DIFFERENCE OF WEIGHT AND LOW-DENSITY LIPOPROTEIN (LDL) LEVEL OF RATTUS NORVEGICUS ON MEDITERRANEAN DIET AND UNSATURATED KETOGENIC DIET

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ABSTRACT

Introduction: Sedentary lifestyle continues to develop, which causes problems such as obesity and coronary heart disease. This is caused by an increase in body weight and LDL levels that can be overcame with a Mediterranean diet or an unsaturated ketogenic diet.
Purpose: To analyze the difference in weight and LDL of Rattus norvegicus on the unsaturated ketogenic diet and the Mediterranean diet
Method: this study was an experimental study on 30 Rattus norvegicus divided into three groups (control, unsaturated ketogenic, and Mediterranean), each group is divided into two, according to the time of administration (two weeks and four weeks). After the intervention was completed, bodyweight was calculated, and LDL levels were checked. Then, the data was analyzed with one-way ANOVA test.
Results: The test results show $p < 0.001$ in the comparison between the three groups and $p < 0.05$ for comparison in the unsaturated ketogenic group. The Mediterranean diet shows $p > 0.05$, and the control diet leads $p > 0.05$. The data show that rapid weight loss (2 weeks) and reduction in LDL (in 4 weeks) in the unsaturated ketogenic diet is better than the Mediterranean diet or control. For medium-term weight loss (4 weeks), the Mediterranean diet is better than the neither saturated ketogenic diet nor control.
Conclusion: There is a difference in weight and low-density lipoprotein (LDL) level of Rattus norvegicus on the Mediterranean diet and unsaturated ketogenic diet. This favorable condition might minimize the probability risk of obesity and coronary heart disease.

Keyword: Weight, LDL, Rattus norvegicus, mediterranean diet, unsaturated ketogenic diet.

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INTRODUCTION

The ketogenic diet is an effort to regulate the intake of low carbohydrate (2% -5%), protein (8% -15%), and high fat (55% -80%) intake into the body, which in the medical field is used to control epilepsy in young children. [1,2] The ketogenic diet is also used to lose weight, which is quite popular among people and can treat obesity. [3] The prevalence of obesity in Indonesia in the last five years has increased by 14.8% -21.8%, and in 2017, the East Java region has a prevalence of obesity of 16.25%. [4,5]

A person can be obese if he has a Body Mass Index (BMI) of 30 kg / m2 as measured by a person's body weight and height. An obese person has a 1.5 times higher risk of developing coronary heart disease than normal. [6] The coronary heart death rate is quite high, which is 12.9% in 2014 [7], and is the biggest cause of death globally. [8] Coronary heart disease can be caused by several factors: high-fat food patterns, lack activity, and changing bodyweight that is increasing and decreasing in a short time. These factors cause increased LDL levels (low-density lipoprotein), which can be a cause of atherosclerosis. [8.9]

The ketogenic diet in several studies with 55-70% fat composition, 8-15% protein, and 5-10% carbohydrates given to humans shows weight loss and decreased LDL levels within two weeks to one year. [3,10-12] In rats, the ketogenic diet can affect body weight and lower LDL levels compared to rats fed normally after one month of administration. [12-14] The ketogenic diet focuses on the condition of ketosis to obtain the effects mentioned above. The ketogenic diet's effect can reduce the risk of coronary heart disease by decreasing risk factors, both reducing the risk of obesity through weight loss or decreasing LDL levels. [16,17] The ketogenic diet also has side effects that need to be considered. The diet's transition period at the beginning of the diet can cause nausea, vomiting, dizziness, fatigue, constipation, and even insomnia, which can cause discomfort that can disappear with time. This diet also has the risk of causing liver damage both in the short and long term. [12,18]

