J. Food Sci. Nutr.
Vol. 2, No. 2, p. 121~128(1997)

Effects of High Cholesterol Feeding on Regulation of Plasma Lipids and Reverse Cholesterol Transport in Rabbits

Myung-Sook Choi†, Jin-Ah Seo, Kyung-Hyun Cho*, Song-Hae Bok and Yong-Bok Park***

Dept. of Food Science and Nutrition, Kyungpook National University, Taegu 702-701, Korea
**Dept. of Genetic Engineering, Kyungpook National University, Taegu 702-701, Korea*
***Bioproducts Research Group, Genetic Engineering Research Institute, KIST, Taejon 305-606, Korea*

Abstract

This study was conducted to examine the atherogenic effect of high cholesterol diet(experimental diet) that influences changes of lipoprotein cholesterol metabolism and arterial wall. Seven NewZealand white rabbits were fed control diet, and the other 7 rabbits 2% cholesterol diet for 10 weeks. Results obtained from this study are as follows: 1) High cholesterol diet resulted in a gradual increase of plasma total cholesterol level, reaching up to 1422mg/dl at the seventh week. 2) CETP(cholesteryl ester transfer protein) activity was significantly higher in high cholesterol group(64.9% at the 7th week) than control group(49.3% at the 7th week) during most of the experimental period except the 6th week. 3) The cholesterol supplementation induced fatty liver and a decrease of hepatic HMG-CoA reductase activities(2.1nmoles vs. 0.3nmoles) compared to control group. 4) Bands of apo B-100 and apo E in plasma lipoprotein were thicker in high cholesterol-fed animals than control animals as visualized by SDS-PAGE. 5) Oxidizability of plasma lipoproteins measured *in vitro* was greater in high cholesterol group than control group, but vitamin E level higher in control group. 6) The effect of cholesterol feeding for 10 weeks also led to early fatty streaks in aortic intima. High choleserol feeding was atherogenic to rabbits, and this seems to be mediated through elevated CETP activities that regulate plasma HDL cholesterol level and decrease an efficiency of reverse cholesterol transport in lipoprotein cholesterol metabolism. The enhanced oxidizability of plasma lipoproteins and lowered vitamin E level may also contribute to the formation of fatty streaks in aorta of choleterol-fed rabbits.

Key words: high cholesterol, CETP, reverse cholesterol transport, HMG-CoA reductase

INTRODUCTION

The relationship between dietary lipid and atherosclerosis has been a subject of extensive investigation over the last six decades. Epidemiologic studies have demonstrated that high cholesterol diet is atherogenic and plasma high-density lipoproteins(HDL) cholesterol concentrations are inversely correlated with coronary heart disease(CHD) incidence(1,2). It is very important to elucidate the mechanism of atherogenesis by high cholesterol diet. Concentrations of plasma HDL, which are negatively correlated with the development of atherosclerosis in both humans and some experimental animals(3,4), vary considerably among individuals. However, little is known about the regulation of plasma HDL levels.

In seeking for a mechanism for the changes of HDL levels, most attention has been directed at the role of HDL in reverse-cholesterol transport(RCT). The protective effect of plasma HDL is thought to be due to its role in reverse cholesterol transport, involving CETP-mediated transfer of lecithin: cholesterol acyltransferase(LCAT)-derived cholesteryl esters from HDL to more rapidly metabolized lipoproteins(i.e. very low density lipoproteins(VLDL) and low density lipoproteins (LDL)), selective hepatic uptake of HDL cholesteryl esters at rates exceeding catabolism of the major HDL apoprotein, apolipoprotein A-I(apo A-I), and uptake and catabolism of intact HDL particles(5,6). Recently, however, the potential of HDL to retard some of the fundamental processes in atherogenesis has received attention. Klimov et al.(4) reported the protective effect of HDL against the oxidative modification of LDL, which was dependent on time of incubation and the concentration of HDL. HDL is effective in preventing the *in vitro* oxidative modification of LDL by transition metal ions in tissue culture. Vitamin E is also the major antioxidant in LDL and is the first antioxidant to be con-

†Corresponding author

sumed during LDL-oxidation(3,4). HDL cholesterol(ester) is a preferential substrate for bile acid synthesis and secretion in liver(7), which eventually contribute to removal of blood cholesterol to liver.

