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EFFECT OF HIGH-FAT DIET ON SERUM TNF-ALPHA LEVELS, A MARKER OF LOW-GRADE INFLAMMATION

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Abstract

A high-fat diet is a pattern of excessive fat consumption that can cause various metabolic function disorders, such as hypertension, dyslipidemia, obesity, and increased glucose levels. The aim of this study was to analyze the effect of a high-fat diet on serum tumor necrosis factor alpha (TNF-α) levels as a marker of low-grade inflammation in Wistar rats. The study was designed using a true experimental randomized posttest-only control group. Sixteen male Wistar rats weighing 150-250g and aged 4-5 months were divided into two groups. Group K1 was a negative control group that was given normal diet, while group K2 was a group that was given a high-fat diet, with a fat content of 66.28%. This treatment was controlled for 50 days. The mean TNF-α levels in K1 (290.912 ± 1.87) pg/mL and K2 (295.149 ± 2.76) pg/mL. Based on the results of independent T-test analysis, TNF-α levels in groups K1 and K2 were significantly different (P = 0.034 or P < 0.05). This means that a high-fat diet increases serum levels of TNF-α, a marker of low-grade inflammation.

***Keywords**: high-fat diet, tumor necrosis factor alpha (TNF-α), low-grade inflammation.*

Abstrak

Diet tinggi lemak adalah pola konsumsi lemak berlebihan yang dapat menyebabkan berbagai gangguan fungsi metabolisme, seperti hipertensi, dislipidemia, obesitas, dan peningkatan kadar glukosa. Penelitian yang dilakukan bertujuan menganalisis efek diet tinggi lemak terhadap kadar TNF-α serum sebagai penanda inflamasi tingkat rendah pada tikus Wistar. Penelitian dirancang menggunakan randomized posttest-only control group eksperimental sejati. Sebanyak 16 ekor tikus Wistar jantan dengan berat 150-250g dan umur 4-5 bulan dibagi ke dalam dua kelompok. Adalah kelompok K1, merupakan kontrol negatif yang diberi pakan normal, sementara kelompok K2 merupakan kelompok yang diberi pakan tinggi lemak, dengan kadar lemak 66,28%. Perlakuan ini dikontrol selama 50 hari. Rerata kadar TNF-α pada K1 (290.912 ± 1.87) pg/mL dan K2 (295.149 ± 2.76) pg/mL. Berdasarkan hasil analisis uji T independen, kadar TNF-α pada kelompok K1 dan K2 berbeda nyata (P= 0,034 atau P< 0,05). Hal ini berarti diet tinggi lemak meningkatkan kadar TNF- serum, sebuah penanda inflamasi tingkat rendah.

***Kata Kunci**: Diet tinggi lemak, tumor necrosis factor alpha (TNF-α), inflamasi tingkat rendah.*

1. INTRODUCTION

Masek & Fabry (1959) were two scientists who first introduced the term "High-Fat-Diet" (abbreviated as HFD) or what is more commonly known as a high-fat diet (Dubey et al., 2013). Various metabolic diseases are often associated with this term (Rani et al., 2016), ranging from obesity, impaired glucose tolerance, hypertension (Moreno-fersn et al., 2018), to insulin resistance which triggers type-2 diabetes (Welty et al., 2016).

A high-fat diet is a pattern of excessive fat consumption that can cause various metabolic function disorders, such as hypertension, dyslipidemia, obesity and increased blood glucose levels (Moreno-fern et al., 2018; Mamikutty et al., 2014; Larsen et al., 2018). This condition is triggered by low-grade inflammation due to excess fat deposits in the tissues (called meta-inflammation) which can progressively increase the risk of cardiovascular disease, type 2 diabetes mellitus (Kaur, 2014), and even death. This is supported by Esser et al in 2014 in their study "Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes" which proves a link between inflammation and obesity, metabolic syndrome and type 2 diabetes.

