THE EFFECT OF TRANS FATTY ACID DIET ON BLOOD GLUCOSE LEVEL OF WISTAR RATS

(A PRELIMINARY STUDY)

PENGARUH PEMBERIAN DIET ASAM LEMAK TRANS TERHADAP KADAR GLUKOSA DARAH PADA TIKUS WISTAR (SEBUAH STUDI PENDAHULUAN)

UNDERGRADUATE THESIS

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The Effect of Trans Fatty Acid Diet on Blood Glucose Level of Wistar Rats (A Preliminary Study)

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Abstract

Background: Trans fatty acids are unsaturated fatty acids that contain at least one double bond in the trans configuration and formed through the process of partial hydrogenation. It is suggested that trans fatty acid may increase the risk of the development of type 2 diabetes mellitus by worsening insulin resistance, thus increasing the blood glucose level. This study is aimed to identify and analyze the effect of trans fatty acid diet on blood glucose level of wistar rats.
Method: A Series of experiment using Pre and Post Test Randomized Controlled Group Design was carried out in six wistar rats which were divided into two groups, one group was control group, received standard diet and the other was intervention group, received additional orally 10 gr/day margarine containing 0.8% (of total energy) trans fatty acids per gram margarine. The blood sampling was obtained from the retro-orbital plexus before and after intervention to determine the blood glucose level of each wistar rats. Blood glucose level was measured with "GOD-PAP" method. The experiment was given for two weeks.
Result: Paired sample T-Test with p=0.056, showed there was an insignificant increment of blood glucose level in rats which have been given TFA diet for two weeks. The result of independent sample T-Test for control group and intervention group, p=0.658 has shown that the increment of blood glucose level between control group and intervention group was insignificant.
Conclusion: Intake of 10 gr/day margarine containing 0.8% (of total energy) trans fatty acids per gram margarine for two weeks did not significantly induce the increment of blood glucose level of wistar rats.
Key Words: Trans fatty acid, blood glucose level

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Pengaruh Pemberian Diet Asam Lemak Trans Terhadap Kadar Glukosa Darah Tikus Wistar (Sebuah Studi Pendahuluan)

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Abstrak

Latar Belakang: *Asam lemak trans merupakan asam lemak tidak jenuh yang memiliki minimal satu ikatan ganda di dalam konfigurasi trans dan terbentuk melalui proses hidrogenasi parsial. Penelitian-penelitian sebelumnya menunjukkan bahwa konsumsi asam lemak trans berhubungan dengan peningkatan resiko menderita diabetes mellitus tipe 2 melalui pengaruhnya terhadap resistensi insulin. Penelitian ini bertujuan untuk mengidentifikasi dan menganalisis pengaruh pemberian diet yang mengandung asam lemak trans terhadap kadar glukosa darah tikus wistar.*
Metode: *Penelitian ini adalah penelitian eksperimental dengan rancangan Pre and Post Test Randomized Controlled Group Design. Sampel terdiri atas enam ekor tikus wistar jantan yang dibagi menjadi kelompok kontrol dan kelompok perlakuan. Kelompok control diberi pakan standard, sedangkan kelompok perlakuan diberi pakan standar beserta 10 gram/hari margarin yang mengandung 0,8% (dari total energi) asam lemak trans dalam 1 gram margarin. Setiap tikus wistar diambil darahnya melalui pleksus retroorbita sebelum dan sesudah perlakuan, untuk diukur kadar glukosa darah dengan menggunakan metode "GOD-PAP". Penelitian dilakukan selama dua minggu.*
Hasil: *Uji T berpasangan dengan p=0.056 menunjukkan bahwa tidak terdapat perbedaan bermakna kadar glukosa darah tikus yang diberi diet asam lemak trans selama dua minggu. Uji T tidak berpasangan dengan p=0.658 menunjukkan bahwa tidak terdapat perbedaan bermakna antara peningkatan kadar glukosa darah pada kelompok control dan kelompok perlakuan.*
Kesimpulan: *Konsumsi 10 gram per hari diet margarine yang mengandung 0,8% (dari total energi) asam lemak trans dalam 1 gram margarin selama dua minggu tidak menyebabkan peningkatan kadar glukosa darah tikus wistar secara bermakna.*
Kata Kunci: *asam lemak trans, kadar glukosa darah*

