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The effect of different types of swimming intensity on increasing serum bone specific-alkaline phosphatase levels of obese male mice (*Mus Musculus*)
El efecto de diferentes tipos de natación de intensidad sobre el aumento de los niveles séricos de fosfatasa alcalina específica ósea de ratones machos obesos (Mus Musculus)

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Abstract. Obesity can interfere with the bone remodeling process, training is one solution to protect bone density. However, training and its intensity on bone remodeling through the biomarker Bone Specific-Alkaline Phosphatase (BALP) with obesity is still not widely studied so it is still unclear its effectiveness. The experimental study involved 24 mice (Mus musculus) that were induced with 30% fructose once a day for 60 days. Mice were grouped into 4 groups according to the intensity of training, namely the control group without training (K1), low intensity with a load of 3% body weight (K2), moderate intensity with a load of 5% body weight (K3), while high intensity was carried out with a load of 7% body weight for 70% (K4) of the maximum duration. All training sessions with a frequency of 3 times/week, and then a post-test comparison examination of BALP levels was carried out. The average number of BALP levels (ng/mL) in each group post-test, the control group had a result of 15.77±3.10, for the provision of low-intensity training with a value of 13.92±2.23, while for the moderate intensity training group of 13.11±1.67, for the highest increase was in high-intensity training with a value of 21.10±3.70. Based on the Tukey honestly significant difference (HSD) test, the post hoc high-intensity training group was significantly different from other groups ($p \leq 0.05$). All types of training intensity analyzed in this study increased BALP levels, especially in the high-intensity training group which had a very significant increase in BALP levels. Therefore, training and its intensity affect bone metabolism increase.
Keywords: Aerobic Training, BALP, Obesity, Swimming, Training intensity.

Resumen. La obesidad puede interferir con el proceso de remodelación ósea, el entrenamiento es una solución para proteger la densidad ósea. Sin embargo, el entrenamiento y su intensidad sobre la remodelación ósea a través del biomarcador Fosfatasa Alcalina Específica del Hueso (BALP) con obesidad aún no está ampliamente estudiado por lo que aún no está clara su efectividad. En el estudio experimental participaron 24 ratones (Mus musculus) que fueron inducidos con 30% de fructosa una vez al día durante 60 días. Los ratones se agruparon en 4 grupos según la intensidad del entrenamiento, a saber, grupo de control sin entrenamiento (K1), baja intensidad con una carga del 3% del peso corporal (K2), intensidad moderada con una carga del 5% del peso corporal (K3). , mientras que la alta intensidad se realizó con una carga del 7% del peso corporal durante el 70% (K4) de la duración máxima. Se realizaron todas las sesiones de entrenamiento con una frecuencia de 3 veces por semana y luego se realizó un examen de comparación posterior a la prueba de los niveles de BALP. El promedio de niveles de BALP (ng/mL) en cada grupo post-test, el grupo control tuvo un resultado de 15,77±3,10, para la realización de entrenamiento de baja intensidad con un valor de 13,92±2,23, mientras que para el de intensidad moderada grupo de entrenamiento de 13.11±1.67, pues el mayor incremento fue en el entrenamiento de alta intensidad con un valor de 21.10±3.70. Según la prueba de diferencias honestamente significativas (HSD) de Tukey, el grupo de entrenamiento post hoc de alta intensidad fue significativamente diferente de otros grupos ($p \leq 0,05$). Todos los tipos de entrenamiento de intensidad analizados en este estudio aumentaron los niveles de BALP, especialmente en el grupo de entrenamiento de alta intensidad que tuvo un aumento muy significativo en los niveles de BALP. Por tanto, el entrenamiento y su intensidad inciden en aumentar el metabolismo óseo.
Palabras clave: Entrenamiento aeróbico, BALP, Obesidad, Natación, Intensidad del entrenamiento.

