RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n1.2651
Majalah Kedokteran Bandung. 2023;55(1):7–12

Majalah Kedokteran Bandung (MKB)

Received: January 27, 2022
Accepted: December 22, 2022
Available online: March 31, 2023

Effect of High Fat and Cholesterol Diet on Total Blood Cholesterol Levels in Pregnant Wistar Rats

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Abstract

Hypercholesterolemia during pregnancy is a physiological condition resulting from increased insulin resistance, lipoprotein synthesis, and lipolysis in adipose tissue, which mobilizes lipids as an energetic substrate for fetal growth. Consumption of foods high in fat and cholesterol may lead to an increase in total blood cholesterol levels during pregnancy due to saturated fat and cholesterol contents that will increases the synthesis of lipoproteins in the blood. The objectives of this study were to determine the effects of high fat and cholesterol diet on the total blood cholesterol levels in pregnant Wistar rats. This study was a true experimental research using a randomized post-test-only control group design conducted from November 2020 to October 2021 on fourteen female Wistar rats that were divided into control and intervention groups. Cow brain was provided as the high fat and cholesterol diet and after the rats gave birth, blood was drawn from the heart. The total blood serum cholesterol levels were assessed using Micro Lab 300 with the CHOD-PAP method and the data were analyzed using an independent t-test. This study showed that the mean total blood cholesterol levels for the control and treatment groups were 80.43±18.512 mg/dL and 142.57±24.786 mg/dL, respectively, which reflected a significant differences in the mean total blood cholesterol level between the control and treatment groups (p-value <0.01). In conclusion, a high fat and cholesterol diet affects the total blood cholesterol level in pregnant Wistar rats..

Keywords: Cholesterol diet, high fat diet, hypercholesterolemia, pregnancy, total blood cholesterol

Introduction

Dyslipidemia is a lipid metabolism disorder in the form of an increase or decrease in the level of lipid fraction in plasma. Total cholesterol is one of the parameters that become the main focus in diagnosing dyslipidemia. Hypercholesterolemia is one of the classifications of dyslipidemia. An increase in total serum cholesterol exceeds 200 mg/dL after nine to twelve hours of fasting without increasing other lipid fractions.[1] Based on the Global Health Observatory (GHO) from WHO data, dyslipidemia caused 2.6 million deaths in the world in 2008, with a prevalence of 37% for men and 40% for women. Based on Basic Health Research 2013, in Indonesia, 35.9% of the Indonesian population aged 15 years and over had hypercholesterolemia conditions, with prevalence based on sex found in men at 30% and in women it was higher, namely 39.6%.2 Women are more at risk for various reasons, including hormonal factors, pregnancy, and menopause.[3]

Gestational hyperlipidemia or hypercholesterolemia is a physiological condition resulting from hormonal increases, insulin resistance, lipoprotein synthesis, and lipolysis in adipose tissue, which mobilizes lipids as energetic substrates for fetal growth.[4,5] Excess cholesterol in pregnancy needs to be considered as a risk factor for pregnancy and fetal development. Gestational hyperlipidemia is associated with metabolic morbidities such as obesity and gestational diabetes mellitus, and several studies have shown an association with post-term, preeclampsia, and preterm birth.[4,6]

Based on research conducted by Siringoringo et al.[7] on pregnant women in Padang, the average total cholesterol in the third trimester reached 247.56 mg/dL in a normal pregnancy. Although several studies have shown that cholesterol levels increase substantially during

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the last two trimesters of pregnancy, the optimal serum cholesterol levels have not yet been determined.[8] According to research conducted by Retnakaran et al.,[9] there was an increase in total cholesterol up to 289.58 mg/dL at the end of the second trimester of pregnancy and three months after birth, a decrease to around 220 mg/dL.

Adequate food intake from the beginning of pregnancy is necessary to support the fetus's physical health and mental development.[10] Unbalanced maternal diet with high-fat content can impact maternal condition and fetal growth due to disturbances in the transportation of placental nutrients.[11] In pregnant women, consuming a high-fat diet can affect offspring with changes in energy balance, cardiovascular dysfunction, neuroinflammation, and an increased risk of metabolic syndrome, including hypercholesterolemia.[12,13] According to research from the Brazilian Diabetes Society Congress in 2015, it was found that as many as 48.4% of pregnant women had inadequate food consumption patterns due to high consumption of fat and cholesterol, and also low consumption of vegetables and dairy products during pregnancy.[14] This aligns with research by Narasiang et al. on 181 pregnant women. The average daily fat consumption during pregnancy was 118.63 grams/day, whereas the maximum fat consumption during pregnancy was 67.3 grams/day.[15]

