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# Pengaruh Pemberian Ekstrak Kulit Melinjo terhadap Aktivitas Enzim Lipoprotein Lipase Pada Tikus dengan Diet Hiperkolesterol

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**Abstrak**

Asupan lemak jenuh dan kolesterol yang berasal dari makanan, yang dicerna di usus, menghasilkan asam lemak bebas, trigliserida, fosfolipid, dan kolesterol. Kadar trigliserida yang meningkat disebabkan aktivitas enzim lipoprotein lipase (LPL) yang terganggu. Aktivitas LPL dapat ditingkatkan dengan flavonoid, seperti kulit Melinjo. Tujuan penelitian ini adalah untuk mengukur aktivitas enzim LPL sebelum dan sesudah diberikan ekstrak kulit melinjo. Jenis penelitian ini adalah eksperimen murni, dengan desain penelitian *pretest posttest control group design*. Diet hiperkolesterolemia (kuning telur puyuh, otak sapi, dan glukosa) diberikan pada tikus dengan sonde sebanyak 1,8 gram selama 14 hari. Ekstrak kulit melinjo diberikan secara sonde selama 14 hari setelah diet hiperkolesterolemia diberikan. Analisis statistik menggunakan *Paired Sample T-Test* untuk membandingkan aktivitas LPL sebelum dan sesudah diberikan ekstrak kulit melinjo. Kemudian, menggunakan uji MANOVA (*Multivariate Analysis of Variance*) untuk melihat perbedaan aktivitas LPL pada setiap kelompok setelah perlakuan. Berdasarkan analisis statistik menunjukkan bahwa ada perbedaan aktivitas LPL pada kelompok diet hiperkolesterolemia, perlakuan 1, perlakuan 2, dan perlakuan 3 sebelum dan sesudah perlakuan ada perbedaan yang signifikan ($p. < 0,05$). Aktivitas enzim LPL dalam kelompok diet normal dibandingkan dengan kelompok diet hiperkolesterolemia dan perlakuan 3 menunjukkan perbedaan yang signifikan ($p. < 0,05$). Aktivitas enzim LPL meningkat setelah diberikan ekstrak kulit melinjo, dikarenakan adanya flavonoid didalamnya.

**Kata Kunci:** hiperkolesterolemia, lipoprotein lipase, kulit Melinjo, flavonoid

# *The Effect of Melinjo Peel Extract in Activity of Lipoprotein Lipase Enzym of the Rats Fed a Hypercholesterolemia Diet*

***Abstract***

*The saturated fat and cholesterol intake from food is digested in the intestine produce free fatty acids, triglycerides, phospholipids, and cholesterol. Increasing of triglyceride levels may caused by presence of impaired lipoprotein lipase (LPL) enzyme activity. LPL activity can be increased by flavonoid in plant, like Melinjo peel. The purpose of this study was to measured LPL enzyme activity before and after being given extract of Melinjo peel treatment. This research used true experimental, with study design of pretest posttest control group design. Hypercholesterolemic diet (quail egg yolk, cattle brain, and glucose) given to rats by oral gavage as much as 1,8 grams for 14 days. Melinjo peel extract were given by oral gavage for 14 days after the hypercholesterolemic diet is given. Statistical analysis used Paired Sample T-*

*Test to compare lipoprotein lipase activity before and after treatment. Then, used MANOVA (Multivariate Analyses of Variance) to see the difference of lipoprotein lipase activity in each group after treatment. Result showed that there were differences of lipoprotein lipase activity in the hypercholesterolemic diet group, HD+54.15, HD+108.30, and HD+216.60 before and after treatment significant (p. < 0.05). The activity of lipoprotein lipase in the normal diet group compared with the hypercholesterolemic and HD+216.60 group showed a significant difference (p < 0.05). Melinjo peel extract can increased activity of lipoprotein lipase enzym after treatment, those can be due to the flavonoid in Melinjo peel extract.*

