Effects of High-carbohydrate and High Fat Diet on Formation of Foam Cells and Expression of TNF-α in *Rattus Novergicus*

Haidar*, Djoko W. Soeatmadji**

ABSTRACT

Aim: to examine the role of high carbohydrate and high fat diet on formation of foam cells and expression of TNF-α, an early stage of atherosclerosis.

Methods: three months old male *Rattus Novergicus* strain Wistar were allocated into 3 groups, normal diet group (GI, n=8), high carbohydrate diet group (GII, n=8), and high fat diet group (GIII, n=8). Those groups received an isoenergetic diet but contained different percentage of carbohydrate and fat for 12 weeks. The rest of the food was measured daily to calculate the calorie intake. The body weight was measured weekly. At the end of study, blood samples were taken using cardiac puncture to examine lipid profiles and random blood sugars.

Results: levels of blood glucose significantly increased in G II compared to the G I (281.87 ± 39.66 mg/dl vs 192.5 ± 1.4 mg/dl, p = 0.002). Group II and G III showed increased of triglyceride compared to GI (138.0 ± 47.15 vs 85.5 ± 20.3, p = 0.02; 163.62 ± 41.77 vs 85.5 ± 20.3, p = 0.00, respectively). Level of LDL significantly increased in G III compared to G I (72 ± 35.6 vs 27.0 ± 8.9, p= 0.00). No statistical difference in level of HDL among the three groups. Level of TNF-α significantly increased in G II and G III compared to GI (19.13 ± 3.68 vs 2.5 ± 1.4, p = 0.00; 23.6 ± 5.58 vs 2.5 ± 1.4, p = 0.00, respectively). The number of foam cells was significantly increased in G II and G III compared to G I (7.18 ± 5.28 vs 1.2 ± 1.4, p = 0.00; 9.91 ± 6.26 vs 1.2 ± 1.4, p = 0.00, respectively). The foam cell had strong correlation with triglyceride level and TNF-α (r=0.696, p= 0.00; r= 0.618, p= 0.00, respectively).

Conclusion: this result shows that high carbohydrate and high fat diet potentially increase the risk factor of atherosclerosis. Both diets induced the inflammatory process and increase foam cells formation, are in the early stage of atherosclerosis.

Key words: high carbohydrate, high fat diet, foam cells, TNF-α, *Rattus novergicus*

INTRODUCTION

Much effort has been done in order to decrease mortality and morbidity related with atherosclerosis disease such as life style modification, decrease in body weight for obese patients and controlling dyslipidemia. Both strategies are profoundly related with dietary regulation. Conventional diet recommended for decrease body weight is calorie restriction with high carbohydrate and low fat component.1,2

It has been so long a time since carbohydrate was considered as a safe dietary component that it is recommended in high percentage. But recently many experts started thinking about the role of carbohydrate in the development of atherosclerosis.2,3 Several factors support the role of high carbohydrate diet in the development of atherosclerosis: 1) The incidence of cardiovascular and cerebrovascular disease is quite high in countries whose residence consume high carbohydrate diet. For example, in Indonesia, the main composition of the diet is carbohydrate, about 67% of energy supply in urban population and 69.6% of total energy in rural population.4 A National Survey of Household Diet conducted in 1992 showed that cardiovascular disease was the most frequent cause of death in 40-year-old people.4 2) Several previous studies revealed that low carbohydrate diet was able to improve lipid profile and decreased body weight better than low fat diet.2,3

This study is intended to investigate the role of high carbohydrate diet and high fat diet on TNF-α expression and the formation of foam cells on aortic wall of *Rattus Novergicus* strain wistar. TNF-α is an inflammatory cytokine involved in the early process of atherosclerosis, so that it was chosen here.