Dietary choices aren't just the ketogenic diet. The Mediterranean diet can also be used in reducing the risk of coronary heart disease. The Mediterranean diet is a diet that uses 25-40% fat from total energy with saturated fat lower than unsaturated fat. [16-18] Monounsaturated fats have effects on increased fat burning, mobilization, and increased energy requirements. These effects cause fat mass in the body to decrease so that it can reduce weight. [21] The Mediterranean diet does not only have fat but also has

sufficient carbohydrate composition. Carbohydrates in this composition are complex carbohydrates that can prevent glucose levels in the blood from rising quickly after meals. This composition can help reduce the inhibition of weight gain. [21] The Mediterranean diet choice seems to have no side effects, but it still needs to be considered for the duration of the Mediterranean diet because the impact of weight loss is difficult to achieve in a short time. Research conducted by Carbonneau É indicated that it takes about five weeks or even six months to show significant weight loss. [22]

The explanation above shows a problem in which dietary choices are more effective in reducing coronary heart disease risk with different nutritional compositions from several studies. This problem attracts researchers' attention to determine whether there are differences in body weight and levels of low-density lipoprotein (LDL) in Rattus norvegicus on the Mediterranean diet and the ketogenic diet.

METHOD

In this study, thirty samples of Rattus norvegicus were treated for four weeks. These experimental animals were divided into six groups, namely control week 2 (K1), ketogenic unsaturated week 2 (P11), Mediterranean week 2 (P21), control week 4 (K2), ketogenic unsaturated week 4th (P12), and Mediterranean 4th week (P22). Each group was weighed after being adapted for one week and then fed according to their respective groups using a feeding tube. After given two weeks of treatment, groups K1, P11, P21 were weighed, and their LDL levels were checked. After the data is obtained, the experimental animals were then mutilated and cremated. The same was done after four weeks of treatment in groups K2, P12, P22. The data were tested statistically using the ANOVA test, and a post hoc LSD test was performed to see comparisons between each group

RESULTS

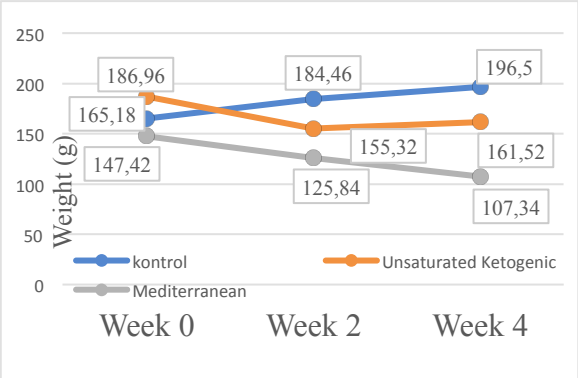

Figure 1. Results of the Average Distribution of Rattus norvegicus Body Weight at the Faculty of Pharmacy Animal Laboratory in 2019

Figure 1 shows the average results of rat body weight per two weeks in each treatment group.

Table 1. Results of the Difference in Average Weight of Rattus norvegicus in the Laboratory of Animal Medicine Faculty of Pharmacy in 2019

Group	Weight Difference Distribution		
	Total (n)	Average weight difference 0-2 week (g)	Average weight difference 2-4 week (g)
Control	5	19,28g ± 11,70158	12,04 g ± 3,01214
Unsaturated Ketogenic	5	-31,64g ± 17,04547	6,2 g ± 7,28595
Mediterranean	5	-21,58g ± 3,42053	-18,5 g ± 18,58922

Table 1 shows the results of the average difference in body weight of rats per week in each treatment group.

Table 2. Results of Post-hoc LSD Test on Body Weight among Groups

Group	Post-hoc LSD Test on Body Weight among Groups on 2nd Week			
	Group	Sig.	Standard error	Information
K_1	$P1_1$	0,00	7,50931	Significant
	$P2_1$	0,00	7,50931	Significant
$P1_1$	$P2_1$	0,193	7,50931	Not Significant

Table 2 shows the results of comparisons between groups of the 2nd week with the information K1 = Control week 0-2, P11 = Ketogenic unsaturated week 0-2, P21 = Mediterranean week 0-2.

Table 3. Results of Post-hoc LSD Test on Body Weight among Groups

Group	Post-hoc LSD Test on Body Weight among Groups on 4th Week			
	Group	Sig.	Standard error	Information
K_2	$P1_2$	0,345	7,50931	Not Significant
	$P2_2$	0,000	7,50931	Significant
$P1_2$	$P2_2$	0,685	7,50931	Not Significant

Table 3 shows the comparison between groups of the 4th week with the information K2 = Control 2-4 weeks, P12 = Ketogenic unsaturated weeks 2-4, P22 = Mediterranean weeks 2-4.