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BACKGROUND

Intake of dietary TFA has been a controversy since several years ago. This is due to TFA adverse effect which may cause diseases such as coronary artery disease, diabetes, dyslipidemia, and heart failure.[1] TFAs are unsaturated fatty acids that contain at least one double bond in the trans configuration and are formed when liquid oils are converted into solid fats, such as shortening and hard margarines, through the process of partial hydrogenation. TFAs formed through this process are considered 'artificially' or 'industrially' produced.[2]

The major source of TFA in our diet are industrially produced and typically found in foods made with partially hydrogenated oil. These foods are fast foods (hamburger, pizza, breaded chicken nuggets), bakery products (doughnuts, cookies, cake, brownie, muffin), packaged snack foods, fried potatoes (appetizer), margarines, and crackers.[3,4,5]

TFAs are also found naturally in meat and dairy products from ruminant animals as a result of normal microbial action in the animal's intestinal tract. These TFAs do not appear to be associated with the health issues attributed to artificially created TFAs. Structural differences, such as the position of the double bonds between artificially created and naturally occurring TFAs, can impact health differently.[6,7]

The mayor source of trans fatty acid diet in Indonesia is fries food consumption ('konsumsi gorengan'). The consumption of fries food is relatively high in Indonesian. The relationship between fries food consumption and TFA intake is based on the evidence that using cooking oil (vegetable oil) for frying more than twice may increase TFA level in the cooking oil. Lacking these information and the high price of the cooking oil itself make many people in Indonesia keep using cooking oil for frying more than twice.[8,9]

High intake levels of industrially produced TFAs may increase the risk of heart disease .[10,11] Other recent studies have led to a justified suspicion that trans fatty acids increase the risk of the development of type 2 diabetes.[12, 13]

Insulin resistance is a growing worldwide phenomenon, which progressively develops over years, and finally, if unchecked, predisposes to

cardiovascular disease and diabetes mellitus type 2. Insulin resistance is a generalized metabolic disorder characterized by insufficient insulin function in skeletal muscle, liver and adipocytes.[14,15]

Controlled studies and observational studies suggest that TFA may worsen insulin resistance, particularly among predisposed individuals with risk factors, for example, preexisting insulin resistance, visceral adiposity or lower physical activity.[13,16] The purpose of this study is to identify and analyze the effect of trans fatty acid diet on blood glucose level of Wistar rats. This research will use Wistar rat because it has been used in previous medical researches which also investigate the administration of trans fatty acid in rats.

RESEARCH METHOD

This study took place in Laboratory of Biochemistry, Medicine Faculty of Diponegoro University at Semarang. The experiment and data collecting were done in two weeks, in July 2011. The type of experimental that was done in this study was the pre and post test randomized controlled group design, by using Wistar rats as the objects. Population in this study was Wistar rats from Laboratory of Biology, Universitas Negeri Semarang. According to WHO formula, the sample size in this study was minimum five in each group. This study used six Wistar rats, that are divided into two group randomly, each group consists of three rats. Sample in this study was gained by consecutive random sampling with these inclusion criteria: male wistar rats, body weight = 180-230 grams, 6-weeks of age, there is no anatomical abnormality. The exclusion criteria were: there is no active movement, there is diarrhea during experiment, there is weight loss (body weight less than 180 grams), dead during experiment.

At the beginning of this study there were 17 rats, 12 rats were used as the sample and 5 rats as a reserved rats. In the period of this research work, 1 rat from control group and 5 rats from intervention group were died. These died rats were excluded and replaced with 5 new rats. However, 3 of these new rats were died, then there were only 3 rats in intervention group can be kept for two weeks of research.