METHODS

This is a complete random design experimental study in animal (*rattus novergicus* strain wistar). The study was conducted in Pharmacology and Biomedical Laboratory, Medical School, Brawijaya University between 1st January 2005 and 1st February 2006. Research sample
consisted of white mice (Wistar strain of rattus novergicus) that belonged to the same breed from Farmacology Laboratory Brawijaya University. The sampling method used was simple random sampling. Inclusion criteria consisted of age 3-4 months, male, 100-120 gr weight, health indicated as having white fur and looked active. Exclusion criteria consisted of mice that did not want to eat during the study, because their using condition was getting worse or dying throughout the study. The mice were divided into three groups i.e normal diet, high carbohydrate diet and high fat diet with similar among of calorie. Each group consisted of 8 rats. Normal diet was a diet usually used in Farmacology lab, Brawijaya University proven not to induce the formation of foam cells. Normal diet was the one of 104.6 calorie consisting carbohydrate 68%, fat 9.7%, and protein 21%. The composition was 90% PAR-s, 9.09% wheat and water as needed. High fat diet was a diet usually used in Farmacology laboratory, Brawijaya University proven to induce the formation of foam cells. High fat diet contained 104.8 calorie consisting of carbohydrate 55.63%, fat 30.12%, and protein 14.25%. The composition was PAR-S 57.27%, wheat 31.81%, cholesterol 1.91%, chotat acid 0.13%, pork fat 8.89% and water as needed. High carbohydrate diet contained 104.7 calorie consisting carbohydrate 81.18%, fat 5.45% and protein 14.12%. The composition was PAR-S 45%, wheat 34%, glucose 21% and water as needed. The duration of intervention was 12 weeks. The unconsumed food was measured everyday in order to calculate the intake of calorie. The increase of body weight was measured every week. At the end of intervention, the animals were put under a 12 hour fasting before being sacrificed. The rats were killed with anesthetizing and cardiac blood sample was obtained in order to check the lipid profile and blood glucose. Foam cells were examined on inner layer of the aorta by using of red oil staining. TNFα was measured on the inner layer of the aorta by using of immunohistochemistry staining. The difference of mean in three groups was studied by the use of anova (parametric data) and kruskall wallis (non parametric data), then post hoc tukey and mann whitney, respectively. It was considered to be significant if p < 0.05. The correlation between foam cells and lipid profile, blood glucose and TNFα was studied by the use of Spearman test. SPSS 10 for window was used as a tool.

**RESULTS**

Characteristic data of research subject were presented on table 1. The intake of calorie was different significantly between three groups. The mean daily intake of calorie was highest in normal diet group (90.09±6.22) and lowest in high carbohydrate diet (55.19±9.03). The initial body weight of the three groups were not different statistically (p=0.275). The body weight of normal diet group was highest at the end of treatment. The final body weight was different significantly between normal diet group and high carbohydrate diet group (p=0.044). If we compare the mean increase of body weight with the mean calorie intake, each calorie of high carbohydrate diet will cause most prominent increase of body weight. But, the difference was not significant statistically.

The highest increase of blood glucose was in high carbohydrate diet group. The difference in mean blood glucose between the high carbohydrate diet group and normal diet group was different statistically (281.87±39.66 mg/dl vs 192.25±1.4 mg/dl, p=0.002). The difference of mean blood glucose between the three groups was not significant statistically.

LDL cholesterol increased significantly in high fat diet compared with normal diet (72±35.6 vs 27±8.9, p=0.00) and with high carbohydrate diet (72±35.6 vs 33.3±11, p=0.15). The LDL cholesterol was not different.
significantly between normal diet group and high carbohydrate diet group. (p=0.279). LDL cholesterol increased significantly in high fat diet compared with normal diet (72±35.6 vs 27±8.9, p=0.00). The LDL cholesterol in high carbohydrate diet was not different significantly compared with normal diet (p=0.279).

Figure 1. Difference of expression of TNFα in intima layer of aorta among 3 groups. Total of cells that expressed TNFα in high carbohydrate diet and high fat diet groups was higher significantly than normal diet group. (p=0.00)

DN: Normal diet, DKH: High carbohydrate diet, DL: High fat diet

The highest trygliceride level was in high fat diet, and the lowest was in normal diet. The mean trygliceride of high fat diet was different significantly with normal diet by the Mann Whitney test (163.62±41.77 vs 85.5±20.3, p=0.00). The trygliceride level in high carbohydrate diet was different significantly with normal diet (138.0±47.15 vs 85.5±20.3, p=0.02). The level of trygliceride in high fat diet and high carbohydrate diet was not different significantly (p=0.248).

The highest level of TNFα was found in high fat diet, the lowest was in normal diet. The mean level of TNFα in high fat diet was different significantly with normal diet (148.8±35.1 vs 15.7±8.8, p=0.00). The TNFα level in high carbohydrate diet was different significantly compared with normal diet (120.3±23.1 vs 15.7±8.8, p=0.00). The difference of TNFα in high fat diet and high carbohydrate diet was not different statistically.

The foam cell level was highest in high fat diet and lowest in normal diet. The mean level of foam cells in high fat diet was different significantly compared with normal diet (9.91±6.26 vs 1.2±1.4, p=0.00). The difference of foam cells in high fat diet and high carbohydrate diet was also significant statistically (9.91±6.26 vs 7.18±5.28, p=0).

The correlation between calorie intake, blood glucose, trygliceride, LDL, cholesterol, HDL cholesterol, TNFα and foam cells was tested by using of spearman correlation test. The result of correlation between several variables and the formation of foam cells is presented in table 2. Table 2 shows that all variables have strong correlation with the formation of foam cells except HDL cholesterol. The variable with strongest correlation to foam cells is trygliceride (correlation coefficient 0.696, p=0.000).