According to Cerf et al.,[11] giving pregnant rats a high-fat diet can increase plasma cholesterol on the 20th day of gestation by about 10% (90 mg/dL) compared with the control diet group. The treatment group was given a high-fat diet with 40% fat, 14% protein, and 46% carbohydrates. While the control group with a composition of 10% fat, 15% protein, and 75% carbohydrates (80 mg/dL). Based on a study conducted by Li et al.,[13] pregnant rats fed a high-fat diet with a composition of 58% fat, there was an increase in body weight, lipid profile, and blood glucose on the 19th day of gestation. In the group that was given a high-fat diet, the average total cholesterol level increased up to 96 mg/dL, while in the control diet group, the average total cholesterol level was 63 mg/dL.

In this study, we will give different doses of a high-fat diet. A high-fat diet was given as mashed beef brain at 3 mL/head/day during pregnancy until delivery. This study aimed to determine the effect of a high-fat and cholesterol diet on total blood cholesterol levels in pregnant Wistar rats.

Methods

This research is experimental research with a post-test-only research control group design. The research was conducted at the Faculty of Medicine, Andalas University's animal house, for the maintenance and treatment of experimental animals, and at the biochemistry laboratory of the Faculty of Medicine, Andalas University, to examine blood samples from November 2020 to October 2021.

The population in this study was the Rattus novergicus Wistar albino strain. The sample was the entire population that met the inclusion and exclusion criteria. Inclusion criteria: healthy female albino Wistar strain rats aged 3–4 months and an average body weight of 200–250 grams, pregnant, and active.

The sampling technique used simple random sampling that met the inclusion and exclusion criteria. The sample size was determined based on WHO criteria, where the minimum number of samples in each experimental research group was five rats. To prevent the existence of exclusion and dropout criteria, the researchers took a total of seven mice in each group. This study consisted of 2 sample groups: one control group without a high-fat and cholesterol diet and 1 group with a high-fat and cholesterol diet, so 14 rats were needed.

Experimental animals were placed in several places according to the number of treatment groups. After acclimatization, the animals were tried to be mated. Mating in mice takes place within 4–5 days. The presence of a vaginal plug or copulatory plug after fusion indicates that copulation has occurred and this day is defined as the first day of pregnancy. The weight of the pregnant experimental animals was recorded, and the treatment started from the first day of the pregnant experimental animals.

A group of female mice (control group, n=7) was randomly selected and placed as a control group, which was fed a standard diet ad libitum during the experiment. Standard food consists of 5% fat, 16% protein, 8% crude fiber, 10% ash content, and 12% moisture content. Another group of rats (treated group, n=7) was given a standard diet supplemented with 2 ml/head/day of bovine brains (high-fat diet (HFD)). The beef brains given have been steamed beforehand and blended with a 1:1 ratio of adding water. Cattle brain is given using a gastric tube once a day and standard food is given ad libitum. The treatment was carried out from the first day the

Table 1 Results of Total Blood Cholesterol Levels' Measurement

No	Control Group mg/dL	Treatment Group mg/dL
1.	75.9	158.2
2.	79.5	137.7
3.	65.4	115.4
4.	106.2	179.9
5.	71.7	162.5
6.	105.2	116.9
7.	59.1	127.4
Mean	80.43	142.57
Standard Deviation	18.512	24.786

rats were pregnant for 21 days.

Blood sampling was carried out after the mice gave birth (the first day after birth) so that the treatment could be given in total for 21 days. Blood sampling was carried out after all rats fasted for 10-12 hours before blood collection. The mice were anesthetized beforehand to make taking samples through the heart easier using a 1cc syringe. Total cholesterol was determined enzymatically using the cholesterol oxidase-peroxidase aminoantipyrine phenol (CHOD-PAP) method.

Data analysis was performed using univariate and bivariate. Univariate analysis was performed to determine the mean total cholesterol level of the sample group. Bivariate analysis was performed to statistically determine the difference in the mean of the two sample groups. The data obtained were first tested for normality using the Shapiro-Wilk test and homogeneity test using the Levene Statistics test. If p> 0.05, then the data is usually distributed and homogeneous. Then an independent t-test was conducted with a significance value of 99% ($p \leq 0.01$). If p 0.01, then the calculation results are statistically significant. The Health Ethics Committee has approved this research of the Faculty of Medicine, Andalas University, with the ethical number 354/UN.16.2/KEP-FK/2021.

