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<https://ejournal.upi.edu/index.php/penjas/article/view/66213>DOI: <https://doi.org/10.17509/jpjo.v9i1.66213>**The Effect of Swimming Various Intensities on Creatine Kinase Activity and Histopathological Features of The Musculus Vastus Lateralis in Obese Rats****Mubayinul Khoeroh¹, Wahyu Siswandari², Susiana Candrawati³, Sindhu Wisesa¹, Fitranto Arjadi^{1*}**¹Departement of Anatomi, Medical Faculty, Jendral Soedirman University, Indonesia²Departement of Clinical Patology, Medical Faculty, Jendral Soedirman University, Indonesia³Departement of Physiology, Medical Faculty, Jendral Soedirman University, Indonesia**Article Info***Article History :**Received January 2024**Revised March 2024**Accepted March 2024**Available online April 2024**Keywords :**creatine kinase, obesity, physical activity, swimming***Abstract**

Obesity is considered a global health problem that has reached epidemic proportions and significantly affects almost all physiological functions of the body. Swimming can reduce fat levels so that it can reduce obesity conditions which can trigger other cases. However, training that exceeds capacity can cause skeletal muscle damage. This study aims to determine the effect of swimming of various intensities (light, moderate and heavy) on creatine kinase (CK) activity and the histopathological appearance of the musculus vastus lateralis in obese rats (*Rattus norvegicus*). This study used a true experimental using a Completely Randomized Design (CRD) posttest-only control group design approach. The sample in this research; white rat (*Rattus norvegicus*) male Wistar strain, body weight 150-200 grams, and aged 6-8 weeks. Obesity induction; Pokphand 551, BOLT, cassavas and carrots. The rats were divided into 5 groups and each group consisted of 4 rats. Swimming is given once per day every morning for 14 days. CK activity was measured using UV-Vis spectrophotometer and histopathological preparations of the musculus vastus lateralis using Hematoxyline Eosine (HE) staining. Swimming significantly ($p < 0.05$) reduced CK activity. The best results were found in group 4 with a reduction of 37.29% from obese controls, and an increase of 11.53% from healthy controls. The best CK activity values were obtained by the group that had values closest to those of healthy controls. The histopathology results of group 4 showed histopathological improvement based on reduced inflammatory infiltration, fiber profile abnormalities and the presence of myofiber hypertrophy as a form of cell adaptation. Group 4 (obesity + moderate intensity swimming) is the best treatment to reduce CK activity and improve the histopathology of the musculus vastus lateralis.

INTRODUCTION

Obesity is considered a global health problem that has reached epidemic proportions, increasing rapidly, and can affect almost all physiological functions of the body (Sinaga et al., 2018). Basic Health Data Research (Riskesdas) findings show that the prevalence of adults in Indonesia, aged 18 years and over, has increased from 15,4.7% in 2013 to 22,0% in 2018 (Balitbangkes RI, 2018). The results of several studies in Asia Pacific countries show that individuals with obesity have a Relative Risk (RR) of more than 3 for developing type 2 DM, dyslipidemia, and metabolic syndrome. Currently, it is estimated that 2-7% of the total health expenditure in developed countries is spent on health problems related to obesity (Kemenkes RI, 2012).

According to the study of Lee & Oh (2014), the recommended physical exercise for obese sufferers is swimming because it does not place weight on the knee or leg joints which can cause injuries. Swimming training using water buoyancy consists of light, moderate, and heavy intensities and can help reduce the body fat while maintaining the stability compared to the on the ground training. In addition, a mild physical exercise can significantly reduce the creatine kinase activity in obesity cases (Ibrahim, 2016). Exercise is a physical stressor, it can cause problems if given incorrectly, such as a tissue damage (Aroziah et al, 2016). One indicator of a tissue damage or the onset of pain is characterized by an increase in creatine kinase (CK) levels in the blood (Koch et al., 2014). The intracellular enzyme known as creatine kinase (CK) is mostly found in the brain, heart, and skeletal muscles, when cell membranes are disrupted by an injury or hypoxia, CK is released from the cellular cytoplasm and enters the bloodstream (Ghosh et al., 2023).