Rabbits are good animal model for studying the effects of dietary factors on regulating plasma HDL concentrations(8). Since they have the high LDL : HDL cholesterol ratio, LCAT-catalyzed formation of cholesteryl esters, and CETP-facilitated cholesteryl ester exchange system, all these factors can constitute significant components of HDL metabolism and the reverse cholesterol transport process(9). Rabbit is known to be one of the experimental animals which is highly susceptible to atherosclerosis and its cholesterol metabolism is closer to human than rats. We previously have looked the changes of lipoprotein metabolism in high cholesterol-fed rats(10). Elevated plasma cholesterol and apo B-containing lipoprotein levels were also observed in cholesterol-fed rats(11). But rats are generally deficient in plasma CETP activities, thus their reverse cholesterol transport can be performed more efficiently than in rabbits or humans by delivering more blood cholesterol to liver for their utilization(22). For this reason rats are generally very resistant to the development of atherosclerosis. We conducted an experiment with rabbits to study the metabolic effects of 10 week cholesterol-feeding and to identify the mechanism of atherogenesis by high cholesterol feeding. This report describes the effects of dietary cholesterol in changes of plasma lipids, lipoproteins profile. CETP and hepatic HMG-CoA reductase activities, oxidizability of lipoproteins and as well as status of aortic wall.

MATERIALS AND METHODS

Chemicals

Ketamin was purchased from Yuhan Yanghang Co. (Korea) and Cholesterol and TG kits from Asan Co. (Korea). 1,2-[^{3}H]-n-cholesteryl oleate was purchased from Amersham life science Co.(Amersham, U.K.). Egg-yolk lecithin(100mg/ml, ethanol), HMG-CoA, NADPH, cholesterol and cholesteryl oleate were purchased from Sigma Co.(St Louis, USA). Phenyl sepharose CL-4B. Sephadex G-200 column media and CNBr-activated Sepharose-4B were purchased from Pharmacia,(Uppsala, Sweden) and protein molecular standards from Bio-Rad Co.(Hercules, California, USA).

Animal experiment

Six-month-old male New Zealand White rabbits weighing 3.2kg on arrival, obtained from Samyuk Nongwon(Suwon, Korea), were used for these studies. The 14 rabbits were fed a ground nonpurified diet(Purina Rabbit Chow) for 7 days, then randomly divided into control and experimental groups. Experimental group was maintained 2% cholesterol Purina rabbit chow diet for 10 weeks, and control group remained on the same Purina lab. chow diet throughout experimental period. Animals were housed in temperature(21~23°C) and light controlled room with a 12-h light-dark cycle beginning at 0800h and at ambient humidity(30~60%). Food and water were provided *ad libitum*. The rabbits were weighed weekly and food intakes were measured daily. Small amount of blood was drawn weekly after 12 hour fasting from ear vein to examine the changes of plasma lipids and CETP activity over the experimental period. Prior to sacrificing animals, food was removed from their cages for about 16 hours. Each animal was anesthetized with Ketamin and blood was removed from heart puncture. Aortae were removed for Oil Red O lipid staining and liver was removed for measurement of HMG-CoA reductase activities.

Plasma lipids and vitamin E measurements

HDL fractions were separated using heparin-$MnCl_2$ precipitation method(12), and total plasma and HDL-cholesterol were measured using Asan-set cholesterol kit and plasma triglyceride using Asan-set triglyceride kit. Plasma vitamin E levels were measured in hexane extract using "Desai's improved spectrophotometric assay for vitamin E"(13). Hexane extract from 2ml plasma was dried under nitrogen gas. The dried lipid residue was dissolved in ethanol and mixed with batho-phenanthroline reagent. After adding $FeCl_3$ and H_3PO_4 solution to the sample mixture, its absorbance was measured at 562 nm. Plasma vitamin E concentration was calculated using α-tocopherol as a standard.