Table 4. Results of Post-hoc LSD Test on Body Weight among Groups

Group	Post-hoc LSD Test on Body Weight among Groups			
	Group	Sig.	Standard error	Information
K_1	K_2	0,345	7,50931	Not Significant
$P1_1$	$P1_2$	0,000	7,50931	Significant
$P2_1$	$P2_2$	0,685	7,50931	Not Significant

Table 4 shows the comparison results in each of the weekly groups with

information K1 = Control weeks 0-2, K2 = Control weeks 2-4, P11 = Ketogenic unsaturated weeks 0-2, P12 = Ketogenic unsaturated weeks 2-4, P21 = Mediterranean weeks 0-2, P22 = Mediterranean weeks 2-4.

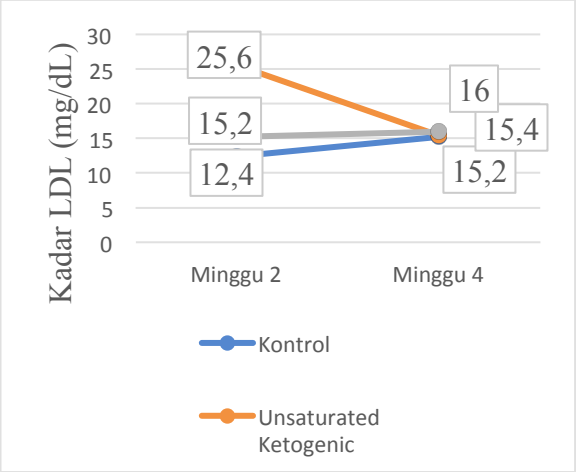

Figure 2. Results of the Average Distribution of Rattus norvegicus LDL Levels in the Laboratory of Animal Medicine Faculty of Pharmacy in 2019

Table 5. Test Results of Post-hoc LSD Test LDL Levels

Table 5 shows the comparison between groups with K1 = Control week 2, P11 = Ketogenic unsaturated week 2, P21 = Mediterranean week 2.

Group	Post-hoc LSD test LDL levels between groups in the 2nd week			
	Group	Sig.	Standard error	Information
K_1	$P1_1$	0,000	2,14165	Significant
	$P2_1$	0,203	2,14165	Not Significant
$P1_1$	$P2_1$	0,000	2,14165	Significant

Table 6. Test Results of Post-hoc LSD Test LDL Levels

Group	Post-hoc LSD test LDL levels between groups in the 4th week			
	Group	Sig.	Standard error	Information
K_2	$P1_2$	0,926	2,14165	Not Significant
	$P2_2$	0,712	2,14165	Not Significant
$P1_2$	$P2_2$	0,782	2,14165	Not Significant

Table 6 shows the comparison between groups with K2 = Control week 4, P12 = Ketogenic unsaturated week 4, P22 = Mediterranean week 4.

Table 7. Test Results of Post-hoc LSD Test LDL Levels

Group	Post-hoc LSD Test LDL Levels Among group			
	Group	Sig.	Standard error	Information
K_1	K_2	0,203	2,14165	Not Significant
$P1_1$	$P1_2$	0,000	2,14165	Significant
$P2_1$	$P2_2$	0,712	2,14165	Not Significant

Table 7 shows the comparison results in each of the weekly groups with

information K1 = Control week 2, K2 = Control week 4, P11 = Ketogenic unsaturated week 2, P12 = Ketogenic unsaturated week 4, P21 = Mediterranean week 2, P22 = Mediterranean week 4.