When swimming, leg movements act as stabilizers (Setiawan, 2007) and one of the muscles that acts as the prime mover is the quadriceps (Lee & Oh, 2014). The musculus quadriceps that continuously contracts at a high intensity has the potential to tire quickly, causing pain (Annafi & Mukarromah, 2021). Musculus quadriceps is the largest muscle, one of the strongest extensors in the body, and included in the quadriceps group with the musculus vastus lateralis as the largest muscle (Lee and Oh, 2014). Based on the aforementioned description, the high prevalence of obesity in the world and in Indonesia will have an impact on various health

problems, so preventive measures are needed. In Indonesia, no research has been conducted on the creatine kinase (CK) activity and histopathological features in musculus vastus lateralis so that researchers were interested in examining the effect of swimming with various intensities (light, moderate, and heavy) on the creatine kinase (CK) activity and the histopathological appearance of the musculus vastus lateralis in obese rats (*Rattus norvegicus*). Previous study had examined the relationship between creatine kinase and blood pressure, physical exercise, or treadmills, but the results of creatine kinase levels had not been confirmed with the histopathological feature of the muscle (Brewster et al., 2019 ; Maigoda et al., 2016 ; Ibrahim et al., 2016). There was also a research on the relationship between obesity and swimming, but it also had not tested the creatine kinase activity and the histopathology of the vastus lateralis muscle (Koch et al., 2014). The vastus lateralis muscle, the part of the strongest extensor muscle in the thigh, is easily damaged after a physical exercise indicated by increased creatine kinase levels. For this reason, the results of this study can provide recommendations for the intensity of physical exercises for obese patients.

METHODS

This research is a true experimental study utilizing a Completely Randomized Design (CRD) with the post-test only control group design approach, considering that the treatment and control groups were equivalent at baseline. External factors were controlled. A pre-test was not possible due to the relatively expensive measurement cost and the mortality as the measured outcome. This research has been approved by the Ethics Commission of the Faculty of Medicine, Jenderal Soedirman University, No. 055/KEPK/PE/V/2023 dated 9 May 2023.

Participants

The samples consisted of 20 male white rats (*Rattus norvegicus* strain Wistar). The calculation used the Federer's formula, $(r-1)(n-1) \geq 15$, whereas r = number of treatments and n = number of samples, so that $n = 3,75 \sim 4$. The samples were divided into 5 treatment groups. Group 1 consisted of healthy control rats. Group 2 were obese model rats. Group 3 were obese rats given light intensity swimming exercises (load 3%

of body weight) (Kanca et al, 2017). Group 4 were obese rats given moderate intensity swimming exercises (load 6% of body weight) (Prasetya et al., 2018). Group 5 were obese rats given heavy intensity swimming exercises (load 9% of body weight) (Kanca et al., 2017).

Sampling Procedures

The inclusion criteria for white rats used in the research were having a body weight of ± 200 grams, 6-8 weeks of age, male gender, and the Wistar strain (*Rattus norvegicus*) given obesity inductions until the Lee obesity index was $> 0,3$. The rat care was carried out in the experimental animal laboratory at Jenderal Soedirman University, Purwokerto. After the rats were declared obese, they immediately carried out swimming for 14 consecutive days every morning. At the end of the study, musculus vastus lateralis samples were taken to check the creatine kinase activity and the histopathology.

Materials and Apparatus

This research utilized experimental animal cages 50 x 40 x 20 cm, feed containers made of glass for food and glass bottles for rats to drink, pools with a height of 60cm and a diameter of 60cm, gloves, dissecting sets, operating trays, analytical balance, rulers, freezers - 20°C and 4°C, cooler boxes, staining places, paraffin cassettes, vacuum machines, water baths, vortex, stopwatch, microtome, brushes, glasses and object covers, beakers, incubators, light microscope, micropipette, tip tracks, centrifuge, scissors, tissues, masks, markers, label papers, mortars, ice packs, organ holders, aluminum foils, polypropylene tubes, sonicators, and UV-Vis spectrophotometers. The materials used were white rats (*Rattus norvegicus*) male Wistar strain, weight of 150-200 grams and age of 6-8 weeks, musculus vastus lateralis of the rats, Pokphand 551, BOLT, cassavas, carrots, 10% formalin, Physiological NaCl, Phosphate Buffer Saline (PBS), ethanol 70%;80%;90%;95%; absolute, xylol, liquid paraffin, distilled water, dye for HE, blue solution, quartz sand, Tris-HCL Ph 6, 125 ppm ATP solution, phosphate buffer, 70 ppm histone solution and 8% TCA solution, blue tip, and yellow tip.