***Keywords:*** *hypercholesterolemia*, *lipoprotein lipase*, *Melinjo peel*, *flavonoid*

## INTRODUCTION

Hypercholesterolemia that occurs associated with eating patterns such as high cholesterol diet or high saturated fatty acids (Setiawan, Tjahyono, and Afifah, 2016). The intake of saturated fat and cholesterol that comes from food digested in the intestine resulting in free fatty acids, triglycerides, phospholipids, and cholesterol (Saputra, 2015). Triglyceride levels increased can be caused by presence of impaired lipoprotein lipase (LPL) enzyme activity, and abdominal obesity and increased levels of small dense low density lipoprotein (LDL) allegedly has a close relationship with the increase triglycerides, decreased high density lipoprotein (HDL) cholesterol and increased ratio cholesterol and HDL cholesterol. This is a risk factor the presence of acute coronary syndrome (Handayani, 2003). The major advances in understanding the mechanisms of action of dietary fatty acids on lipoprotein metabolism have focused on *n*-3 polyunsaturated fatty-acids (PUFA). This knowledge provides insights into the importance of regulating lipoprotein metabolism as a means to improve the plasma lipid profile and lower cardiovascular disease (CVD) risk (Ooi, *et al*, 2015).

Triglyceride can be decreased if activity of lipoprotein lipase enzyme is increasing. It had relation with one of active substance in plant, like flavonoid in Melinjo peel. Flavonoids can increase LPL enzyme activity so as to decrease blood triglyceride levels by activating Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ) (Beekmann, *et al*, 2015). The purpose of this study is to measure LPL enzyme activity before and after being given extract of Melinjo peel treatment.

## MATERIAL AND METHODS

This research was true experimental, with study design of pretest posttest control group design. Samples were male rats strain Wistar. The rats included the

following group (n = 5 per group): normal diet (ND), hypercholesterolemic diet (HD), hypercholesterolemic diet and Melinjo peel extract (54.15 mg/kg) (HD+54.15), hypercholesterolemic diet and Melinjo peel extract (108,30 mg/kg) (HD+108.30), and hypercholesterolemic diet and Melinjo peel extract (216.60 mg/kg) (HD+216.60). Hypercholesterolemic diet contains quail egg yolk, cattle brain and pure glucose which are mixed together, and given to rats by oral gavage as much as 1,8 grams for 14 days. Melinjo peel extract were given by oral gavage for 14 days after the hypercholesterolemic diet is given.

At the end of the treatment, the rats were drawn the blood, then it was collected in a serum vacutainer and centrifuged at 4000 rpm for 20 min and the serum was then used to determine rat lipoprotein lipase activity. Statistical analysis used Paired Sample T-Test to compare lipoprotein lipase activity before and after treatment. Then, used MANOVA (Multivariate Analyses of Variance) to see the difference of lipoprotein lipase activity in each group after treatment. The ethical clearance of this research had been accepted by ethical approval certificate from Health Research Ethics Committee Faculty of Public Health Airlangga University.

## RESULT

### Lipoprotein Lipase Activity Before and After Treatment

The mean of lipoprotein lipase activity among groups before and after treatment in normal diet group were 0.853 (pretest) and 0.872 (posttest), hypercholesterolemic diet were 0.853 (pretest) and 0.435 (posttest), hypercholesterolemic diet and Melinjo peel extract (54.15 mg) were 0.858 (pretest) and 0.871 (posttest), hypercholesterolemic diet and Melinjo peel extract (108.30 mg) were 0.866 (pretest) and 0.910 (posttest), and hypercholesterolemic diet and Melinjo peel extract (216.60 mg) were 0.854 (pretest) and 0.944 (posttest).

**Table 1.** The mean difference of lipoprotein lipase activity before and after treatment of rats fed hypercholesterolemic diet

| Group | n | Pretest | Posttest | *p.* |
|---|---|---|---|---|
| | | Mean ± SD (mg/dl) | Mean ± SD (mg/dl) | |
| ND | 5 | 0.853 ± 0.022 | 0.872 ± 0.031 | 0.141 |
| HD | 5 | 0.853 ± 0.026 | 0.435 ± 0.051 | 0.000 |
| HD+54.14 | 5 | 0.858 ± 0.028 | 0.871 ± 0.035 | 0.003 |
| HD+108.30 | 5 | 0.866 ± 0.012 | 0.910 ± 0.022 | 0.025 |
| HD+216.60 | 5 | 0.854 ± 0.039 | 0.944 ± 0.030 | 0.033 |

The mean difference of Lipoprotein Lipase Activity in each group before and after treatment is shown in Figure 1. below.

