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Original Article

Omega-3 Fatty Acids and Vitamin D Combination Affected TG Levels in Rattusnorvegicus with Limited Fat Intake

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ABSTRACT

Vitamin D is a group of secosteroids that have fat-soluble properties. Vitamin D regulates calcium absorption, bone growth and remodeling, and regulates metabolic processes and immunity. Omega-3 fatty acids are a type of polyunsaturated fatty acids (PUFAs) that are essential fatty acids for humans. Omega-3 fatty acids have various positive effects on health, especially cardiovascular-related ones. This study aims to determine the effect of omega-3 fatty acid and vitamin D combination on the TG/HDL-C ratio in high fat fed Rattus norvegicus. The research design is experimental study with a post-test-only control group design. This study used 24 male rats aged 3–4 months with a body weight of 250–300 grams which were divided into four groups; negative control group, positive control group; treatment group one; and treatment group two. The high-fat diet (HFD) is an additional (emulsion) feed added to standard feed with increased fat composition. The results showed that increased triglyceride (TG) levels of 83.40 mg/dL and HDL levels of 62.60 mg/dL after consumed high-fat diet. There was a significant decrease in TG levels of 54.15 mg/dL (p=0.026) and a decrease in HDL of 53.00 mg/dL (p>0.05, α=0,05) after administration of Omega-3 and Vitamin D combination. Conclusions in this study is the intake Omega-3 and Vitamin D combination has a positive effect on TG levels. Still, this positive effect must be accompanied by limiting the fat intake to the body. Meanwhile, combining Omega-3 and Vitamin D did not significantly affect HDL levels.

Keywords: *Lipid Profile, High Fat, Omega-3, Vitamin D*

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INTRODUCTION

Fat is one of the food components that has various effects on the human body. One of the functions of fat in the body is as a source of energy reserves that can be stored in cells, especially in adipocytes. Fat has a vital function in various metabolic processes in the body [1,2]. Although it has an essential role in various metabolic processes, consuming excess fat can cause various diseases, including an increased risk of developing metabolic syndrome. One of the occurrences of metabolic syndrome is characterized by dyslipidemia, which can lead

to more serious diseases such as heart disease and diabetes. [3–5].

Dyslipidemia is a condition of imbalanced lipid levels in the body. Dyslipidemia is characterized by an increase in triglyceride (TG) and cholesterol levels or both and is also characterized by low levels of *high-density lipoprotein* (HDL-C) cholesterol. [6]. High TG levels, also known as Hypertriglyceridemia, can increase the risk of developing coronary artery disease (CAD) [7]. Besides TG, HDL levels are also associated with various diseases, especially metabolism-related diseases. Research states that the ratio between TG/HDL-C is closely related to an increased risk of various diseases related to metabolism, one of them is fatty liver. Fatty liver can increase the risk of other diseases such as type 2 diabetes mellitus (type 2 DM) and chronic kidney disease [8–10].

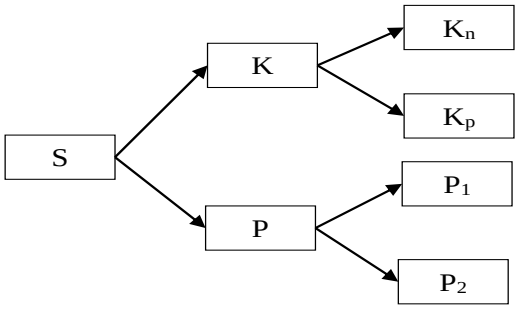
Omega-3 fatty acids are a type of polyunsaturated fatty acids (PUFAs) that are essential fatty acids for humans [11]. Omega-3 fatty acids have been found to have a various positive effects on health, especially those related to cardiovascular [12]. Some literature also states that Omega-3 fatty acids can lower TG levels which is a good effect, especially for people with high plasma TG levels (hypertriglyceridemia) [13,14].

