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Metabolic Syndrome Induced by Hipercalory Diet in Wistar Rats: impact on diabetic risk, dyslipidemia and hypertension complication

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A B S T R A C T

Objective: Metabolic syndrome is a group of metabolic disorders that are usually found in individuals with a high risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease. Comparison of diet composition, especially macronutrients, over a certainperiode in both humans and experimental animals can allegedly increase the risk of metabolic syndrome. This study aims to determine the use of a hypercaloric diet that can cause metabolic syndrome in male Wistar rats. Methods: The hypercaloric diet was administered orally in pellets and oral solution to rats for 16 weeks. Analysis of the condition of the metabolic syndrome was carried out by looking at the increase in serum levels of total cholesterol, triglycerides and decreased HDL levels. Fasting blood sugar and fasting insulin levels were also seen to increase and blood pressure testing was carried out to see an increase in blood pressure after 16 weeks of giving a hypercaloric diet. Results: Administration of a hypercaloric diet showed an increased risk of metabolic syndrome in rats. Administration for 16 weeks showed a significant difference in the improvementofs conditions of dyslipidemia, insulin resistance and hypertension. Conclusion: Based on the results, a hypercaloric diet for 16 weeks can cause metabolic syndrome in rats.

Kata kunci:

Diet hiperkalori
Sindrom metabolik
Tikus Wistar jantan

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A B S T R A K

Objektif: Sindrom metabolik adalah sekelompok kelainan metabolik yang biasanya ditemukan pada individu dengan risiko tinggi diabetes militus tipe 2 (T2DM) dan penyakit kardiovaskular. Perbandingan komposisi diet khususnya makronutrien, dalam jangka waktu tertentu baik pada manusia maupun hewan coba disinyalir dapat meningkatkan resiko dari sindroma metabolik. Penelitian ini bertujuan untuk menentukan penggunaan diet hipercalori yang dapat menyebabkan kondisi sindrome metabolikpada tikus Wistar jantan. Metode: diet hiperkalori diberikan secara oral dalam bentuk pelet dan larutan oral pada tikus selama 16 minggu. Analisis terhadap kondisi syndrome metabolic dilakukan dengan melihat peningkatan kadar serum kolesterol total, trigliserida dan penurunan kadar HDL. Kadar gula darah puasa dan insulin puasa juga dilihat peningkatannya serta dilakukan pengujian tekanan darah untuk melihat terjadinya peningkatan tekanan darah setelah 16 minggu pemberian diet hiperkalori. Hasil: Pemberian diet hiperkalori menunjukkan adanya peningkatan risiko sindrom metabolic pada tikus. Pemberian selama 16 minggu menujukkan adanya perbedaan signifikan pada peningkatan kearah kondisi

dyslipidemia, resistensi insulin dan hipertensi. Kesimpulan: Berdasarkan hasil, pemberian diet hiperkalori selama 16 minggu dapat menyebabkan terjadinya kondisi sindrom metabolic pada tikus

INTRODUCTION

Metabolic syndrome is a group of metabolic disorders that are usually found in individuals with a high risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease. Common abnormalities include obesity, insulin resistance (hyperinsulinemia), dyslipidemia (hypertriglycerides and low high-density lipoprotein (HDL)) hypertension and hyperglycemia (Wiley & Sons, 2011). The pathogenesis of the metabolic syndrome involves both genetic and acquired factors which play a major role in inflammation and lead to atherosclerotic cardiovascular disease and insulin resistance. In a state of insulin resistance, the process of lipolysis increases and results in increased production of fatty acids. Free fatty acids, mainly from triglyceride stores in adipose tissue, are released via cyclic adenosine monophosphate during lipolysis, but under normal circumstances this process is actually inhibited by insulin by reducing the activity of cyclic adenosine monophosphate (Roberts et al., 2014).