Isolation of whole lipoprotein fraction

For isolation of plasma lipoproteins, density of plasma was adjusted to 1.225g/ml with solid KBr and these samples were overlayed with a d=1.225g/ml KBr solution in cellulose-nitrate tubes(12ml)(14). Ultracentrifugation was carried out using a Ti 70.1 rotor at 38.000 rpm for 40 hours at 10°C in a Beckman L5-50 ultracen-

trifuge(Beckman, Fullerton, CA, USA). Lipoproteins isolated in the d<1.225g/ml supernatant were obtained by pipetting the upper 1~2ml of the tubes with careful handling.

SDS-PAGE analysis of plasma apolipoproteins

Electrophoresis was performed on polyacrylamide slab gels using Laemmli's method(15). Electrophoresis was performed at 120v in Tris/glycine buffer, pH 8.8. After electrophoresis the gels were stained directly with 0.05% Coomassie Brillant Blue(R 250) in methanol-glacial acetic acid-water 5 : 1 : 4(v/v). The gels were destained for 4hr with methanol-glacial acetic acid-water 5 : 1 : 5(v/v) and then overnight with methanol-glacial acetic acid-water 1 : 1 : 8(v/v).

Measurement of oxidizability in isolated whole lipoproteins

Lipoprotein peroxides formed *in vitro* were measured in oxidized lipoprotein samples using method of El-Saadani et al.(16). Plasma lipoproteins obtained from ultracentrifugation were dialyzed extensively against degassed phosphate-buffered saline(PBS) to remove any salts which were present throughout the preparation. Formation of lipid peroxides was measured during copper-induced oxidation of rabbit lipoprotein. Lipoproteins were incubated with 5μM $CuCl_2$ in phosphate-buffered saline containing 2.5μM ascorbic acid to generate the lipid peroxides. Concentrations of lipid peroxides were calculated with absorbance at 365nm. Cumene hydroperoxide was used for standard.

Lipid staining of aortic tissues

At time of sacrificing animals, the aortae were removed, cleaned of periadventitial tissue, and opened longitudinally. The incision was on the anterior wall of the thoracic aorta, and on the posterior wall of the abdominal aorta, in order to avoid damaging the branch orifices. The aorta was stained with a saturated solution of Oil Red O in 60% isopropyl alcohol for 10min, rinsed with 60% isopropyl alcohol to remove excess staining, and then returned to phosphate-buffered saline. The pressure-fixed aorta was pinned on a cork board to expose the intimal surface, which was photographed in color.

Assay of HMG-CoA reductase and CETP activities

Liver was homogenized and microsome pellets were obtained by ultracentrifugation. Activities of HMG-CoA reductase were measured by the method of Hulcher and Oleson(17). The level of released coenzyme A was measured spectrophotometrically during the reduction of 3-hydroxy-3-methyl CoA to mevalonate. Plasma CETP activities were measured using artificial HDL as cholesteryl ester donor and artificial LDL as cholesteryl ester(CE) acceptor which was previously described (18). Radioactivities of CE transferred from HDL to LDL were counted by Liquid Scintillation counter, and CETP activities were calculated as percent of transferred radiolabeled CE/total radiolabeled HDL-CE.

Statistic analysis

Mean and standard error were computed by SPSS statistical package. Comparison between diet groups was made using student t-test(19).

