The Effect of Vitamin A on Insulin Sensitivity in Mice Models of Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disease caused by elevated levels of blood glucose (hyperglycemia) due to the degradation of the sensitivity of peripheral tissue to insulin (insulin resistance). The state of chronic hyperglycemia leads to the formation of free radicals causing oxidative stress through multiple pathways that can increase the severity of diabetes. Oxidative stress causes a decrease in insulin sensitivity. Vitamin A as an antioxidant is thought to improve insulin sensitivity. The purpose of this study is to determine the effect of vitamin A supplementation on insulin sensitivity in T2DM. Research using experimental study with post test only randomized controlled group design in male Wistar rats (Rattus norvegicus). The samples is divided into 5 groups, each comprises 4 rats, namely negative control (KN), positive control T2DM model (KP), the treatment group of T2DM that were given vitamin A dose of 50 mg/kg (VAP1), a dose of 100 mg/kg (VAP2), and a dose of 150 mg/kg (VAP3). Insulin sensitivity index (ISI) was measured using the formula of QUICKI by involving insulin levels and fasting blood glucose levels. The data was analyzed by using ANOVA and Post Hoc Test. The results showed ISI group KN higher than KP group ($p=0.329 > p=0.276$). There was an increase in ISI after the administration of vitamin A between treatment group of vitamin A and KP group, but it was not different significantly ($p=0.087$), as well as KN and VAP1 group ($p=0.043$). The conclusion of this study is that vitamin A affects insulin sensitivity in T2DM, but the data is not statistically significant.

Keyword: vitamin A, insulin sensitivity, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a metabolic disease that is characterized by elevated blood sugar level or hyperglycemia accompanied by impaired metabolism of carbohydrate, fat, and protein.[1] The hormone of insulin that is released by the pancreas is the main substance in the defense of blood sugar level. The decrease of insulin due to the damage of pancreas can cause the elevated of glucose in the blood.[2] In general, there are several types of diabetes mellitus (DM), namely type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus, and other types of diabetes mellitus. The most common type is type 2 diabetes mellitus. Type 2 diabetes mellitus or also called Non-Insulin-Dependent Diabetes Mellitus (NIDDM) has onset in adults.[3]

In 2010, the report of global status NCD World Health Organization (WHO) reported that as many as 60% dead cause of all ages in the world is due to non-communicable diseases. DM has ranked the 6th leading cause of death. About 1.3 million people has died from diabetes and 4% died before the age of 70 years.[4] WHO projects that diabetes will be the 7th leading cause of death in 2030. More than 80% of diabetes death occur in middle- and low-income countries.[5] WHO predicts a rise in the number of people with DM from 8.4 million in 2000 to 21.3 million in 2030. International Diabetes

Federation (IDF) in 2009 also predicted a rise in the number of people with DM from 7 million in 2009 to 12 million in 2030.[6]

Some experts have revealed some relationship between vitamin A with a diabetic condition. In the plasma, the concentration of vitamin A (retinol), retinol-binding protein (RBP), and transthyretin (TTR) is decreased in human subject with diabetes.[7] The vitamin and mineral are including micronutrient group. The micronutrient has been investigated as a potential preventive and therapeutic agent for type 1 diabetes, type 2 diabetes, and general complication of diabetes.[8] Vitamin A, C, and E act as antioxidant. On the subject of diabetes, the vitamin antioxidant declines because of the increased need to control the excess oxidative stress produced by the abnormality in metabolism of diabetic glucose.[9] In children with diabetes, there is a significant decrease of erythrocyte glutathione, total glutathione, α-tocopherol plasma, and β-carotene plasma. The decline of antioxidant is related to the compound formation as a marker of oxidative stress.[10]

According to Dr. Lorraine Gudas, the chairman of the Department of Pharmacology at Weill Corner – along with his colleagues, the activity of β cell can be induced by vitamin A, in other words, vitamin deficiency can increase the development of type 2 diabetes mellitus.[11] Based on study conducted by Dr. Jean Louis Chiasson, retinoic acid induces the normalization of blood glucose and reduction of obesity, with the result showing a new metabolic effect on retinoid and it enables as an antiobesity and antidiabetic medicine. The deficiency of vitamin A dietary can lead to decreased vitamin A of pancreas, hyperglycemia, as well as decreased insulin secretion.[12]

From researches that have been done above, there is no study showing how the effect of vitamin A to insulin sensitivity in people with type 2 diabetes mellitus. Therefore, the purpose of this study is to determine the effect of vitamin A supplementation on insulin sensitivity in male Wistar rats (*Rattus norvegious*) with type 2 diabetes mellitus. The vitamin A supplementation is expected to increase the insulin sensitivity in type 2 diabetes mellitus.