Procedures

The obesity induction was given ad libitum in the form of manufactured feed (Pokphand 551 and BOLT)

at 30g/rat given alternately. Then, cassavas at 70g/rat and carrots at 50g/rat were given three times a week. Rats were declared obese after reaching the Lee obesity index requirement value > 0.3 after inductions. Swimming was carried out for 14 days, every morning, in a pool with a height of 60 cm, a diameter of 60 cm, and a water level of 30 cm. The weight was tied using a 5 cm long raffia rope and attached to the base of the rat tail.

The final procedure of the study was the decapitation by cervical dislocations in all groups. The musculus vastus lateralis was taken and divided into two, left parts for measuring creatine kinase activity values using UV-Vis spectrophotometry at the Plant Physiology Laboratory, Faculty of Biology, Jenderal Soedirman University. The procedure for measuring the CK activity started from isolating protein kinase, determining the maximum wavelength, and testing the activity of the creatine kinase enzyme based on the amount of ATP reacting to transfer phosphates (using the ATP standard curve) (Ariyono, 2015):

$$\text{Activity unit of CK} = \frac{(\text{ATP initial} - \text{ATP residue})}{\text{BM ATP}} \times \frac{v}{p \cdot q} \times \text{fp}$$

The dextra side of the musculus vastus lateralis sample was used to see the damage caused by obesity and to see improvements after being given swimming exercises in various intensities (light, medium, and heavy). The examination of the musculus vastus lateralis was carried out using histopathological examinations to see the muscle size conditions, such as atrophy, hypertrophy, necrosis, and regenerations (Ibrahim, 2016). The histopathological examination was carried out at the Waskitha Laboratory in Yogyakarta using an Olympus CX23 microscope with a magnification of 400x.

Design or Data Analysis

The results of the creatine kinase activity data were processed using a normality test employing the Shapiro-Wilk test and a homogeneity test using the Levene's test. The analysis was then continued by using the One Way Anova test. The further test used the LSD post hoc test. The result of the histopathological features of musculus vastus lateralis were processed descriptively.

RESULT

Table 1. The calculation of increases and decreases of the creatine kinase activity (95% CI)

Treatments	Average of CK ($\mu\text{g/mL}$)	Increased CK from Group 1 (%)	Decreased CK from Group 2 (%)	P-value
Normal group (K-1)	0,104 \pm 0.013	-	-	0,234
Obesity group (K-2)	0,185 \pm 0.007	77,88%	-	0,630
Obesity + swimming with light intensity (K-3)	0,150 \pm 0.012	43,26%	19,45%	0,122
Obesity + swimming with moderate intensity (K-4)	0,117 \pm 0.007	11,53%	37,29%	0,678
Obesity + swimming with heavy intensity (K-5)	0,137 \pm 0.011	31,73%	29,94%	0,485

Note: It is considered that the closer the value to the normal group value (K-1), the closer the CK value to the normal value. The data normality test using the Shapiro-Wilk test and the homogeneity test using the Levene's test showed that the data were normally distributed and homogeneous ($p > 0.05$).

Table 2. Results of One Way ANOVA test for creatine kinase activity values

Source of Diversity	Degrees of Free (df)	F count	Sig.
Between Groups	4.0	37.214	0.000
Within Groups	15.0		
Total	19.0		

Statistical analysis using the One Way ANOVA test showed that swimming with various intensities (light, moderate, and heavy) in obese rats was able to reduce the CK activity significantly ($p < 0.05$) in the musculus vastus lateralis.