**DISCUSSION**

The body weight of the Wistar mice increased each time the body weight was measured (every 7 days). The highest increase of body weight was in normal diet group and the lowest was in high carbohydrate diet group. The highest increase of Wistar mice given normal diet had linear correlation with the intake of calorie. In this study, overweight mice were not found considering the normal weight of male adult wistar is 450-520 gram, female adult wistar is 250-350 gram.

The highest increase of body weight per 1-calorie intake was in high carbohydrate diet, although this result was not significant statistically. This result fits the study by Samaha et all in which the decrease of body weight in obese women with low carbohydrate diet was higher than those with low fat diet.

The highest mean blood glucose was in the high carbohydrate diet. The blood glucose in high carbohydrate diet was different significantly with normal diet. The underlying mechanism is suspected to be insulin resistance and decrease of the pancreas b cell density. In the high carbohydrate diet, glucose as a carbohydrate with high glycemic index causes increase in blood glucose fast after meal causing higher increase insulin production by β-cell pancreas. The increase activity of
β cell causes increase of reactive oxygen species (ROS) production. The increase of ROS causes autooxydation of β-cell pancreas. These cells are sensitive cells against oxidation. Several animal studies revealed that chronic hyperglicemia is able to decrease the density of β-cell suspected to be the consequence of autooxydation.6,7

The increase of trygliceride that was significant in high carbohydrate diet rather than normal diet fitted the theory.8,9 The increase of trygliceride (fatty acid) in high carbohydrate diet is suspected to be involved in the increase of blood glucose. In several studies in mice infused by the use of trygliceride and heparin there was increase of blood glucose. The increase of glucose is suspected to be the result of decrease oxidation of glucose and the decreased uptake of glucose by the liver and skeletal muscle (Randle hypothesis).6,7 The decrease of glucose uptake is caused by competition with fatty acid in tissue using glucose and fatty acid.6,7 Although it was not significant statistically, there was increase of blood glucose in high fat diet more than in normal diet. The increase of blood glucose in high fat diet is suspected to be caused by the same mechanism as Randle hypothesis.

The formation of foam cells is an early phase of the sequence event of atherosclerosis.10 The mean foam cells were highest in high fat diet, which was statistically significant. The formation of foam cells in high carbohydrate diet was different from normal diet although the LDL cholesterol level between those two group was not significantly different. The high trygliceride level and suspicion of insulin resistance seem to be a factor playing a role in the formation of foam cells in high carbohydrate diet. The high trygliceride level and insulin resistance cause the formation of small dense LDL particles.11 This small dense LDL particle is more atherogenic than ordinary LDL particle. It is suspected that in high carbohydrate diet, there is the formation of many small dense LDL.

Trygliceride and TNFα have strong correlation with the formation of foam cells. The role of trygliceride in the formation of foam cells is not direct. In high trygliceride level, there is an increase in lipoprotein-rich the trygliceride that will be transformed to small dense LDL in condition of insulin resistance. This small dense LDL is a very atherogenic lipoprotein.11,12 Besides trygliceride, TNFα also has strong correlation with the formation of foam cells. The expression of TNFα is higher in high fat diet and high carbohydrate diet significant than in normal diet. The increase of the expression of TNFα correlated with the increase of the formation of foam cells in high carbohydrate diet and high fat diet fitted theory that atherosclerosis is an inflammatory process.10

There are several obstacles in these study that should be paid attention in future study, i.e:

1). The difference of calorie intake in the three groups. The diet formula with the same palatability should be considered.

2). In this study insulin resistance and the decrease of the density of foam cells were based on rough assumption. The insulin level and apoptosis of β cell pancreas should be examined.

3). This study did not examine small dense LDL level because of the lack of facility.

4). This study only considered the influence of carbohydrate percentage in dietary formula without considering glicemic index of carbohydrate contained in the food.

5). This is an experimental study-using animal, so it needs to be considered further before applied in humans.
CONCLUSION

It has been revealed in this study that high carbohydrate diet and high fat diet potentially increase the risk factor of atherosclerosis. Both diet induce inflammatory process on the vessel wall and increase the formation of foam cells, an early stage of atherosclerosis. Insulin resistance and high levels of small dense LDL are two major parts thought to be the underlying mechanisms in the formation of foam cells in high carbohydrate diet. However, the effect of high carbohydrate diet, especially in humans, should be studied further.

REFERENCES

2. Samaha FF. A low carbohydrate as compared with a low fat diet in severe obesity. NEJM. 2003;21(348):2074-81.