碩士學位論文

고콜레스테롤 식이를 먹인 쥐의 혈장과 간 중 콜레스테롤, 중성지방 수준 및 혈소판 응집성에 있어 암·수 차이

濟州大學校 大學院

食品營養學科

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Sex differences in cholesterol and triglyceride levels the plasma and liver, and platelet aggregation in rat fed a hypercholesterolemic diet.

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摘　　要

이 연구는 0.5% cholesterol과 0.2% cholate을 함유한 식이의 급여가 쥐의 성별간 혈장과 간 중 cholesterol, 중성지방의 수준 및 혈소판 응집에 미치는 영향을 알아보고자 실시 되었다. Sprague Dawley 암쥐 20마리 (평균체중 165g)와 숫쥐 20마리 (평균체중 203g)를 2×2 요인 실험으로 배치 하였고 대조식이와 0.5% cholesterol과 0.2% cholate를 함유한 식이를 4주간 무제한 급여하였다. 4주동안 사육한 쥐들을 12시간 절식시켜 chloroform으로 마취하여 Cardiac puncture방법으로 혈액, 간 시료를 채취하였다.

하루식이 섭취량 (ADFI)과 하루 증체량 (ADG)에는 식이로 인한 차이가 없었으나 숫쥐가 암쥐보다 높았으며 ($P<0.001$) 사료 전환율 (F/G)은 낮았다 ($P<0.001$). 체중에 대한 간 무게 비율 (Liver/B.W ratio)에서는 대조식이를 급여한 쥐에서 보다 cholesterol이 첨가된 식이를 급여한 쥐에서 2배 정도 증가 하였으나 ($P<0.001$) 숫쥐와 암쥐간에는 차이가 없었다. 혈장과 간 중의 총 콜레스테롤 수준은 cholesterol이 첨가된 식이를 급여한 암·숫쥐에서 2배 이상 증가 되었으며 ($P<0.001$) 특히, 혈장의 콜레스테롤 수준은 숫쥐 (265.7㎎/㎗)에 비해 암쥐 (569.9㎎/㎗)가 2배 이상 높았다 ($P<0.001$). HDL-cholesterol은 대조식이를 급여한 쥐들에 비해 cholesterol이 첨가된 식이를 급여한 숫쥐 ($P<0.05$)와 암쥐 ($P<0.01$)에서 감소 하였으나 성별 간에는 차이가 없었다. 혈장 중성지방의 수준은 기본식이를 급여한 쥐에 비해 cholesterol이 첨가된 식이를 급여한 숫쥐에서 감소 ($P<0.01$) 하였지만, 암쥐에서는 ($P<0.05$) 증가하

였다. 간 중 중성지방의 수준에는 식이에 의한 영향이 없었다. 혈소판 응집초기의 응집곡선은 대조식이를 급여한 쥐에 비해 cholesterol이 첨가된 식이를 급여한 쥐들이 증가 ($P<0.05$) 하였으나 최대 응집치는 식이의 영향을 받지 않았다.

본 실험의 결과를 종합하여 보면 cholesterol이 첨가된 식이를 급여하는 경우, 대조식이에 비해 혈장과 간 중의 cholesterol 수준은 증가하고 숫쥐에 비하여 암쥐가 cholesterol 첨가 식이에 아주 높은 반응을 나타냈다. 따라서 암쥐와 숫쥐간에 따른 cholesterol 흡수-배설 그리고 합성-분해의 대사 차이에 대하여 좀더 다양하고 폭넓은 연구가 필요하다고 사료된다.

Ⅰ. Introduction

Hypercholesterolemia and hyperlipidemia are significant risk factors in the development of cardiovascular diseases (CVD) or reciprocally related to risk of other causes of morbidity and mortality. In 1985, 48% of all deaths resulting from a form of CVD such as stroke, heart attack or vascular disease. In 1992, 65 millions of Americans are in some way afflicted by CVD (Beitz, 1994). However, it is still controversial that lowering serum cholesterol is always beneficial; it may increase overall mortality due to increased suicide, homicide and accidents (Hibbeln, 1995). Cholesterol is one of the major components of all animal cell membrane, myelin and bile acid, and is a precursor of steroid hormones.

In the normal population at any age, there are no significant differences in plasma total cholesterol and LDL-cholesterol between sexes. Levels of plasma triglyceride and HDL-cholesterol in childhood are similar between sexes, while adult men have generally lower HDL-cholesterol (41.8 ㎎/dℓ vs 50.3 ㎎/dℓ, $p<0.001$), and higher triglyceride (207.1 ㎎/dℓ vs 149.8 ㎎/dℓ, $p<0.001$) than adult women (Pugeat, 1995; Crowley, 1994).

