ROLE OF HIGH CHOLESTEROL AND HIGH FAT DIET ON LIPID PROFILES IN SPRAGUE DAWLEY RATS

PERAN KOLESTEROL DAN DIET LEMAK TINGGI TERHADAP PROFIL LIPID PADA TIKUS SPRAGUE DAWLEY

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ABSTRAK

Empat puluh lima ekor tikus putih Sprague Dawley umur 2 bulan dengan berat rata-rata 100 gram digunakan dalam penelitian ini untuk mempelajari peran kolesterol dan lemak tinggi dalam diet terhadap profil lipid (trigliserida, HDL, LDL dan kolesterol total). Tikus putih dibagi menjadi 3 kelompok masing-masing 15 ekor. Kelompok I, sebagai kelompok kontrol diberi ransum diet normal. Kelompok II adalah kelompok dengan pemberian diet lemak tinggi, dan kelompok III adalah kelompok dengan pemberian diet lemak tinggi dan kolesterol tinggi. Pada minggu ke-3, ke-6 dan ke-12 setelah perlakuan, dari masing-masing kelompok diambil 5 ekor tikus putih secara acak, kemudian diambil darahnya guna pemeriksaan profil lipid meliputi total kolesterol, trigliserida, HDL dan LDL. Hasil analisis statistik dengan menggunakan multifactorial randomized design pada tingkat kepercayaan 95% menunjukkan bahwa baik jenis ransum, waktu dan interaksi antara waktu dan ransum berpengaruh nyata terhadap konsentrasi kolesterol total dalam darah. Jenis ransum, waktu dan interaksi antara waktu dan jenis ransum berpengaruh nyata terhadap konsentrasi trigliserida total dalam darah. Jenis ransum, waktu dan interaksi antara waktu dan jenis ransum berpengaruh nyata terhadap konsentrasi kolesterol total dalam darah. Jenis ransum dan waktu berpengaruh nyata, tetapi tidak ada interaksi antara waktu dan ransum terhadap konsentrasi trigliserida LDL total dalam darah. Dari hasil penelitian ini dapat disimpulkan bahwa: (1) diet kolesterol dan lemak tinggi dapat meningkatkan konsentrasi total kolesterol dan trigliserida, (2) tidak ada pengaruh interaksi antara periode penelitian dengan diet terhadap konsentrasi HDL-kolesterol dan LDL-kolesterol.

Kata kunci: tikus putih Sprague Dawley, trigliserida, HDL, LDL dan kolesterol.

ABSTRACT

Fourty five-male Sprague Dawley rats, weighing about 100 g of 2 month old were used as experimental animals to study the role of high cholesterol and high fat diets on blood lipid profiles, triglyceride, HDL, LDL, and total cholesterol. Before this research began, rats were adapted for a week and were fed basal diet. The rats were then randomly allotted into three groups (I, II, III) of 15 each. Group I as control was fed normal (basal) diet, group II was fed diet containing high fat diet, and group III was fed diet containing high cholesterol and high fat diet. After 3, 6, and 12 weeks on experimental diets, blood specimen from 5 rats of each group were collected to determine triglyceride, HDL, LDL, and total cholesterol concentration. The statistical analyses using multifactorial randomized design for blood lipid, showed that experimental time periods caused significant increased (p<0.05) in the total cholesterol concentrations, which 12 weeks on experimental diet was the highest concentration. Diet and experimental time periods showed significant increased (p<0.05) in the total triglyceride
concentrations, after 12 weeks of treatments was the highest concentration. Significantly increased (p<0.05) in HDL-cholesterol concentrations were caused by diet and experimental time periods, however, there was no significant effect by interaction between experimental time periods and diet. Significantly increased (p<0.05) in LDL-cholesterol concentrations were caused by diet and experimental time periods. However, there was no interaction between experimental time periods and diets in total LDL-cholesterol concentration. In this study, high fat and high cholesterol diet group (group III) and six weeks in experimental diet had the greatest influenced in total LDL-cholesterol concentration. Based upon the experimental results, it can be concluded that: (1) high cholesterol and high fat diet could increase total cholesterol concentration and total triglyceride concentration, (2) there was no interaction between experimental time periods and diet on HDL-cholesterol and LDL-cholesterol concentration.

**Key words:** Sprague Dawley rats, triglyceride, HDL, LDL, and cholesterol

**INTRODUCTION**

Atherosclerosis is a major cause of morbidity and mortality in nations with Western lifestyles (Black et al., 2000). The major risk factor were age hyperlipidemia (Lakatta, 2003), diabetes mellitus, cigarette smoking (Tithof et al., 2001). Obesity, physical inactivity, and behaviour pattern are also risk factors (Steinberg, 1989).

