THE EFFECT OF INTERMITTENT FASTING ON TRIGLYCERIDE LEVELS IN THE WISTAR STRAIN WHITE RATS *(RATTUS NORVEGICUS)* DIABETES MELLITUS MODEL

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ABSTRACT

Background *: In Indonesia, there is an increasing population prevalence with abnormal triglyceride levels. Reducing triglyceride levels in the blood can be done in various ways including taking drugs andchanging healthy lifestyles. Diet intervention methods that can be done are very diverse, one of which is Intermittent Fasting 5:2.*

Methods*: Experimental it uses this type of analysis quantitative laboratory research in vitro using a pre-test and post-test group design. The population used in this study were 30 (Rattus norvegicus) Wistar with a body weight of 150-250 grams. Which were divided into 3 group,*

Results*: From the results of the paired T-Test. it prove that there was no significant difference in treatment groups A, B and C which was marked by the statistical test results obtained p> 0.05 (not significant).*

Conclusion*: There was no difference in triglyceride levels before and after treatment with intermittentfasting type 5:2 white Wistar rats.*

Keywords*: Triglyceride levels, intermittent fasting 5:2, Wistar rats*

INTRODUCTION

Triglycerides are a type of fat that is found in the organs of the body and also in the bloodstream. Triglycerides are formed from fat and glycerol which come from food consumed using insulin stimulation or excess calories due to excessive eating. Excess food calories are converted into triglycerides and stored under the skin as fat. Based on Basic Health Research (RISKESDAS) in 2018, the proportion of triglyceride levels in the population ≥15 years 13.3% are included in the high borderline category (150-199 mg/dl), 13.8% are included in the high category (200-499 mg/dl) and as much as 0.8% are included in the very high category (≥500 mg/dL)[2].

Reducing triglyceride levels in the blood can be done in various ways including taking drugs and changing healthy lifestyles. Changes in healthy lifestyles such as setting a food diet and setting physical activity. Diet intervention methods that can be done are very diverse. One of the Intermittent Fasting methods that is often used is Intermittent Fasting type 5: 2, namely by limiting intake for 2 days a week, of which 2 days are fasting and the other 5 days are not fasting. This

fasting method has many effects including anti-inflammatory effects, can increase insulin sensitivity, and prevent cardiovascular disorders Research conducted by Ruth E. Patterson and Dorothy D. Sear from the University of California with the research title Metabolic Effects of Intermittent Fasting in 2017 stated that Intermittent Fasting in rats is proven to reduce total cholesterol in the blood and reduce triglyceride concentrations in the blood. Also, based on research by Ayudia EI, regarding the Effect of Intermittent Fasting on triglyceride levels in Sprague Dawley white rats in 2021 obtained significant results for reducing triglyceride levels in the blood.

METHOD

This study used a type of in vitro experimental laboratory quantitative analysis using a pre-test and post-test group design. Data collection was carried out before and after the treatment was carried out by comparing the results in the control group and the treatment group. The research was conducted in two places, namely: the Biomedical Laboratory, Faculty of Medicine, University of Jambi, in the intermittent diet treatment and monitoring stages. As well as at the Jambi Provincial Health Office Laboratory in the process of collecting sample data and measuring Serum triglyceride levels of Wistar rats. The population used in this study were 11 male Wistar white rats (Rattus norvegicus) with a body weight of 150-200 grams obtained from the Faculty of Medicine and Health Sciences, University of Jambi.

Preparation of Tools and Materials

The tools used in the research were owned by the researchers themselves and some of the tools were borrowed from the Biomedical Laboratory of the Faculty of Medicine, University of Jambi. The tools used include digital scales, hand scoops, masks, 1 cc syringes, razor blades/razors, alcohol swabs, and blood serum tubes. The materials used included Wistar Strain White Rat (Rattus novergicus), Rat food and drink, 5% dextrose, distilled water, and rat anesthetic (xylazine and ketamine).

Adaptation of experimental animals

Mice were acclimatized about 2 weeks before the study. Maintenance of white rats is carried out once every three days including cleanliness of the cage, arrangement of shelves, and addition of iron matting on the cage wall to maintain the security of the cage if there are rats that have escaped or there are predators that are feared to enter the cage. As well as cleaning around the cage environment. Experimental animals were acclimated for approximately 1 week before treatment. Adapted sample with his new residence, with the provision of food and drink. This treatment was the same for all mice. This adaptation is intended for all research objects so that the experimental animals are not stressed and in the same condition as when the research was started.

Stages of DM Induction in Mice

DM induction was carried out in the DM control group and the treatment group by manipulating the rats' drinks with a 5% dextrose solution. The dextrose solution used is 5% dextrose solution, the dextrose solution is prepared by mixing 100 ml of 5% dextrose solution with 100 ml of distilled water, with a ratio of 1:1. Drinking is given for 5 consecutive days. Confirmation of hyperglycemia was carried out on the 6th day after previously the rats were fasted for 12 hours. Mice with GDP levels ≥135mg/dL were considered hyperglycemia and used as research objects.

Intermittent Fasting Treatment Stage Type 5:2

Each experimental group was given a different treatment. The first group was treated with a diet with the composition and time of eating according to need, the second group was given treatment with a diet given the composition and time of eating according to need in white Wistar rats with a model of diabetes mellitus, the third group treated with a fasted diet for 24 hours by consuming 20-25% of energy requirements according to the fasting schedule, namely 2 days of fasting and 5 days of normal eating in white Wistar rats with a model of diabetes mellitus. The diet is carried out for 30 days. The type of food and drink given to each group is the same, the food used is RatBio feed pellets with the nutritional composition being: Water content max 12%, Protein min 20%, Fat MAX 4%, Crude Fiber max 4%, Calcium 12 %, Phosphorus 0.7%. The need to drink a mouse every day is approximately 15-30 ml of water. This amount can be reduced if the feed consumed already contains a lot of water.