Table 3. Results of One Way ANOVA test for creatine kinase activity values

Treatments 1(I)	Treatments 2(J)	Mean Difference (I-J)	SE	Sig.
K-1	K-2	-.081*	0.007	0.000
	K-3	-.045*	0.007	0.000
	K-4	-0.01	0.007	0.110
	K-5	-.033*	0.007	0.000
K-2	K-1	.081*	0.007	0.000
	K-3	.036*	0.007	0.000
	K-4	.069*	0.007	0.000
	K-5	.048*	0.007	0.000

Note: there is a significant difference if the Sig. is smaller than 0.05 (sig. < 0.05).

The results of further tests using the LSD post hoc test showed that, in groups 3, 4, and 5, there were significant differences among groups (Post Hoc LSD; $p < 0.05$). It shows that swimming with various intensities (mild, moderate, and heavy) in obese rats has significant differences among the treatment groups 3, 4, and 5. This study also found no significant differences in

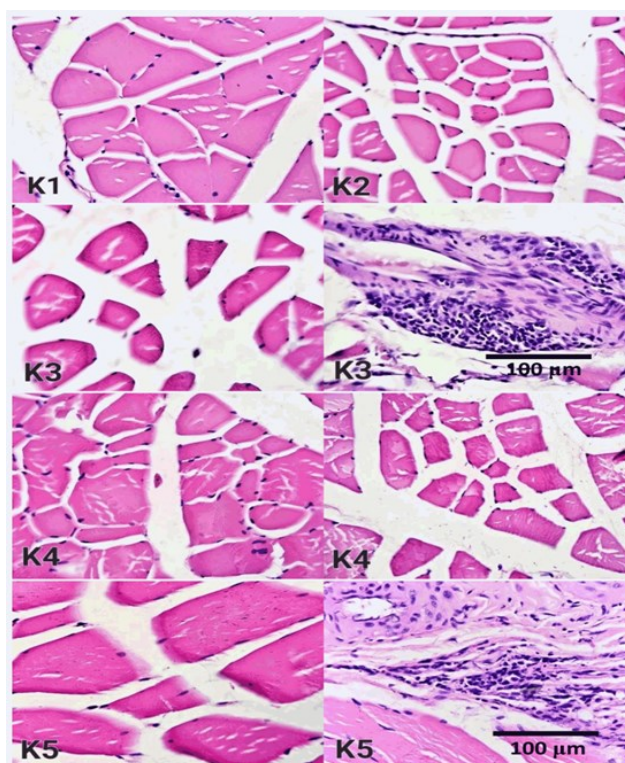


Fig 1. Musculus vastus lateralis of obese rats after being given swimming treatments in various intensities. 400x magnification. Hematoxylin-Eosin (HE) staining. Description: K1 (Group 1), K2 (Group 2), K3 (Group 3), K4 (Group 4), and K5 (Group 5). The 100 μm scale

Group 4 and Group 1 (Post Hoc LSD; $p < 0.05$).

Histopathological images show that there is no muscle necrosis found in all groups. K-1 shows the normal muscle where the epimysium, endomysium, and perimysium are intact. Muscle cells are monomorphic,

with sufficient and intact cytoplasm, round-oval nuclei, located peripherally, and smooth chromatin. K-2 and K-3 look atrophic, but there is a slight lymphocyte infiltrate on K-3. Images K-4 and K-5 show hypertrophy, but there is a slight infiltrate of lymphocytes and leukocytes in the epimysium on K-5 indicating a muscle damage.

DISCUSSION

The results of Group 1 were the healthy controls used as a basis for the comparison of the CK activity increase in other treatment groups. Group 2, the obese group, was used as a comparison for the CK activity decrease in other treatment groups. The results of Group 2 had a mean value of creatine kinase activity of 0.185 ± 0.007 and experienced an increase in CK activity of 77.88% from Group 1 (healthy controls). Obesity creates conditions that endanger the capillary beds and causes fat infiltrations of the tissues, thereby reducing blood circulations and/or oxygen extractions (Johnsen et al., 2014). This condition triggers ischemia causing damages to muscle membranes and increased CK activity (Kumar et al., 2013). Group 4 experienced a decrease in CK activity of 37.29% from Group 2 (obese group) and an increase of 11.53% from Group 1 (healthy controls). The CK was not significant compared to groups 2, 3, and 5. Groups 3 and 5 respectively experienced a decrease in CK activity of 19.45% and 29.94% from Group 2 (obese group). The increase in CK activity amounted to 43.26% and 31.73% from Group 1 (healthy controls). It shows that an excessive physical exercise can increase the CK activity.