Excessive fat consumption (high-fat diet, HFD) and fat accumulation in adipocytes, are the initial triggers for meta-inflammation, namely low-grade chronic inflammation mediated by the immune system and various substances derived from adipocyte tissue. Unesterified fats such as cholesterol, fatty acids, and their derivatives are responsible for this inflammatory response (Walther & Farese Jr, 2012). This inflammation is the result of continuous activation of the innate and adaptive immune system. Under these conditions, there is an increase in various pro-inflammatory cytokines in the form of leptin (Brzeska et al., 2013), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) (Siriwardhana et al., 2013; Patterson et al., 2015; Shen et al., 2015; Shen et al., 2013; Patterson et al., 2015; Shen et al., 2013), interleukin-18 (IL-18) (Pugia, 2015) and decreased anti-inflammatory cytokine (IL-10) (Poret et al., 2018). Adipocyte tissue macrophages (ATM) in this case, are responsible for almost all expression of TNF-α which triggers the activation of other immune cells (Engin, 2017). This is in line with a study conducted by Telle-hansen et al in 2017 which proved that a high-fat diet can have an effect on the emergence of inflammatory markers in overweight and obese individuals. A number of researchers have also proven that accumulated fat will secrete a number of cytokines (adipokines) such as leptin (Ameer et al., 2018; Brzeska et al., 2013), MCP-1, TNF-α, IL-6, IL-1β (Siriwardhana et al., 2013; Patterson et al., 2015; Shen et al., 2013), and IL-18 (Pugia, 2015) to attract macrophages, or act as antigens that can be recognized by immune cells, such as Th1 cells, CD8+ T cells, and NKT cells (Kammoun et al., 2014; Satoh & Iwabuchi, 2018). The accumulation of these conditions can cause various damages, such as decreased expression of IRS-1 and GLUT-4, the main factors causing various metabolic function disorders (Leguisamo et al., 2012; Poletto et al., 2015). As for other meta-inflammatory effects, they can also arise from an imbalance in the metabolism of the gut microbiota (dysbiosis) (Fawley & Gourlay, 2016), recognition of free fatty acids (FFA) by a number of pattern recognition receptors (PRR), and mitochondrial dysfunction (Marchi et al., 2014).

This study aimed to analyze the effect of a high-fat diet on serum TNF-α levels, a marker of low-grade inflammation. This research provides additional knowledge for the community to maintain a healthy diet to avoid various diseases, especially those related to metabolic diseases.

2. HIGH-FAT DIET AND METABOLIC SYNDROME

The term 'High-Fat-Diet' (HFD) or high-fat diet, was first introduced by Masek & Fabry in 1959 (Dubey et al., 2013). HFD is often associated with metabolic syndrome (Rani et al., 2016). Currently, many studies have proven that HFD can trigger various disorders, such as obesity, dyslipidemia, hypertension, impaired glucose tolerance (Moreno-fersn et al., 2018) and insulin resistance (Welty et al., 2016).

According to Rini & Wahyuni (2012), to increase triglyceride levels from 41.28 ± 6.72 mg/dL to 62.77 ± 7.19 mg/dL can be done by administering HFD which consists of 1 part lard and 2 parts yellow chicken eggs for 14 days (Rini & Wahyuni, 2012). This is supported by the statement of Hendra et al (2011) that administration of HFD with a composition of 100 g of egg yolk and 50 g of lard in white rats was able to increase cholesterol by 91% from the 14th day and triglycerides by 87% from the 30th day (Hendra et al., 2011).

As for 100 g of lard, there is as much as 100 g of fat (Marcus, 2013), while egg yolk contains 31.9 g (Ministry of Health & Ministry of Agriculture, 2010). The amount of fat that can be given once sonde should not exceed 1% of the rat's body weight (Brown et al., 2000). This is in accordance with a study by Turner et al., (2012) that rats that were sond with a liquid substance of 5 ml/kg bw or 1 ml/200 g bw did not cause negative effects. Thus, for

mice with an average weight of 216 g, the amount that can be sonde is 1.08 ml (1 ml). If converted to g, then the composition of the feed that is weighed is 0.86 g with a respective weight of lard and egg yolk which is 0.43 g and the total fat concentration is 66.28%.