Cholesterol from diets (about 335mg/day) and synthesis (800mg/day) are two sources for the body pool cholesterol (about 120g), which is relatively constant (Beitz, 1994). Cholesterol synthesis is tightly regulated by various enzymes involved in response to dietary cholesterol (Grundy, 1969). Americans are obtaining about 37% of their energy intake from fat (Beitz, 1994). About a half of which is animal fat known to be associated with cholesterol. Cholesterol is known to influence triglyceride metabolism and vice versa.

The present study was conducted to examine the effects of sex and feeding a diet supplemented with 0.5% cholesterol on cholesterol levels in the plasma and liver. We also examined the effect of a high cholesterol diet on triglyceride levels in the plasma and liver and platelet aggregation that are known to be associated with cardiovascular disease.

Ⅱ. Literature Review

1. Cholesterol metabolism(synthesis and excretion) in vivo

Cholesterol is an extremely important biological molecule comprising cell membrane structure as well as being a precursor of steroid hormones and bile acids. Epidemiological studies have shown a direct relationship between the high plasma cholesterol level and premature development of atherosclerosis (Lipid Research Clinics Program, 1984). Cholesterol absorption efficiency (Kesäniemi and Miettinen, 1987) and the excretion of products of cholesterol oxidation are contributing factors to plasma cholesterol level and thus can be a risk factor for the development of atherosclerosis.

Cholesterol synthesis occurs in the cytoplasma and microsomes from acetyl-CoA, beginning with the transport of acetyl-CoA from the mitochondria to the cytosol. An early product, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is converted to mevoalonate, which is catalyzed by HMG-CoA reductase and is a rate limiting step for cholesterol biosynthesis. HMG-CoA reductase is subject to complex regulatory controls. Hepatic HMG-CoA reductase activity was increased in rats fed a 15% lard

diet (Fernandez, 1994) but was reduced by 50% with diet containing 5% cellulose (Basu, 1993). The rate of cholesterol synthesis is regulated in part by dietary cholesterol intake. Liver cholesterol metabolism is sensitive to dietary cholesterol which inhibits cholesterol synthesis by a negative feedback action at the level of the HMG-CoA reductase and increases cholesterol conversion to bile acids (Shefer, 1973). Dietary cholesterol significantly increases hepatic cholesterol concentration, and slightly liver bile acid (Sklan, 1979).

Cholesterol is transported in the plasma predominantly as cholesteryl esters which are associated with lipoproteins. The rate of intestinal cholesterol absorption in related to pancreatic cholesterol esterase activity (Kumar, 1996). Dietary cholesterol is transported from the small intestine to the liver within chylomicrons and the excess cholesterol over hepatic needs is transported in the plasma within low density lipoproteins (LDLs). The liver synthesizes very low density lipoproteins (VLDLs) which are converted to LDLs through the action of endothelial cell-associated lipoprotein lipase. The cholesterol acquired from peripheral tissues by high density lipoproteins (HDLs) can then be transferred to VLDLs and LDLs by the action of cholesteryl ester transfer protein (apo-D) which is associated with HDLs.

Feeding large amounts of cholesterol reduced cholesterol

synthesis but low dietary cholesterol induced the synthesis (Grundy, 1969). Cholesterol absorption was reduced by dietary pectin (Kelley, 1978) and cholesterol synthesis was reduced by dietary taurine (Becker, 1993). Cholesterol alone did not cause cholesterolemia but did when fed with cholate or any of its conjugates except aspartocholate (Story, 1974). Marlett et al., (1990) reported that ingestion of 0.2% cholic acid with 1% cholesterol significantly elevated serum and liver cholesterol and triglyceride levels with an increased liver weight.

Ultimately, cholesterol is excreted in the bile as neutral sterols or as bile salts. (Choi, 1992). Story and Kritchevsky (1978) reported that dietary fiber increased bile acid excretion as well as pool size and turnover of bile acids. The hepatic cholesterol levels tended to decrease with alginates (free acid, sodium and calcium salts) feeding, while the fecal excretion of cholesterol increased (Nishida, 1993)

The end products of cholesterol metabolism are the bile acid, which synthesis is the predominant mechanism for the excretion of excess cholesterol. However, the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess dietary intake of cholesterol.

2. Sex differences in cholesterol and lipid metabolism .

The incidence of coronary heart disease (CHD) is significantly higher in men than in women, and it has been thought that young women are protected from CHD by ovarian hormones and they lose this protection after the menopause. The mortality rate from cardiovascular disease increased in older men compared to older women and the early mortality rate from CHD was also higher among men than among women (Buring, 1995; Cohen, 1959).

