

DIFFERENCES OF MASS AND DIAMETER MUSCLE RATTUS NORVEGICUS IN GIVING KETOGENIC DIETS AND EXERCISE

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ABSTRACT

Introduction: Saturated ketogenic diet without exercise can lead to muscle mass decrease and disturb body metabolism. Therefore, the researcher is interested in discovering the differences between a saturated and unsaturated ketogenic diet with exercise to muscle mass and diameter.

Purpose: This study aimed to analyze the difference in muscle mass and diameter of *Rattus norvegicus*, which were given a ketogenic diet and exercise.

Method: This experimental research used *Rattus norvegicus* that had been adapted for two weeks. Rats are divided into five groups; control (K), a saturated ketogenic diet (P1), a saturated ketogenic diet with exercise (P2), an unsaturated ketogenic diet (P3), and an unsaturated ketogenic diet with exercise (P4). Upright running wheel exercise was given to the group with exercise for 10 minutes daily. The ketogenic diet was given with oral gavage. Muscle mass was examined in the second and fourth weeks. Muscle diameter was examined before treatment, second week, and fourth week.

Results: There are differences in mass and diameter muscle *Rattus norvegicus* that given ketogenic diet and exercise ($p=0,000$). In second week, there are significant increase in muscle diameter for saturated ketogenic diet without exercise (P1($p=0,000$)) and significant decrease in muscle diameter for three other group (P2($p=0,000$), P3($p=0,001$), P4($p=0,000$)). In the fourth week, there is a significant increase in muscle diameter for an unsaturated ketogenic diet with exercise (P4).

Conclusion: An unsaturated ketogenic diet with exercise could increase the mass and diameter of quadriceps muscle at the end of this research.

Keyword: *Rattus norvegicus*; ketogenic diet; exercise

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INTRODUCTION

Obesity is one of the problems in this modern era—the WHO reported that in 2016 there are 650 million people with obesity. If people don't change their habits, obesity will increase two times in ten years.¹

Ketogenic diets consist of high fat (70-80%), adequate protein (5-10%), and low carbohydrate (10-20%). The purpose of proper protein in this diet is to maintain muscle mass and blood glucose. There are two primary sources of fat for the ketogenic diet; saturated fat, which is from lard, butter, fatty meats, and processed meat, and unsaturated fat, which are from nuts, seeds and grains, avocado, vegetable oil, and fish oil.^{2,3}

A ketogenic diet uses fat as the primary source of energy. In the human body, fat is changed into acetyl-CoA and then transformed into a ketone body by the liver and transported throughout the body. Ketogenic diets have a hypoglycemic effect, and then the body will react through gluconeogenesis mechanism to produce glucose. This whole mechanism occurs in the liver.⁴

In previous studies, the provision of exercise is often made. Hence, we want to know about the effects of the ketogenic diet with saturated and unsaturated fats and exercise on quadriceps diameter and

quadriceps muscle mass. Therefore, we are interested in researching with the title "Differences in Mass and Diameter of the *Rattus Norvegicus* Muscle in Giving the Ketogenic Diet and Exercise."

METHOD

Materials

The food for the control group is 6.62-gram wheat flour, 0.45-gram coconut oil, and 4-gram white eggs. The unsaturated ketogenic diet contains 1.52-grams almond flour, 1.3-gram olive oil, and 6.3-gram chicken eggs. Meanwhile, the saturated ketogenic diet contains 1.4-grams almond flour, 6.6-gram chicken eggs, and 1.84-gram margarine. An upright running wheel (diameter 35 cm and width 10 cm) was used for exercise in this study.

Preparation of Research Animal

Animal for this research is 90 males Wistar rats (2-3 months) with 125-250 grams and placed in one cage containing each one of the rats.

Male Wistar divides into five groups; control group (K), unsaturated ketogenic diet (P1), unsaturated ketogenic diet with exercise (P2), saturated ketogenic diet (P3), saturated ketogenic diet with exercise (P4).

Experiment Procedure

Rats were given diets using oral gavage at 6 a.m. and 3 p.m. All of the rats in this research got free access to tap water.

Rats in the exercise group were given exercise for 10 minutes every day with upright running wheels.

Data Analysis

Test of normality performed with Skewness test, Kurtosis test, Kolmogorov-Smirnov test, Histogram test, Normal QQ-Plot test, and Detrended Normal QQ-Plot and Observed Value test. Meanwhile, the Levene test was for the homogeneity test.

RESULTS

Quadriceps Diameter and m. Quadriceps Mass of Rattus norvegicus

Average data for diameter and muscle mass are done by compare means test, and the results are available in Table 1 and Table 2, as well as in Figure 1 and Figure 2.