3. HIGH-FAT DIET AND LOW-GRADE INFLAMMATION

Unesterified fats such as cholesterol, fatty acids, and their derivatives, can trigger an inflammatory response or what is called meta-inflammatory (Walther & Farese Jr, 2012). This inflammation is the result of continuous activation of the innate and adaptive immune system. Under these conditions, there is an increase in various pro-inflammatory cytokines in the form of leptin (Brzeska et al., 2013), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL- 6), interleukin-1 beta (IL-1β) (Siriwardhana et al., 2013; Patterson et al., 2015; Shen et al., 2013), interleukin-18 (IL-18) (Pugia, 2015) and decreased anti-inflammatory cytokine (IL-10) (Poret et al., 2018). Adipocyte tissue macrophages (ATM) in this case, are responsible for almost all expression of TNF-α which triggers the activation of other immune cells (Engin, 2017).

3.1 Immune Response In Adipocytes

It has long been known that SM is closely related to chronic inflammation involving innate immune cells such as macrophages, neutrophils, mast cells, eosinophils and adaptive immune cells such as T helper 2 cells (Th2), regulatory T cells (Treg), T cells. helper 1 (Th1), CD8+ T cells and B cells. In adipocyte tissue of healthy people, these immune cells are in a state of balance, where immune cells such as M2 macrophages, Treg cells, eosinophils (Huh et al., 2014) and Th2 cells play a role in anti-inflammatory and insulin sensitivity (Kammoun et al., 2014). However, when consuming excess fat, the situation becomes unbalanced and triggers an increase in immune cells such as macrophages, neutrophils, mast cells, B cells, Th1 cells and CD8+ T cells (Choe et al., 2016; Boutens & Stienstra, 2016) which produce various pro-inflammatory cytokines (Kammoun et al., 2014). These cytokines then migrate to the liver, muscle, and pancreatic tissue through blood vessels and can trigger insulin resistance (IR) (Pugia, 2015; Choe et al., 2016).

The anti-inflammatory response is generally played by M2 macrophages which produce various anti-inflammatory cytokines. M2 macrophages are polarized via signal transducer and activator of transcription 6 (STAT6) signaling which is activated by cytokines from Th2 cells and eosinophils, such as IL-4. Apart from Th2 cells, NKT cells are also known to play a role in the anti-inflammatory response because they can produce the cytokines IL-2, IL-4 and IL-10 (Figure 2.11) (Satoh & Iwabuchi, 2018; Choe et al., 2016).

3.2 Polarization of M1 Macrophages by CD8+ T Cells, Th1 Cells and NKT Cells

Adaptive immune cells, such as T cells, are known to play a role in the early stages of meta-inflammatory development in HFD-treated mice (Morin et al., 2017). These mice showed an imbalance of inflammatory response in which there was an increase in Th1/Th17 cells and CD8+ T cells (Kammoun et al., 2014), while Th2 cells, M2 macrophages (Becker et al., 2017) and Treg (IL-10 producers), tends to decrease (Figure 2.23) (Chatzigeorgiou et al., 2012). This can trigger the development of SM (Kammoun et al., 2014; Mclaughlin et al., 2014).

Several aspects that affect T cell activation in adipocytes are through the role of T cell cost-stimulating molecules, namely CD28 and CD154 and the ability of adipocytes as APCs (Morin et al., 2017). When fat accumulation occurs in adipocyte tissue, the response of T cells (especially CD4+ and CD8+), will be activated in response to the presentation of polypeptides

by APC (Kammoun et al., 2014). ATM is known to be the main APC in T cells. However, it was recently discovered that adipocytes may also act as APC (Fig. 2.23) (Huh et al., 2014).

Activated CD8+ T cells, Th1 cells, and NKT cells will then produce IFN-γ, a pro-inflammatory cytokine that can induce migration and polarization of M1 macrophages (Chatzigeorgiou et al., 2012; Satoh & Iwabuchi, 2018).