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Introduction

Obesity is a health problem that is often ignored in Indonesia, from Basic Health Research (Riskesdas) and Central Bureau of Statistics (BPS) data the prevalence of overweight and obesity in Indonesia tends to increase as much as 10.5% in 2007 than 14.8% in 2013 and 21.8% in 2018, while the prevalence rate of obesity in the population aged ≥ 18 years from 2013 was 26.25% and in 2018 it was 35.5%. The long-term effects of obesity can result in increased oxidative stress, inflammation, and changes in bone metabolism (Balitbangkes RI, 2007, 2018; Riskesdas, 2013; Savvidis et al., 2018; Statistik, 2022). Obesity can disturb bone metabolism through mechanical, hormonal, and inflammatory factors, which can lead to various bone disorders (Welsh et al., 2024). Obesity can also affect the secretion or activity of Bone Alkaline Phosphatase (BALP), resulting in disruption of the bone remodeling process (Ning et al., 2023; López-Gómez et al., 2016; Roy et al., 2016).

A 2018 review article on the effectiveness of physical training on bone density in osteoporosis patients, concluded that there are several training recommendations to increase bone density (Benedetti et al., 2018), the most effective intervention for Bone Mineral Density (BMD) is a multicomponent training program, one of which is weight-bearing aerobic training and training with a vibrating platform can also have an impact on increasing BMD (Stanik et al., 2019). A systematic review on the effects of physical training on bone biomarkers Bone-specific alkaline phosphatase (bone formation biomarker) and Amino-terminal Cross Linked Telopeptide of type 1 collagen (bone resorption biomarker) states that there is a possible benefit of training in increasing bone formation and reducing bone resorption biomarkers in osteoporotic populations (Ma et al., 2018), both studies were conducted on humans with normal body mass index. Meanwhile, 12 weeks of interval training were reported increasing BMD in the femur and serum total alkaline phosphatase (TALP) in animal (Le et al., 2021).

Research on the effects of training on bone biomarkers in obese body conditions is still very rare. In this study, we used BALP because it is more specific for understanding bone metabolism (Fragala et al., 2017). To see the effectiveness of various training intensities on bones with obese conditions, experimental research was conducted on fructose-induced experimental animals which were then divided into several groups to be given several kinds of training intensity. This is because the effect of exercise intensity on BALP levels has not been clearly explored. For that reason, the purpose of this study is to analyze the protective potential of training intensity on BALP levels in mice.

Materials and Methods

Study design

This study is a true experimental research, utilizing a '*randomized posttest-only control group design*'. The study involved 24 male mice (*Mus musculus*) aged eight weeks and weighing around 20±5 grams. Sampling was carried out using a consecutive sampling technique, while the division of mice into groups was carried out randomly. Group K1 (n=6) was a control group that received no intervention. Group K2 (n=6) was a group that performed low-intensity swimming. Group K3 (n=6) was a group that performed moderate-intensity swimming, and Group K4 (n=6) was a group that performed high-intensity swimming. All procedures for this research have received approval from the Health Research Ethics Commission, Faculty of Medicine, Universitas Airlangga, with number: 100/EC/KEPK/FKUA/2023.

Animals that met the inclusion criteria were acclimatized for seven days. This process includes training and familiarizing the test animals to the intervention to be given. The test animals were placed in room conditions with a temperature of 26±2°C, humidity between 50-60%, and lighting arranged in a 12-hour light and 12-hour dark cycle (Sari et al., 2024). The mice cages were made of transparent acrylic material with dimensions of 20 x 20 x 25 cm. Each cage was filled with 1-3 mice, depending on the size and condition of the test animal. Feed and drink were provided every day at 06.00 a.m. Cage cleaning was carried out every 2-3 days to ensure a clean and healthy environment for the test animals. To make mice obese before intervention all animals were exposed by high carbohydrates using 30% fructose solution, per-oral, ad libitum for a period of 1 weeks. Meanwhile, observe weight gain in mice, the mice were induced with a 30% fructose solution ad-libitum orally from day-1 to day-60 (Ali et al., 2006).

Study organization

The acclimation process was completed 7 days before the training program (day-1 to day-60). Light intensity training was swimming training with a load of 3% body weight, moderate intensity was done with a load of 5% body weight, while heavy intensity was done with a load of 7% body weight. All training were performed for 70% of the maximum duration/training session with a frequency of 3 times/week for 8 weeks. Swimming was held start from 17.00 p.m (Upadhyay et al., 2015).

Before and after the completion of the entire 8-weeks training intervention, following the measurement of body weight, blood glucose, and Lee's obesity index the test animals used in the research were measured then sacrificed in an appropriate manner according to ethical guidelines and blood samples (post-test) were collected from the left ventricle of the mice, amounting to 1-1.5 ml, 24 hours after training (Sari et al., 2024). Mice were fasted overnight before blood sampling.