Vitamin D was first discovered by Elmer McCollum by accident while researching vitamin A in cod liver oil [15,16]. Vitamin D is a fat-soluble vitamin. It has various critical metabolic functions in the body, especially in the mechanism of calcium homeostasis and bone metabolism [17]. Several studies have found that the role of Vitamin D is not only related to calcium and bone metabolism but also has been found to be related to lipid profiles levels in the body [18]. High levels of Vitamin D were found to have a positive relationship with serum HDL-C levels [19].

Vitamin D and Omega 3 have been widely studied regarding their effects on the body, including those related to fat metabolism. However, very few studies have discussed the effect of combined Omega-3 and Vitamin D intake on plasma lipid levels. Therefore, this research will examine the effect of the combination of Omega-3 and Vitamin D on TG and HDL levels in the plasma of experimental animals.

METHOD

This research is an experimental study with a post-test-only control group design. This study used 24 male rats aged 3–4 months with 250–300 grams of weigh. The rats were divided into four groups; negative control group (Kn, n=5), positive control group (Kp, n=5), treatment group one (P1, n=7), and treatment group two (P2, n=7). The HFD feed given as an additional feed other than standard feed with increased fat composition. The HFD used in this study was an emulsion of a mixture of lard and chicken egg yolk. The ratio of chicken egg yolk and lard is 1:1. The HFD given as much as 1 mL to each rat with an estimated fat content of 66.28%.



Description:
S: Research Subject
K: Control
P: Treatment

Figure 1. Group division scheme.

The negative control group (Kn, n=5) was the group given only standard feed, while the positive control group (Kp, n=5) was the group that were given standard feed and HFD. The standard feed used in this study was "Pokphand 593", while the HFD used was a mixture of lard and egg yolks and was given as much as 1 mL/rat by sonde.

Treatment group one (P1, n=7) was a group that given a HFD + standard feed and given a combination of Omega-3 and Vitamin D. In contrast, treatment group two (P2, n=7), was a group that were only given combined intake of Omega-3 and Vitamin D as well as standard feed, without giving HFD.

The Omega-3 fatty acids used were Omega-3 fatty acid supplements containing 180 mg of docosahexaenoic acid (DHA) and 120 mg

of eicosapentaenoic acid (EPA). As much as 400 IU Vitamin D supplements were used in this study. The dose of Omega-3 fatty acids is 75 mg/rat, while the dose of Vitamin D is 200 IU (5µg)/rat [20,21].

Before giving treatment, the Kp dan P1 group was given high-fat feed for two weeks (14 days). Each group was treated according to the scheme with a duration of two weeks (14 days), and measurements of lipid profile levels (TG and HDL) were carried out on the 15th day. Lipid profile examination carried out at the Department of Clinical Pathology, Diagnostic Center (GDC), Dr. Soetomo Hospital, Surabaya, Indonesia. The data were then tested using Shapiro-wilk test for normality test and *Anova* and *Posthoc* to find out the differences in the TG and HDL levels in each group.

RESULTS

After four weeks of administration HFD in Kp group, it was found that the TG levels in Kp did not differ significantly compared with Kn. While, there was a significant different in TG levels in P2 group compared with Kp.

In this study, the duration of HFD induction in the Kp group was 28 days, this duration did not significantly increase TG levels. Likewise with HDL, HFD were not able to produce significant different in HDL levels in the Kp and Kn group. This shows that the HFD given has not been able to have a significant effect on TG and HDL levels in experimental animals

However, Table 1 shows that there was a significant difference in the TG levels of the experimental animals in each group after being tested with One-Way Anova (p=0.034). From each of these groups, a *Posthoc* Bonferroni test was carried out to find out which groups had significant differences.