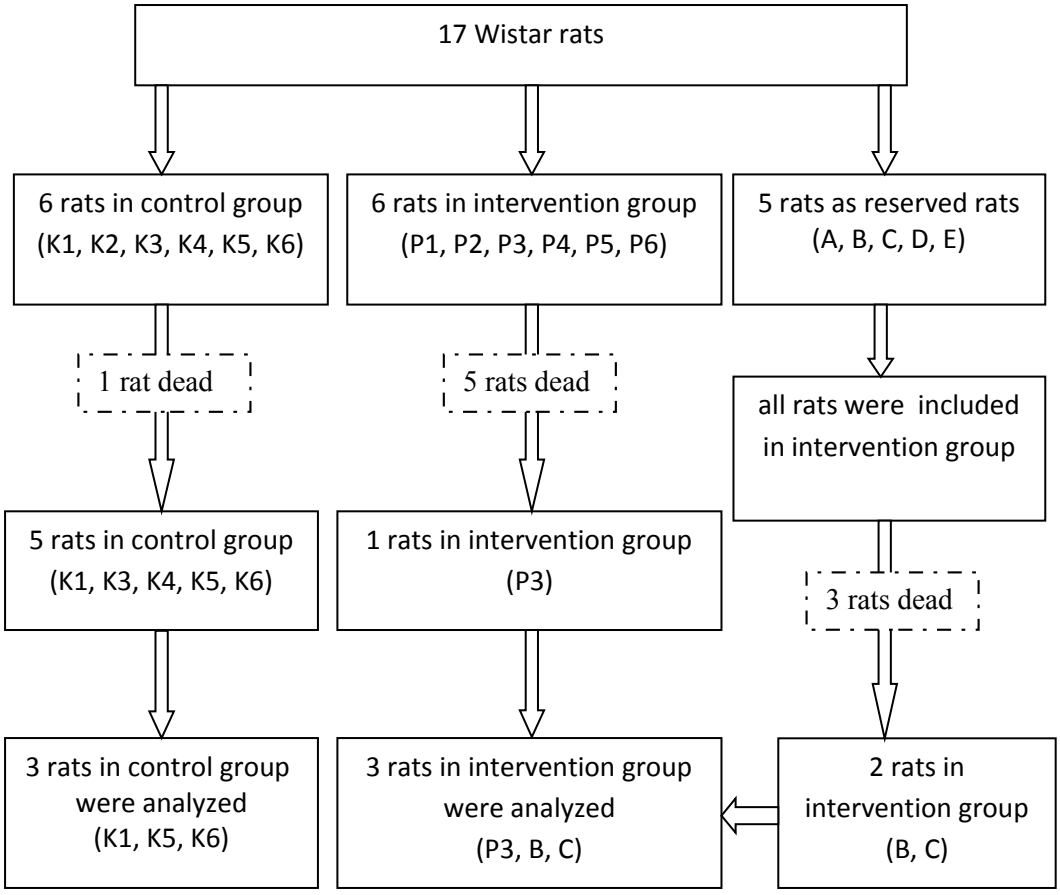

Figure 1 The flowchart of sample analysis during the period of research

Male Wistar rats, 8 weeks of age, were allowed free access to standard, semipurified chow diet and water upon arrival and are individually housed under controlled conditions for one week (12:12 h light–dark cycle; 50–60% relative humidity, 22–23 °C). The blood sampling was obtained from retro-orbital plexus and the blood glucose level is measured by GOD-PAP method.

All Wistar rats (n =12 rats) is divided into two group (control group and intervention group) randomly. Each group consists of six rats. Rats in control group were administered with standard diet while rats in intervention group were administered with 10 gram margarine containing 0,8% per gram TFA for two weeks. After two weeks, the blood glucose level of Wistar rats were measured

again to observe the effect of dietary trans fatty acid on blood glucose level of Wistar rats.