Stages of Measuring Blood Sugar Levels

Checking blood glucose levels uses an enzymatic method, this method reacts specifically with glucose in the blood. Glucose molecules that are oxidized by the Glucose Oxidase (GOD) enzyme produce electrons that are captured by the electrodes so that the glucose level is directly proportional to the electronic signal received. The amount of blood needed to measure blood glucose levels is 1-2 ml of blood which is put into a blood serum tube and checked in the laboratory.

Stages of Measuring Triglyceride Levels

Rat triglyceride levels were measured by the enzymatic color gas method using a serum tube containing rat blood and then centrifuged with a 2 ml sample. Blood is taken from the rat orbital area. Triglyceride levels will be measured 2 times, namely, before treatment (type 5:2 Intermittent Fasting diet) and after treatment (Intermittent Fasting type 5:2 diet).

Data on blood triglyceride levels will be analyzed using data processing software and presented in the form of the mean and standard deviation (SD) of each

group. To analyze the relationship data between the 2 groups will use the Paired T-test.

RESULTS

This study conducted a comparison between the 5:2 intermittent fasting diet group on triglyceride levels in Wistar white rats to know whether there was a comparison between the 5:2 intermittent fasting diet group on triglyceride levels in Wistar white rats which is presented in the following table1.

Table 1. Mean measurement of triglyceride levels in white Wistar rats before and after the 5:2 intermittent fasting diet

Test Group	*Rerata Kadar TG (mg/dl)*		*Sig (p value)*
	Pre-Treatment	*Post Treatment*	
Group 1 (Control Negative)	*122.33*	*114.00*	*0,895*
Group 2 (Control Positive)	*59.75*	*59.75*	*1.000*
Group 3 (Group Treatment)	*70.33*	*84.25*	*0,631*

DISCUSSION

This study showed that there was no significant difference in the treatment groups 1, 2, and 3 which was indicated by the statistical test results obtained p> 0.05 (not significant). So that there was no significant difference between triglyceride levels before and after treatment for each group, even the triglyceride levels of rats after treatment tended to increase.

The results of this study are similar to a recent study by Klempel et al., researchers found that intermittent fasting only increased HDL levels, and did not decrease LDL, triglyceride, and cholesterol levels. The mechanism of intermittent fasting for increasing lipoprotein, cholesterol, and serum triglycerides is the same as the mechanism for losing fat mass. Most importantly, the quality of the food must be considered

According to research conducted by Surabhi Bhutani et al., the application of intermittent fasting for 2-3 weeks showed a 3% reduction in body weight, in addition, triglyceride levels of total cholesterol (TC) and low-density cholesterol (LDL) were also reduced. 6

High levels of triglycerides and fasting glucose are two components of the metabolic syndrome. The triglyceride-glucose index (TyG) combines both triglyceride and fasting glucose levels and has been reported to significantly correlate with insulin resistance and to be a surrogate marker of insulin resistance.

A meta-analysis of time-restricted eating by Marianna et al. and a meta-analysis of intermittent energy limitation by the meta-analysis of Mirmiran et al. revealed no effect of intermittent fasting on triglycerides. Cioffi et al. also showed no effect on triglyceride concentrations. In contrast, and agreement with these findings, the meta-analysis of Ramadan fasting by Faris et al. shows a slight decrease in triglyceride concentrations, where intermittent fasting can improve blood lipid profiles

According to these researchers, nuclear expression of peroxisome proliferator-activated receptor alpha (PPAR-y) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-19) in the liver causes increased oxidation of fatty acids and production of apolipoprotein A (apo A), as well as decreased apo B synthesis. In addition, increased fatty acid oxidation occurs concomitantly with decreased hepatic triglyceride levels and the production of very low-density lipoprotein (VLDL).8

In a recent study by Klempel et al., researchers found that intermittent fasting only increased HDL levels, and did not decrease LDL, triglyceride, and cholesterol levels. The mechanism of intermittent fasting for increasing lipoprotein, cholesterol, and serum triglycerides is the same as the mechanism for losing fat mass. Most importantly, the quality of the food must be considered

High fasting triglycerides are generally the result of increased VLDL-triglyceride secretion (commonly associated with hepatic steatosis) and/or impaired triglyceride clearance by lipoproteins. These metabolic changes that lead to elevated fasting triglycerides have been linked to many factors including insulin resistance, and dietary and lifestyle behaviors that promote obesity and ectopic fat accumulation. The most accepted mechanism for increasing fasting triglycerides is adipose tissue insulin resistance, leading to uninhibited lipolysis, increased free fatty acid flux to the liver, and ultimately increased VLDL-triglyceride secretion.

The homeostatic model of insulin resistance (HOMA-IR) correlates strongly with triglyceride-rich VLDL1 secretion and the size of the VLDL1 apoB pool. While visceral adipose tissue is more insulin resistant than subcutaneous fat, and consequently is frequently cited as a contributor to increased triglycerides, it should be noted that the majority of free fatty acids that reach the liver originate from peripheral depots. In addition, increased de novo hepatic lipogenesis in the fasting state may contribute to hepatic fat accumulation and VLDL secretion in obese and hyperinsulinemic individuals.

CONCLUSION

A study has been carried out on the effect of intermittent fasting type 5:2 diet on triglyceride levels conducted on 11 subjects

of white Wistar rats with the result that there was no significant decrease between triglyceride levels before and after intermittent fasting type 5:2 diet on the third mean triglyceride level. treatment group and there was no difference in triglyceride levels before and after the Intermittent Fasting diet type 5:2 Wistar rats.

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