RESEARCH METHOD

Research Design

This study uses true experimental design in vivo in laboratory using Randomized post test only controlled group design, comparing the result obtained after treatment with the control group.[13]

Tools and Materials

The tools that used in this research are cage, water bottle, weigher, measuring cup, gauge for measuring FBG level (*Easy Touch*), blender, syringes, eppendorf, and vacutainer. The materials that used are tablet of vitamin A (IPI 6000 IU) with a dose of 50 mg/kg body weight, 100 mg/kg body weight, 150 mg/kg body weight, normal diet, high-fat diet, drinking water, and husk.

Research Subject

The subjects are white Wistar rats (*Rattus novergicus*) with the inclusion criteria as follows: male, aged 6-8 weeks, weighing 120-160 grams, in a healthy condition characterized by an active movement, and has white and clean fur, while the exclusion criteria in this study is white rat that dies during the study period.

The sample is devided into 5 groups, as follows:

1. The negative control group (KN): given normal diet
2. The positive control group (KP): given a high-fat diet and injected by STZ
3. The treatment group I (VAP1): given a high-fat diet, injected by STZ, and given vitamin A with a dose of 50 mg/kg body weight
4. The treatment group II (VAP2): given a high-fat diet, injected by STZ, and given vitamin A with a dose of 100 mg/kg body weight
5. The treatment group III (VAP3): given a high-fat diet, injected by STZ, and given vitamin A with a dose of 150 mg/kg body weight

The rats are kept in a cage with given husk and drink. In the beginning, the adaptation is conducted by giving a normal diet as much as 25

grams for each rat in the first week for all groups. The treatment of experimental animal is in accordance with the Ethical Clearance No. 142/EC/KEPK/05/2016.

The Provision of High Fat Diet

In the second week, it begins to give a high fat diet of 25 grams/rat in the positive group (KP) and the treatment group (VAP1, VAP2, VAP3). High fat diet is made with the composition of BRI 221.75 grams, 123.25 grams of wheat flour, 0.098 grams of cholic acid, 7.105 grams of cholesterol, and 184.25 grams of lard.[14]

Streptozotocin Injection (STZ)

In the 7th week, the injection of STZ 30 mg/kg body weight is conducted via intraperitoneal. The steptozotocin (Calbiochem, No. Catalog 572201) 100 grams is dissolved into 3 ml citrate buffer pH 4.5, then in the vortex until homogenous, so that it results STZ stock solution. The solution of STZ stock is stored at 40ºC.[14,15]

The Measurement of Fasting Blood Glucose (FBG)

After being injected with STZ, 1 week later (week 8) the blood glucose level is measured to confirm to state of type 2 DM.[15] The rat is considered to have DM because of the FBG > 126 mg/dl and has symtoms of polyuria, poliphagia, polydipsia, and weight loss. The second measurement of FBG is at the end of week 11 (after vitamin A supplementation). This is to determine the improvement of the insulin resistant state.

The blood glucose level is measured through the blood of rat's end tail (lateral vein). The blood glucose is measured by using a tool (*Easy Touch*) and strips for blood glucose measurement.

Vitamin A Supplementation

Vitamin A (IPI 6000 IU) is smoothed by using a blender. Vitamin A supplement is given by using such a rubber tube (vitamin A is reconstituted with 2 ml of distilled water) according to the dosage of each treatment group. The vitamin A supplementation is given for 4 weeks after the first FBG measurement (week 8 until week 11).

Blood Sampling and Measurement of Insulin Level

At the beginning of 12th week, before getting dissection the rat is injected with 0.2 ml ketamine (100 mg/ml). Then the blood sampling is done via intracardiac with a syringe and inserted into vacutainer and centrifuged for 10 minutes at speed of 3000 rpm. The serum which has been separated is inserted into the eppendorf.

The next step is measuring the insulin serum level by using immunoassay method, namely ELISA (Enzyme-Linked Immunoabsorbent Assay, Rat Insulin ELISA Kit (RayBio), No. Catalogue ELR-Insulin).