Fat is one of the three main macronutrients and the most calorically dense macronutrient (Brown et al., 2010). Glycerol and fatty acids diffuse freely into the bloodstream, free fatty acids in plasma are the main substrate for hepatic production of VLDL triglycerides (Nielsen & Karpe, 2012). High levels of VLDL cholesterol in the blood can cause obesity, dyslipidemia and cholesterol accumulation. Accumulation of triglycerides in the liver can also cause insulin resistance (Daniels et al., 2012). Fructose is a monosaccharide that enhances the taste of food. The entry of large amounts of fructose into the liver causes the accumulation of triglycerides and cholesterol due to its lipogenic (lipid-synthesis) properties, which in turn causes reduced insulin sensitivity, insulin resistance and glucose intolerance. One possibility of insulin resistance in fructose-fed rats is insulin receptor downregulation with lower levels of insulin receptor mRNA in skeletal muscle but not kidney. This effect has been found to be associated with decreased insulin-stimulated glucose utilization and decreased insulin sensitivity (Abdulla et al., 2011). Insulin resistance that occurs due to high fructose intake can also cause hypertension through its effect on nitric oxide levels in endothelial cells. Nitric oxide is produced in endothelial cells and regulates vasodilation. In a state of insulin resistance, there is a decrease in NO production due to an increase in reactive oxidative stress (ROS) which causes endothelial dysfunction and hypertension (Wiley & Sons, 2011).

Comparison of diet composition, especially macronutrients, over a certain period of time in both humans and experimental animals can allegedly increase the risk of metabolic syndrome. Research conducted on experimental animals (male Wistar rats) showed that administration of a high-carbohydrate diet (50% carbohydrate; 24% fat) and a high-fat diet for 8 or 16 weeks induced obesity, dyslipidemia, hypertension, impaired glucose tolerance, and endothelial dysfunction (Aziz et al., 2009). Another study showed that giving a high-carbohydrate diet (45% carbohydrates, 35% fat and 20% protein) for 7 weeks, and a high-fat diet (20% carbohydrates, 59% fat and 21%) for 4 weeks, both showed an increase in insulin response. , but the disturbance of glucose tolerance only occurred in the high - fat diet group . A high-carbohydrate and high-fat diet for 16 weeks in male Wistar rats showed that there was an increase in body weight, abdominal fat, impaired glucose metabolism, dyslipidemia, hyperinsulinemia, and increased leptin plasma concentrations, increased systolic blood pressure and endothelial dysfunction (Panchal et al., 2011).

In this study, male Wistar rats were induced with a diet of hypercaloric pellets and hypercaloric solutions for 16 weeks. Hypercaloric pellets are made with the following ingredients: ground Labina (371 g/kg), roasted peanut (185 g/kg), casein (99 g/kg), corn oil (68 g/kg) instant noodles (185 g/kg), grated cheese (92 g/kg), vitamins, and minerals are known to cause metabolic syndrome in male Wistar rats (Nascimento et al., 2008). Giving a diet of 15% goat fat for 4 weeks is known to increase the values of serum cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), atherogenic indices, glucose and lactate dehydrogenase (LDH). were significantly increased, while the levels of high density lipoprotein cholesterol (HDL-C) and HDL-C ratio were statistically decreased (Al-Attar 2010). Another study stated that giving a high-calorie diet with 27.5% fructose, 10% quail egg yolk and other mixtures also caused rats to experience metabolic syndrome (Maryani et.al. 2016). 2.5% mixed salt, 35% sweetened condensed milk, 20% lard, 17.5% fructose, 20% rat feed, 5% water in a study conducted by Senaphan et al. (2015) had the effect of increasing blood pressure and conditions of cardiovascular disorders and diabetes in male Sprague-Dawley rats.

