HIGH-FAT DIET INCREASES SERUM HDL, BUT NOT FOR LDL AND HDL/LDL RATIO IN MICE

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ABSTRACT

The use of ketogenic diet with an increased proportion of fat is rampant lately, both as a therapy or even lifestyle. The use of ketogenic diet is feared to cause dyslipidemia and will lead atherosclerosis and thrombosis. This study aimed to determine effect of high-fat diet on HDL, LDL and HDL/LDL ratio in mice. Thirty male Mus Musculus, 2-3 mo, 15-25 g divided into five groups diet. K1 (12% fat, 20% protein, 62% carb), K2 (30% fat, 60%protein), K3 (45% fat, 45% protein), K4 (60% fat, 30% protein) and K5 (75% fat, 15% protein) for 4 weeks. Feed and water by adlibitum. Blood for serum was taken from ventricle, while the measurement of serum level of HDL, LDL used ELISA. Normality of data analyzed by Saphiro Wilk, and homogeneity by Levene test. HDL serum was analyzed using equality test followed by Games Howell, LDL serum and ratio of HDL/LDL was tested by Anova followed by LSD with significance 0,05. HDL serum in K1 (62,50±9.94) mg/dL, K2 (78,40±18,76) mg/dL, K3 (79,00±3,81) mg/dL, K4 (80,00±2,16) mg/dL, and K5 (83,50±5,62) mg/dL with p<0,05 in K1, K2 to K3, K4 and K5. LDL serum in K1 (21,67± 4,80) mg/dL, K2 (23,00±12,70) mg/dL, K3 (18,40±4,34) mg/dL, K4 (24,00 ±1,83) mg/dL and K5 (22,00 ± 4,08) mg/dL with p>0,05. Rasio HDl/LDL K1 (3,01±0,91), K2 (4,10±1,86), K3 (4,53±1,5), K4 (3,35±0,34), and K5 (3,96 ± 1,25) with p>0,05.

***Keywords**: high fat diet; mice; HDL; LDL; HDL/LDL ratio; obesity*

ABSTRAK

Penggunaan diet ketogenik dengan peningkatan proporsi lemak akhir-akhir ini semakin populer, baik sebagai terapi ataupun gaya hidup. Penggunaan diet ketogenik dikhawatirkan menyebabkan dislipidemia dan akan menyebabkan aterosklerosis dan trombosis. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh diet tinggi lemak pada rasio HDL, LDL dan HDL/LDL pada tikus. Tiga puluh Mus Musculus jantan, usia 2-3 bulan, 15-25 gram dikelompokan kedalam lima kelompok diet. K1 (12% lemak, 20% protein, 62% karbohidrat), K2 (30% lemak, 60% protein), K3 (45% lemak, 45% protein), K4 (60% lemak, 30% protein) and K5(75% lemak, 15% protein) selama empat minggu. Pakan dan air secukupnya. Normalitas data dianalisis dengan Shapiro Wilk, sedangkan homogenitas dengan uji Levene. Serum HDL dianalisis dengan uji kesetaraan dan Games Howell. LDL serum dan rasio HDL/LDL diuji oleh Anova dan LSD dengan signifikansi 0,05. Serum HDL pada K1 (62,50±9.94) mg/dL, K2 (78,40±18,76) mg/dL, K3 (79,00±3,81) mg/dL, K4 (80,00±2,16) mg/dL, and K5 (83,50±5,62) mg/dL dengan p<0,05 pada K1, K2 to K3, K4 and K5. Serum LDL pada K1 (21,67± 4,80) mg/dL, K2 (23,00±12,70) mg/dL, K3 (18,40±4,34) mg/dL, K4 (24,00 ±1,83) mg/dL and K5 (22,00 ± 4,08) mg/dL dengan p>0,05. Rasio HDl/LDL K1 (3,01±0,91), K2 (4,10±1,86), K3 (4,53±1,5), K4 (3,35±0,34), and K5 (3,96 ± 1,25) dengan p>0,05.