The Measurement of Insulin Sensitivity Index (ISI)

By knowing the result of insulin serum measurement which is obtained from laboratory, we can get the insulin sensitivity index by using the formula QUICKI (Quantitative Insulin Sensitivity Check), in which the index is based on mathematical formula as well as logarithmic function of insulin and fasting blood sugar level on the individual whose the insulin sensitivity is measured. The formula of QUICKI as follows:

$$\frac{1}{\log\left(fasting\ insulin \frac{\mu U}{mL}\right) + \log\left(fasting\ glucosa \frac{mg}{dL}\right)}$$

The Data Analysis

The data is analyzed by using program of SPPS for windows Versi 16.0. The result and the treatment of control rat measurement are analyzed statistically with a level of confidence of 95% (α=0,05). The data is first tested by using normality and homogeneity test. If the data was normally distributed and homogeneous ($p > 0.05$), then it would be continued with *One Way* ANOVA and *Post Hoc* LSD test to determine the significant differences between groups.

RESEARCH RESULT

The Characteristics of Experimental Animal

The result of rat's body weight measurement is conducted to the 5 treatment groups which is taken an average value of each week, during 11 weeks of treatment. The average weight measurement of each group as follows:

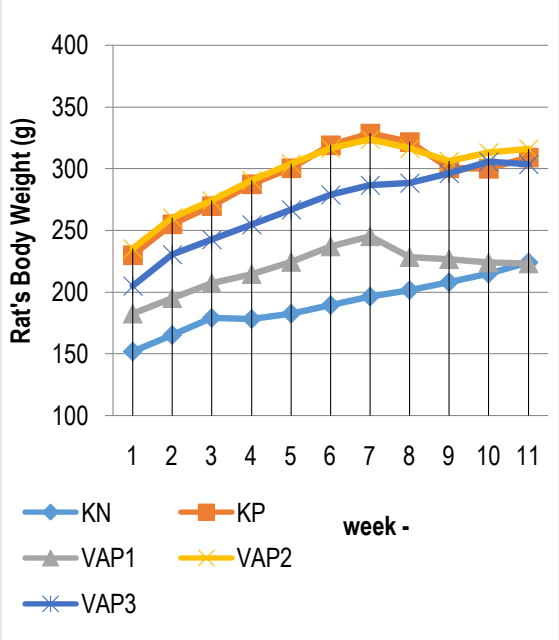

Figure 1. The Average of Weight Measurement

Fasting Blood Glucose

The normal fasting blood glucose level in rat is ranged from 90-110 mg/dl.[17] Below is the average of fasting blood glucose before and after the treatment of each group:

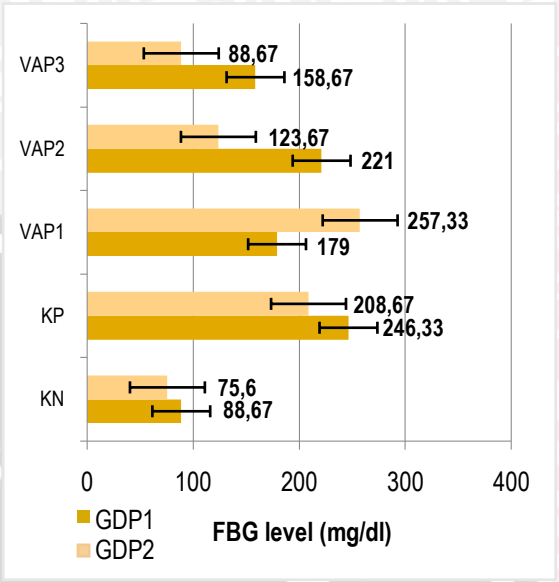

Figure 2. The Average of Rat's Fasting Blood Glucose Before and After the Treatment

The Level of Insulin Serum

The average of insulin of each group as follows:

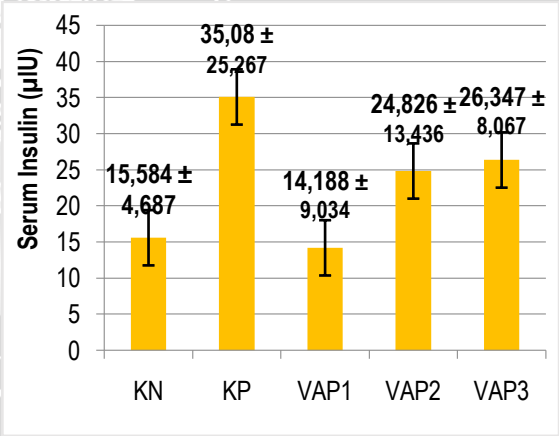

Figure 3. The Average of Insulin After the Treatment

The Index of Insulin Sensitivity

Below is theaverage of insulin sensitivity index (ISI) of each group:

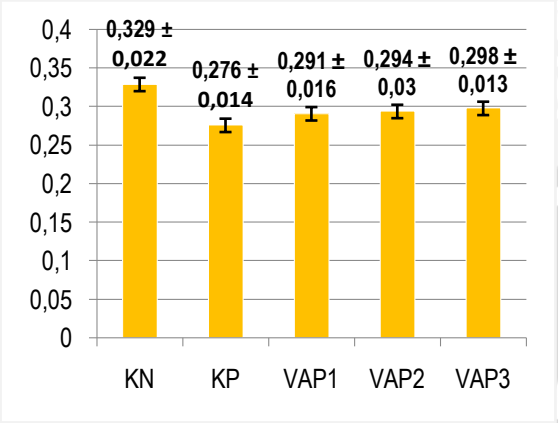

Figure 4. The Average of Insulin Sensitivity Index

The data is then tested for the normality by using Kolmogorov-Smirnov and the result shows that the data is distributed normally with KN p=0.797; KP p=0.752; VAP1 p=0.891; VAP2 p=0.831; VAP3 p=0.59 (p > 0.05). Next, it is proceed with the homogeneity test using *Levene Test* and the results indicates that the data is homogeneous with p=0.642 (p > 0.05). Because the data obtained is distributed normally and homogeneous, it can be used a statistical test of comparison with parametric method, namely *One Way* ANOVA. In *One Way* ANOVA test, the result shows that the treatment did not give significant difference to the insulin sensitity index (0.087 > 0.05).

Table 1. The Result of *Post Hoc* LSD Test Analysis

	KN	KP	VAP1	VAP2	VAP3
KN		0,009	0,043	0,063	0,094
KP	0,009		0,392	0,290	0,204
VAP1	0,043	0,392		0,828	0,652
VAP2	0,063	0,290	0,828		0,814
VAP3	0,094	0,204	0,652	0,814	

DISCUSSION

The Effect of High Fat Diet and STZ Injection to the Body Weight and Blood Glucose

Based on the study result, the body weight of all groups is consistently increased in 2nd week to 7th week by giving high fat diet to the positive control group and treatment group, whereas normal diet to negative control group, so that the body weight of the KN group is lower than the other groups. Feeding a high fat diet is a method to increase body fat. In average, the groups that are given high fat diet have higher level of total cholesterol, LDL cholesterol, triacylglycerol (TG) than those that are given standart diet.[18] Providing high fat diet can be used to initiate insulin resistance which is an important feature of type 2 DM, or it can be concluded that a high fat diet can be used as an agent of prediabetes.[15]

The change in body weight is influenced by the amount of energy expended against the amount of the used energy. Therefore, if the energy expenditure is low, while the diet consumed is excess, there will be an increase in the body weight.[19] In the 8th week, based on the data, the rat's body weight decreases. The weight loss is one of the symptoms of type 2 DM, after examinating the previous blood glucose (FBG1). In the people with uncontrolled diabetes, it can lead to the weight loss. It is caused by the process of lipolysis. Lipolysis is the decomposition or breakdown of fat, which produces FFA (free fatty acid). The spending of FFA from adipose tissue/fat is influenced by several hormones that affect the rate of esterification/lipolysis, one of them is insulin hormone. The insulin obstructs the release of free fatty acid from adipose tissue followed by a decrease in free fatty acid in plasma. The increase of FFA in plasma strengthens the development of insulin resistance and improves the gluconeogenesis in hepatic, accelerates the hyperglycemia and the FFA oxidation can increase ROS (Reactive Oxygen Species) which causes the damage of endotel.[20]

The Effect of High Fat Diet and STZ Injection to the Insulin Sensitivity

From the research that has been done, it shows that there is a significant decrease in insulin sensitivity index (ISI) in which the lowest average is in the positive control group, that is 0.276. ISI is an index that shows how the body responds to the insulin that that functions as a hormone that controls glucose in the plasma to be absorbed by the body cells. The low of ISI also indicates the body sensitivity to the insulin is low or resistant to the insulin. This is the mechanism of the occurence of type 2 DM.[21]