The Lee Obesity Index (LOI) was measured by dividing the cube root of the body weight (g) by the naso-anal length (mm) and multiplying the whole expression by 10 (Bernandis & Patterson, 1968). Obese mice were considered if their weight was ≥ 0.3 g/mm.

Post-test blood samples were centrifuged to separate red blood cells, plasma, and serum. The centrifugation was performed for 15 minutes at a speed of 3000 rpm. The serum was stored at a temperature of -80°C for the analysis of BALP levels the next day using the Colorimetric Assay Kit (Cat.No: E0199Mo, Bioassay Technology Laboratory, Inc., Shanghai, China P.R.).

Statistical analysis

Data analysis in this study was conducted using the Statistical Package for Social Science (SPSS) software version 21. The first step was descriptive statistical calculation to get a general picture of the data. Then, a normality test was performed using the Shapiro-Wilk test to check whether the data was normally distributed. The third step was a homogeneity test using the Levene test to determine whether the variance from different groups was homogeneous. Then a Paired t-test was used for pre and post data of body weight, Lee Obesity Index, and blood glucose. One-way ANOVA test was used, followed by Tukey's honestly significant difference (HSD) post hoc test. All statistical analyses were performed with a significance level of 5%.

Results

Based on the research results, the following are the average of body weight, Lee Obesity Index, and blood glucose levels of the low, moderate, and high intensity swimming groups before and after 8-week intervention (Table 1). The normality and homogeneity of body weight, Lee Obesity Index, and blood glucose levels data in each group need to be known by conducting a Shapiro-Wilk test and a Levene test before the difference test using a paired sample t-test.

After treatment on experimental animals for 8 weeks, the results show that the distribution of body weight, Lee

Obesity Index, and blood glucose levels data was normal (p ≥ 0.05) and homogenous (p ≥ 0.05). The difference test using a Paired t-test was conducted with the result showing a significant difference in body weight pre and post-training data (p ≤ 0.05) however, there was no significant difference in Lee Obesity Index and blood glucose data (p ≥ 0.05) as depicted in Table 1.

Table 1.
Analysis Results of Body Weight, Lee Obesity Index, and Blood Glucose

Variable	Training Intensity				p-value
	K1	K2	K3	K4	
Pra BW (gram)	25.17±2.14	22.17±1.17	22.83±2.14	22.17±1.17	
Post BW (gram)	35.33±4.18	29.83±3.19	29.67±3.33	29.67±5.57	0.000*
Difference (gram)	10.16±2.14	7.66±2.02	8.62±0.38	8.58±0.50	
Pra LOI (g/mm)	0.33±0.02	0.31±0.01	0.33±0.01	0.32±0.02	
Post LOI (g/mm)	0.32±0.01	0.33±0.01	0.32±0.01	0.32±0.02	0.927
Difference (g/mm)	-0.01±0.01	0.02	-0.01	0.00	
Pra BG (mg/dL)	65.17±21.91	68.67±41.87	73.67±19.94	76.17±20.77	
Post BG (mg/dL)	68.17±18.15	71.17±36.94	69.83±26.14	80.00±13.51	0.981
Difference (mg/dL)	3±3.76	2.50±4.93	-3.84±6.20	3.83±7.26	

Description: Data are expressed as mean ± SD. K1 – Control group; K2 – Low intensity swimming training group; K3 – Moderate intensity swimming training group; K4 – High intensity swimming training group. BW: Body weight; LOI – Lee Obesity Index; BG – blood glucos. (*) p ≤ 0.05 pra vs post. p-value was obtained by paired sample t-test.

Based on the research results, the following are the average BALP levels of the low, moderate, and high intensity swimming groups after 8-week intervention. The distribution and homogeneity of BALP data in each group needto be known by conducting a normality test and homogeneity test before the difference test using one-way ANOVA.

Based on the results of the normality test using Shapiro-Wilk, it was shown that the distribution of BALP level data was normal (p ≥ 0.05) and the results of the homogeneity test analysis using the Levene test are homogenous (p ≥ 0.05), as depicted in Table 2. After obtaining the result of data analysis being normally distributed and homogeneous, the difference test using one-way ANOVA was conducted with the result showing a significant difference in the obtained BALP levels (p ≤ 0.05).