METHOD

Animals

Animals used in this study were male Wistar rats, weighing 150-250 grams and aged 2-3 months obtained from the National Food and Drugs Administration, Republic of Indonesia (BPOM RI). Animals were bred and housed at Faculty of Pharmacy, Universitas Indonesia in standard housing (12 h light/dark cycle), temperature 24-26℃, humidity 60-65%, in groups of 5 rats per cage with ad libitum access to standard water and chow. Before the study, rats were acclimatized in laboratory condition for at least 1 weeks. The experimental protocols and animal handling procedures were approved by the Ethics Committee Faculty of Medicine, Universitas Indonesia (approval number KET-182/UN2.F1/ETIK/PPM.00.02/2021).

Equipment and apparatus

1 ml syringe (Terumo, Philippines) with oral sonde, animal scale (Mettler Toledo, Indonesia), plate reader GloMax® (Promega, USA), surgical board, single channel

micropipette (Socorex, Switzerland), multichannel micropipette (HWLAB®, China), UV-Vis spectrophotometer (Shimadzu, Japan), rats blood pressure meter (Coda® Kent Scientific Corporation, USA).

Experimental Design

The animals were randomly divided into 2 (two) groups. The first group served as a normal or vehicle group and was treated with 0.5% CMC-Na solution and normal pellet, the second group served as a control group and was orally administered hipercalory pellet and hipercalory solution.

Experimental Procedures

The first group was a normal group given 0.5% CMC-Na and standard pellet. The second group given hipercalory pellet and hipercalory solution orally once daily for 16 weeks. The group received water *ad libitum*. Food and water consumtion were controlled every day.

Hipercalory pellet was design based on Nascimento et al. (2008) research. The ingredient (g/kg) used to prepare the hypercalory pellet were: ground Labina (371 g/kg), roasted peanut (185 g/kg), casein (99 g/kg), corn oil (68 g/kg), instant noodles (185 g/kg), grated cheese (92 g/kg), vitamin dan mineral. Hipercalory pellet produced by PT Indofeed with nutritional value:

Table 1. Hipercalory Pellet Nutritional Value (Nascimento et al., 2008)

Component	Value
Protein	28%
Carbohidrate	36%
Fat	23%
Others	13%
Kalori	4.6 Kcal/g

The hypercaloric solution was made based on a combination of research from Al-Attar (2010), Maryani et.al. (2016), Mohamed & Bahgat (2009), Ruslin et.al. (2019), Senaphan et al. (2015) so it was made with the composition: quail egg yolk (40%), goat fat (25%), fructose (20%), pure salt (4%), coconut oil (5%) and Na-CMC solution (ad 100 %). The hypercaloric solution is made daily by weighing each ingredient and then all the ingredients are mixed until homogeneous. The amount of material needed for one day is made with the assumption that the Oral Absorption Volume (VAO) given to rats is 3 mL for rats weighing 200 g. Calculation of the weight for each material needed multiplied by its specific gravity. VAO for each mouse is calculated by the formula:

$$\text{VAO for every rats} = \frac{\text{Rat body weight}}{200\text{g}} \text{x } 3 \text{ mL}$$

Assuming the average body weight of rats is 250 g, so the calculation of the number of calories for the hypercaloric solution is attached in table 2.

Table 2. Total Chaloric of Hyperchaloric Diet

Ingridient	Weight (gram)	Chaloric (kkal)
Quail egg yolk	1,55	4,99
Goat fat	0,66	5,95
Fructose	1,19	4,17
Salt	0,33	0
Coconut oil	0,42	3,30
Total Chaloric		**18,41**

Note: Weight of hypercaloric diet (grams) = amount of ingredients used x (250 gr BW/200 gr x 3 mL) x specific gravity (gr/mL). Calories = weight of high-fat diet (grams) / weight of total serving sizes (grams) x total calories in serving sizes (kcal). Yolk specific gravity = 1.03 gr/mL (AVCalc LLC, 2020); animal fat = 0.7 gr/mL (Charrondiere et al., 2012); fructose = 1.59 gr/mL; salt = 2.16 g/mL (Merck, 2020) and coconut oil = 0.903 gr/mL (Bp et al., 2005).