In the Framingham study, premenopausal women rarely developed or died of myocardial infarction or coronary heart disease which is very common in postmenopausal women, and the surgical menopausal women had higher risk than the natural menopuasal women (Kannel, 1976; Gordon, 1978). The postmenopausal women on hormones had a doubled risk of CHD (Gordon, 1978), whereas Hennekens (1991) and others reported the cardioprotective effect of estrogen in postmenopausal women. Ross (1981) also reported that estrogen replacement therapy was effective on ischaemic heart disease in postmenopausal women. The male treated with estrogen had less atherosclerosis than the normal male (Rivin, 1954; Katz, 1952).

In prepubertal children, plasma high density lipoprotein

cholesterol (HDL-C) and triglyceride levels were similar between sexes, while adult men had generally lower HDL-C and higher triglyceride levels than premenopausal adult women (Pugeat, 1995). Average men had higher triglyceride and VLDL and lower HDL concentrations than the women (Heiss, 1980; CannonⅢ and Guetta, 1996). Crowley et al. (1994) reported that there is a correlation between sex and LDL-C, and LDL-C is the principal lipoprotein independently influencing whole blood viscosity.

The serum total cholesterol and very low density lipoprotein (VLDL-C) were lower in women treated with estrogen (Stender, 1991), and the estrogen users had higher mean levels of HDL-C and apolipoprotein A-1 than nonusers (Nabulsi, 1993). Estrogen decreased plasma triglyceride and LDL-C and increased HDL-C. This hormone stimulated the uptake of VLDL-C and the catabolism of LDL-C in liver and affected several enzymes involving in HDL-C and triglyceride metabolism in animals treated with estrogen (Eberhard, 1980) and in men (Eriksson, 1989). Estrogen increased the concentration of hepatic mRNA for the LDL-C receptor (Goldstein, 1986). Colvin (1996) reported that the residence time of LDL-C tracer in plasma was decreased by estrogen. It was reported that increased glucose and insulin concentrations were associated with increased free testosterone and decreased its binding protein in both premenopausal women

and postmenopausal women. In contrast to women, increased androgen concentrations in men did not seem to be associated with cardiovascular risks (Haffner, 1995).

Female sex hormones had substantial effects on both lipoprotein metabolism and the arterial estrogen receptor (Gevers Leuven, 1994). Plasma apolipoprotein-A (apo A) levels showed a stratification for age and sex; apo A levels being higher in females than in males and increased with age (Igarashi, 1995). The estrogen appears increase apo A-1 of HDL-C and apo A-1 synthesis (Bernard, 1990; Lindheim, 1994). Kushwaha et al., (1990) reported that VLDL-apo B production was not affected by estrogen but was increased by progesterone, whereas LDL-apo B production was increased by both estrogen and progesterone. The reduction of plasma cholesterol was primarily because of decreased LDL-C and LDL apo B levels. Recent studies demonstrated that estrogen inhibited LDL oxidation induced by cupric ions, macrophages, or endothelial cells in vitro (Masziere, 1991; Subbiah, 1993) and in vivo (Sack, 1994). Equine estrogen was effective in inhibiting both fatty acid and cholesterol oxidation. Recently published data suggest a possible involvement of arterial estrogen receptor in the beneficial action of estrogen (Mendelsohn, 1994; Isner, 1994).

3. Cholesterol and platelet function

The blood platelets are concoid discs of about 2 ㎛ in diameter in human beings and are present at the concentration of 250,000 (range ; 140,000 - 400,000) per ㎣. They are produced in megakaryocytes of the bone marrow and circulate in the blood for about 10 days before they aggregate each other or adhere to vascular endothelial cells upon the damage of the blood vessel. Platelets can aggregate and adhere to collagen, basement membrane or microfibrils in response to the activating substances such as adenosine diphosphate (ADP), serotonin and epinephrine which are released from disrupted red blood cells or extravascular tissues. These platelet activating substances can also induce the release reaction of platelet in the primary phase aggregation, secreting ADP, serotonin and other substances that potentiate the platelet to cause the secondary phase of aggregation (Weiss, 1975). Platelet adherence especially to collagen causes to release the platelet granule contents including platelet-derived growth factor (PDGF) and activate the arachidonic acid pathway to form thromboxane A_2 (TXA_2). PDGF is known to stimulate the migration and proliferation of smooth muscle cells and the accumulation of LDL-cholesterol in the atherosclerotic plaque and

TXA_2 causes the contraction of vessel walls and spasm (Packarm, 1986).