***Kata kunci**: high fat diet; tikus; HDL; LDL; rasio HDL/LDL; obesitas*

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INTRODUCTION

Nowadays, obesity is one of serious problems around the world. Its prevalence increases every year (Oyekale 2019). In 2013, Indonesia had a prevalence of obesity reached 26% of the total population of Indonesia (Ministry of Health 2013). The ketogenic diet is one of methods that requires people to change their eating habits with special rules (Masood et al 2020). In the ketogenic diet, a person will consume foods with high in fat and protein, but very low in carbohydrates (Kosinki & Jomayvaz 2017). The ketogenic diet initially only became a non-pharmacological therapy for people with epilepsy, but it is now widely used as a therapy for obese patients (Paoli et al 2013), diabetes mellitus and cancer. Now, this diet even leads to a lifestyle.

The high proportion of fat in the ketogenic diet raises a polemic, especially an increasing risk of cardiovascular disease. High fat is thought will trigger dyslipidemia as the pathophysiology of atherosclerosis. The ketogenic diet leads physiological process of ketosis (Paoli et al 2013). In many ketogenic diet using as therapy, there is an increase high density lipoprotein (HDL), and a decrease low density lipoprotein (LDL) blood (Dashti 2004). High HDL and low LDL level affect the risk of cardiovascular diseases, such as atherosclerosis (Hu & Bazzano 2015). However, the proportion of energy sources in this diet that influences HDL and LDl levels in the blood has not been revealed clearly. This study was to determine the effect of the proportion of energy sources in a high-fat diet on HDL and LDL levels.

MATERIALS AND METHODS

This study had been approved by the Committee of Animal Care and Use, Faculty of Veterinary Universitas Airlangga under a decree No 2.KE.124.07.2018. The design of the study was posttest only control group design conducted at the Faculty of Veterinary Medicine, Universitas Airlangga during December 2018 to February 2019.

Fifty male Mus Musculus balb/c, 2-3 aged month, 120-150 gram, and acclimatized for one week. Mice were divided into 5 groups; K1 (control, 12% fat, 20% protein, 62% carbohydrate), K2 (30% fat, 60% protein, 0% carbohydrate), K3 (45% fat, 45% protein, 0% carbohydrate), K4 (60% fat, 30% protein, 0% carbohydrate) and K5 (75% fat, 15% protein, 0% carbohydrate). Diet was administrated for 4 weeks; ad libitum and mice were purified. Body weight weighed before and after treatment. Serum was obtained from the blood taken from the ventricle. The measurement of HDL and LDL levels was using ELISA. The serum obtained when less than 1.5 ml was not refractive in HDL and LDL measurement.

The data were analyzed using SPSS included normality test using Saphiro Wilk ($p>0.05$), and homogeneity using Brown-Forsythe robust homogeneity test ($p<0,05$), Anova, and post Hoc using Games Howell test.

RESULTS

The initial number of mice before diet treatment was 50. Some mice died during study took place. Weight before and after 4 weeks of feeding can be seen in Table 1. In addition to the samples excommunicated due to death, the samples were also excommunicated due to other factors, such as blood volume that was insufficient to be able to perform HDL and LDL measurements.

Table 1. Body weight in pre and posttest

Group	n	Pre BW (gram) X ± SD	Post BW (gram) X ± SD
K1	9	23.56 ± 2.46	26.22 ± 4.66
K2	5	27.20 ± 2.39	21.40 ± 3.65
K3	5	24.00 ± 4.24	14.40 ± 3.78
K4	4	24.00 ± 3.37	14.25 ± 2.06
K5	4	26.50 ± 1.30	18.00 ± 1.16

Table 1 showed that overall mice at K2 – K5 group occurred weight loss, while at K1 the body weight increased.