The biochemical tests, blood preasure measurement and serum insulin test were conducted on weeks 0 and 16. The evaluation is performed using spektrofotometer UV-Vis (Shimadzu, Jepang) for biochemical test, Coda® (Kent Scientific Corporation, USA) with tail-cuff method for blood pressure measurement and for serum insulin were measured by the Elisa method using assay kits from PT. Bioenzy Indonesia (Bogor, Indonesia).

Statistical analysis

All data were tested for normality and homogeneity. After that, the data were analyzed using one-way ANOVA, followed by the post hoc Tukey's HSD test. All data were analyzed using SPSS software version 23. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Results of the research consisted of descriptive statistics, test results of the assumptions and this study investigated the effects of hypercaloric diet on metabolic syndrome markers. Metabolic syndrome markers were evaluated by looking at conditions of insulin resistance (hyperinsulinemia), dyslipidemia (hypertriglycerides, hypercholesterolemia and low high-density lipoprotein (HDL), hypertension and hyperglycemia (Wiley & Sons, 2011). Dyslipidemia is shown by the increase in blood levels of triglycerides and cholesterol, also by decreasing blood levels of HDL cholesterol.

Triglyceride, cholesterol and HDL levels were calculated before the hypercaloric diet was induced (week 0) and after the hypercaloric diet was induced (weeks 16). The levels obtained were then compared to the significance of the difference between the groups at week 0 and week 16. Hypercaloric diet induction was carried out for 16 weeks to obtain dyslipidemia conditions with increased cholesterol levels (≥ 140 mg/dL), triglyceride levels (≥ 150 mg/dL) and decreased HDL levels (< 40 mg/dL). The results showed that after the 16 weeks the group of rats that were given a hypercaloric diet experienced an increase in cholesterol levels, triglyceride levels and a decrease in HDL which was significantly different from the group of mice that were not given a hypercaloric diet ($P<0.05$).

