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Physical Exercise and High-Calorie Diet on Kidney Histopathology

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ABSTRACT

High calorie diet without proper balance of physical excersises is a major factor to many health issues such as obesity, fat accumulation and organs failure. Fat accumulation in kidney can lead up to atherosclerosis and cause major problem in the organ through its mechanism that causes fibrogenesis. The aim of this research is to determine the effect of physical exercise and high- calorie diet on kidney histopathology. This study is conducted with 27 Mus musculus that were divided to 3 groups (negative control, positive control and experiment) with each group got different treatment. Negative control group was given no D40 nor physical exersice, positive control was given D40 without physical exersice and experimental group was given D40 and physical exercise. The kidneys from these mice then microscopically tested to see the histopathological changes in the form of inflammatory cell infiltration, hemorrhage, cell necrosis, glomerular adhesion, and microscopic swelling. Data was analyzed with Kruskal-Wallis Test to determine the difference between each test group using the help of Statistical Package for the Social Science (SPSS) Version 25. 30 mice were observed histologically with HE stain. The results of the Kruskal-Wallis test showed a value of $p < 0.05$ for glomerular adhesion, swelling, and inflammatory cell infiltration. The only histopathological changes that had significant difference in all of its group was inflamatory cell infiltration. Physical exercise and high-calorie diet showed an effect on the kidney in the form of glomerular adhesion, swelling and inflammatory cell infiltration as evidenced by the results of the analysis test.

Keywords: Physical Exersice, High-Calorie Diet, Kidney Histopathology, Mus musculus, Diet

INTRODUCTION

According to Indonesian Central Bureau of Statistics (2018), the average calorie intake of Indonesian people have increased 304,34 kcal from 2013 to 2018. This increase can be explained by the lifestyle changes in these past years to a more sedentary lifestyle with no sufficient exercise. A lifestyle with high consumption of calories and supported by a lack of activity can lead to numerous health issues such as obesity, diabetes and hypertension (1).

Every individual have different amount of calories needed each day and all of these nutrients have to be consumed to meet up the minimum intake of calories, but a problem occurs when a person has an unbalanced diet and consumed too many foods. While less intake of calorie causes disturbance in physical health and daily activity, over intake of calories causes overweight that eventually leads to increased risk of metabolic and cardiovascular diseases (2).

A research by Yuliantini et al. (2015), shows that a high calorie consumption leads to an excessive amount of fat in body and will be followed by an increased ratio of total cholesterol/HDL (High Density Lipoprotein). This will ultimately cause atherosclerosis in blood vessels and become a reason for many more health problems (3). Atherosclerosis if occured in the kidney will cause fibrogenesis to happen and induces both acute and chronic kidney injury. A good balance between food intake and energy outtake can minimize the possibility of this (4).

Physical exercise has been proven to be a solution to counter fat accumulation in the body, fight obesity and maintain a good health in general. A well-planned physical exercise with a proper intensity have a wide range of benefits from decreasing blood pressure and trigliserid to increasing HDL level (5,6). These effects of physical exercise will directly and indirectly prevent atherosclerosis (7,8). This research was conducted to determine the effect of physical exercise and high-calorie diet on kidney histopathology.

MATERIAL AND METHODS

2.1 Ethics

This study was approved by the Medicine Faculty Ethics Committee of Airlangga University by considering research principles in animals like replacement, reduction, and refinement.

2.2 Animal and surgical procedures

A total of 27 female Mus musculus Balb/C strain (8 weeks old), weighing 20-25 g were used in present study. The animals had full access to standard food and water ad libitum throughout the duration of study. Animals were divided into 3 groups, negative control (without D40 and physical exercise), positive control (given D40 without physical exercise), and experimental group (given D40 and physical exercise moderate intensity). Moderate-intensity physical exercise was given in the form of swimming with a weight of 6% of the mice's body weight tied to the base of the tail which is carried out for 15 minutes in stages and is carried out 3 times a week on Monday, Wednesday, and Friday. The animals were then sedated and dissected to remove their kidney. After it was removed, the kidney was cleaned and fixated with 10% formalin.