Table 2.
Analysis Results of Differential Test of BALP Levels of Each Group Based on Blood Sampling Time

Group	BALP (ng/mL) Post-training (mean±SD)	p-value
K1	15.77 ± 3.10^{ɣ}	
K2	13.92 ± 2.23^{ɣ}	0.000*
K3	13.11 ± 1.67^{ɣ}	
K4	$21.10\pm3.70^{\wedge\#\dagger}$	

Description: Data are expressed as mean ± SD; K1 – Control group; K2 – Low intensity swimming training group; K3 – Moderate intensity swimming training group; K4 – High intensity swimming training group. ^ significantly different from K1; # significantly different from K2; † significantly different from K3; ɣ significantly different from K4. p-value was obtained by one-way ANOVA and Tukey's honestly significant difference (HSD) post hoc test.

Meanwhile, to identity the difference in levels of BALP in each group, a Tukey's Honestly Significant Difference (HSD) post hoc test was performed, as shown in (Table 3).

Table 3.
Results of Tukey's Honestly Significant Difference (HSD) Post Hoc Test for BALP Levels

Group	K1	K2	K3	K4
K1		0.644	0.373	0.017*
K2			0.957	0.001*
K3				0.000*
K4				

Description: K1 – Control Group; K2 – Light intensity swimming training group; K3 – Moderate intensity swimming training group; K4 – High intensity swimming training group. (*) p ≤ 0.05 indicates a significant difference.

Based on the analysed data from the Tukey's Honestly Significant Difference (HSD) post hoc test, it was found that there were significant differences (p ≤ 0.05) in BALP levels between K4 and K1, K4 and K2, K4 and K3, while the other groups did not have significant differences (p ≥ 0.05).

Discussion

The results of the study showed that there was an increase in body weight in all groups, both the control group and the intervention group (K2, K3, and K4). But in the control group the increase in body weight occurred the highest. So it can be seen that the intensity of training has an effect on slowing down weight gain. Several previous studies have recommended training for weight control. Training influences changes in body weight and obesity (De Lorenzo et al., 2018; Gerosa-Neto et al., 2016; Hunter et al., 2018; Osiński & Kantanista, 2017; Soh et al., 2020). Training can increase metabolism (de Matos et al., 2019), increase oxidation in muscles and cause changes in total fat in the body (de Matos et al., 2018). Weight loss will result in a decrease in the Lee Obesity Index (LOI). The significant decrease in LOI values in the moderate and high intensity training groups was thought to be caused by a reduction in the size and number of adiposity cells resulting in a decrease of fat tissue mass. This is proven by slowing down in weight gain (Dunn, 2009; Shahram et al., 2011).

The results of this study are in line with research by Antoni et al. (2022) which reported that moderate intensity swimming training activity in female mice reduces body weight and the Lee Obesity Index (LOI). Training carried out at moderate intensity can increase the production of free fatty acids as an energy source (Karstoft et al., 2016), thereby potentially reducing the accumulation of visceral fat and body weight (Wewege et al., 2017). In addition, regular physical exercise also plays an important role in reducing obesity by increasing energy output (Houdebine et al., 2019) which is mediated by the hormone irisin (Boström et al., 2012). The release of irisin in the blood circulation induces the browning process in white adipose tissue. Thus, increases energy output and reduces fat accumulation, which can be a factor in weight loss (Antoni et al., 2022). Over weight has a high level of adipocytes, where these adipocytes secrete adipokines associated with weight loss associated with the consequent normalization of different metabolic parameters, reinforcing the idea that adipokines are essential for whole body metabolic homeostasis. In addition, the amount of adipokines secreted can influence various body organ systems that are important for energy homeostasis (Uranga & Keller, 2019).

In this study, the results of blood glucose (BG) levels were also obtained between the training and control mice, where no significant BG levels were found in all groups. This could happen because all groups were obese mice, which condition has a risk of developing hyperglycemia and if it continuely occur can caused of type 2 diabetes (Schaller & Mons, 2018). However, it can be seen that BG in the moderate intensity training group decreased.

