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 HDL total dalam darah. Jenis ransum dan waktu berpengaruh nyata, tetapi tidak ada interaksi antara waktu dan ransum terhadap konsentrasi kolesterol 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 alloted 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 cholestrol 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, trigly-ceride, 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 – triglgyseride/5-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 cholestrol 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

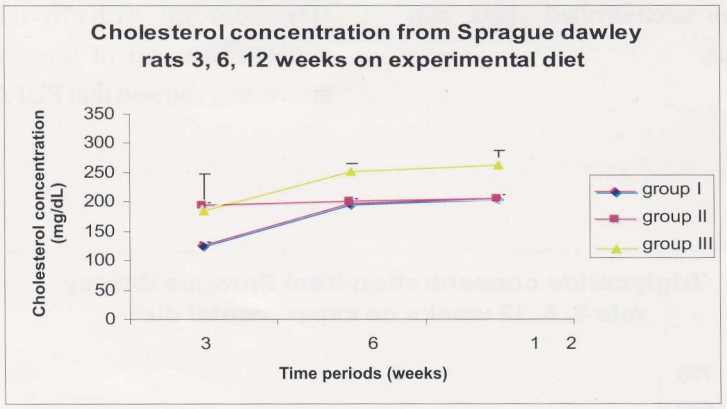

Figure 1. Total cholesterol concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

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 timeperiods, 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 tu 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.

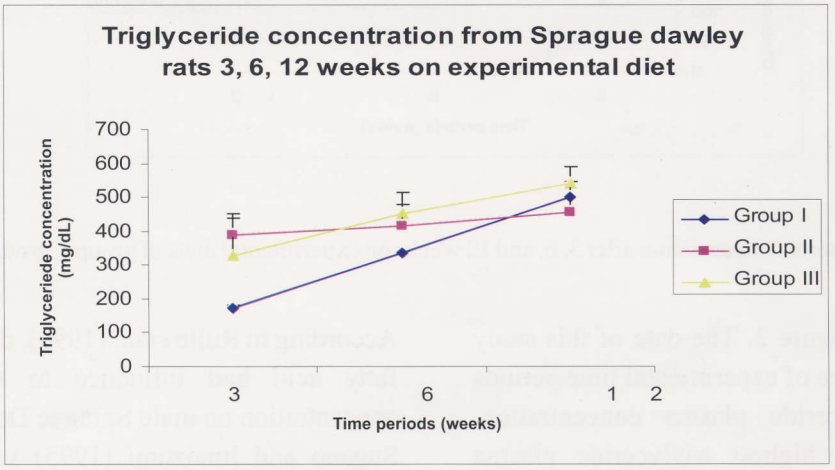

Figure 2. Total triglyceride concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

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 experi-mental 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 con-centration.

This results were consistent with previous studies in African green monkeys, rat, gunea 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 triglyceride (chylo-micron and VLDL) metabolisms. All of those studies that mentioned above also showed that PUFA caused the lowest total 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 AI 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 AI, 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.

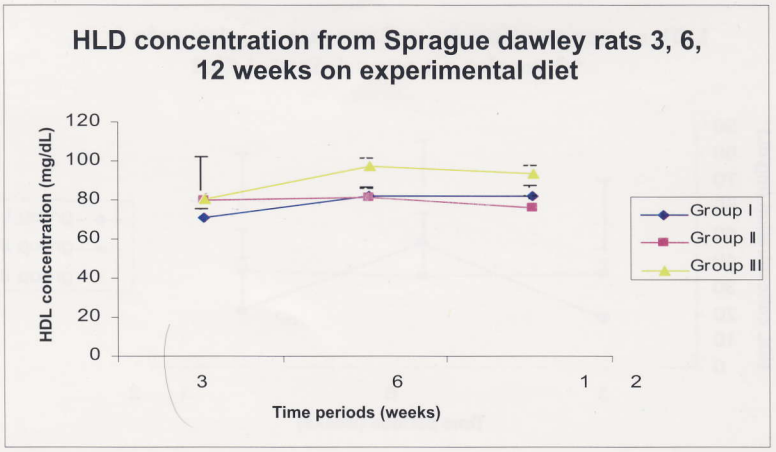

Figure 3. Total HDL- cholesterol concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