Lipid diets play an important role in the development and progression of atherosclerosis (Kreisberg and Oberman, 2003). The lipid hypothesis of atherosclerosis originally related to total and low density lipoprotein (LDL)-cholesterol. Increasing evidence suggest that atherosclerosis is an inflammatory disease promote by hypercholesterolemia (Robertson et al., 2003). Numerous animal studies such as mice and rabbit (Staprans et al., 1998), showed that increasing dietary cholesterol content and duration of the exposure to cholesterol-rich diets resulted in augmented atherosclerosis (Cortes et al., 2002).

The present study was designed to evaluate the role of high fat and high cholesterol diet (atherogenic diet) in lipid profiles using Sprague Dawley rats as experimental animals.

**MATERIALS AND METHODS**

Forty five male Sprague Dawley rats, 150-200 grams of body weight and three months of age were used as experimental animals. They were housed individually, and then randomly assigned to three diet groups with fifteen rats in each group. Tap water and diets were freely available. Group I as control was fed normal diet, group II was fed diet containing high fat (tallow: 20%), and group III was fed diet containing high cholesterol and high fat diet (pure cholesterol; 4,5% and tallow; 20% or atherogenic diet). After 3, 6, and 12 weeks on experimental diet, 15 rats were selected randomly (5 rats of each group), and blood samples were withdrawn for blood lipid analyses (total cholesterol, triglyceride, HDL, and LDL).

Total cholesterol was determined using spectrophotometry (wavelength 546 nm). Ten (10 μL) plasma sample were mixed with 1,000 μL cholesterol reagents with vortex and incubated for 20 minutes at room temperature. After incubation, the absorbance was measured against reagen blank within 60 minutes (Buccolo and David, 1993).

Triglyceride was determined using spectrophotometry (wavelength 546 nm). Ten (10 μL) plasma sample were mixed with 1,000 μL high density lipoprotein reagents with vortex and incubated for 20 minutes at room temperature. After incubation, the absorbance was measured against reagen blank within 60 minutes (Buccolo and David, 1993).

High density lipoprotein was determined using precipitation of LDL, VLDL, and chylomicrons methods. Two hundred microliter (200 μL) plasma sample were mixed with 500 μL HDL reagents with
vortex and incubated for 10 minutes at room
temperature and centrifuge for 10 minutes at 4,000
G. After centrifugation, 100 µL supernatant were
mixed with 1,000 µL and then incubated for 10
minutes at room temperature the absorbance was
measured against reagen blank within 60 minutes at
546 nm wavelength (Buccolo and David, 1993).

Low density lipoprotein was obtained with
formula LDL= cholesterol total – triglgyseride5-
HDL (mmol/L) (Buccolo and David, 1993):

Data from the experiments were analyzed
using multifactorial randomized design. Differences
was considered statistically significant at P<0.05.

RESULTS AND DISCUSSION

Total cholesterol concentration in the blood of
rats given experimental diets for 3, 6 and 12 weeks
were presented in Figure 1. Statistical analysis with
multifactorial randomized design (p<0.05) showed
that there were significant difference effects of diets
and experimental time periods, and interaction
between diet and experimental time period on total
cholesterol level.

This research demonstrated that total cholesterol
level were influenced by experimental time periods
and diets. These data showed that high cholesterol
and fat diet had the greatest effect on total cholesterol
levels compared to group I and group II. This result
are similar to the previous studies in mice, transgenic
mouse model, rats (Rahman et al., 2001), and rabbits
(Rong et al., 1999), that hypercholesterolemia is due
to high fat and high cholesterol diets. This research
suggested that the longer of the experimental time
periods, the greater of the total cholesterol levels.

This findings are similar to the previous studies in
male zucker rats, Sprague Dawley rats, Zew Zealand
White Rabbits and mice (Black et al., 2000) as
experimental animals.

The causal relationship between blood
cholesterol concentration and atherosclerosis is no
longer in doubt (Anonim 1993). More than 20
epidemiological studies in many countries showed
that single cholesterol levels measurement was a
strong predictor for coronary artery disease in the
following years (Grundy, 1999). The following
studies also showed that hypercholesterolemia was
one of the coronary artery disease risk factors
(Steinberg, 1989). The report came from Multiple
Risk Factor Intervention Trial (MRFIT) in 356,222
man for 6 years follow-up showed a linear
relationship between cholesterol serum and mortality
due to coronary heart disease (Stamler et al., 1986).