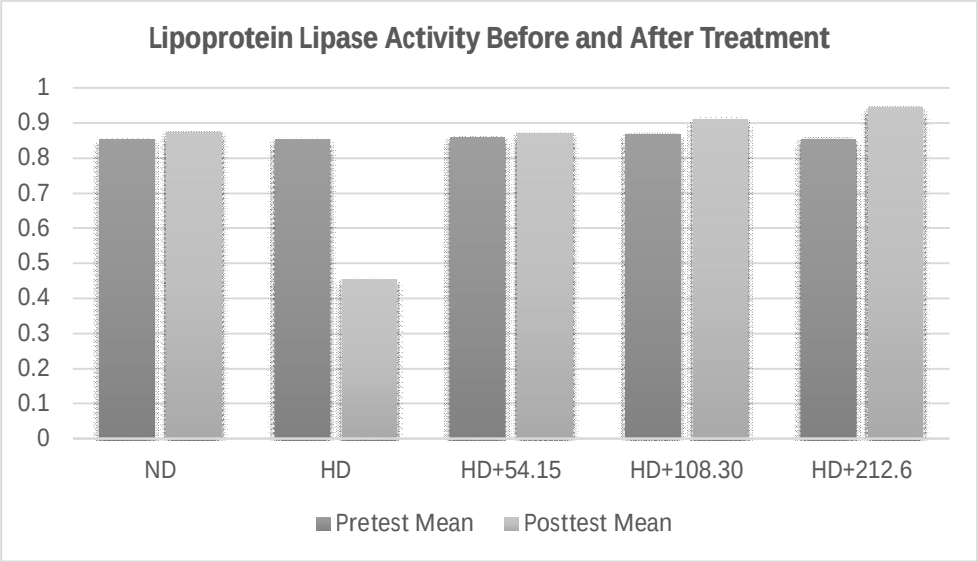


**Figure 1.** Graph of lipoprotein lipase activity before and after treatment among groups

Based on the Paired T-Test showed that there were differences of lipoprotein lipase activity in the hypercholesterolemic diet, hypercholesterolemic diet and Melinjo peel extract (54.15 mg), hypercholesterolemic diet and Melinjo peel extract (108.30 mg), and hypercholesterolemic diet and Melinjo peel extract (216.60 mg) group before and after treatment significant (*p.* < 0.05). However, in the normal diet group, there was no significant difference (*p.* > 0.05).

**Lipoprotein Lipase Activity Changes Analysis**

Based on the MANOVA test, the significance value obtained was 0.000 (*p.* < 0.05). Consequently, there are differences in lipoprotein lipase activity in among groups (ND, HD, HD+54.15, HD+108.3, HD+216.6). Then, to know the different groups significantly based on *p.* value can be seen in the following table 2.

**Table 2.** The comparison of lipoprotein lipase activity among groups

| Group | ND | HD | HD+54.14 | HD+108.3 | HD+216.6 |
|---|---|---|---|---|---|
| ND | - | 0.000 | 1.000 | 0.957 | 0.039 |
| HD | 0.000 | - | 0.000 | 0.000 | 0.000 |
| HD+54.14 | 1.000 | 0.000 | - | 0.941 | 0.038 |
| HD+108.3 | 0.957 | 0.000 | 0.941 | - | 1.000 |
| HD+216.6 | 0.039 | 0.000 | 0.038 | 1.000 | - |

Based on the Paired T-Test showed that there were differences of lipoprotein lipase activity in the hypercholesterolemic diet, hypercholesterolemic diet and Melinjo peel extract (54.15 mg), hypercholesterolemic diet and Melinjo peel extract (108.30 mg), and hypercholesterolemic diet and Melinjo peel extract (216.60 mg) group before and after treatment significant (p. < 0.05). However, in the normal diet group, there was no significant difference (p. > 0.05).