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 (Rong *et al.,* 2001). For the past three decade, epidemiological studies were consistently demonstrated the lower HDL-cholesterol was the one of the risk factor of atherosclerosis incidence (Cohen, 2003). Low level of HDL-cholesterol are strongly associated with 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

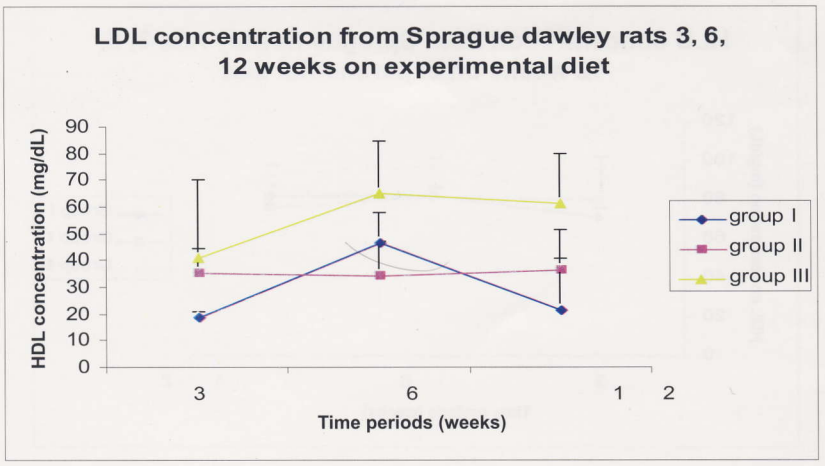

Figure 4. Total LDL- cholesterol concentration after 3, 6, and 12 weeks on experimental diets of group I, group II, and group III.

high fat in rabbits (Rodrigueza *et al.,* 1998). Studies on Cebus monkeys, African green monkeys, Cynomolgus monkeys, *Sprague Dawley* rats (Csont *et al.,* 2002), and human (Marks *et al.*, 2003) also showed the similar results.

According to Brown and Goldstein (1986), regulation of LDL-cholesterol concentration was affected by LDL-cholesterol receptor on the cell membrane. However, the precise mechanisms for the regulation LDL-choles-terol concentration in the blood was remains unclear (Park and Snook, 1995). Other studies indicated that dietary lipids had effect on LDL-cholesterol receptors. Studies in *Macaca fascicularis*, Cebus monkeys and Rhesus monkeys, hamster (Ohtani *et al.,* 1990), guinea pig (Fernandez *et al.,* 1992), as an experimental animals model showed that PUFA diets could decrease LDL cholesterol concentration in part through enhanced LDL-cholesterol receptor activities in the liver. Moreover, in human, MUFA diets could decrease LDL cholesterol concentration in part through enhanced LDL-cholesterol receptor activities, and the LDL-cholesterol receptor-mediated pathway could be increased in part by changes in fatty acid composition. (Park and Snook, 1995).

In this study, the increased LDL cholesterol concentration is probably due to LDL-cholesterol uptake through receptor dependent pathway (Goldstein and Brown 1984; Spady *et al.,* 1983). Dependent receptor pathway of plasma LDL-cholesterol uptake *in vivo* is saturable. Rats, rabbits, and dogs, 90% of Plasma LDL-cholesterol uptake was through dependent receptor pathway (Spady and Dietschy, 1985). Thus, in this study, abundant on cholesterol intake caused LDL-cholesterol receptors on surface cell membrane became saturable, hence it increased plasma LDL-cholesterol.

In this study, six weeks on experimental diet had the greatest influenced 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 respons on experimental animals species, and the concentration of cholesterol as a challenge diet (Grundy, 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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