According to Quintao et al. (1971), high
cholesterol intake increased bile cholesterol and the
most important, total cholesterol levels increased
whenever excessive cholesterol was given in the
diet. Total cholesterol absorbed from gut resulted in a
linear relationship with total cholesterol diet and
plasma cholesterol concentration (Simon et al.,
1978). Reducing cholesterol consumption also
decrease incidence of coronary heart disease (Lee
and Libby, 1997), provided plaque stabilization, and
increased endothelial function (Anderson et al.,
1995). Several studies showed that every 10% in
reducing cholesterol level, lowered mortality due to
coronary heart disease 15% (Gould et al., 1998).

Dietary lipids elevated plasma cholesterol
concentration. Previous studies indicated that
saturated fatty acid (SFA) will increased total
cholesterol concen-tration, respectively whereas
polyunsa-turated fatty acid (PUFA) will decreased
total cholesterol concentration (Albert et al., 1996).

However, not all SFA affect total cholesterol
concentration in the same manner. For instance,
stearic acid (18:0) has little effect on total cholesterol
concentration, whereas myristic (14:0) and palmitic
acids (16:0) have been reported to have the greatest
cholesterol-raising potential (Grundy, 1981). Many
foods from animal products containing large amount
SFA have strong correlation to higher levels of total
cholesterol concentration (Kromhout et al., 1995)
and coronary heart disease (Tell et al., 1994). Several
studies suggested that beef, pork, poultry (especially
skin) and cheese had a great cholesterol-raising
potential (Nelson, 1998). On the other hand, PUFA-
rich in omega-3 from fish oil reduced incidence of
cardiovascular diseases (Ando et al., 1999).

Total triglyceride concentration in the blood of
rats given experimental diets for 3, 6 and 12 weeks
were presented in Figure 2. The data of this study illustrate the influence of experimental time periods on elevated triglyceride plasma concentration. Group III had the highest triglyceride plasma concentration compared to group I and group II. Statistical analysis illustrated that both diets and experimental time periods, and interaction between experimental time periods and diets had significant differences to total triglyceride concentration. In this study, group III after 12 weeks on experimental diets had the greatest effect on total triglyceride concentration.

This study showed that group III showed the highest elevated of total triglyceride concentration compared to group II and group I. However, after 12 weeks on experimental diets, group I had higher on total triglyceride concentration than group II. Although high fat diet could increased total triglyceride concentration (Anonim 2001), in contrast, several studies showed that not high fat diet but low fat diet and high carbohydrate would increased total triglyceride concentration (Letexier et al., 2003). However, Cominacini et al. (1988) indicated that low fat diet and high carbohydrate would decreased total triglyceride concentration. These contrary results, according to Ullman et al. (1991) probably due to altering low fat and high carbohydrate diets too fast, thus increased total triglyceride concentration was temporarily. The different results of these studies probably due to kind of fatty acids used on the experimental diets.

According to Rulle et al. (1996), degree of saturated fatty acid had influence to total triglyceride concentration on male Sprague Dawley. In contrast, Sugano and Imaizumi (1995) suggested that the degree of saturated fatty acid had no effect on total triglyceride concentration of Syrian hamster. The role of triglyceride serum concentration in the development of atherosclerosis is still controversial. Various studies showed that as a risk factors for atherosclerosis, triglyceride was not inde-pendent. Based on analyses univariat, increased in triglyceride concentration had a relationship with incidence of atherosclerosis, in contrast, multivariat analyses failed to show the relationship between triglyceride concentration and incidence of atherosclerosis (Anonim, 1993). Epidemiological studies showed that risk of cardiovascular disease increased two times higher in person who had high level of triglyceride concentration (Zilversmit, 1995). However, triglyceride concentration is not independent, there always accompanied by increasing of atherogenic lipoprotein, such as VLDL and LDL-cholesterol (Grundy, 1999), lower of HDL-cholesterol concentration, and obesity. Nevertheless, hypertriglyceridemia was considered as an independent risk factor (Hennig et al., 2001).

In this study, 12 weeks on experimental diets had the greatest effect on triglyceride concentration. This results were consistent with previous studies (Blankenhorn et al., 1990), triglyceride concentrations increase significantly with age, and it
was considered due to uncontrolled diets and reduced physical activities.

Triglyceride (chylo-micron and VLDL) metabolisms. All of those studies that mentioned above also showed that PUFA caused the lowest total triglyceride concentration from Sprague dawley rats 3, 6, 12 weeks on experimental diet

Total HDL-cholesterol concentration in the blood of rats given experimental diets for 3, 6 and 12 weeks were presented in Figure 3.

The data illustrated that increased of HDL-cholesterol concentrations were influenced by experimental time periods. Group III showed the greatest increased in total HDL-cholesterol concentration compared to group I and group II. Statistical analysis indicated that both experimental time periods and diets, and interaction between experimental time periods and diet had a significant differences on total HDL-cholesterol. In this study, group III and six weeks on experimental diet had the greatest influenced on total HDL-cholesterol concentration.