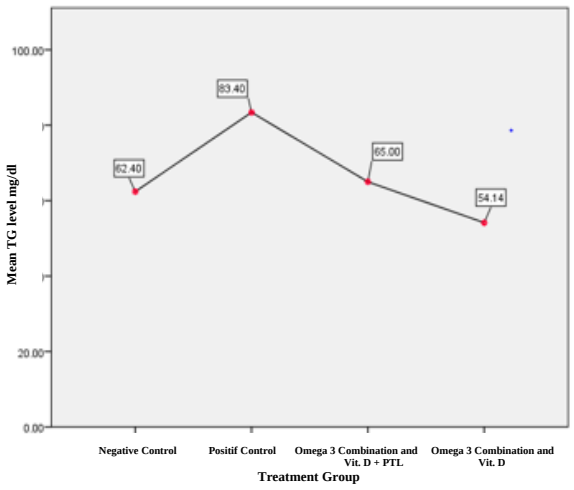
Table 1. The results of examining the TG levels of each group

Group	Total (N)	Fasting TG Levels (mg/dL)	Std. Deviation
K_p→ Negative Control	5	62.40	10.64
K_n→ Positive Control (High-fat diet)	5	83.40	29.38
P_1→ Treatment 1 (High fat diet + Omega-3 + Vit. D)	7	65.00	9.36
P_2→ Treatment 2 (Omega-3 + Vit. D)	7	54.14	8.23
Total	24	65.12	17.92

Table 2. The results of examining the HDL levels of each group

Group	Total (N)	Fasting HDL Levels (mg/dL)	Std. Deviation
K_p→ Negative Control	5	57.60	6.18
K_n→ Positive Control (High-fat diet)	5	62.60	9.86
P_1→ Treatment 1 (High fat diet + Omega-3 + Vit. D)	7	53.86	6.12
P_2→ Treatment 2 (Omega-3 + Vit. D)	7	53.00	6.76
Total	24	56.20	7.70

Post hoc data showed that the group of experimental animals that were given a HFD for 28 days (Kp) had a significant difference from the group of animals that only received a combination of Omega-3 and Vitamin D (P2) with a significance value of p=0.026. This result shows that combined intake of Omega-3 and Vitamin D has the potential to have an effect on lowering TG levels in serum.



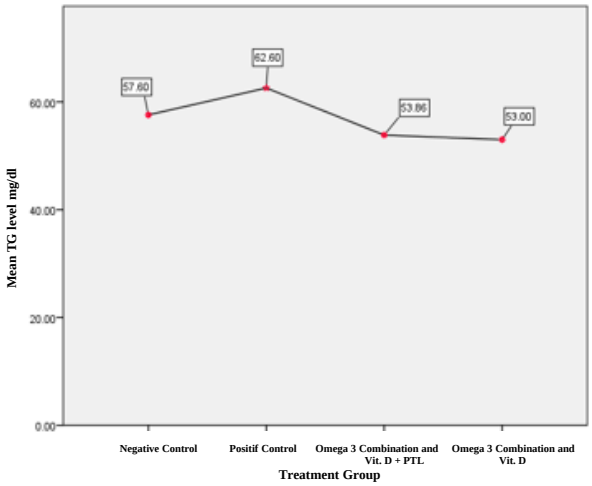


Table 2 shows data on HDL levels of experimental animals in each group. After the *One-Way Anova* test was carried out on the HDL levels of each experimental group, no significant difference was found in the mean HDL levels in each group (p=0.135).

DISCUSSION

TG Levels and Omega-3 and Vitamin D Combination

Triglycerides (TG) are one of the four groups of lipids found in lipoproteins. In plasma TG accounts for about 16% of total plasma lipids [11]. Increased TG levels in the blood are often associated with excessive food intake, especially foods that contain lots of fat and carbohydrates, although several studies also state that TG levels in the blood can also increase in certain circumstances such as pregnancy [22,23].