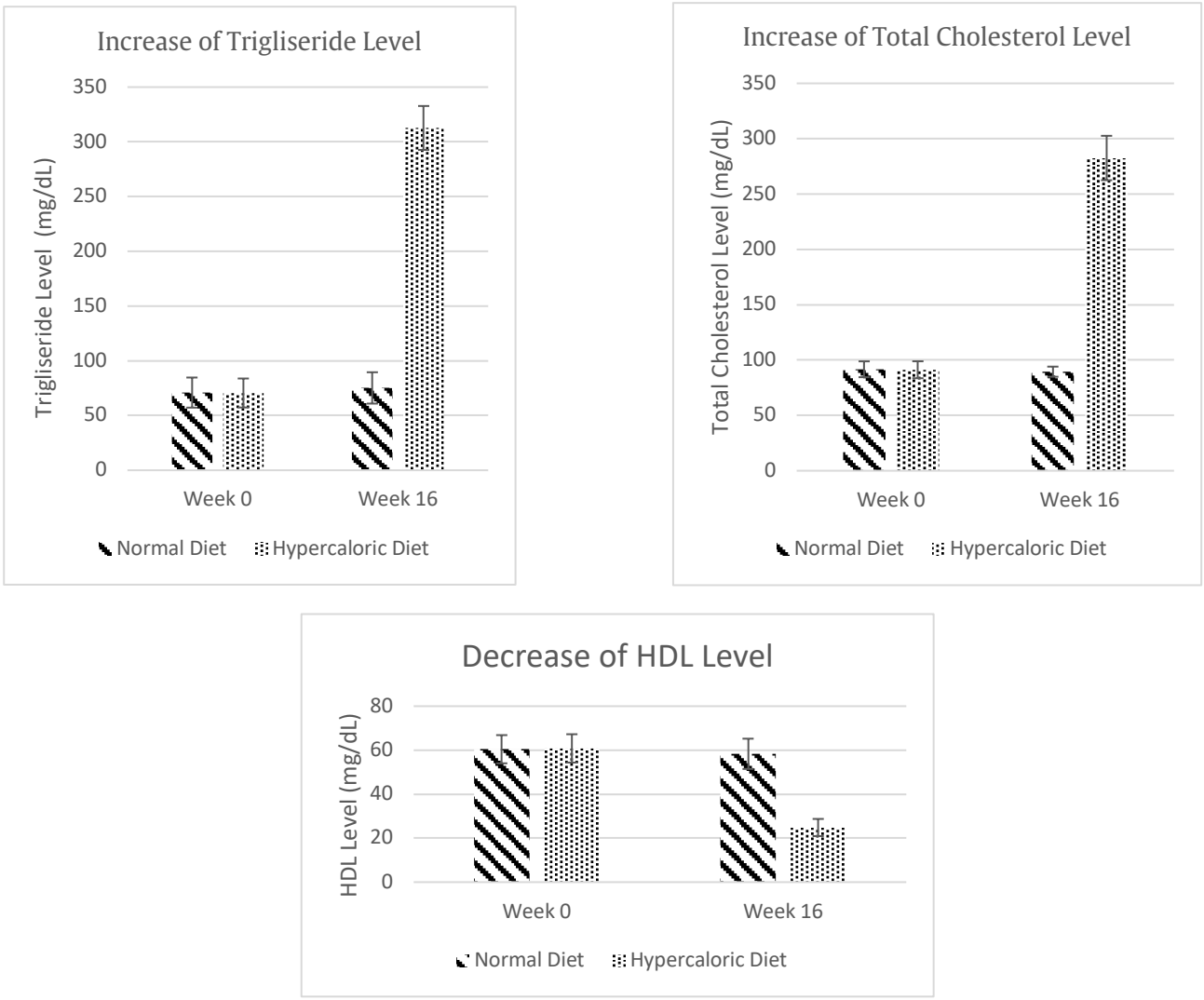

Figure 1: The dyslipidemic in the normal and hyperchaloric diet groups. The values are expressed as the means ± SEM of each group (n = 10). # significant difference compares to normal and hyperchaloric diet groups in week 16 (P< 0.05).

Average total cholesterol, triglyceride and HDL levels of the normal group at week 16 were 89.48 ± 4.58 mg/dL, 75.19 ± 13.23 mg/dL and 58.35 ± 6.87 mg/dL, respectively. These levels are normal levels (normal values for male rats: total cholesterol = 68.42 – 112.40 mg/dL; triglycerides = 46.12 – 79.48 mg/dL; HDL = 41.06 – 70.11 mg/dL) for 7 month old male white (Ihedioha et.al., 2013). In the hyperchaloric diet group, after induction for 16 weeks, the rats experienced a dyslipidemic condition with an average value of total cholesterol, triglyceride and HDL levels of 282.56 ± 20.05 mg/dL, 312.66 ± 20.06 mg/dL, 24.83 ± 3.90 mg/dL.

The hyperinsulinemic and hyperglycemic parameters showed the insulin resistance condition of the animal. In normal condition, rat fasting serum insulin level is <25 mIU/L and for blood glucose normal level is 50 – 135 mg/dL (Hidayaturrahmah et al., 2020). Figures 2 showed increased the level of insulin and blood glucose level after administration of hyperchaloric diet for 16 weeks. Both of parameters, the increased was significant different compare to normal groups with standard diet and hyperchaloric diet group (P<0.05). The results mean the animal glucose metabolisme was abnormal, there was a insulin resistance condition.