Results

The results of measuring total cholesterol levels from blood serum samples of female Wistar rats from both groups can be seen in Table 1. It shows that the mean total cholesterol level of pregnant Wistar rats in the control group is 80.43±18,512 mg/dL, and the mean total cholesterol level of pregnant Wistar rats in the treatment group is 142.57±24,786 mg/dL.

Data analysis was carried out using the independent sample T-Test based on the data obtained. Independent sample T-Test requires several rules: the data is normally distributed, the variance between groups of homogeneous data, the two groups of independent data, and the associated variables are numerical (with only two groups).

The normality test results conducted with the Shapiro-Wilk test showed that the data were normally distributed with a significance value of the control and treatment groups of 0.221 and 0.500, respectively ($p>0.05$). Furthermore, a homogeneity test was carried out with the Levene test, which showed that the data was homogeneous with a significance of 0.264 ($p>0.05$).

An Independent T-Test was conducted to determine the difference in the mean of two

Table 2 Differences in Cholesterol Levels in the Control Group with the Treatment Group

Group	Cholesterol Level		p
	Mean	SD	
Control	80.43	18.512	0.001
Treatment	142.57	24.786	

independent data groups. The data obtained were tested with a 99% confidence interval and a significance level of 0.01 (p=0.01). The test results showed a significant difference between the total blood cholesterol levels of the treatment group and the control group (p<0.01). The results of the data analysis are presented in Table 2.

Table 2 shows that the mean total blood cholesterol level of female Wistar rats in the treatment group was higher than in the control group. According to the results of statistical analysis, it was found that there were significant differences between the two groups.

Discussion

Based on the results of the average total cholesterol levels of pregnant Wistar rats in table 1, it was found that the average total cholesterol level in the control group was in the normal range of 80.43 mg/dL. This result is in line with research conducted by Cerf and Herrera. The total cholesterol levels of pregnant rats fed standard diets were in the normal range of 80 mg/dL.11 According to research by Xie et al., there was an increase in total cholesterol levels in pregnant rats to 81.05 mg/dL compared to 77.30 mg/dL before pregnancy.[16]

There is an increase in total cholesterol levels physiologically during pregnancy. However, not all pregnant women experience hypercholesterolemia. There is a relative increase in cholesterol, especially in the second and third trimesters of pregnancy. Hypercholesterolemia in pregnancy is mostly experienced by women who have familial hypercholesterolemia.[17] Familial hypercholesterolemia is an autosomal dominant inherited disorder of lipid metabolism. Mutations in the LDL receptor gene cause HF. During pregnancy, women with familial hypercholesterolemia show relative changes in plasma lipid levels similar to those in healthy women. However, the absolute increase is higher in familial hypercholesterolemia.[18]

A significant effect was found in the treatment group's average increase in total cholesterol levels compared to the control group. The treatment group was given standard feed plus high-fat and cholesterol foods using beef brains with a total fat content of 63% and cholesterol of 3100 mg per 100 grams of material. In the treatment group, the average total cholesterol level was 142.57 mg/dL, and five out of seven rats had hypercholesterolemia.

This result is in line with research conducted by Cerf and Herrera. There was an increase in the average total cholesterol level of pregnant rats fed a high-fat diet and cholesterol with a total fat content of 40% compared to the control group up to 90 mg/dL. However, it did not reach a hypercholesterolemic condition.[11] Based on research conducted by Li et al.,[13] pregnant rats fed a high-fat diet with a composition of 58% fat experienced an increase in the average total cholesterol level of up to 96 mg/dL, compared with the control diet group with only 63 mg/dL.A study by Xie et al.[16] also showed a significant increase in total cholesterol levels of pregnant rats given a high-cholesterol diet of 117.05 mg/dL compared to the control group of 77.30 mg/dL.