Table 1. Diameter Quadriceps Average of Rattus norvegicus: 2019

Groups	Diameter <i>Quadriceps</i> (Mean±SD)		
	Zero week (cm) (n=18)	Second week (cm) (n=18)	Fourth week (cm) (n=9)
K	0.91±0.08	0.93±0.06	0.96±0.03
P1	0.86±0.08	0.98±0.05	0.94±0.02
P2	1.1±0.07	0.99±0.06	0.98±0.02
P3	1.03±0.05	0.98±0.03	0.96±0.02
P4	1.12±0.08	0.98±0.03	1.07±0.03

K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

Table 2. m. *Quadriceps* and Femur Mass Average of *Rattus norvegicus*: 2019

Groups	Massa m. <i>Quadriceps</i> dan tulang femur	
	Second week (g) (n=9)	Fourth week (g) (n=9)
K	7.11±0.49	6.76±0.72
P1	4.99±0.38	5.55±0.63
P2	4.75±0.41	5.18±0.45
P3	6.71±0.75	6.48±0.92
P4	5.55±0.87	6.77±0.69

K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

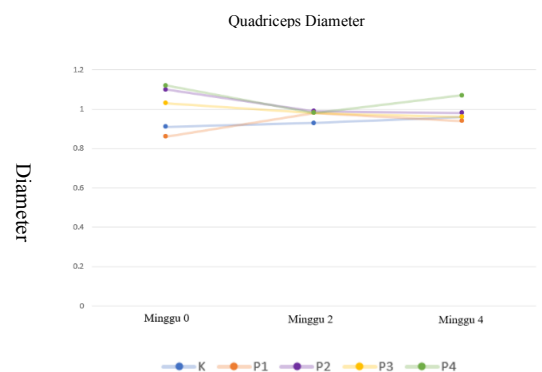


Figure 1. Quadriceps Diameter Graphs

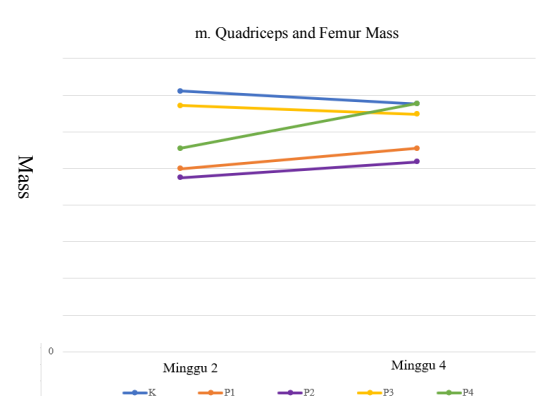


Figure 2. m. *Quadriceps* dan Femur Mass
Graphs

Effect of Ketogenic Diet and Exercise to Muscle Diameter

The result of hypothesis Kruskal Wallis test on the effect of ketogenic diet and exercise on muscle diameter showed significant with $p=0.000$, which means that there is an effect of ketogenic diet and exercise to muscle diameter. The result of Mann-Whitney U is available in Table 3 to Table 6.

Table 3. Mann-Whitney U Results on Quadriceps Diameter in Fourth Week of *Rattus norvegicus*: 2019

Groups	Groups	Sig.	Description
K (0.96±0.03) (n=9)	P1 (0.94±0.02)	0.111	Not significant
	P2 (0.98±0.02)	0.168	Not significant
	P3 (0.96±0.02)	0.563	Not significant
	P4 (1.07±0.03)	0.001	Significant
P1 (n=9)	P2	0.005	Significant
	P3	0.069	Not significant
	P4	0.000	Significant
P2 (n=9)	P3	0.061	Not significant
	P4	0.001	Significant
P3 (n=9)	P4	0.000	Significant

K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

The result of this post-hoc test showed that K (0.96±0.03 cm) has significant differences with P4 (1.07±0.03 cm) with $p=0,001$. Likewise, P1 (0.94±0.02 cm) have significant

differences with P2 (0.98±0.02 cm) with $p=0.005$ and P4 (1.07±0.03 cm) with $p=0.000$. These results show exercise given in this study has an impact on muscle diameter. Meanwhile, there isn't a significant difference between the saturated ketogenic diet and the unsaturated ketogenic diet (P1 and P3), which indicates that unsaturated and saturated ketogenic diet doesn't affect muscle diameter the fourth week, as shown in Table 3.