3.3 Gut Microbiota Dysbiosis

The gut microbiota has long been known to play an important role in obesity and other metabolic diseases, such as T2DM, NAFLD, dyslipidemia, and metabolic syndrome (Chávez-Carbajal et al., 2019). The composition and activity of the gut microbiota is strongly influenced by environmental and lifestyle factors. Diet, in this case, greatly influences the balance of the gut microbiota (Mehal, 2013; Festi et al., 2014). HFD is known to change the pattern of interaction of the intestinal microbiota and increase pathogenic bacteria containing lipopolysaccharide (LPS) (Fawley & Gourlay, 2016).

A number of pieces of evidence have also been proposed to explain the effect of HFD on the gut microbiota, but in general, through increased production of chylomicrons (CM) and bile acids (BA) as illustrated in figures 2.25 and 2.26 (Liu et al., 2015), decreased intestinal enzyme activity alkaline phosphatase (IAP) (Moreira et al., 2012) and changes in energy intake by the gut microbiota (Tomas et al., 2016).

The CM molecules produced can integrate with LPS through micelles so that LPS can indirectly penetrate the intestinal epithelium, enter the bloodstream and cause endotoxemia and inflammation (Moreira et al., 2012). In addition, the CM-LPS complex can also increase intracellular pressure and reduce tight junction integrity between enterocytes, even impacting on the rupture of the basement membrane (Moreira et al., 2012; van den Brand, 2014). This then leads to increased intestinal permeability and metabolic endotoxemia, which is characterized by increased plasma LPS. (Carreira et al., 2018).

Besides functioning to destroy fat, BA can also change the composition of the gut microbiota (van den Brand, 2014). Recent evidence suggests that changes in the composition or metabolic activity of the gut microbiota can trigger the development of obesity and metabolic syndrome (Xiao & Zhao, 2014). BA is known to have anti-microbial effects against bacteria in the gut. The active substances produced, such as DCA, have the potential as antimicrobial agents because they have hydrophobic and detergent properties against bacterial cell membranes (Ridlon et al., 2015). Through activation of the farnesoid x receptor (FXR), BA can regulate host genes and increase innate defense against microbes (Torres et al., 2018). However, abnormal BA metabolism can actually disrupt the balance of the gut microbiota (Jones et al., 2014), especially against the decrease in Bacteroidetes, Actinobacteria (Ridlon et al., 2015) and Bifidobacter bacteria which function to maintain intestinal integrity (Mehal, 2013). The decrease in these bacteria can lead to an increase in pathogenic bacteria belonging to the Firmicutes, Proteobacteria, Verkomikrobia (Tomas et al., 2016) and Enterobacter (Mehal, 2013) groups.

The active substances contained in BA, include; cholic acid (CA), deoxycholic acid (DCA) and chenodeoxy cholic acid (CDCA). These three substances can induce increased intestinal epithelial permeability through activation of the epidermal growth factor receptor (EGFR) and occludin phosphorylation in colon cells (Caco-2) (Murakami et al., 2016).

Over the past few decades, IAP has been known to play an important role in maintaining intestinal hemostasis (Bilski et al., 2017). IAP especially plays an important role in intestinal pH regulation, lipid absorption, detoxification of free nucleotides and LPS, and modulation of gut microbes (Lallès, 2014). The IAP enzyme works by dephosphorylating the lipid-A group of LPS (Fawley & Gourlay, 2016;

Bilski et al., 2017), a substance in the cell walls of gram-negative bacteria that can cause endotoxemia (Moreira et al., 2012).

Actually, the decrease in IAP due to HFD is still being debated. Some say HFD can reduce IAP, and vice versa. However, according to several studies, HFD has been shown to reduce the expression of IAP enzymes by increasing pro-inflammatory cytokines (Figure 2.28) (Bilski et al., 2017). This is in line with a study conducted by de La Serre et al., (2010) which proved that rats that consumed a high-fat diet compared to those who did not, showed a significant decrease in IAP enzymes, increased inflammation in enterocytes, increased TLR4 activation, increased intestinal permeability and increased plasma LPS (de La Serre et al., 2010). In addition, Malo (2015) also showed that mice lacking IAP (Akp3 knockout, Akp3−/−) can trigger T2DM (Malo, 2015). Therefore, IAP enzyme deficiency can increase intestinal inflammation, dysbiosis, endotoxemia to systemic inflammation.