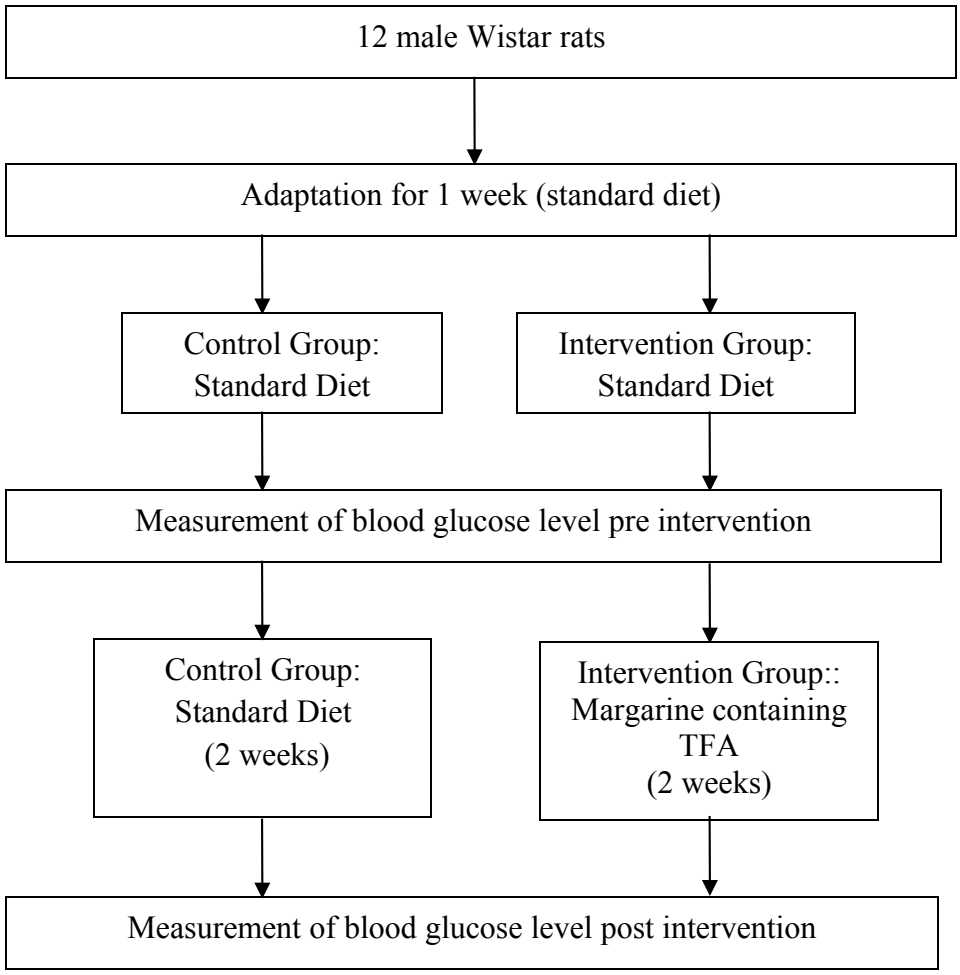

Figure 1 Research Flow

The data of this study were performed with test of normality using the *Shapiro-Wilk* method to determine whether the data had a normal distribution or not, then used to determine parametric test or nonparametric tests that would be used. Test of hypothesis were performed to determine how the effect of trans fatty acids increase the blood glucose levels rats. According to *Shapiro-Wilk* test, the distribution of data was normal then the data was processed with statistical parametric test, *paired t test*. The next stage was to perform an *independent t test* to determine whether the intervention group experienced a significant increase in glucose levels compared with the control group. The obtained data were analyzed

by using SPSS 19.00 for windows. True confidences of this test is 95%. It can be concluded there is significant difference if $p < 0.05$.

RESULT

Research data obtained from this study are as follows:

Table 5.1 Effects of trans fatty acids on blood glucose levels of wistar rats after intervention for two weeks

Group	Blood Glucose Level (mg/dl) Before Intervention	Blood Glucose Level (mg/dl) After Intervention	The Difference of Blood Glucose Level (mg/dl) Before and After Intervention
Control (n=3)	73.000 ± 8.2286	96.067 ± 7.4842	23.0667 ± 0.86217
Intervention (n=3)	63.067 ± 12.5540	89.500 ± 23.6246	26.4333 ± 11.3298

Data of blood glucose levels are mean ± SD

From the analysis of *Shapiro-wilk*, the value of *significancy p* obtained for blood glucose level of intervention rats before and after intervention, and the difference of blood glucose level of both control and intervention rats before and after intervention are > 0.005, then it can be concluded that the distribution of data on these variables is normal.

According to *Shapiro-Wilk* test, the distribution of data is normal then the data was processed with statistical parametric test, *paired t test*. Based on paired t test analysis, the average blood glucose levels in the intervention group after being fed trans fatty acids (89.500 ± 23.6246 mg/dl) is not significantly increased compared to before administration of trans fatty acids (63.067 ± 12.5540 mg/dl) with a value of *significancy,* p>0.005 (p=0.056).

The next stage is to perform an *independent t test* to determine whether the intervention group experienced a significant increase in glucose levels compared with the control group. Based on the analysis of independent t test, by comparing the difference of blood glucose levels before and after intervention between control group and treatment group, the value of *significancy* p=0.658 (p>0.05). It

showed that there was no significant increase in blood glucose levels in the administration of trans fatty acids for two weeks.

Table 5.2 Value of the difference of blood glucose level before and after TFA feding for two weeks

Group	Mean ± SD
Control	23.0667 ± 0.86217
Intervention	26.4333 ± 11.3298

The table shows that the increment of blood glucose levels in intervention group was higher than the increment of blood glucose levels in control group after administration of trans fatty acids for two weeks. However, according to the analysis of independent t test, the difference of the blood glucose level between before and after intervention in both control and intervension group is not significant ($p=0.658$; $p>0.05$).