In this study, the treatment group that received an Omega-3 and Vitamin D combination and was still given a high-fat diet (P1) had an average TG level that did not differ much from the positive (Kp) and negative (Kn) control groups. Meanwhile, the group that only received the Omega 3 and Vitamin D combination (P2) showed a significant decrease in TG levels compared to the control group (Kp). This shows that combined intake of Omega-3 and Vitamin D cannot have the maximum effect on TG levels if it is not accompanied by limiting fat intake.

Data on TG levels in this study showed that the intake of a combination of Omega-3 fatty acids and Vitamin D could reduce TG levels in experimental animals, both in the treatment group 1 (P1) and in the P2 group. However, the intake of Omega-3 and Vitamin D combination in the P1 group did not produce as good results as in the P2 group. The TG levels of these two groups were not significantly different (p=1.00).

The TG level of P1 group was lower (65.00 mg/dL) but not different significantly (p=0.342) compared to the positive control (Kp). However, P2 group had a significant (p=0.026) lower of TG levels by 54.14 mg/dL. Based on these results, it can be noted that the intake of an Omega-3 and Vitamin D combination had a better effect on the group that received standard feed (P2) compared to the group that was given high-fat feed (P1). This is consistent with previous research which showed a relationship between decreased TG levels with intake of Omega-3 fatty acids and serum vitamin D levels [14,18]. However, this study did not compare the effect of reducing TG levels by Omega-3 and Vitamin D separately.

Omega-3 fatty acids are known to reduce TG levels in the blood by modifying various mechanisms related to lipid metabolism, especially those related to decreasing the activity of enzymes that synthesize TG. [14]. Omega-3 fatty acids are known to modify various enzymes involved in TG formation by binding to various nuclear receptors. There are four core receptors that can be affected by Omega-3 fatty acids; liver X receptor (LXR); hepatocyte nuclear factor–4α (HNF-4 α); farnesol X receptor (FXR); and peroxisome proliferator–activated receptors (PPARs). The bond between Omega-3 and

these receptors can reduce TG levels in the blood [24].

In contrast to Omega-3 fatty acids, the mechanism underlying the effect of Vitamin D intake on reducing TG levels is remain unclear. However, some literature states that Vitamin D can have indirect effects on lipid profiles, including reducing lipid absorption in the intestine and lipid synthesis [25].

HDL Levels and Omega-3 and Vitamin D Combination

High-density Lipoprotein (HDL) is a form of Lipoprotein in the body. HDL Lipoprotein is known as Lipoprotein which has anti-atherogenic effect. Lipoprotein HDL is a lipoprotein with a high protein content, which is around 45-55% of the total HDL content itself [26]. Various studies have found that HDL plays a role in various mechanisms in the cholesterol transport process [26,27]. In addition, HDL has also been studied for its role in inflammatory mechanisms and oxidative signaling. In the inflammatory mechanism, HDL was found to be able to suppress the inflammatory response, by inhibiting the induction of cytokines which play a role in increasing the inflammatory response. Meanwhile, in oxidative signaling, HDL was found to inhibit lipid peroxidation [27].

In several studies, intake of Omega-3 was found to reduce the risk of suffering from cardiovascular disease and diabetes. Omega-3 fatty acids, both EPA and DHA, were found to increase HDL-Cholesterol levels. Increased HDL by Omega-3 intake is mediated by increased activity of lipoprotein lipase (LPL). Increased LPL activity by Omega-3 can reduce levels of Intermediate-density lipoprotein (IDL), and Very low-density lipoprotein (VLDL), which causes an increase in TG hydrolysis and then increases HDL levels [28].

Although several studies have stated that there is an effect of intake of Omega-3 fatty acids on HDL, in this study there was no significant difference in HDL levels in the group that received Omega-3 and Vitamin D combination when compared to the control group (Kn and Kp). This can be caused by the source of Omega-3 given. Research by Zibaeenezhad (2017) found that there were differences in the effect of Omega-3 intake from different sources on lipid profiles. In the research, it was found that intake of Omega-3 obtained through consumption of fresh fish will have a better effect on reducing LDL levels, and other lipid profiles, including the ratio of LDL/HDL. The study was also found an increase in LDL levels in the group consuming Omega-3 supplementation, but on the contrary there was a decrease in LDL levels in the group consuming fresh fish [29]. However, this needs to be investigated further in order to find out the reasons for this difference.