DISCUSSION

Bodyweight in the control group continued to increase, and the difference between the two did not show a significant difference (p = 0.345). Growth in these control mice follows with age growth, which is around 30-50g / month. This weight growth was almost the same in a study conducted by Sihombing, 2010 in 30 mice fed ad-libitum for three months whose weight increased by about 50g / month. [23]

Weight in the unsaturated ketogenic group showed a decrease at the beginning of the treatment until the 2nd week. This initial decrease in treatment was caused by the combined effects of an unsaturated ketogenic diet. The process of gluconeogenesis and ketosis in this diet forces the body's fat reserves to be broken down so that its weight also decreases. Still, before the ketone body is used by the body effectively, the body carries out the process of glycogenolysis as a temporary source of energy so that glycogen in the muscles and liver is broken down. [13] In 1g of glycogen, there is 3g of water, so that if glycogen is used, then the water in it also comes out. This is accompanied by ketone bodies, which can be osmotic diuretics causing loss of body fluids. [24–26] The breakdown of fat and the increase in diuresis causes weight in the ketogenic group to decrease considerably. This effect caused the unsaturated ketogenic group's weight at 2 weeks to be lower than the control group for two weeks, and when compared, both showed significant results (p = 0.00). Research carried out by B. Eiya conducted in 40 Wistar rats, given the ketogenic diet for three weeks, also showed lower body weight than controls. [27]

The difference between unsaturated ketogenic bodyweight in the 2nd week and 4th week showed a significant difference (p = 0.00) caused by a difference from the initial decrease, which was quite large in the 2nd week then slightly increased in the 4th week. This increased weight seen after week four is still lower than the average body weight in the week after adaptation (week 0). This slight increase is due to the body, which has begun to adapt to high-fat feeding and effective use of ketone bodies. When measured regularly, ketone body levels will decrease with time. It shows the body starts using the ketone body effectively, so that body fat breakdown is slightly reduced. [24] The reduced-fat breakdown causes a slight increase in body weight from the 2nd week to the 4th week

even though the weight compared to the week after adaptation still shows weight loss. Weight at week 4 of the unsaturated ketogenic group showed slightly lower results than the control. When compared, the two did not show a significant difference (p = 0.44). Research conducted by Bielohubby, 2013 that used 30 rats fed the ketogenic diet for four weeks also showed lower body weight than controls. [28]

The Mediterranean group's weight decrease at week 2 was not significantly different from the Mediterranean week 4 (p = 0.68). When compared at each week (week two and week four) with the control, it is showing a significant difference. Unsaturated fats contained in this diet have a fairly high Monounsaturated Fatty Acid (MUFA) content. Oleic acid can function to activate Adenosine Monophosphate Protein Kinase (AMPK) increase fat oxidation. [29] This is followed by complex carbohydrate compositions with a longer glucose absorption effect in the intestine than simple carbohydrates and fiber composition, which help increase intestinal motility and volume deflation. Less is absorbed, and less is stored as food reserves. [21,30]. Research conducted by Carbonneau, 2017 on giving a Mediterranean diet as early as five weeks before giving a low-calorie diet shows a lower body weight than controls. [22]

Weight loss in the 2nd week of the Mediterranean is slightly lower than unsaturated ketogenic, and when compared, the two do not have significant differences (p = 0.19). This is due to the diuresis effect found in the ketogenic diet, which is different from the Mediterranean. The opposite was confirmed in the 4th week; namely, Mediterranean body weight decreased while unsaturated ketogenic experienced an increase that had significant differences (p = 0.01). This is caused by the adaptation experienced by the ketogenic diet, so that fat breakdown is reduced. In contrast, in the Mediterranean diet, the effects of MUFA and complex carbohydrates and fiber continue. [24]

CONCLUSION

The results of this study indicate that the unsaturated ketogenic diet and the Mediterranean diet have effects that can be used by the public in terms of weight loss.

- Weight loss in large enough quantities and in a short amount of time is better found in the unsaturated ketogenic diet
- Better weight loss over the medium term was seen in the Mediterranean diet

This study also shows that to reduce LDL levels was better in the unsaturated ketogenic diet because only in the unsaturated ketogenic diet a decrease

was observed compared to the Mediterranean diet and control.

ACKNOWLEDGEMENT

1. Faculty of Pharmacy Widya Mandala Surabaya Catholic University has provided an animal laboratory for use.
2. Faculty of Veterinary Medicine, Airlangga University has provided an animal laboratory for use.
3. The Surabaya Center for Health Laboratory has helped test blood samples in this study.

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