3.4 Adipocyte Hypertrophy

Excessive fat consumption can trigger hypertrophy in AT which causes cells to experience hypoxia (Wu et al., 2010). Actually, there are many hypotheses about the causes of hypoxia, one of which is the understanding of low blood flow (Pugia, 2015). According to Engin (2017), blood flow in AT of obese subjects is around 30-40% lower than non-obese subjects. As a result, asympocyte tissue does not have an adequate supply of oxygen. Therefore, adipocytes will increase the process of angiogenesis in order to supply more oxygen to the tissues (Wu et al., 2010).

Cells experiencing hypoxia can secrete a number of adipokines in the form of pro-inflammatory cytokines such as TNF-α, IL-1β, IL6, IL-8, leptin (Esser et al., 2014; Welty et al., 2016), IL-18, TGF-β and several chemokines such as MIC-1, MCP-1, and CINC-1 (Pugia, 2015), MIF, MMP1 and MMP2, VEGF (Rajan, 2016) which can cause local insulin resistance (Zubiria et al., 2017) and also systemic such as in the liver, muscle, heart and pancreas (Rajan, 2016).

In addition, hypoxia can also cause ER stress and increase oxidative stress (Netzer et al., 2015; Ferranti et al., 2008) which can progressively trigger necrosis or non-programmed cell death (Francisqueti et al., 2017). Through necrosis, cells will release various substances that cause inflammation or so-called DAMPs which can activate M1 macrophages and dendritic cells through TLR signaling and other mechanisms (Nagata & Nakano, 2017).

3.5 FFA Increase and Activation of The TLR4/2 and NLRP3 Pathways

Apart from being used as energy, it turns out that FFA can also play a role in inflammation. The structure of SFA, which is similar to the endotoxin LPS (Lipid A), is known to induce TLR4/2 signaling and stimulate the production of pro-inflammatory cytokines (Fritsche, 2015). SFA recognized by TLRs can trigger activation of JNK and NFκB signaling. Activated JNK and NFκB can then increase pro-inflammatory cytokines (Xiao & Zhao, 2014), serine phosphorylation and IRS, inhibit P13K and decrease GLUT-4 expression so that they can increase insulin resistance (Pugia, 2015; Welty et al., 2016; Rajan, 2016).

In addition, SFA can also activate the nucleotide-binding domain, leucine-rich repeat and pyrin domain pathways containing protein 3 (NLRP3). Palmitic acid (PA) (16:0) and stearic acid (SA) (C18:0), the main sources of SFA, can cause intracellular crystallization and activate NLRP3 inflamasomes (Karasawa et al., 2018; Micaelo et al., 2016) via lysosomal dysfunction in macrophages and dysregulation of autophagy (Karasawa et al., 2018). This pathway is required for the maturation of pro IL-1β and IL-18 which play a role in the development of IR and T2DM (Legrand-poels et al., 2014).

Apart from FFA, various dangerous molecules or so-called danger associated molecular patterns (DAMPs) such as ATP,

cholesterol, urate crystals and ceramides, can also activate the NLRP3 pathway (Shirato et al., 2017). Apart from adipocytes, these DAMPs can also be produced by non-adipocyte cells that experience necrosis such as the liver, heart, pancreas and muscles due to the buildup of triglycerides. It can also trigger the development of insulin resistance (IR) and type 2 diabetes (T2DM). In the pancreas, for example, β-cell dysfunction occurs which leads to failure of insulin secretion (Saponaro et al., 2015).

The products of the NLR pathway are generally IL-1β and IL-18. Initially PAMP or DAMP will form inflamasome formation, a multimeric protein complex containing a Nod-like receptor family pyrin domain containing protein 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and procaspase-1 . Inside the inflamasome, procaspase-1 is converted to caspase-1 and cleaves pro-IL-1β and IL-18 into an active form (Figure 2.31) (Shirato et al., 2017).

