, will affect the shape, size, and structure of the bone (Z. Liu et al., 2019).

CONCLUSION

Based on our study, it concludes that moderate and high intensity exercise could affect the bone morphology of rat femur and increase bone density. In this study, the treadmill intervention (aerobic exercise and strength training), 5 times each week for 30 minutes each day for 8 weeks, was sufficient to give a positive impact on the bones of 24-26 weeks old rats, or around 18 years when converted to human age. Moderate-high intensity exercise can improve bone health in adolescents. Exercise interventions should be provided early to improve bone quality in the adulthood and elderly. This study did not examine bone density before the treatment due to limited equipment. Further research examining the effect of exercise interventions, in a shorter period, on bones is necessary. Protein examination is also needed to see which part of the osteogenesis pathway is affected by the intervention.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

H.G., L.L. and R.L. participated in the planning and oversight of the project, F.N.S. and F.K. carried out the measurements, F.N.S. and H.G. processed the experimental data, carried out the analysis, prepared the text, and created the figures. The results and the text were worked on by F.N.S., H.G., and R.L. The findings were discussed and the text was reviewed by all authors.

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