RESULTS AND DISCUSSION

Fig. 1 shows the weekly changes in plasma total cho-

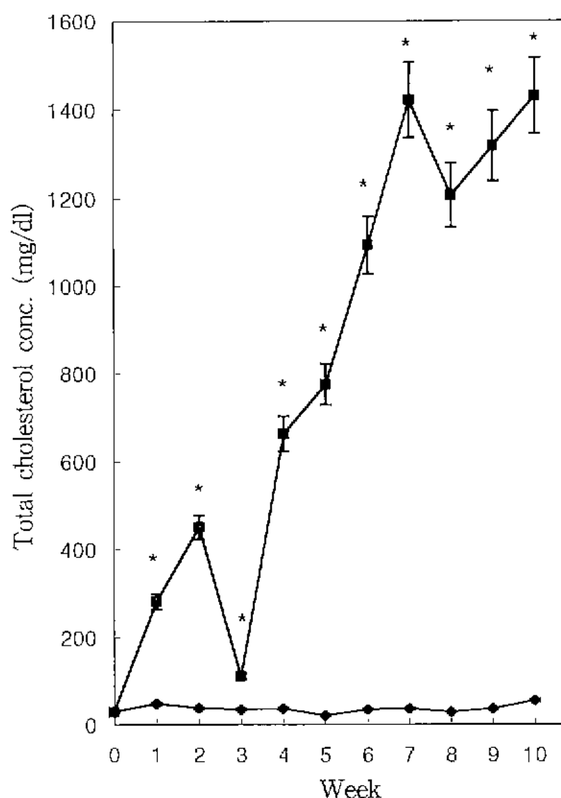

Fig. 1. Weekly changes in plasma total cholesterol concentrations of rabbits fed control and high cholesterol diet.
Values are expressed as mean±SEM. *$p<0.05$
-◆-: Control group, -▣-: High cholesterol group

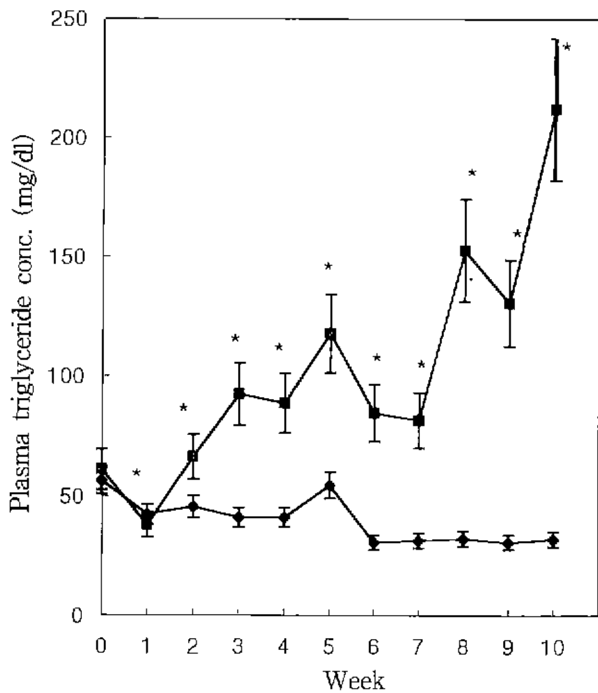

Fig. 2. Weekly changes in plasma triglycerides concentrations of rabbits fed control and high cholesterol diet.
Values are expressed as mean±SEM. *p<0.05
-◆-: Control group, -▩-: High cholesterol group

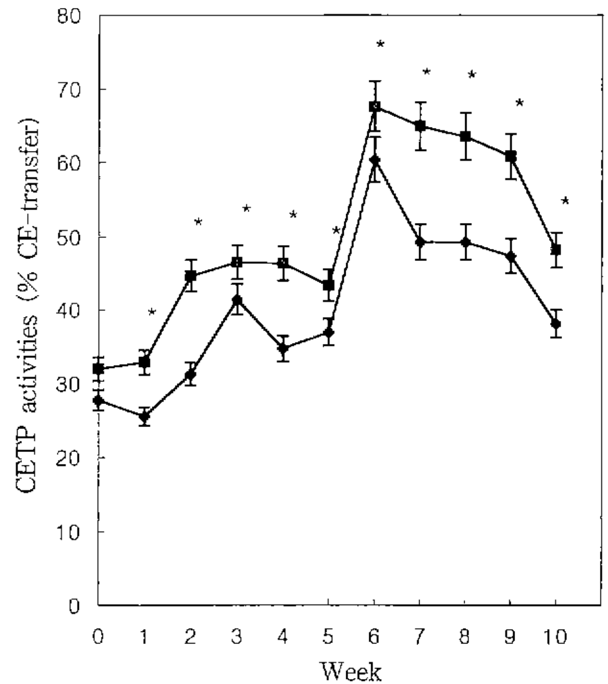

Fig. 3. Weekly changes in plasma CETP activities of rabbits fed control and high cholesterol diet.
Values are expressed as mean±SEM. *p<0.05
-◆-: Control group, -▩-: High cholesterol group