In contrast to SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) cannot induce TLR4/2 signaling (Fritsche, 2015), but can directly produce inflammatory mediators, such as PUFA omega-6 linoleic acid (LA) (18:2 n-6) and its product, arachidonic acid (ARA) (20:4 n-6), which becomes a substrate for the enzymes cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 to form prostaglandins (PG) and leukotriene (LT) (Innes & Calder, 2018). Activation of COX-2 and PGE2 signaling in hypertrophic and hypoxic adipocytes has been shown to increase pro-inflammatory adipokines and decrease adiponectin through activation of NFκB (Chan et al., 2016) (Fig. 2.33), which progressively contributes to insulin resistance and insulin resistance syndrome. metabolic (Welty et al., 2016; Chan et al., 2016).

3.6 Mitochondrial Dysfunction and Increased Oxidative Stress

One of the causes of inflammation in SM is mitochondrial dysfunction. It is well known that mitochondria are where most of the energy is produced in cells, especially eukaryotic cells (Mendrick et al., 2018). Excessive energy intake will increase the burden of mitochondrial metabolism and result in excess activation of the electron transport chain (ETC) (Rani et al., 2016) thereby causing disruption of lipid metabolism (Saponaro et al., 2015).

In essence, mitochondrial dysfunction is a very complicated process, but a number of assumptions have been advanced. Excessive fat accumulation in WAT is thought to result in disruption of redox homeostasis and increased oxidative stress (Jha et al., 2017). Oxidative stress is a condition in which the production of free radicals is higher than their degradation by the antioxidant system which is characterized by an increase in reactive oxygen species (ROS) in tissues (Velasquez, 2015). Oxidative stress is also considered to be a major cause of tissue toxicity. During respiration, the release of high energy and unpaired electrons by mitochondria can increase ROS in cells (Gagne, 2014). In addition, several studies have also reported that ROS has a positive correlation with metabolic syndrome (Avelar et al., 2015).

ROS production is triggered by the formation of free radicals in mitochondria, which include superoxide anions ($O2^-$), hydrogen peroxide (H2O2), hydroxyl radicals (OH^-), and nitric oxide radicals (NO^-) (Yadav & Ramana, 2013) which can cause cell dysfunction, atherosclerosis, DM and IR (Avelar et al., 2015).

Increased oxidative stress due to a diet high in fat or carbohydrates can be activated through various pathways such as NADPH oxidase (NOX), oxidative phosphorylation (ATP formation), glycoxidation, protein kinase C (PKC) and the polyol pathway. This process then produces excess superoxide ions ($O2^-$), which can react to produce ROS and RNS (Figure 2.34) (Rani et al., 2016).

Increased ROS will in turn lead to the progressive development of

inflammation by increasing various redox signaling pathways and changing the expression of inflammatory marker genes (Yadav et al., 2016). These pathways are through NADPH oxidase, inflamasome, MAPK and NF-κB (Figure 2.34) (Tan et al., 2016).

Oxidative stress caused by HFD is evidenced by increased lipid peroxidation products, protein carbonylation, and decreased antioxidant systems and glutathione (GSH) levels (Rani et al., 2016). This is in accordance with research conducted by Bhale (2014) that obese men have higher MDA levels than non-obese men (Bhale et al., 2014).

4. RESEARCH METHOD

The study was designed using a randomized posttest-only control group design (true experiment). Sixteen male Wistar rats weighing 150-250g and aged 4-5 months were divided into two groups. Group K1 was a negative control group that was given normal diet, while group K2 was a group that was given a high-fat diet, with a fat content of 66.28%. This treatment was controlled for 50 days. Data were analyzed using independent t test. The procedures for taking and collecting data are described as follows:

4.1 Rat Acclimatization Stage

Adult male wistar rats obtained from Lab. Biochemistry, Airlangga University, was first acclimatized for approximately 1 week to a temperature of 22 ± 2°C and 40-60% humidity, on light and dark cycles for 12-12 ± 1 hour and placed in a large and hygienic container (Parasuraman et al. al., 2010). During the acclimatization period, food and drink are provided ad libitum (Nurmasitoh et al., 2018).