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Figure 2: The insulin resistance in the normal and hyperchaloric diet groups. The values are expressed as the means ± SEM of each group (n = 10). # significant difference compares to normal and hyperchaloric diet groups in week 16 (P< 0.05)

The normal diet groups showed no increase in fasting insulin levels or fasting blood sugar levels. The average fasting blood sugar and fasting insulin levels in the normal group at week 16 were 67.33 ± 8.42 mg/dL and 12.12 ± 1.39 mIU/L, respectively. The hypercaloric diet group showed an increase in fasting insulin levels and fasting blood sugar in rats which were significantly different from the normal diet group. The average fasting blood sugar and fasting insulin levels for hypercaloric diet groups at week 16 were 226.81 ± 12.41 mg/dL and 41.96 ± 3.22 mIU/L, respectively.

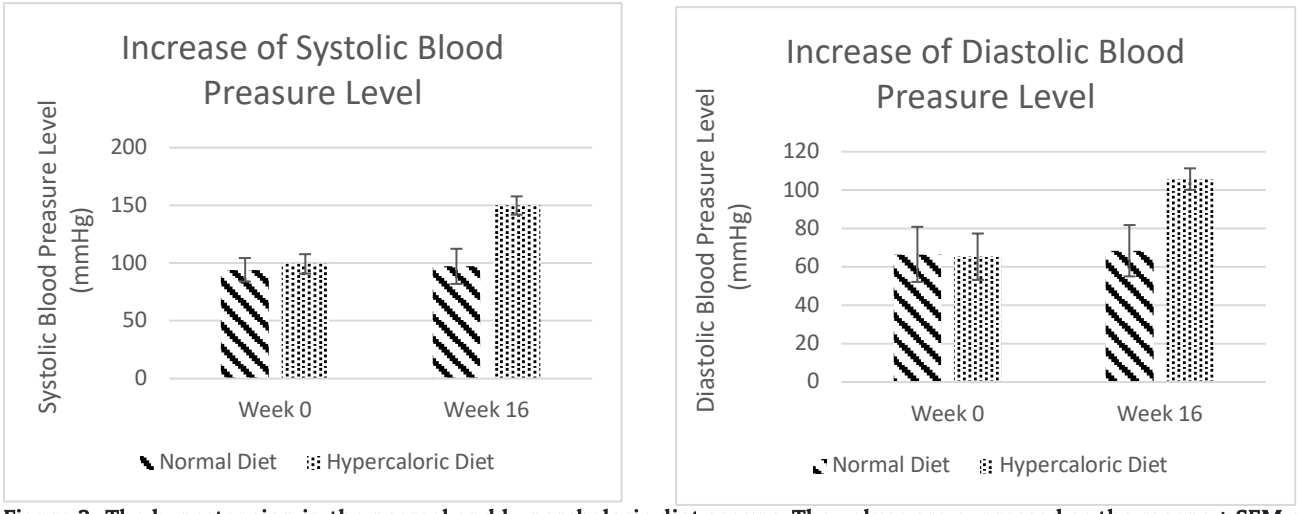

Figure 3: The hypertension in the normal and hyperchaloric diet groups. The values are expressed as the means ± SEM of each group (n = 10). # significant difference compares to normal and hyperchaloric diet groups in week 16 (P< 0.05)

Hypercaloric diet also caused an increase in blood pressure in rats which was significantly different when compared to rats given a standard diet (P<0.05). Administration of hypercaloric diet for 16 weeks caused systolic blood pressure of the rats to increase to an average of 149.9 ± 7.92 mmHg and the average diastolic blood pressure of the rats also increased to an average of 105.7 ± 5.61 mmHg. The mean systolic and diastolic blood pressure in normal male rats is 124 mmHg and 82 mmHg (Luo et al., 2013).