An epidemiological study (Meade, 1985) showed platelet aggregability increased with age in both sexes and was greater in women than men, but showed no relation between aggregability and current or past oral contraceptive use, menopausal state, or plasma cholesterol and triglyceride concentrations. However, most workers have reported an enhanced platelet activity in certain hyperlipidemia and hypercholesterolemia (Carvalho, 1974; Jamieson, 1985; Brook, 1988; Tomizuka, 1990). Cholesterol-rich human platelet had hypersensitivity to TXA_2 with an increased serotonin release (Tomizuka, 1990) and human platelet incubated with cholesterol had higher production of TXA_2 than that incubated without cholesterol (Stuart, 1980). Jamieson (1985) observed that normal platelets incubated in cholesterol enriched medium or platelets from the type II_b hypercholesterolemia (VLDL and apo B_{48} rich) had an increased adhesion to subendothelium of rabbit aorta and an increased thrombus size. Carvalho et al. (1974) reported that the platelets of patients with the type II hyperlipoproteinemia had hypersensitivity to the aggregating agents such as ADP, epinephrine and collagen. Type IIa hyperlipoproteinemia especially rich in LDL-cholesterol had an increased platelet adhesion to collagen without change in platelet-platelet interaction, which may

be involved in the accelerated development of athrosclerosis (Sakariassen, 1993). Baumgartner (1987) reported an excessive deposition of fibrin, platelet and platelet thrombi during contraceptive drug use. Crowley et al. (1994) reported that women in the normal population had higher platelet counts and plasma fibrinogen than men.

The impedance method of determining platelet aggregation in whole blood was introduced in 1980 by Cardinal and Flower. This method has the advantage of measuring platelet aggregation under nearly physiological conditions with the aggregation performed within minutes of cardiac puncture and in the presence of other blood components. Platelet aggregation is measured with an increase in impedance across two platinum electrodes in the whole blood upon applying the activating agents such as ADP, arachidonic acid, collagen and epinephrine that are physiologically involved in the platelet aggregation.

Ⅲ Materials and Methods

1. Animals and diets

A total of 40 female and male Sprague-Dawley rats at age of 8 weeks were divided into four groups by sex, and 0% and 0.5% cholesterol supplementation in 2 × 2 factorial design. Rats were individually housed in stainless steel cages in a room maintained at 20-25 °C with a 12-hour dark-light cycle. Rats had ad libitum access to their respective diets (Table 1) and water for 4 weeks. Food intake for individual rats was monitored every 3 days and animals were weighed weekly during the feeding period.

2. Sample collection

At the end of 4 weeks, rats were anesthetized with chloroform after fasted for 16 hours, and blood was collected by cardiac puncture into vacuum tubes containing heparin. Plasma was obtained by centrifugation of blood sample at 2000×g and stored at -20 °C for later analysis. Hematocrit was determined by centrifugation of blood at 3000 ×g for 15 minutes. The liver was quickly removed and weighed and frozen at -20 °C until analyzed.

Feces was collected weekly during the feeding experiment and was frozen at -20 °C until analyzed.

3. Platelet aggregation

Platelet aggregation was measured using a Chronolog Model 500-Ca Whole Blood Aggregometer (Havertown, Pennsylvania, USA). The whole blood was diluted with isotonic saline (1:2) to give a platelet concentration of approximately 400,000/μl. Two μM adenosine diphosphate (ADP) was added to initiate aggregation, and three readings of impedance changes were taken for each rat and the mean value was used. Platelet aggregation caused an increase in impedance across two platinum electrodes in the whole blood and the impedance gain was set 20Ω in recorder response. The impedance method using the fresh whole blood has the advantage of measuring platelet aggregation under nearly physiological conditions in the presence of other blood components.

4. Analyses of cholesterol, HDL-cholesterol, glucose and triglyceride concentrations in the plasma

Total cholesterol and glucose were determined by using

commercial assay kit (Wako Pure Chemical Ind., Osaka, Japen). The wako kit test is an in vitro enzymatic colorimetric method for the quantitative determination. For total cholesterol, HDL-cholesterol and glucose assay, 20, 50 and 20㎕ plasma samples were used respectively. The concentrations in all samples were determined by measurement of the absorbance at the wavelength of 505 nm using a spectrophotometer. Triglyceride (20 ㎕) concentration was determined by using commercial assay kit (International Reagent Co, Tokyo, Japan), and the absorbance was measured at 535 nm.

5. Analyses of cholesterol, glucose and triglyceride in the liver

Liver samples were prepared by modifying the method described in Folch et al. (1956) to determine cholesterol and triglyceride. Liver tissue (1g) was homogenized in 6㎖ chloroform/methanol mixture (2/1, v/v) and 2㎖ distilled water using a tissue homogenizer and centrifuged at 2000 × g for 10 minutes. The chloroform fraction containing cholesterol and triglyceride is the bottom layer after centrifugation. Half ㎖ of chloroform fraction was taken and dried under nitrogen gas. Fifty ㎕ Triton X-100/chloroform solution (1/1, v/v) was added to the dried

residue, diluted to 0.5㎖ with chloroform and throughly mixed. Then 50㎕ sample was transferred to a test tube and chloroform was evaporated under flowing N_2 gas. Total cholesterol in the residue was determined as described for the determination of plasma cholesterol.