The results of this study indicate that the treatment in Group 4 (K-4) was more effective for reducing the CK activity in the musculus vastus lateralis of obese rats (*Rattus norvegicus*). This situation was because the CK activity value of K-4 (a group of obese rats given a moderate intensity swimming) did not have a significant difference from K-1 as the healthy control. The CK activity value was considered to have decreased if it approached the value of healthy controls (K-1). Obesity causes the accumulation of fats in adipose tissues, resulting in inflammations of the adipose and skeletal muscles. The results of research from Group 1, the control of healthy rats, showed that the histopathology of the musculus vastus lateralis appeared normal. Groups 2 and 3 showed atrophy of some myofibers. These re-

sults are in line with the research of Le et al. (2014), which found musculus gastrocnemius atrophy in rats after being given obesity inductions. Obesity causes an increase in free fatty acids in skeletal muscles, where the binding of free fatty acids to inflammatory receptors, Toll Like Receptors (TLRs), triggers the activation of the inflammatory signaling pathway. The binding of FFA (Free Fatty Acid) to TLR4 activates inflammatory signal molecules, such as MAP kinase ERK and p38, thereby inducing the expression of Atrogin-1/MuRF1 (atrophy gene) and triggering the loss of muscle mass ($p < 0.05$) (Le et al., 2014).

The histopathological results of the obese group with the moderate and heavy intensity swimming (K-4 and K-5) showed an increase in the muscle cell size, called hypertrophy. The occurrence of hypertrophy indicates a cell adaptation response to the physiological stress and pathological stimuli to maintain a normal homeostasis. Muscle cells have a permanent shape that cannot divide so that the muscles will adapt by experiencing hypertrophy (Kumar et al., 2013). Images K-3 and K-5 show the presence of inflammatory cells characterized by infiltrates of lymphocytes and leukocytes which are signs of a cell damage. The inflammatory response functions to attract plasma proteins and phagocytes to the site of injury to isolate, destroy or clear debris, and prepare the tissue for the healing process. The damaged tissue, due to a physical injury, will cause inflammations so that the damaged tissue will be destroyed and eliminated by macrophages and neutrophils to initiate the tissue repair process (Darmono, 2015). The histopathological images of all groups did not show any necrosis characterized by karyolysis, pyknosis, and karyorrhexis.

The treatment in Group 4 (K-4), the moderate intensity swimming treatment group with a load of 6% of body weight, is a more effective treatment for improving the histopathological picture of the musculus vastus lateralis in obese rats (*Rattus norvegicus*). In the histopathological picture of K-4, there was no visible infiltrations of lymphocytes and leukocytes, but myofiber hypertrophy, one of the adaptation processes of muscle cells when the tissue damage occurs, was observed. The histopathological feature of the vastus lateralis muscle in K-4 was associated with a significant decrease in CK activity values, showing that the moderate intensity swimming training, analogous to giving a load of 6% of

the body weight of the experimental animals when swimming, did not cause any damage to the skeletal muscle tissue. The result of this research recommends that swimming athletes should be given a moderate intensity swimming training to avoid a serious damage to their skeletal muscles, especially the muscles functioning as the prime mover. The study only used samples from one muscle so it cannot describe the condition of creatin kinase activity throughout the body and does not represent the histopathological picture of the quadriceps muscles and lower extremities.

CONCLUSION

The moderate intensity swimming treatment with a load of 6% of body weight is a more effective intensity for reducing the creatine kinase activity of the musculus vastus lateralis and improving the histopathological condition of the musculus vastus lateralis in obese rats. Further studies on the creatin kinase activity and histopathological features after physical exercises in obesity cases need to be expanded to more diverse organs or muscles with more diverse physical exercises to get the best and more effective results so that it can be used as a preventive measure for pre-obese individuals and/or therapeutic measures for individuals with obesity.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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