4.2 Grouping Mice Stage

After the acclimatization period, the rats were then weighed and grouped according to the research design (randomization).

4.3 A High-Fat Diet Stage

On the 1st day after acclimatization, the K1 group rats were given standard feed while the K2 group rats were given standard feed plus high fat feed which was given by sonde. The amount of fat that is sonde does not exceed 1% of the rat's weight (Brown et al., 2000) or 5 ml/kg of rat's weight (Turner et al., 2012). Because the average weight of the mice obtained was 216 g, the amount that had to be sonde was 1.08 ml or 1 ml. Based on calculations, the concentration of fat that is sonde is around 66.28%. To provide the same treatment, control rats were also given sonde treatment in the form of plain water.

4.4 Analysis of TNF-α Levels

TNF-α levels were measured on the 50th day (after HFD administration). TNF-α examination was carried out using the ELISA-Sandwich method according to the TNF-α Kit procedure obtained from Elabscience. The principle of this assay is that micro wells that have been coated with TNF-α specific antibodies (capture antibodies) bind to the TNF-α present in the sample, forming an antigen-antibody complex. Furthermore, the added biotin-labeled antibodies (detection antibodies) will bind to the complex and the enzyme-labeled avidin-HRP conjugate will bind to biotin. The added substrate then reacts with the enzyme, producing a blue color and turning yellow when given a stop solution. The color concentration was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of the color formed is proportional to the concentration of TNF-α in the sample (Elabscience, 2019).

5. RESULTS AND DISCUSSION

The total number of rats was 16 which were divided into two groups, namely

Kel. K1 and K2. Both groups were then acclimatized. After that, the two groups were given different treatment, where Kel. K1 was given standard feed and K2 was given high-fat feed.

5.1 Standard Curve Test Results

Examination of TNF-α levels using the sandwich ELISA method was preceded by determining the standard curve or calibration curve and its equations. This curve equation is used to determine the level of TNF-α present in the sample.

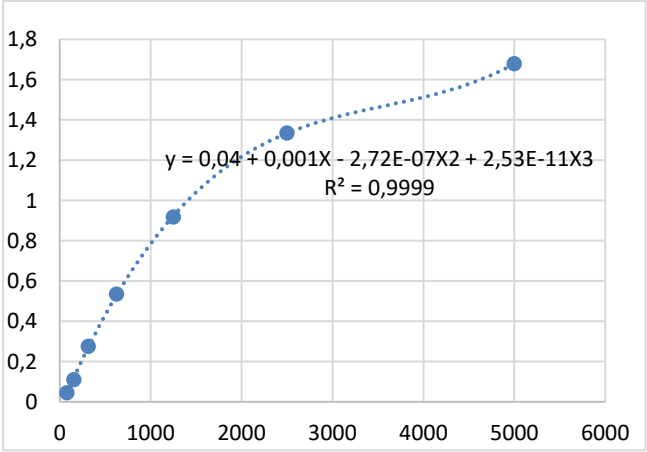

Figure 1. Standard curve of TNF-α levels

5.2 Normality and Homogeneity Test Results

Each variable was tested for normality using the Shapiro Wilk test, and specifically for TNF-α levels, a homogeneity test was carried out using the Levene test. Based on the results of the analysis, the majority of the data is normally distributed (P>0.05). The homogeneity test showed a significance of 0.680 (P> 0.05), which means that the data for each treatment group was homogeneous.

5.3 Test Results for TNF-α Levels

Based on the results of matching the concentration and absorbance standards using the SPSS and Microsoft Excel data processing applications, the closest approach is the cubic regression equation, Y= a + b1X + b2X 2 + b3X3, where: a = intercept , and b = slope.

The results of the analysis of the mean and standard deviation of TNF-α levels in groups K1 and K2 can be seen in Table 1.

Table 1. Analysis of mean and standard deviation of TNF-α levels

Kel.	N	Kadar TNF-α (X ± SD) (pg/mL)	Uji T Independen (*p-value*)
K1	8	290.912 ± 1.87	0,034
K2	8	295.149 ± 2.76	

Based on the results of the independent T-test analysis, the levels of TNF-α in groups K1 and K2 differed significantly (P = 0.034 or P <0.05).