Organized and measured physical exercise can improve blood glucose levels, health, and reduce body weight. The process of improving glucose control through physical exercise can occur through three mechanisms, namely stimulation of glucose transport to muscles, increased insulin action on organ cells involved in physical exercise, and positive regulation stimulated by insulin as an effect of organized physical exercise. Physical exercise has been indicated as an insulin-like activity. This is due to increase in muscle capacity to capture plasma glucose due to a decrease in intramuscular fat reserves (Ratna et al., 2021; Teixeira-Lemos et al., 2011). Previously it was also stated that high-intensity swimming training can reduce body weight and LIO in obese female mice (Antoni et al., 2022). So it seems that high-intensity training is more suitable for therapy to reduce LIO and BG in cases of obesity.

This research sought to find the relationship between bone metabolism and training intensity, which we analysed from the calculation of the bone biomarker, bone-specific alkaline phosphatase (BALP). The results of this study indicate changes in BALP levels post-treatment, with the highest BALP levels in high intensity training. A cross-sectional study in geriatrics explained that there is an increase in serum BALP levels in subjects post-training, indicating that training can influence calcium and bone homeostasis (Aly et al., 2017). Another study in menopausal women showed the same results that sub maximal traning can increase BALP (Roghani et al., 2013). This significant increase in BALP indicates that training can stimulate new bone synthesis (Bakhtiyari et al., 2021). Alkaline phosphatases (ALPs) are a group of isoenzymes located on the outer layer of the cell membrane (Sharma et al., 2014). The enzyme BALP is commonly found in tissue nonspecific form produced in the liver, bone, and kidney, involved in the removal of phosphate minerals from molecules and inflammatory conditions (Hashim, 2024; Fragala et al., 2017).

BALP levels increase significantly after weight training (Fragala et al., 2017). Other studies say that the weight training group and the walking group also increase BALP, although very minimally (Gombos et al., 2016). The most significant increase in BALP occurs after long duration of cycling (Maïmoun et al., 2006; Wallace et al., 2000). In the in vivo studies on experimental animals, it was found that BALP levels also increase post-training (Qi et al., 2008; Qi et al., 2009). In our study, all training intensities (low, moderate, and high) grup serum BALP levels post-test measured. However in our study, high-intensity training was able to significantly increase BALP. Based on this, we can conclude that type of training and intensity play a role in increasing bone metabolism.

A systematic review study explained that walking does not effectively prevent osteoporosis in older patients; resistance training acts as a strong stimulus to increase and maintain bone mass during the aging process. A multi-component training program, which includes strength training, aerobic, and impact and/or weight-bearing, can help increase, or at least prevent, the decline in bone mass that occurs with age, especially in postmenopausal women (Gómez-Cabello et al., 2012). Regular and long-term training can influence bone metabolism indices, prevent further bone resorption, reduce secretion of parathyroid hormone, increase calcitonin, and prevent bone tissue from losing calcium and other minerals (Troy et al., 2018).

Training also reduces the concentration of leptin and decreases body fat, thus playing a role in weight loss (Izquierdo et al., 2019). Leptin has a positive relationship with adiposity, and reducing body fat will decrease its concentration. The accumulation of adipocytes in the microenvironment of bone marrow (BM) promotes inflammation that leads to increased bone resorption by enhancing osteoclastogenesis and inhibiting the development of osteoblasts (Yu et al., 2021). Adiposity within the BM is associated with proinflammatory cytokines such as TNF-a and IL-6 (Córdova et al., 2015). The cytokine TNF-α stimulates osteoclastogenesis through the activation of NFκB by increasing the expression of RANKL and M-CSF (Marahleh et al., 2019).

The strength of this study is that it examined the direct effects of swimming training or aerobic training at different intensities on BALP. However, our study also has several limitations. The research was carried out on mice that were obese without comparing mice with normal weight or thin weight. In addition, we also focused on BALP, rather than measuring other bone metabolism parameters such as serum osteocalcin. Therefore, it is recommended that further research add these parameters to confirm the findings.

Conclusions

Based on the results of this study, we can conclude that all training intensity analysed in this study resulted in an increase in BALP levels compared to the control group. High-intensity training demonstrated a significantly highest increase in BALP levels compared to the other treatment groups, further emphasizing the vital role of training in enhancing bone health and strength, particularly in individuals classified as obese.

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Conflict of interest

The authors declare that there is no conflict of interest.

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