Table 2. Table 2. Serum HDL, LDL, HDL/LDL ratio

Group	n	HDL (mg/dL) X ± SD	LDL (mg/dL) X ± SD	HDL/LDL ratio
K1	9	62,50 ± 9.94[a]	21,67 ± 4,80[a]	3,01 ± 0,91[a]
K2	5	78,40 ± 18,76[a]	23,00 ± 12,7[a]	4,10 ± 1,86[a]
K3	5	79,00 ± 3,81[b]	18,40 ± 4,34[a]	4,53 ± 1,25[a]
K4	4	80,00 ± 2,16[b]	24,00 ± 1,83[a]	3,35 ± 0,34[a]
K5	4	83,50 ± 5,62[b]	22,00 ± 4,08[a]	3,96 ± 1,25[a]

HDL and LDL measurement results, HDL/LDL ratio could be seen in Table 2. There was no significant difference in HDL serum between K1 and K2, but different from K3, K4 and K5. Yet, this difference did not happen to LDL and HDL/LDL ratio. There were no differences among K1-K5.

DISCUSSION

This study also purposed to know the effect of nutrient composition on high fat diet to these parameters. High fat diet (the ketogenic diet) in K3-K5 was increasing HDL serum, but not in K2. This result was in line with a study conducted by Dashti et al (2004) and Salvador et al (2021) which revealed that the ketogenic diet had an effect on increasing HDL levels. The ketogenic diet which was commonly called as a high-fat and low-carbohydrate diet had provided a significant increase in HDL levels (Lima et al 2015). When undergoing ketogenic diet treatment, the body would be in ketosis state (Jensen et al 2020).

The state of ketosis continuous glucose and insulin level might be low, which lowered lipoprotein lipase (LPL) and increased hormone sensitive lipase (HSL), encouraged an increased triacylglycerol hydrolysis (TAG) and fatty acids. The increased free fatty acids would be taken by the liver then diverted from esterification to TAG and towards oxidation of mitochondria to acetyl-CoA. The accumulation of acetyl-CoA that exceeded the capacity would then be converted into a ketone body. The reduction of TAG production in the liver resulted in the effect of secretion of VLDL in the decreased circulation. The lipolysis VLDL mediated by LPL results in transfer of un-esterified cholesterol, phospholipid (PL), apolipoprotein (apo) E, apoC-II, and apoC-III to form mature HDL-C. The remaining particles were taken by the liver or converted to LDL (Volek et al 2005).

The results of this study regarding the effect of the ketogenic diet on LDL levels carried out for one month did not have a significant effect for both clinically and statistically. LDL levels in all groups were not much different and tend to be the same. Giving the ketogenic diet less than three months would not have the effect of changing LDL levels (Foster et al 2003). The ketogenic diet only had a very minimal effect on LDL levels (Westman et al 2008). Similar results were also found in other study that the ketogenic diet only provided insignificant changes in LDL levels (Chiu et al 2017). This was different from the results of a study conducted by Dashti et al (2004) which stated that after undergoing a long-term ketogenic diet program LDL levels in the sample decreased.

Other study had also compared the effects of changes in LDL levels before and after undergoing ketogenic therapy (Noain et al 2020, Burén et al 2021). The samples were given therapy in the form of a ketogenic diet for three months and six months, stating that the samples treated with the ketogenic diet for three months did not change their LDL levels, while the samples treated with the ketogenic diet for six months found an average decrease in LDL levels only around .06 nm (Lima et al 2015).

The remaining particles from the formation of HDL were taken by the liver or converted to LDL. The reduced circulation of VLDL, especially in the post-prandial period results in less transfer of cholesterol ester protein (CETP) – exchange of neural lipids with LDL-C. The reduction in hepatic lipase (HL) prevented a greater LDL-C from being converted to solid (atherogenic) LDL. This made the ketogenic diet in the short-term not too significant for LDL levels (Volek et al 2005).

CONCLUSION

High fat diet increased HDL and its increase occurred starting in high-fat diet with a proportion of 45% fat or more.

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