2.3 Histological evaluation

The mice kidneys were processed into histological preparate through the manufacture of paraffin blocks and given HE staining. Observations were made by looking at histological changes in the form of inflammatory cell infiltration, hemorrhage, cell necrosis, glomerular adhesion, and microscopic swelling which was then scored. The assessment was carried out on 5 fields of view with a magnification of 400x.

2.4 Statistical analysis

Data was analyzed with Kruskal-Wallis Test to determine the difference between each test group using the help of Statistical Package for the Social Science (SPSS) Version 25.

RESULT AND DISCUSSION

30 mice survived until the end of experiment and then underwent surgery to take their kidney for the histopathological microscopic sample with the help of HE staining. The results are shown in Figure 3.2-3.4 and the scoring results are shown in Table 3.1-3.5.

Table 3.1 Glomerular Adhesion Microscopical Changes Result

Group	**N**	**Glomerular Adhesion**		
		Score 0	Score 1	Score 2
K-	10	3 (30%)	7 (70%)	0 (0%)
K+	10	0 (0%)	6 (60%)	4 (40%)
P	10	0 (0%)	10 (100%)	0 (0%)

Table 3.2 Swelling Microscopical Changes Result

Group	**N**	**Swelling**		
		Score 0	Score 1	Score 2
K-	10	5 (50%)	5 (50%)	0 (0%)
K+	10	0 (0%)	10 (100%)	0 (0%)
P	10	0 (0%)	10 (100%)	0 (0%)

Table 3.3 Inflammatory Cell Infiltration Microscopical Changes Result

Group	**N**	**Inflamatory Cell Infiltration**		
		Score 0	Score 1	Score 2
K-	10	10 (100%)	0 (0%)	0 (0%)
K+	10	0 (0%)	7 (70%)	3 (30%)
P	10	3 (30%)	7 (70%)	0 (0%)

Table 3.4 Hemorrhage Microscopical Changes Result

Group	N	Hemorrhage		
		Score 0	Score 1	Score 2
K-	10	10 (100%)	0 (0%)	0 (0%)
K+	10	8 (80%)	2 (20%)	0 (0%)
P	10	10 (100%)	0 (0%)	0 (0%)

Table 3.5 Necrosis Microscopical Changes Result

Group	N	Necrosis		
		Score 0	Score 1	Score 2
K-	10	10 (100%)	0 (0%)	0 (0%)
K+	10	10 (100%)	0 (0%)	0 (0%)
P	10	10 (100%)	0 (0%)	0 (0%)

The observation data was then tested for normality using Saphiro-Wilk because the number of samples is 30 (<50) and it was found that the data were not normally distributed ($p<0.05$). Since the data were not normally distributed, then a non-parametric difference test was carried out using Kruskal-Wallis. The results of the Kruskal-Wallis test in Table 3.6 showed that there were statistically significant differences in the variables of glomerular adhesion, swelling, and inflammatory cell infiltration ($p<0.05$), while no statistically significant difference found in the variables of hemorrhage and necrosis ($p>0.05$).

Table 3.6 Kruskal-Wallis Test Result

	Glomerular Adhesion	Swelling	Inflammatory Cell Infiltration	Hemorrhage	Necrosis
df	2	2	2	2	2
P value	0,006	0,003	0,0001	0,126	1,000

Table 3.7 shows the result of mean value from Kruskal-Wallis test. The highest mean value in all variables belongs to the K+ group, while the lowest value belongs to the K- group.

Table 3.7 Kruskal-Wallis Test Mean Result.