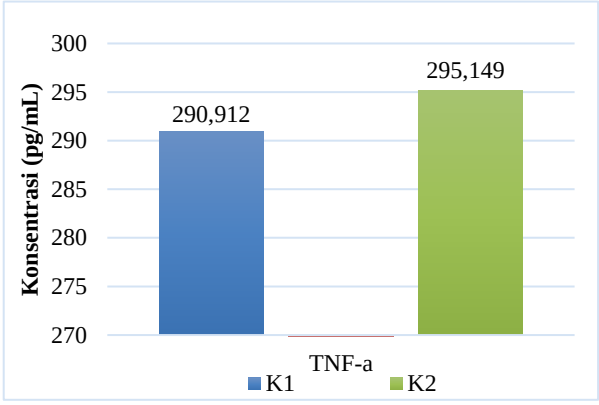

Figure 2. Diagram of TNF-α levels for groups K1 and K2

Based on the results of the independent T-test analysis, it appears that the K2 group (rats on a high-fat diet) showed a significant increase in TNF-α levels (P <0.05) compared to the K1 group (control group). This indicates that low-grade inflammation has occurred.

Low-grade chronic inflammation is mainly caused by macrophage cells, especially in adipocytes or other tissues such as intestine, liver and muscle. In adipocyte tissue, infiltrating immune cells, such as macrophages, B cells, T cells, constitute the second largest population after adipocytes and play an important role in regulating

tissue function and hemostasis (Li et al., 2018). These cells are in a balanced state, but due to stress induction (fat accumulation), the infiltration of these cells will also increase, so that apart from maintaining hemostasis, they can also cause various damages, one of which is damage to the insulin receptor.

The increased infiltration of these cells and other damage is triggered by the production of various pro-inflammatory cytokines, especially TNF-α which is secreted by the macrophages or adipocytes themselves. Adipocytes that experience hypoxia due to fat accumulation, secrete a number of adipokines such as IL-6, MCP-1, and TNF-α. The secreted TNF-α will then recruit and activate other immune cells such as neutrophils and mast cells, activating NFkB signals which can also induce the polarization of M2 macrophages to M1, where M1 macrophages provide positive feedback by secreting TNF-α again. Continuous activation of NFkB signaling by TNF-α can reduce IRS-1 (insulin receptor) expression through increased serine phosphorylation and decreased tyrosine phosphorylation, which can lead to glucose intolerance, after metabolic syndrome. Apart from being triggered by adipokines, there is some assumption that in adipocytes, macrophages act as APCs that can respond to ovalbumin antigens and present them to T cells to induce adaptive immune responses.

Apart from macrophages and adipocytes, increased TNF-α production can also be triggered by various signals from other immune cells such as CD8+ T cells, Th1 cells, and NKT cells which can recognize antigens presented by adipocytes, produce IFN-γ and increase M1 macrophage polarization, following TNF-α secretion. As for other signaling, such as that mediated by endotoxemia, FFA, and ROS, also play a role. As a result of this signaling, some anti-inflammatory cells (those that mediate regulation or homeostasis), such as eosinophils, Tregs and NKT-2 and the resulting cytokines, are decreased. In addition, changes in the circadian clock due to excessive fat consumption can also exacerbate this condition by increasing TNF-α and IL-6 levels (Comas et al., 2017).

6. CONCLUSIONS AND SUGGESTIONS

Based on the results of the analysis, it was concluded that a high-fat diet had an effect on increasing serum levels of TNF-α, a low-level inflammatory marker that can trigger various metabolic diseases.

The suggestions that can be given should be considered during the sampling process, so as not to cause gastric reflux or lung injury which can cause the death of the rats. Limitations of researchers regarding the variables examined, can be a consideration for future researchers if they want to conduct related studies. It is recommended to analyze a wider range of variables, such as pro-inflammatory and anti-inflammatory cells, and the various cytokines produced.

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