A 20㎕ aliquot of the chloroform fraction for the liver triglyceride was taken and dried under flowing N_2 gas. The residue was dissolved in 100 ㎕ methanol, and the concentration of triglyceride was determined as described above for plasma triglyceride assay.

6. Statistical analysis of experimental data

Values were analyzed using the SAS package (SAS, 1988). Analyses of variance (ANOVA) were conducted in a completely randomized block design (Table 2). Duncan's multiple range test was applied to compare individual means when F-value was significant ($P < 0.05$).

Ⅳ. Results and Discussion

1. Effect of sex on growth rate and feed intake

Composition of the experimental diets are shown in Table 1. Males had significantly higher ($P<0.001$) weight gain and feed intake than females in the both control and rats fed the diets supplemented with cholesterol. (Table 3). The feed/gain ratio was significantly higher ($P<0.01$) in female than male rats. No differences were found in weight gain, feed intake and feed/gain ratio between the diets.

Male Wistar rats fed diet containing 0.5% cholesterol and 0.5% cholate for 2 weeks had significantly lower weight gain than those fed a control diet (Story, 1974), whereas female Sprague Dawley rats fed a diet containing 2% cholesterol and 0.5% cholate for 6 weeks (Spady, 1992), or male Sprague Dawley rats fed 1% cholesterol with 0.2% cholate for 20 days (Marlett, 1990) were not different in weight gain as shown in the present study. The effect of dietary cholesterol on growth rate may differ between rat strains.

Liver weight / body weight (Liver/B.W) ratio was significantly ($P<0.001$) higher in rats fed the cholesterol diet than in the control

(Table 3) and the liver of rats fed the hypercholesterolemic diet was hypochromic as shown in Figure 1. An increased liver weight has been observed in male (Marlett, 1990) and female (Spady, 1992) Sprague Dawley rats fed hypercholesterolemic diet. However, Story et al. (1974) reported that a diet containing 0.5% cholesterol and 0.5% cholate did not increase the liver weight, but significantly elevated the liver cholesterol level in Wistar male rats. It is very interesting that different rat strains differently respond to hypercholesterolemic diets in growth and liver metabolic activity. Marlett et al. (1990) microscopically observed fatty infiltration in the rat liver by the induced hypercholesterolemia. Visual observations, the hypochromic tissue and the large size of the liver in hypercholesterolemic rats in the present study may or may not be related to fatty liver. Microscopic examination of the liver is necessary in future experiments.

2. Cholesterol and triglyceride levels in the plasma and liver

Plasma total cholesterol level was significantly ($P<0.001$) higher in rats fed the cholesterol diet than in rats fed the control (417.8 ㎎/㎗ vs 161.9 ㎎/㎗), and the increased plasma cholesterol level was much greater in female (569.9 ㎎/㎗) than male rats (265.7㎎/㎗) (Table 4). The liver total cholesterol content was also significantly ($P<0.001$) higher in rats fed the cholesterol diet than in control (37.9mg/㎗ vs 9.2mg/㎗) in male and (54.9mg/㎗ vs 8.4mg/㎗) in female. The plasma HDL cholesterol level was significantly lower in rats fed the cholesterol diet than in the control (43.6mg/㎗ vs 33.6mg/㎗ in male, $P<0.05$ and 42.5mg/㎗ vs 24.2mg/㎗ in female, $P<0.01$). No significant difference was found between sexes.

Plasma triglyceride levels were significantly decreased (61.9mg/㎗ vs 32.4mg/㎗, $P<0.05$) in male rats and significantly increased (46.0mg/㎗ vs 77.0mg/㎗, $P<0.05$) in female rats by feeding the cholesterol diet compared to those in the control (Table 4). Liver triglyceride level was not different between sexes or between diets

The control male rats had higher plasma total cholesterol level (176.6mg/㎗ vs 147.3mg/㎗) and higher HDL-cholesterol (43.6mg/㎗

vs 42.5mg/dℓ) than the control female rats. Average normal men have slightly higher total cholesterol (209.4mg/dℓ vs 206.8mg/dℓ) and slightly lower HDL-cholesterol (41.8mg/dℓ vs 50.3mg/dℓ) than women (Crowley, 1994). Before menopause, plasma LDL-cholesterol was lower and HDL-cholesterol was higher in women than menopause women. (Cannon III, 1996). Women taking estrogen had higher triglyceride and higher total cholesterol than women not taking estrogen. HDL-cholesterol was higher in women taking estrogen (Heiss, 1980) than women not taking. Men with high risk of CVD were observed to have a low level of HDL-cholesterol and high LDL-cholesterol, which were associated with a low level of apo A-1 due to its high catabolic rate and a high level of apo B due to its high production (Ramakrishnan, 1994). Significant decrease in catabolic rate of VLDL apolipoprotein B was observed in baboons fed a high cholesterol diet (Kushwaha, 1978). Dietary cholesterol may influence the plasma and liver cholesterol levels through the synthesis and degradation of apo A-1 and apo B.