Table 4. Mann-Whitney U and Compare Means Results on Quadriceps Diameter in Zero Week and Second Week of *Rattus norvegicus*: 2019

Groups	Zero week (cm)	Second week (cm)	Sig.
K (n=18)	0.91±0.08	0.93±0.06	0.389
P1 (n=18)	0.86±0.08	0.98±0.05	0.000*
P2 (n=18)	1.1±0.07	0.99±0.06	0.000*
P3 (n=18)	1.03±0.05	0.98±0.03	0.001*
P4 (n=18)	1.12±0.08	0.98±0.03	0.000*

*: Significant; K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

The test shows a comparison between the zero weeks and the second week on muscle diameter. P1 zero weeks compared with P1 second week significantly increased from 0.86±0.08 cm to 0.98±0.05 cm with $p=0.000$. Then, P2 zero week has a substantial decrease than P2 second week from 1.1±0.07 cm to 0.99±0.06 cm with $p=0.000$. Next, P3 zero week and P3 second week have a significant downturn from 1.03±0.05 cm to 0.98±0.03 cm with $p=0,001$. Last, P4 zero week compared with P4 second week

have a considerable decline from 1.12 ± 0.08 cm to 0.98 ± 0.03 cm with $p=0.000$, as shown in Table 4.

Table 5. Mann-Whitney U and Compare Means Results on Quadriceps Diameter in Second Week and Fourth Week of *Rattus norvegicus*: 2019

Groups	Second week (cm) (n=18)	Fourth week (cm) (n=9)	Sig.
K	0.93 ± 0.06	0.96 ± 0.03	0.047*
P1	0.98 ± 0.05	0.94 ± 0.02	0.098
P2	0.99 ± 0.06	0.98 ± 0.02	0.777
P3	0.98 ± 0.03	0.96 ± 0.02	0.055
P4	0.98 ± 0.03	1.07 ± 0.03	0.000*

*: Significant; K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

The Mann-Whitney U test shows a comparison of muscle diameter in the second week and fourth week. P4 second week compared to P4 fourth week has a significant increase of 0.98 ± 0.03 cm to 1.07 ± 0.03 cm with p score 0.000, as shown in Table 5.

Table 6. Mann-Whitney U and Compare Means Results on Quadriceps Diameter in Zero Week and Fourth Week of *Rattus norvegicus*: 2019

Groups	Zero week (cm) (n=18)	Fourth week (cm) (n=9)	Sig.
K	0.91 ± 0.08	0.96 ± 0.03	0.035
P1	0.86 ± 0.08	0.94 ± 0.02	0.005*
P2	1.1 ± 0.07	0.98 ± 0.02	0.000*
P3	1.03 ± 0.05	0.96 ± 0.02	0.001*
P4	1.12 ± 0.08	1.07 ± 0.03	0.050

*: Significant; K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

Table 6 shows a comparison in the zero weeks and the fourth week on muscle diameter. P1 zero weeks compared with P1 fourth week shows a significant increase

from 0.86 ± 0.08 cm into 0.94 ± 0.02 cm with $p=0.005$. Next, P2 zero weeks have a substantial decrease than P2 fourth week from 1.1 ± 0.07 cm into 0.98 ± 0.02 cm with $p=0.000$. Last, P3 zero week compared with P3 second week significantly declined from 1.03 ± 0.05 cm to 0.96 ± 0.02 cm with $p=0.001$.

Effect of Ketogenic Diet and Exercise on Muscle Mass

In this study, a hypothesis test to see the effectiveness of the ketogenic diet and exercise on muscle mass. The hypothesis used was Kruskal Wallis. The effect of ketogenic diet and exercise on muscle mass showed significant with $p=0.000$, which means that there is an effect of ketogenic diet and exercise on muscle mass. The result of Mann-Whitney U is available in Table 7 and Table 8.

Table 7. Mann-Whitney U m.Quadriceps and Femur Mass in Second Week of *Rattus norvegicus*: 2019

Groups	Groups	Sig.	Description
K (7.11±0.49) (n=9)	P1 (4.99±0.38)	0.000	Significant
	P2 (4.75±0.41)	0.000	Significant
	P3 (6.71±0.75)	0.216	Not significant
	P4 (5.55±0.87)	0.001	Significant
P1 (n=9)	P2	0.216	Not significant
	P3	0.001	Significant
	P4	0.133	Not significant
P2 (n=9)	P3	0.000	Significant
	P4	0.024	Significant
P3 (n=9)	P4	0.012	Significant

K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

The post-hoc test on muscle mass using Mann-Whitney U shows K have significant differences than P2 with $p=0.000$ and P4 with $p=0,001$ and P3 compared to P4 has substantial differences with p score 0.012, which means the exercise that given in this study have an effect on muscle mass in the second week, as shown in Table 7.