	N	Glomerular Adhesion	Swelling	Inflamatory Cell Infiltratio n	Hemorrhage	Necrosis
K-	10	11,10	10,50	7,00	14,50	15,50
K+	10	20,40	18,00	23,05	17,50	15,50
P	10	15,00	18,00	16,45	14,50	15,50

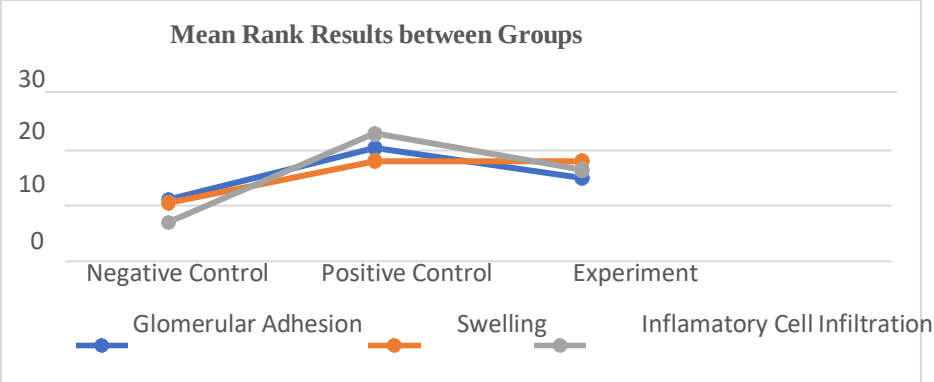

Figure 3.1 Mean Rank Results between Groups.

Table 3.8 shows the comparison of glomerular adhesion, swelling and infiltration of inflammatory cells between the 2 research groups using the help of Mann-Whitney. The only histopatological changes that had significant difference in all of its group was inflamatory cell infiltration.

Table 3.8 Kruskal-Wallis Test Mean Result.

	Group	**P Value**
Glomerular Adhesion	Negative Control-Positive Control	0,010
	Negative Control-Experiment	0,067
	Positive Control-Experiment	0,029
Swelling	Negative Control-Positive Control	0,012
	Negative Control-Experiment	0,012
	Positive Control-Experiment	1,000
Inflamatory Cell Infiltration	Negative Control-Positive Control	0,000
	Negative Control-Experiment	0,001
	Positive Control-Experiment	0,017

The histopathological results of glomerular adhesion, swelling, and inflammatory cell infiltration showed that the positive control group (K+) had the highest score of histopathological changes when compared to the negative control group (K-) and treatment (P), this was due to the administration of D40 as A high-calorie diet can lead to atherosclerosis. Atherosclerosis can cause pressure on the renal glomerular capillaries which will cause stenosis or adhesions to the glomerulus, the emergence of an inflammatory reaction characterized by the infiltration of inflammatory cells, and decreased renal filtration function which will result in cloudy swelling (swelling) in the epithelial cells (6,9).

A high-calorie diet derived from both carbohydrates and fat increases blood glucose directly, as a result of which there is an increase in pull and mesangial pressure due to cell polyferation so that the glomerular mesangium expands triggering cellular hypertrophy that stimulates the dilation of glomerular cells and damages the walls of the vessels (10). Energy consumption that is greater than the energy expended will result in excessive accumulation of nutrients in the body which in the long term will cause metabolic syndrome. This metabolic

syndrome can increase the risk of diseases such as type 2 diabetes mellitus, cardiovascular disease, kidney disease, and lead to death (11). In rat kidney pieces given a high-carbohydrate and high-fat diet, glomerular and tubular damage were found, which consisted of hyaline degeneration and/or hydropic degeneration and necrosis (12).

High-calorie diet stimulates the α1- and β-adrenergic receptors, resulting in increased sympathetic nerve activity and a long-term increase in blood pressure that is the cause of decreased kidney function. Large blood pressure and kidney function are related cause and effect to each other. An increase in blood pressure in the long term has the potential to cause glomerular damage that leads to decreased kidney function. Decreased kidney function causes blood filtration of the glomerulus is not maximal so that it activates the mechanism (RAAS) rennin-angiotensin- aldosterone-system to meet the oxygen needs of tissues by increasing the blood supply to the kidneys.