The high fat diet causes the hyperinsulinemia which will cause an increase in the working of the part of adipocytes leading to hypertrophy. Then it will form a new adipocytes continuously until it becomes adipocytes hyperplasia and FFA will be increased. The increase of FFA in the plasma will affect the working of insulin, lower the uptaking of glucose, glycolysis, and glycogen synthesis. Based on the previous research, the giving of high fat diet for 8 weeks can lead to the insulin resistance due to the accumulation of visceral fat, causing an increase of FFA in the hepatic, triglycerides circulation, and also the production of hepatic glucose.[22]

STZ is a substance that can interfere the metabolism of insulin, so that it can occur diabetes mellitus by damaging the cells of pancreas. STZ works by preventing the synthesis of DNA. STZ enters the cells of pancreas through the GLUT2 (Glucose transporter 2) and it causes the alkalization on DNA. In order to induce the diabetes, STZ will induce the activation of ribosylation of poliadenosinediphospate and release of the nitric oxide that causes the damage of pancreas cells.[23,24]

The Effect of Vitamin A to the Insulin Sensitivity

Based on the study result, the therapy with vitamin A supplementation has a positive impact in improving the insulin sensitivity that has been injected by STZ. This is consistent with the statement that the retinol target in type 2 diabetes mellitus is free radical and gene involved in the metabolism of insulin and obesity. The vitamin A is a powerful antioxidant that has the potential to alter the gene expression. In addition, vitamin A regulates the release of insulin and energy homeostasis.[25]

The formation of free radical occurs in diabetes mellitus type 2 through the process of hyperglycemia. Hyperglycemia causes the nonenzymatic protein glycation (advanced glycation end products = AGEs), pathway of sorbitol polyol (aldose reductase), pathway of protein kinase C (PKC), and glucose autooxidation. It is a process that can accelerate the formation of reactive oxygen compounds that causes an increase in modification of lipid, DNA, and protein networks. That modification results the inequality between the protective antioxidant and an increase of free radical production. It is the beginning of the formation of oxidative damage (oxidative stress). Besides, the oxidative stress has also contribution to the deterioration and development of complication of DM.[26] Vitamin A in the form of retinoic acid (RA) can detain or decrease the activity of protein kinase C (PKC) pathway. If the activity of PKC pathway is detained, it will add the insulin sensitivity.[26,27]

Based on the analysis using statistical test *One Way* ANOVA, it shows that the insulin sensitivity index (ISI) is not significantly different to the average ISI of white rat per group (0.087 > 0.05). It could be because one of them is the variety of data that is too large due to the food supply (high fat diet) is not consumed as a whole according to the proportion. Based on the analysis test *Post Hoc* LSD shows that there is a significant difference in the negative control group (KN) with vitamin A treatment group with a dose of 50 mg/kg body weight (VAP1), ie 0.043 ($p < 0.05$). Vitamin A with a dose of 50 mg/kg body weight is minimal dose in this study, in the calculation, the average body weight of rat is ± 300 grams (0.3 kg), so that the dose in the group VAP1 is 15 mg (50,000 IU).

In the previous study, the vitamin A supplementation with a dose of 100,000 IU (30 mg) on alternate days for five days in rats resulted a decrease in vitro of lipid peroxidation in the network,

but the excessive supplementation can cause toxic effect.[28] The dose of acute toxic of vitamin A is 25,000 IU/kg body weight, while the chronic toxic dose of vitamin A is 4000 IU / kg body weight.[29] It is proved that with a low dose (15 mg) is sufficient to provide a significant difference to the negative control group (KN).

CONCLUSION

The conclusion from this research as follows:

1. The insulin sensitivity index in the positive control group of rat (KP) is lower than the negative control group (KN), but it is not significant. The insulin sensitivity index in rat of treatement group (VAP1, VAP2, and VAP3) is lower than the negative control group (KN) and higher than the positive control group (KP), but it is not significant.
2. The vitamin A can improve the insulin sensitivity index in rat with type 2 diabetes mellitus model, in which the VAP1 group gives significant difference to the negative control group (KN).

SUGGESTION

The suggestion that can be drawn from this research as follows:

1. Further research is needed by using other parameters, such as HOMA (Homeostatic Model Assessment), a method for assessing the β cell function and insulin resistance (IR) by involving insulin and blood glucose.
2. It is required prior insulin sensitivity measurement before doing a treatment to the experimental animal.

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