## DISCUSSION

Based on statistical analysis, lipoprotein lipase activity before and after treatment is significant (p.< 0.05), except on normal diet group. The difference mean of lipoprotein lipase activity of those among group are increased. Lipoprotein lipase activity decreased in hypercholesterolemic diet group because the rats consume high diet cholesterol every day without treatment. Activity of lipoprotein lipase enzyme is decreased because in Melinjo peel extract contain flavonoids. Flavonoid compounds bioactive group which is found in foods that come from plants. Regular consumption of flavonoids is associated with a reduced risk of a number of chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders.

Flavonoids are grouped into subgroups based on their chemical structure: flavanon, flavones, a flavonol, flavan-3-ol, anthocyanin and isoflavones. Its action on the molecular level includes antioxidant effects, and the ability to modulate some of the trails main enzymatic (Kozlowska and Szostak-Węgierek, 2014). Flavonoids can increased the activity of LPL by activate Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ). PPARγ belongs to the core receptor group defined as a ligand-activated transcription factor (some fatty acid or lipid metabolite). PPARγ activation involves adipocyte differentiation and storage of fatty acids in adipose tissue. PPARγ is a nuclear hormone receptor that forms heterodimers with retinoid X receptors and binds to the recognition site, proliferator response element (PPRE), which has been identified in some adipocyte-specific genes (Fan, et.al., 2006). LPL is a critical determinant of plasma triglycerides clearance and resultant tissue uptake of fatty acids. The activity of LPL needs to be carefully regulated in order to match the rate of uptake of plasma triglycerides-derived fatty acids to the needs of the underlying tissue and the ability of the tissue to dispose of the fatty acids, all while being confronted with huge fluctuations in the production of

triglyceride which rich lipoproteins. The activity of it is extensively regulated through multiple mechanisms, which primarily operate at the transcriptional and post-translational level. Regulation of DNA transcription is responsible for the upregulation of LPL gene expression and activity during (cardio)myogenesis and adipogenesis. Most of the physiological variation in LPL activity, such as during fasting and exercise, appears to be driven via post-translational mechanisms by extracellular proteins (Kersten, 2014).

LPL-regulatory proteins can be grouped as either LPL-stimulatory or LPL-inhibitory proteins. Apoprotein C-2 (APOC2) and APOA5 have been described to have LPL-stimulatory properties with APOC2 recognized as a required cofactor for hydrolytic activity of LPL. APOC2 is required for maximal rates of triglycerides-rich lipoprotein lipolysis. The C-terminal helix in APOC2 guides lipoproteins to the active site of LPL. APOC2 deficiency is associated with marked elevation of plasma triglyceride, very low density lipoprotein (VLDL) and chylomicron levels and decreased LPL activity, LDL, intermediate density lipoprotein (IDL) and high density lipoprotein (HDL) levels. As opposed to APOC2 and APOA5, APOC1, APOC3 and APOE inhibit LPL-dependent triglyceride clearance. APOC3 is characterized for its LPL inhibitory activity with known human heterozygous carriers of null mutation in the APOC3 gene exhibiting lower plasma triglycerides levels (Geldenhuys, 2016).

The first event in catabolism of TRLs is that they dock at the vascular endothelium. This requires LPL and glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1), the endothelial transporter of LPL. Kinetic studies in rats with label chylomicrons showed that once a chylomicron has docked in the heart it stays for minutes and a large number of triacylglycerol molecules are split. The distribution of binding between tissues reflects the amount of LPL, as evident from studies with mutant mice. Clearance of triglycerides-rich lipoprotein is often slowed down in metabolic disease, as was demonstrated both in mice and men. In mice, this was directly connected to decreased amounts of endothelial LPL (Olivecrona, 2016).

Based on statistical test among groups, after were given melinjo peel extract, in the normal diet group, there was no different significance between HD+54.14 and HD+108.3 groups. It means that with the smallest dose of melinjo peel extract had been able to increase LPL enzyme activity.

## CONCLUSION

In this study, Melinjo peel extract can increased lipoprotein lipase activity after treatment. These effects can be due to the flavonoid of Melinjo peel, which is can affect triglyceride levels.

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