Physical exercise can improve the function of endothelium in blood vessels which is useful in providing protection during filtration. Physical exercise can also increase the production of nitric oxide (NO) and reduce reactive oxygen species (ROS) which are very involved in tissue inflammatory processes (13). This is in accordance with research in 2009 that showed exercise improved homeostasis of fasting and postpandrial blood sugar levels, maintain weight loss, increase HDL, lower LDL and triglycerides, lower blood pressure, reduce inflammation, and improve endothelial function (14).

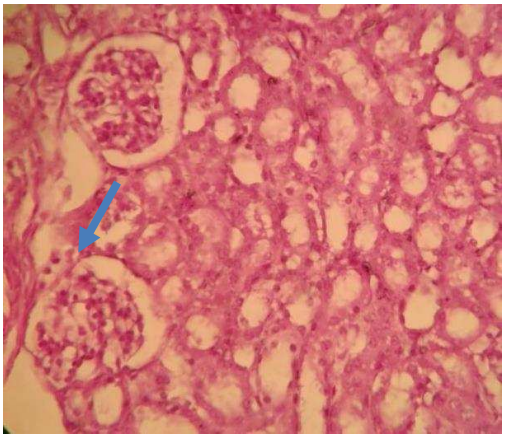

Figure 3.2 Histological Finding of Glomerular Adhesion in Negative Control Group

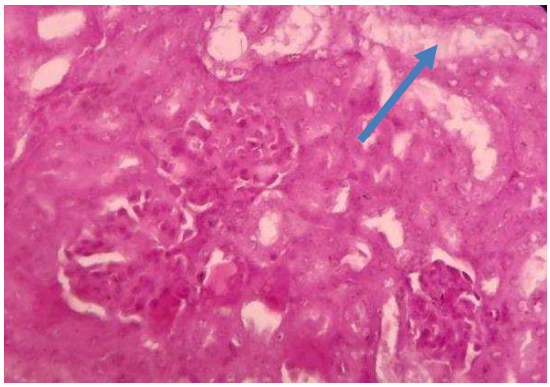

Figure 3.3 Histological Finding of Kidney Swelling in Positive Control Group

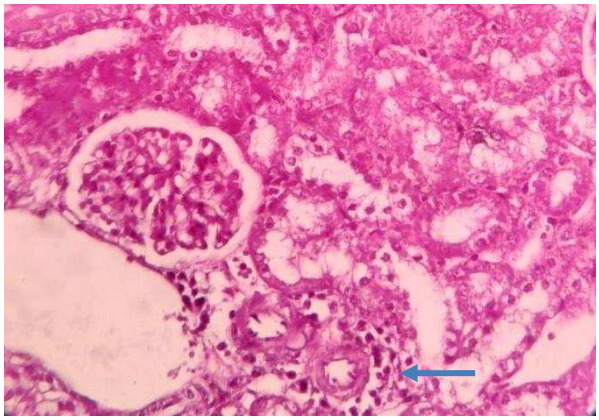

Figure 3.4 Histological Finding of Inflammatory Cell Infiltration in Experimental Group

CONCLUSION

Physical exercise and high-calorie diet showed an effect on the kidney in the form of glomerular adhesion, swelling and inflammatory cell infiltration as evidenced by the results of the analysis test. While histopathological changes in the form of hemorrhage and necrosis did not have a significant difference in it.

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Peneliti Utama : **Ahmad Rukhani Lutfi, S. Or**
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Alocation of Research

Dengan judul:
Title

"Pengaruh Kombinasi Restriksi Kalori dan Latihan Kontinu Intensitas Sedang Terhadap Kadar *Free Fatty Acid* (FFA) Serum Dan Berat Lemak Visceral Pada Mencit Betina Yang Dipapar Diet Tinggi Kalori"

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Pernyataan Laik Etik ini berlaku selama kurun waktu tanggal 6 Januari 2020 sampai dengan tanggal 6 Januari 2021.

This declaration of ethics applies during the period January 6, 2020 until January 6, 2021.

January 6, 2020
Professor and Chairperson,

Prof. Dr. H. Eddy Bagus Wasito, dr, MS., Sp.MK (K)