A prolonged hypercaloric diet with high fat and high fructose content will lead to metabolic syndrome conditions through the path of increasing free fat levels and insulin resistance (Aydin et al., 2014). Fat is one of the three main macronutrients and the most calorically dense macronutrient (Brown et al., 2010). Fats, also known as triglycerides, are composed of 3-chain esters of fatty acids and glycerol. Lipid metabolism begins with the process of lipolysis. Many glycerol and fatty acids diffuse freely into the bloodstream, free fatty acids in plasma are the main substrate for hepatic production of VLDL, triglycerides (Nielsen & Karpe, 2012). About 70% of the released free fatty acids will be re-esterified (lipogenesis) to form triglycerides. The rate of re-esterification depends on the rate of production of glycerol-3-phosphate via glycolysis and the rate of release of fatty acids from adipose. Re-esterification of free fatty acids (triglycerides) to form VLDL helps fat to circulate in the blood (Chen Y, 2012).

As a conclusion, increased of VLDL formation helps to redistribute the triglycerides synthesized by the liver as a result of excess consumption of a high-fat diet. High levels of VLDL cholesterol in blood flow can cause obesity,

dyslipidemia and cholesterol accumulation. Accumulation of triglycerides in the liver can cause insulin resistance (Daniels et al., 2012). Excessive production and accumulation of triglycerides in non-adipose tissues such as muscle, liver and pancreas are common criteria seen in the development of the metabolic syndrome (Lehnen et al., 2013).

Fructose is commonly known as fruit sugar, and is one of the monosaccharides along with glucose and galactose. Currently fructose is often used as a food flavor enhancer. There is no biological requirement for fructose in food, only an intermediate molecule during glucose metabolism. The concentration of fructose (~ 0.01 mmol/L) circulating in the blood is very low compared to glucose (~ 5.5 mmol/L) (Bray, 2007).

Theoretically, the entry of large amounts of fructose into the liver causes the accumulation of triglycerides and cholesterol due to its lipogenic (lipid-synthesis) properties, which in turn causes reduced insulin sensitivity, insulin resistance and glucose intolerance. One possible explanation for insulin resistance in fructose-fed rats is insulin receptor downregulation with lower levels of insulin receptor mRNA in skeletal muscle but not kidney. This effect has been found to be associated with decreased insulin-stimulated glucose utilization and decreased insulin sensitivity (Abdulla et al., 2011). Fructose will be taken up by adipose tissue, intestinal epithelium, and liver tissue to be converted into 1-phosphate which is broken down into triose by aldolase. This pathway leads to the synthesis of fatty acids. Through the same mechanism, fructose also supplies carbon atoms to long-chain fatty acids. Thus, fructose causes metabolic syndrome because of the increased amount of triacylglycerol (Sánchez-Lozada et al., 2007).

Two liver enzymes that use fructose as a substrate are hexokinase and fructokinase. The affinity of hexokinase for fructose is about 1/20 that of glucose. When hexokinase is saturated with glucose (in a satiety state), fructose will be phosphorylated by fructokinase leading to the phosphofructokinase-1 step because that major step in the regulation of glycolysis is missed. All fructose intake is quickly metabolized to acetyl Coenzyme A (CoA) and because cells are already rich in energy, acetyl CoA is not catabolized through the tricarboxylic acid cycle but diverted to fatty acid synthesis which will induce metabolic syndrome (Sherwood, 2016). Insulin resistance that occurs due to high fructose intake can cause hypertension through its effect on nitric oxide (NO) levels in endothelial cells. Nitray oxide is produced in endothelial cells and regulates vasodilation. In a state of insulin resistance, there is a decrease in NO production due to an increase in reactive oxidative stress (ROS) which causes endothelial dysfunction and hypertension (Wiley & Sons, 2011).

CONCLUSIONS AND SUGGESTIONS

The administration of hypercaloric diet for 16 weeks orally can lead to metabolic syndrome in male rats Wistar.

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Conflict of Interest Statement

There is no conflict of interest in this study.

ABBREVIATIONS

BW	Bodyweight
CMC-Na	Sodium Carboxymethyl Cellulose
CoA	Coenzyme A
HDL	High Density Lipoprotein
VLDL	Verry Low Density Lipoprotein

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