Cholesterol supplementation increased the plasma and liver cholesterol levels in rats only when cholate is added to the diet (Story 1974; Marlett 1990). Dietary cholesterol depressed hepatic and intestinal cholesterol synthesis and enhanced the conversion of cholesterol to bile acids (Sklan, 1979)

Roach et al (1993) reported strain differences in response to

hypercholesterolemic diets. Sprague Dawley rats fed a cholesterol diet had lower plasma total cholesterol and higher HDL-cholesterol than other strains of rats. Unlike Albino Wistar or Sprague Dawley, Hooded Wistar rats showed hypercholesterolemic response to a diet with 1% cholate alone.

The excess triglyceride accumulation in the liver of rats fed 1% cholesterol supplemented was resulted from increased synthesis and decreased secretion of triglyceride, resulting in increased liver triglyceride and decreased plasma triglyceride (Huang, 1995). In type II hyperlipoproteinemia, especially type II_b, high plasma total cholesterol is accompanied by high plasma triglyceride, which is attributed to a large fraction of VLDL (Sakariassen, 1993). In the present study, decreased plasma triglyceride with an increased liver triglyceride in male rats fed the cholesterol diet may result from the reduced release of liver triglyceride, and an increased plasma triglyceride level in female fed the cholesterol diet have may resulted from disturbance in hepatic clearance of the newly absorbed cholesterol.

Plasma glucose was decreased (128.9mg/dℓ vs 102.0mg/dℓ) in male rats and increased (92.6mg/dℓ vs 127.7mg/dℓ) in female rats fed the cholesterol diet compared to those of the control diet, but significant difference was not found between any two groups. A direct relationship between blood glucose and the plasma level of

cholesterol has not been reported. Acetyl coA is a building block in cholesterol synthesis and a basal substance in citric acid cycle as energy source. Disorder in cholesterol metabolism can be associated with a metabolic disorder of triglyceride and glucose in one way or another. Mykkanen et al (1994) reported total cholesterol, VLDL cholesterol and triglyceride positively correlated and HDL cholesterol inversely correlated with fasting insulin level. A decreased insulin sensitivity in hyperlipoproteinemia may be the cause of an elevated blood glucose in hypercholesterolemic female rats in the present study.

3. Effect of sex and diet on platelet aggregation

Hematocrit value was decreased by feeding the cholesterol diet, but the difference was significant ($P<0.05$) only in female rats (Table 5). The decreased value of hematocrit in male (48.2%) and female (44.8%) still remain the normal range so that hematocrit in this study may not be significantly related with hypercholesterolemia. Sakariassen (1993) reported that human type II_b hyperlipoproteinemia had an increased hematocrit.

The maximum aggregation was not significantly different between the dietary groups (Table 5). The initial slope of aggregation was significantly ($P<0.05$) higher in rat fed the cholesterol diet than in the control. Aggregation with a high initial slope in this experiment tended to show a rapid dissociation after a maximum aggregation.

It has been reported that an increased platelet function is related to hyperlipoproteinemia (Carvalho, 1974; Jamieson, 1985; Tomizuka, 1990; Sakariassen, 1993). Platelets from type II hyperlipoproteinemia were activated in response to a low concentrations of ADP, epinephrine and collagen (Carvalho,

1974). Platelet membranes from hypercholesterolemic rats had higher thrombin receptors (Jamieson, 1985). Cholesterol rich human platelet had hypersensitivity to TXA_2 (Stuart, 1980), and platelets incubated with cholesterol increased the in vitro production of TXA_2 (Tomizuka, 1990). Brook (1988) reported that increased binding of LDL to platelet was associated with an increase in platelet cholesterol, and platelet cholesterol concentration was positively correlated with plasma cholesterol concentration. Because platelet activation is considered as a membrane-associated phenomenon, changes in platelet membrane lipid appear to determine platelet responsiveness. Lipid composition of platelet membrane apparently is influenced by plasma lipid profile, and therefore plasma triglyceride and cholesterol may ultimately be responsible for the degree of platelet activation.

V. Summary

This study was conducted to examine the effects of sex and feeding a diet supplemented with 0.5% cholesterol on cholesterol levels in the plasma and liver.

Twenty female (165g) and 20 male (203g) Sprague Dawley rats were divided into 4 treatment groups: 2 sexes and 2 diets in a 2× 2 factorial arrangement. Rats were fed ad libitum for 4 weeks. Blood samples were collected by cardiac puncture and livers were removed.