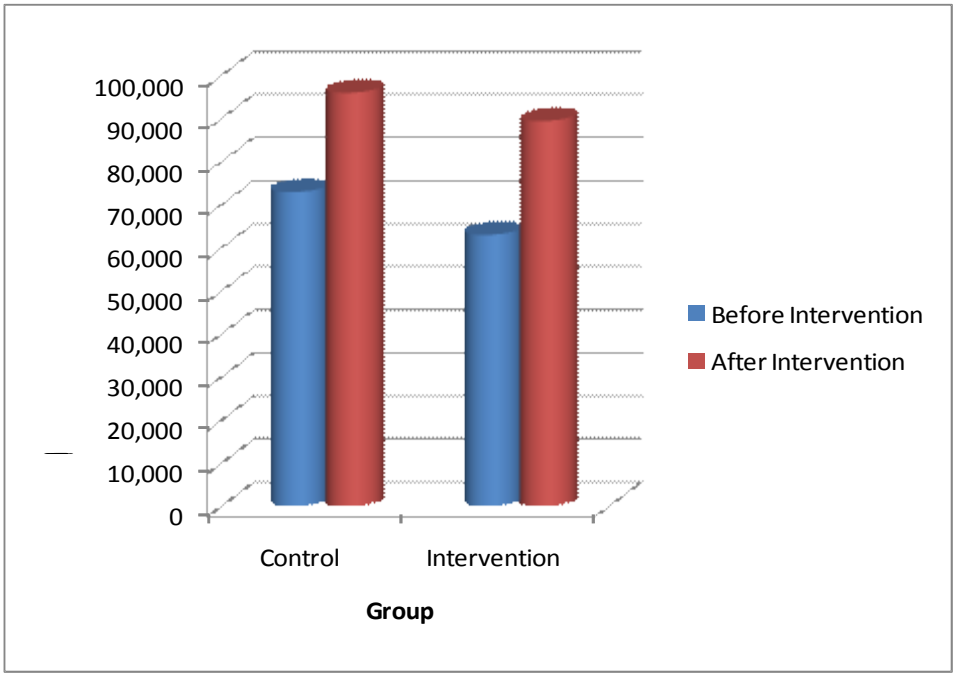

Figure 5.2 The Effect of trans fatty acid diet for two weeks on blood glucose level of wistar rats

Based on the results above, trans fatty acids are not shown to increase blood glucose levels of wistar rats. The increment of blood glucose levels in groups fed with trans fatty acids compared with the increment of blood glucose levels in groups of rats which have not been fed with trans fatty acids is not significant. However, there are still limitations in this study that will be described in the discussion session.

DISCUSSION

Most trans fatty acids in Indonesian diet are found in fries food (*gorengan)* and fast foods.[3,8,17,18] Dietary fatty acids have been shown to lead to insulin resistance and glucose metabolism changes. Intake of these fatty acids is known to have detrimental effects on glucose metabolism and insulin resistance through several mechanisms. It is widely recognized that both the amount and type of dietary fatty acids modify insulin sensitivity in muscle, liver, and pancreas of experimental animals. In addition, intracellular glucose metabolism suppression might precede and/or cause insulin resistance of rats fed with a high-fat diet.[15,19,20,21]

Fatty acids may induce the activities of Acetyl-Coenzyme Carboxylase 1 (ACC1) and Acetyl-Coenzyme Carboxylase 2 (ACC2) in animal tissues. ACC1 and ACC2 are two distinct enzymes that evolved in fatty acid synthesis and oxidation. By inducing the activity of ACC1and ACC2, the translocation of GLUT4 to the plasma membrane is decreased, thus lowering glucose uptake and increasing the plasma blood glucose level.[21,22]

Increased fatty acids consumption is associated with the development of obesity and the reductions in insulin release. In obese individuals, adipose tissue releases increased amounts of proinflammatory cytokine, such as TNF-α and leptin, which are clearly involved in the induction of insulin resistance. TNF-α and leptin decrease the insulin sensitivity and reduce the activity of GLUT4,

thereby lowering glucose uptake and increasing the plasma blood glucose level.[15,23]

Increased fatty acid intake leads to increased triglyceride storing that results in net spillover of fatty acids to nonadipose tissue, which further increases extraadipocytic triglyceride storage, such as muscle, liver, and pancreas. Triglyceride accumulation in pancreas induces β-cell dysfunction and reduces insulin clearance in liver. These mechanisms lead to insulin resistance and type 2 diabetes mellitus.[15,20]

In view of these assumptions, this study evaluated the blood glucose level of wistar rats after fed with margarine containing 0.8% per gram TFA for two weeks. The variables were: (1) dietary margarine containing 0.8% per gram TFA; (2) the blood glucose level of wistar rats. Retro-orbital blood samples for measurement of glucose were collected at the start of the experiment and 2 weeks thereafter. Serum after centrifugation at 3000 g at 4°C assayed for biochemical analysis by Hettich MIKRO-200 then were determined using GOD-PAP method.