Administration of Omega-3 and Vitamin D combination for only 2 weeks is also thought to have an effect on the results obtained. Several studies have stated that giving Omega-3 intake for more than 1 month can have a beneficial effect, both on the immune response and against cardiovascular disease [29,30]. Research regarding the duration of Omega-3 intake on lipid profiles needs further investigation.

Vitamin D is a type of vitamin that is widely consumed by the public. Vitamin D is generally associated with calcium homeostasis. Vitamin D in the form of *calcidiol* (25(OH)D), circulates in the blood and will pass through the kidney proximal tubules to become calcitriol(1,25(OH)2D), where calcitriol is the active form of Vitamin D [16,17].

Apart from playing a role in the process of calcium homeostasis, Vitamin D also has various effects on processes in the body, including the inflammatory response and various processes related to an increased risk of metabolic syndrome [17,31]. Research has also found that Vitamin D is related to serum lipid profiles. Increased levels of Vitamin D in the form of calcidiol (25(OH)D), were found to reduce TG and LDL-C levels, both in men and women [32].

Although it was found to have a significant effect on lipid profiles, especially those associated with metabolic syndrome, the administration of Vitamin D combined with Omega-3 in this study was found to have no significant effect on HDL levels. The mechanism underlying the effect of vitamin D on serum lipid levels is remain unclear [33]. The data in this study can be used as a reference and additional data for subsequent research to reveal the mechanism underlying the relationship between Vitamin D and lipid profile

In this study, intake of Vitamin D in the form of *calcidiol* (25(OH)D) of 400 IU for 2 weeks was not able to have a significant effect on serum HDL levels in experimental animals. In addition, this study also did not examine other lipid levels, such as LDL, and Total

Cholesterol (TC), which can be used as a comparison for HDL levels.

HDL-TG Levels and Combination Omega-3 + Vitamin D

A high-fat diet can reduce the activity of the lipoprotein lipase enzyme in the blood circulation. A decrease in the lipoprotein lipase enzyme can reduce the breakdown of triglycerides into fatty acid and glycerol molecules. This decrease results in the accumulation of visceral fat thereby increasing TG levels[34].

The high-fat diet also increases the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). HMG-CoA Reductase is an enzyme in the liver that plays a role in synthesizing cholesterol levels, Very Low-Density Lipoprotein (VLDL) apolipoprotein-B100 (ApoB100), and increasing Low-Density Lipoprotein (LDL) receptors found on the surface of the liver and cells. Increased HMG-CoA Reductase due to a high-fat diet can result in increased levels of cholesterol, VLDL, LDL and Apolipoprotein B [35].

A high-fat diet can increase TG levels [35]. through a decrease in the enzyme lipoprotein lipase and the accumulation of visceral fat. As a result of this accumulation, it can increase Free Fatty Acid (FFA) thereby inducing an increase in the concentration of glucose produced by the liver into the blood circulation which results in insulin resistance [36]. Insulin resistance can also be caused by excessive food intake, causing glucose intolerance and hyperglycemia. In the pre-diabetic stage, there is a decrease in β-cell levels due to several risk factors, such as glucotoxicity, lipotoxicity, and increased inflammation. [37]. Previous literature studies have shown an increase in triglyceride (TG) levels, fasting blood sugar (GDP) and a decrease in HDL cholesterol in high-fat animal models [38].