The male rats had higher ($P<0.001$) average daily feed intake (ADFI), average daily gain (ADG) and lower feed/gain than did female rats. Liver weight / body weight (Liver/B.W) ratio was significantly ($P<0.001$) higher in rats fed the cholesterol diet than in the control.

Total cholesterol levels in the plasma and liver were significantly higher ($P<0.001$) in rats fed the cholesterol diet than in the control, and the increased plasma level was much greater in female than male (569.9mg/dℓ vs 265.7mg/dℓ). Plasma HDL-cholesterol level was significantly higher in rats fed the cholesterol diet than the control, the response being higher in female than male rats. No significant difference in HDL cholesterol

was found between sexes. Plasma triglyceride level in male rats was significantly decreased ($P<0.05$), whereas that in female rats was significantly increased ($P<0.05$) by feeding the cholesterol diet compared to the control. Liver triglyceride levels was not influenced by diet. The initial slope of aggregation was significantly higher ($P<0.05$) in rats fed the cholesterol diet than the control, but the maximum aggregation was not influenced by diet.

Results indicated that dietary cholesterol increases plasma and liver cholesterol levels and the effect was much greater in female than male rats. The sex difference in the dietary hypercholesterolemic effect has yet to be studied in view of cholesterol absorption - excretion and cholesterol synthesis - degradation.

Table 1. Composition of experimental diets

Ingredient	Control	Cholesterol
Casein[a]	20.0	20.0
L-methionine[b]	0.3	0.3
Lard[c]	9.0	9.0
Soy bean oil[d]	1.0	1.0
Choline chloride[a]	0.2	0.2
Vitamin mix[e]	1.0	1.0
Mineral mix[e]	3.5	3.5
sucrose[d]	20.0	20.0
Corn starch[e]	45.0	44.3
Cholesterol[b]	–	0.5
Cholic acid[b]	–	0.2
Total	100.0	100.0

[a] United States Biochemical Corp., Cleveland, Ohio
[b] Sigma Chemical Co., St. Louis, MO.
[c] Samlip Yugi Co.
[d] Jeil Jedang Co.
[e] AIN-76, Harlan, Madison, WI.
[f] Sunil Pododang Co.

Table 2. Analysis of variance (ANOVA)

	MS			
	Treat	Sex	Treat×Sex	MS_{ERROR}
Final weight, g	50.63	148474.23**	55.23	509.18
ADFI,[1] g	0.70	90.90**	0.21	3.73
ADG,[2] g	0.07	88.36**	0.08	0.48
Feed/Gain ratio	0.15	49.11**	0.37	0.37
Liver/B.W ratio	34.63***	0.25	0.42	0.07
Hematocrit	49.51**	79.81**	2.76	5.59
Plasma mg/dℓ				
Total cholesterol	654653.63***	188817.83**	278008.94***	20558.08
HDL-cholesterol	1997.56***	275.99	167.73	127.03
LDL-cholesterol	724123.80***	200971.70**	294917.0***	20219.18
Triglyceride	6.20	2069.43*	9130.97*	396.11
Glucose	168.26	280.79	9626.13	890.01
Liver mg/g				
Total cholesterol	14141.74***	649.72**	795.57***	55.55
Triglyceride	193.95	76.67	3.83	67.38
· Aggregation				
Maximum (Ω)	18.22	24.02	7.22	21.28
Initial Slope (Ω/min)	156.02*	0.02	11.0	27.05

[1] ADFI = Average daily feed intake [2] ADG = Average daily gain

* P<0.05 , ** P<0.01 , *** P<0.001

Table 3. Average daily gain (ADG), feed intake (ADFI), feed/gain and relation liver weight in rats feed control or cholesterol-supplemention diet

	Control			Cholesterol			Sex class		SD
	male	female	mean	male	female	mean	male	female	
Initial	203.9	165.1	184.5	203.8	165.3	184.5	203.8	165.2	20.5
Final	374.8	255.3	315.0	374.9	250.7	312.8	374.8^{A}	253.0^{B}	65.4
ADG	6.1	3.2	4.6	6.1	3.0	4.5	6.1^{A}	3.1^{B}	1.6
ADFI	19.7	16.5	18.1	19.2	16.4	17.8	19.4^{A}	16.4^{B}	2.4
Feed/Gain ratio	3.2	5.2	4.2	3.1	5.5	4.3	3.1^{A}	5.4^{B}	1.2
Liver/B.W ratio	2.8	2.4	2.6^{b}	4.4	4.5	4.5^{a}	3.6	3.4	0.9

Values are means of 10 rats and pooled standard deviation(SD).

^{a,b}Significantly different($P<0.001$) between treatments.

^{A,B}Significantly different($P<0.01$) between sexes.