The major findings of this study showed TFA did not induces significant increment of blood glucose level in rats which have fed with TFA for two weeks. This result did not give evidence that intake of high level of TFA is associated with increment of blood glucose level. The TFA used in this study was *MeadowLea original* margarine, which containing 0.8% per gram TFA.

The result of this study showed that blood glucose level was not significantly different between intervention and control group. The increment of blood glucose level was higher in intervention group (26.4333 ± 11.3298) than the increment of blood glucose level in control group (23.0667 ± 0.86217) but was not indicated as significant difference ($p=0.658$; $p>0.05$).

The results indicated that TFA diet might not increase the blood glucose level of wistar rats significantly. This result may be consistent with previous results from Huang Z *et. al.* Two groups of Wistar rats were fed with a diet containing 4.5% trans fat or a control diet containing no trans fat for 16 weeks. Blood glucose level was monitored every 2 weeks. The plasma glucose level, insulin level, and insulin sensitivity index were not significantly different between

the trans fat and control groups. The results indicated that trans fat intake might not be related to insulin resistance.[24]

Another study by Bernal CA *et al* examined whether the level of dietary cis fatty acid (cFA), or the isomers (trans or cis) and/or the saturation of the fatty acids at high dietary fat levels altered the intracellular glucose metabolites and certain regulatory enzyme activities in the skeletal muscle and liver of rats. The animals were fed for 30 days on either a recommended control diet or a high-fat diet. The high-fat diet was enriched with either cFA, trans fatty acid (tFA), a moderate proportion of saturated fatty acid (MSFA), or a high proportion of saturated fatty acid (HSFA). One of the conclusions in this study show the amount TFA led to no great differences in glucose metabolism as compared with the respective control group.[25]

A study that investigated the metabolic effect of dietary TFA in Sprague-Dawley rats by Suzanne ED *et al* show that a low-fat diet enriched with TFA were associated with hyperphagia, increased hepatic and visceral fatness, and diminished whole-body glucose disposal. This study as well concluded that TFA are uniquely handled by liver, adipose, and muscle in such a way as to induce mild insulin resistance by undiscovered mechanisms.[26]

Study population conducted by Hu FB *et al* suggested the intake of trans fatty acids was significantly related to the risk of diabetes among 84,941 female nurses who were followed for 16 years and in whom self-reported diabetes was validated and information on dietary intake was periodically updated.[27]

The insignificant increment of blood glucose level of wistar rats in intervention group might because of insufficient sample size (n=3). According to WHO formula, the sample size in this study is minimum five in each group. However, some rats were died during the research work unknownlingly. The period of intervention with TFA diet (2 weeks) might be an insufficient time to induce an increment of blood glucose levels of wistar rats. In addition, 8% TFA diet per day for two weeks might not have been able to increase blood glucose levels of wistar rats significantly.

This result is presumably as well because of unpredictable metabolic and hormonal factors of these wistar rats. The homeostatic mechanism of rat keeps blood glucose levels within a normal range. It is composed of several interacting systems, of which hormone regulation is the most important. Liver, extrahepatic tissue, and several hormones (insulin, glucagon, cortisol, catecholamine) play important roles in the regulation of blood glucose levels.[15,20,28]

A variety of physiological stressors such as hypoglycemia, hypoxia, trauma, illness, pain, or fear can affect blood glucose level. Physiological stress may induce release of epinephrine hormone (one of catecholamine hormones) from adrenal medulla and this hormone will stimulate glycogenolysis in the liver, thereby increase blood glucose level.[29,30]

In spite of the conclusion of this study show that TFA diet did not increase blood glucose level of wistar rats significantly, further research on the effects of TFA diet on blood glucose level should be done to evaluate the mechanism between dietary TFA intake with the increment of blood glucose levels.

Conclusion

According to this study result, intervention with 10 gram margarine containing 0.8% TFA per 1 gram margarine for two weeks did not increase the blood glucose level of wistar rats significantly. In addition, the increment of blood glucose levels in groups fed with trans fatty acids compared with the increment in groups of rats which have not been fed with trans fatty acids was not significant.

Suggestion

Further research on the effects of TFA diet on blood glucose level should be done with more sample size, different dose of TFA, and longer period of research thereby the mechanism between dietary trans fatty acids with the increment of blood glucose levels can be clearly explained.

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