Table 8. Mann-Whitney U m.Quadriceps and Femur Mass in Fourth Week of Rattus norvegicus: 2019

Groups	Groups	Sig.	Description
K (6,76±0,72) (n=9)	P1 (5,55±0,63)	0,004	Significant
	P2 (5,18±0,45)	0,001	Significant
	P3 (6,48±0,92)	0,566	Not significant
	P4 (6,77±0,69)	0,965	Not significant
P1 (n=9)	P2	0,402	Not significant
	P3	0,052	Not significant
	P4	0,005	Significant
P2 (n=9)	P3	0,004	Significant
	P4	0,000	Significant
P3 (n=9)	P4	0,508	Not significant

K: Control; P1: Saturated ketogenic diet; P2: Saturated ketogenic diet with exercise; P3: Unsaturated ketogenic diet; P4: Unsaturated ketogenic diet with exercise

Table 8 shows the result of the Mann-Whitney U test on muscle mass in the fourth week. P2 has a significant difference from P3 with a p-score of 0.004, which means the variations of the ketogenic diet (unsaturated and saturated)

have a different effect on muscle mass in the fourth week. Comparing P1 and P4 also supports that and shows a significant difference with $p=0.005$ on muscle mass in the fourth week.

DISCUSSION

In this study, quadriceps muscle and femur cannot be separated, but recent research showed the effect of the ketogenic diet on muscle mass appeared on 12 weeks⁵; meanwhile, this research only is done for four weeks.

From the result of this study for four weeks, found the most effective diet is the unsaturated ketogenic diet with exercise. It is supported by the research conducted by Zajac et al. that an unsaturated ketogenic diet, in particular, omega-3 that found in chicken eggs and almond flour, have an ergogenic effect (enhance the performance on the body) that can maintain the integrity of the membrane of erythrocytes and lower blood viscosity.⁶ Blood viscosity is a direct measure of blood resistance. If the unsaturated ketogenic diet can reduce the thickness of the blood, more erythrocytes can reach the tissue so that more oxygen enters the working muscles.⁷ Omega-7 in olive oil can reduce inflammation in the body by deactivating NF-kappaB. This protein is useful for controlling the production of pro-inflammatory cytokines⁸. Suppose the unsaturated

ketogenic diet is given with exercise. In that case, it can increase the diameter and muscle mass quickly compared to the saturated ketogenic diet because the unsaturated ketogenic diet does not need to go through the inflammatory phase. In contrast, the saturated ketogenic diet needs to go through the inflammatory phase first.

The saturated ketogenic diet in this study experienced an increase in quadriceps muscle diameter at week two and decreased again in week 4. For phenomena that occur in week 0 to week 2, that is, the muscle diameter of the rat can rise due to inflammation in the muscle of the mouse. This phenomenon is supported by research conducted by Sitnick et al. and Kennedy et al. that a saturated ketogenic diet can cause inflammation in muscles because saturated fat increases the expression and secretion of pro-inflammatory cytokines^{9,10}, so the saturated ketogenic diet is not proper to use if you want to increase muscle tone.

The ketogenic diet group saturated with exercise in this study experienced decreased muscle diameter from weeks 0, 2, and 4 and an increase in m. quadriceps and femur mass in week 4. It indicates that exercise is effective in this research. A saturated ketogenic diet can be an alternative diet if given along with exercise because exercise has an anti-inflammatory effect. It is supported by

research conducted by Starkie et al. and Kohut et al., which says if endurance-type exercise has an anti-inflammatory effect by inhibiting the formation of $TNF\alpha$ so that inflammation does not occur by stimulating an increase in IL-6.¹¹⁻¹³ Endurance exercise also reduces oxidative stress by increasing the endogenous antioxidant defense system.¹⁴

In this study, the unsaturated ketogenic diet group experienced a decrease in quadriceps muscle diameter every week and decrease in m. quadriceps and femur mass. It descends due to the sedentary life of rats so that no activity can increase muscle mass.^{15,16}

CONCLUSION

Based on the study results, it can be concluded that the unsaturated ketogenic diet with exercise is the recommended diet of this study because it can increase the diameter and muscle mass.

A saturated ketogenic diet with exercise can be an alternative because it can increase muscle diameter even though it is not significant and can increase muscle mass at the end of the study.

A saturated and unsaturated ketogenic diet without exercise is not recommended because a saturated ketogenic diet without exercise can cause muscle inflammation. An unsaturated ketogenic diet cannot increase muscle diameter and muscle mass

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