Vitamin D is a group of *secosteroids* which have fat soluble properties. Vitamin D plays a role in regulating calcium absorption, bone growth and remodeling, regulates metabolic processes and immunity. Recent studies support that vitamin D plays a relevant role in islet dysfunction and insulin resistance [39]. The results of this study indicated that there was a decrease in TG levels after administration of omega 3 and vitamin D combination. The results of this study were supported by previous studies which showed that experimental animals of Rattus norvegicus were induced on diets rich in vitamin D3 within 3 weeks, there was an increase in subcutaneous and visceral fat. [40], whereas mice fed calcitriol via continuous pump showed reduced adipose weight. While the experimental animal Rattus norvegicus model of Vitamin D Receptor (VDR) showed a decrease in white adipose tissue mass, serum triglyceride (TG) concentration and total cholesterol [41].

Vitamin D plays a role in stimulating insulin secretion in obesity due to a high-fat diet [40]. Both Vitamin D and Vitamin D Receptors (VDR) directly play a role in regulating functional genes, including genes that play an important role in the metabolism, secretion and action of insulin. Vitamin D also acts as an anti-inflammatory hormone that helps tissues to reduce local and systemic inflammation, thereby preventing islet, liver and muscle dysfunction. [37]. Trans fatty acids contained in high-fat diets can increase LDL (Low Density Lipoprotein) cholesterol concentrations, triglycerides (TG), reduce HDL (High Density Lipoprotein) cholesterol concentrations, and increase the LDL/HDL cholesterol ratio. Trans fatty acids can also increase the LDL/HDL cholesterol ratio two times higher than saturated fatty acids [42]. Previous literature studies showed that there was a decrease in glucose levels in experimental animals that had been given a combination of vitamin D and L-cysteine supplementation [43].

Omega-3 can increase the activity of the lipoprotein lipase enzyme [35]. Increased activity of lipoprotein enzymes can increase the breakdown of triglycerides (TG) into fatty acids and glycerol which results in a decrease in TG levels circulating in the blood [44]. The fatty acids and glycerol that are formed are then needed as fuel by cells to form energy (ATP) such as water (H_2O) and carbon dioxide (CO_2) to produce energy [34]. Increased lipoprotein lipase activity also increases the inhibition of exogenous pathways of lipid metabolism by increasing the absorption of fat in the small intestine so that the concentration of cholesterol and triglycerides in the blood decreases [45].

This event can increase the risk of atherosclerosis and CHD, compared to

measurements of total cholesterol or LDL cholesterol concentrations, respectively. In a 1% increase in total energy derived from trans fatty acid intake, there is a decrease in HDL cholesterol concentration of 0.013 mmol/liter and an increase in LDL cholesterol concentration (LDL-C) of 0.04 mmol/liter [46]. Previous literature studies have shown that in experimental animals that are made to lose Vitamin D Receptors (VDR) can increase insulin resistance in the liver [47]. In addition, vitamin D can reduce the expression of pro-inflammatory cytokines (IL-1β, IL-6) [48]. secreted by adipocytes thereby suppressing the inflammatory response. A number of studies have shown that vitamin D is involved in lipid metabolism by regulating adipogenesis, lipolysis, and lipogenesis [37].

CONCLUSION

In this study, it was found that a combination of Omega-3 and Vitamin D had a positive effect on TG levels. Still, this positive effect must be accompanied by limiting fat intake. In contrast to TG levels, serum HDL levels after administration of Omega-3 and Vitamin D combination did not provide a significant difference between groups. This study did not examine the other cholesterol, such as LDL, VLDL, and iLDL. Thus, HDL level has not been compared to these types of cholesterol to see if the different cholesterol levels were affected.

The effects of Omega-3 and Vitamin D separately were not investigated in this study, so their respective effects on lipid levels cannot be compared. Omega-3 intake has been extensively studied for its relation to lipid profile. Vitamin D was found to be related to lipid profile. Vitamin D intake can affect lipid absorption in the intestine. Nevertheless, much research on Vitamin D still needs to be done in order to answer the mechanisms underlying the effect of Vitamin D on lipid levels.

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