Dietary factors and pregnancy factors influenced the treatment group's total cholesterol levels. Beef brains contain high levels of saturated fat and cholesterol, which affect fat metabolism in the body. The content of saturated fatty acids can lead to the overexpression of ACAT 2 in catalyzing the formation of cholesterol esters released into the bloodstream with ApoB in the form of VLDL, increasing VLDL levels.[19]

Cholesterol content causes high intracellular cholesterol levels in the liver. Increased liver intracellular cholesterol levels result in increased VLDL production. Both of these mechanisms can increase LDL levels in the blood. The high level of intracellular cholesterol in the liver causes the liver to stop the mechanism of returning LDL, which transports cholesterol esters in the blood so that LDL levels increase, leading to an increase in total cholesterol levels.[20]

The mechanism of this increase in cholesterol is also influenced by lipid metabolism in pregnancy, where there is an increase in lipogenesis in the early two-thirds of the phase and the process of lipolysis in the late third of pregnancy.[12] In the early two-thirds of pregnancy, an increase in insulin production and sensitivity is caused by an increase in maternal hormones, especially estrogen, and progesterone, which triggers pancreatic cell hyperplasia.[21] These changes affect maternal hyperphagia and increase the performance of lipoprotein lipase, resulting in increased fat synthesis and hypertrophy of adipocytes. It can be seen from the increased body weight of the mice weighed from the beginning of pregnancy to the end.

In weighing the rats' body weight twice a week, there was an increase in body weight every time it was weighed. It is in line with research conducted by Mathias et al.[22] that there was an increase in body weight in pregnant rats, where the body weight of rats fed a high-fat diet was higher than that of pregnant rats in the control group.

In the final third of pregnancy, there is a decrease in insulin sensitivity due to a spike in local and placental hormones such as estrogen, progesterone, leptin, cortisol, human placental lactogen (HPL), and placental growth hormone. Those trigger lipolysis of triglycerides in adipocytes and decrease LDL receptors. This mechanism stimulates cells to use energy intake other than glucose, such as free fatty acids, to increase the supply of nutrients to the fetus.[23]

The increase in adipose cells from the first and second trimesters also leads to increased secretion of tumor necrosis factor (TNF-α) and leptin in the local circulation, which triggers mechanisms of insulin resistance. TNF-α interferes with insulin performance by inhibiting insulin receptor signaling, namely tyrosine kinase, resulting in the failure of phosphorylation of insulin receptor substrates (IRS) on tyrosine. Reduced IRS phosphorylation causes failure of phosphatidylinositol (PI) 3-kinase activation, so insulin fails to distribute it to the glucose transporter (GLUT4) that contains vesicles. PI 3-kinase is thought to play a role in the activity of vesicle fusion with the cell surface, resulting in glucose uptake from the cell surface to the intracellular. The decrease in PI 3-kinase activity causes the vesicles in GLUT4 not to fuse with the cell surface, so glucose cannot enter the cell.[24]

Insulin resistance results in decreased lipoprotein lipase performance resulting in a decrease in chylomicron catabolism, thereby increasing triglyceride synthesis in the liver. This increase in triglycerides can increase VLDL in the blood, so hypertriglyceridemia occurs. In addition, triglycerides are closely related to the formation of sdLDL, which can increase total cholesterol levels in the blood. The decrease in LDL receptors due to insulin resistance also affects the increase in total blood cholesterol due to a decreased *clearance* in the blood.[25]

The components studied were only total blood cholesterol levels and did not measure other lipid components such as HDL, LDL, and triglycerides. They could not see the effect of a diet high in fat and cholesterol on these lipid components. In addition, the dosage in this study was only one variant, as much as three cc/day, so it could not see variations in the increase in total cholesterol levels in pregnant rats.

The cow brain consists of 63% fat, 33% protein, and 4% carbohydrates (per 100 grams). In 1 gram of beef brain, there are 31 mg of cholesterol and 0.1 gram of fat. The maximum cholesterol requirement in humans is 300 mg/day, and the fat requirement in pregnant women is 67.3 grams/day. If converted to mice, 5.4 mg/day of cholesterol and 1.2 grams/day of fat is needed. The fat content in 1 gram of beef brain does not meet the maximum fat requirement, while it has exceeded the cholesterol requirement. Therefore, the researchers gave a dose of 3 ml/day of the beef brain, which was based on previous studies, where giving cow brain at that dose could increase total cholesterol levels by up to 70% in two weeks. [26]

In this study conclude a significant difference in the mean total blood cholesterol levels of pregnant Wistar rats between the group given a standard diet and the group given a high-fat and cholesterol diet during pregnancy. This study emphasizes that consuming a diet high in fat and cholesterol can increase cholesterol levels higher than physiological ones during pregnancy. Further research is needed regarding the measurement of others lipid fractions of each rat before and after pregnancy.

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