This results were consistent with previous studies in African green monkeys, rat, guinea pig and hamster (Listenberger et al., 2003), where total HDL-cholesterol concentration increased on experimental diet containing high fat and high cholesterol. The raising of total HDL-cholesterol concentration was probably due to lipoprotein-rich HDL-cholesterol concentration comparing with MUFA and SFA. These findings supported by and Grundy (1999) PUFA caused decrease in total HDL-cholesterol concentration, through reducing concentration of apoprotein A1 as a precursor of HDL-cholesterol synthesis. According to (Listenberger et al. 2003), chylomicron derived from PUFA had larger particle size and its surface had a potential to carry Apo A1, as a precursor of HDL-cholesterol synthesis. In contrast, Yokogoshi et al. (1999) showed that high cholesterol diet on rats decreased HDL-cholesterol concentration. Nevertheless, Gardner and Kraemer (1995) showed no different in HDL-cholesterol concentration between enriched PUFA diet and enriched MUFA diet. In this study, HDL-cholesterol of group I in agreement with the previous studies that low fat and low cholesterol diet had no raising effect in HDL-cholesterol concentration (Garg et al., 1994). More surprisingly, Knopp et al. (1997) showed that low fat and low cholesterol diet decreased HDL-cholesterol concentration.

Figure 2. Total triglyceride concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.
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Figure 3. Total HDL-cholesterol concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

According to Packard and Shepherd (1997), the contrary results of these studies were likely due to heterogeneity of HDL-cholesterol. Castellani et al. (1997) also showed that heterogeneity of HDL-cholesterol had different effects in incidence of atherosclerosis. Moreover, in the research using mice as animals model, increased atherosclerosis resistance was not only caused by elevated HDL-cholesterol concentration, but also by removal of cholesterol from peripheral tissue that called reverse cholesterol transport. Thereby, although HDL-cholesterol measurement was important, however, it could not be used to predict the presence of atherosclerosis accurately (Silverman et al., 1993).

According to Kotke (1986), the level of apo AI might be predicted to be better marker for the presence of atherosclerosis disease than the level of HDL-cholesterol, because HDL-cholesterol measurements include HDL-cholesterol particles that are fully saturated with bound free cholesterol. Epidemiologic prospective studies showed that the plasma level of either HDL-cholesterol or the major structural protein of HDL-cholesterol, apolipoprotein AI (Apo AI) was inversely correlated with the risk of cardiovascular heart disease risk, with each 1 mg/dl decrease in HDL-cholesterol level accompanied by a 2-3% increase in risk (Weng and Breslow, 1996). Although many studies were supported the claimed that mentioned above, however was still unclear, whether HDL-cholesterol had the direct or indirect effect to inhibit atherosclerosis development process (Boisfer et al., 1999), thus, none of studies can explained in details the causal effect between atherosclerosis and HDL-cholesterol concentration (Harper and Jacobson, 1999).

Total LDL-cholesterol concentration in the blood of rats given experimental diets for 3, 6 and 12 weeks were presented in Figure 4.

The data illustrated that increased of LDL-cholesterol concentrations were not influenced by experimental time periods. Group III showed the greatest increase of total LDL-cholesterol concentration compared to group I and group II. Statistical analysis indicated that experimental time periods and diets had a significant difference effect on LDL-cholesterol concentrations, however, there was no interaction between experimental time periods and diets on total LDL-cholesterol. In this study, group III after six weeks on experimental diet had the greatest influenced on total LDL-cholesterol concentrations.

This results were similar with previous studies that LDL-cholesterol concentration increased on experimental diets containing high cholestrol and...
Figure 4. Total LDL-cholesterol concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

In this study, six weeks on experimental diet had the greatest influence in total LDL-cholesterol concentration. According to Schaefer et al. (2002), increasing plasma LDL-cholesterol was correlated with aging, and was likely due to delayed of chylomicron remnant clearance in elderly compared to the young. However, according to Schaefer et al. (1995) in very old persons, LDL-cholesterol concentration was lower than in middle-aged persons, and its probably due to decreased apo B-100 production, the main protein of LDL, thus, LDL-cholesterol concentration decreased. However, other studies showed various effects of dietary cholesterol, depended on individual, different responses on experimental animals species, and the concentration of cholesterol as a challenge diet (Grunedy, 1999).

Based upon the experimental results, it can be concluded that: (1) high cholesterol and high fat diet could increase total cholesterol concentration and total triglyceride concentration, (2) there is no interaction between experimental time periods and diet on HDL-cholesterol and LDL-cholesterol concentration.
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