Table 4. Sex differences in cholesterol, triglyceride and glucose levels in the plasma and liver of rats fed a control or a cholesterol-supplemented diet

	Control			Cholesterol			Sex class		SD
	male	female	mean	male	female	mean	male	female	
Plasma mg/dℓ									
Total cholesterol	176.6	147.3	161.9^{b}	265.7	569.9	417.8^{a}	221.9^{B}	358.6^{A}	218.4
HDL-cholesterol	43.6	42.5	43.0^{a}	33.6	24.2	28.9^{b}	38.6	33.3	13.4
LDL-cholesterol	134.7	104.8	119.7^{b}	232.1	545.6	388.8^{a}	183.4^{B}	325.2^{A}	223.4
Triglyceride[1]	61.9^{a}	46.0^{d}	54.0	32.4^{b}	77.0^{c}	54.7	47.2	61.5	46.6
Glucose	128.9	92.6	110.8	102.0	127.7	114.9	115.5	110.2	32.8
Liver mg/g									
Total cholesterol	9.2	8.4	8.8^{b}	37.9	54.9	46.4^{a}	23.6^{B}	31.6^{A}	21.2
Triglyceride	21.4	24.8	23.1	26.4	28.6	27.5	23.9	26.7	8.3

Values are means of 10 rats and pooled standard deviation(SD).

^{ab}Significantly different($P<0.001$) between treatments

^{AB}Significantly different($P<0.01$) between sexes.

[1]Significantly different($P<0.05$) between control and cholesterol in both sexes, according to Student-t test.

Table 5. Sex differences hematocrit and platelet aggregation in rats fed a control or a cholesterol-supplemented diet

	Control			Cholesterol			Sex class		SD
	male	female	mean	male	female	mean	male	female	
Hematocrit	49.9	47.6	48.7[a]	48.2	44.85	46.5[b]	49.0[A]	46.2[B]	2.9
Aggregation									
Maximum(Ω)[1]	16.5	15.8	16.1	18.7	16.3	17.5	17.6	16.0	4.5
Initial slope(Ω/min)[2]	17.8	16.7	17.2[b]	21.7	20.7	21.2[a]	19.2	19.2	5.4

[1] Maximum aggregation in ohm at the point where aggregate dissociated.

[2] Initial slope is ohm change for the first one minute.

Values are means of 10 rats and pooled standard deviation(SD).

[ab]Significantly different($P<0.05$) between treatments

[AB]Significantly different($P<0.01$) between sexes.

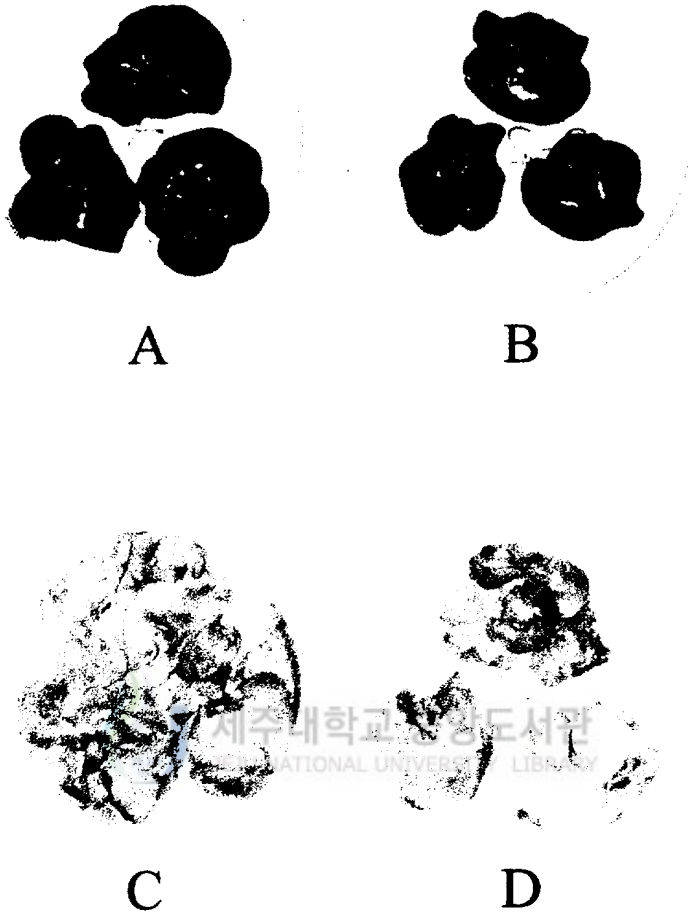

Figure 1. Comparison in size and color of the liver between rats fed a control or a cholesterol-supplemented diet

A : Male, Control diet　　B : Female, Control diet
C : Male, Cholesterol diet　　D : Female, Cholesterol diet

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