lesterol concentrations of rabbits fed normal and high-cholesterol diet for 10 weeks. Total cholesterol level of control group was maintained quite constantly(30mg/dl to 55mg/dl), but that of high cholesterol group was dropped 450.8mg/dl to 110.2mg/dl at the 3rd week for unknown reason and sharply increased to 1422mg/dl at the 7th week and slightly declined at the 8th and 9th weeks, then increased back to 1431mg/dl at the 10th week. Plasma triglyceride level, as shown in Fig. 2, in high cholesterol-fed rabbit(220mg/dl) was significantly ($p<0.05$) higher than control group(40mg/dl) even though the difference between two groups was less than that of plasma total cholesterol level. The peak of plasma triglyceride and cholesterol concentration appeared at slightly different period in high cholesterol group over 10 weeks. In fact, the relationship between plasma triglyceride levels and dietary cholesterol levels has failed to show consistent association according to other findings(20,21). HDL-cholesterol level was higher in high-cholesterol group than control group, but the ratio of HDL- C/total-C was lower in high-cholesterol group than that of control group as shown in Table 1. When HDL-cholesterol level was expressed in terms of atherogenic indices, it was significantly higher in high cholesterol group(39.48) than control group(5.08).

Fig. 3 shows the weekly changes in plasma CETP activities of both groups. These were significantly higher in high-cholesterol group(64.9% at the 7th week) than control group(49.3% at the 7th week) during most

Table 1. Concentration of total cholesterol, HDL-cholesterol, percentage of HDL-cholesterol and atherogenic index of rabbits fed control and high cholesterol diet for 10 weeks

	Total-C (mg/dl)	HDL-C conc. (mg/dl)	HDL-C/Total-C (%)	Atherogenic Index
Control group(n=7)	55.5± 4.1	9.1±0.8[a]	16.4±1.1[a]	5.08±0.2[a]
High cholesterol group(n=7)	1416.9±19.5	35.4±5.8[b]	2.5±0.6[b]	39.48±4.6[b]

Each values are expressed as a mean±SEM. Different superscripts in the same column indicate significant differences ($p<0.05$) between groups
Atherogenic Index=[(Total-C-HDL-C)/HDL-C]

Table 2. Effects of dietary cholesterol on hepatic HMG-CoA reductase activity, oxidizability of whole lipoproteins and plasma vitamin E concentration

	Control group(n=7)	High cholesterol group(n=7)
HMG-CoA reductase activities (nmoles of CoA-SH formed/min)	2.1±0.6[a]	0.3± 0.1[b]
Lipid peroxides conc. (nmol/ml plasma lipoprotein)	29.6±3.9[a]	190.4±38.2[b]
Vit E conc. (μg/ml)	5.1±0.4[a]	3.3± 0.4[b]

Each values are expressed as a mean±SEM. Different superscripts in the same column indicate significant differences ($p<0.05$) between groups

of the experimental period. The activity of HMG-CoA reductase which regulates the rate limiting step of cholesterol synthesis was measured by using liver tissues. It was significantly decreased in high cholesterol-fed animals(0.3nmoles) compared to control animals(2.1 nmole CoA-SH formed/min) as shown in Table 2. Oxidation of isolated lipoproteins was carried out to compare oxidizabilities of lipoprotein samples from these two groups, which is shown in Table 2 along with plasma vitamin E levels. When lipoprotein samples were oxidized *in vitro*, more lipid peroxides were formed in high-cholesterol group(190.4nmole/ml) than control group (29.6nmoles/ml). But level of plasma vitamin E was lower in high-cholesterol group than control group.

Profiles of plasma apolipoproteins were compared by visualized bands from SDS-PAGE as shown in Fig. 4. Apo A-I which is a major apoprotein in HDL was thicker in control group, but apolipoprotein B which is a major apoprotein in LDL was thicker in high-cholesterol group according to the Coomassie Blue-stained bands. Arterial intima walls were compared between these two groups in Fig. 5. These lipid-stained aorta displayed fatty streaks only in high-cholesterol group. Cholesterol feeding also induced fatty livers in high-cholesterol group as shown in Fig. 6.

In other studies, cholesterol-added diet in 2% resulted in a rapid development of hypercholesterolemia and an increase of CETP activities in hamsters(23) and men (24). It is well known that cholesterol is generally the strongest factor in changing blood cholesterol among various dietary components, and it raises particularly

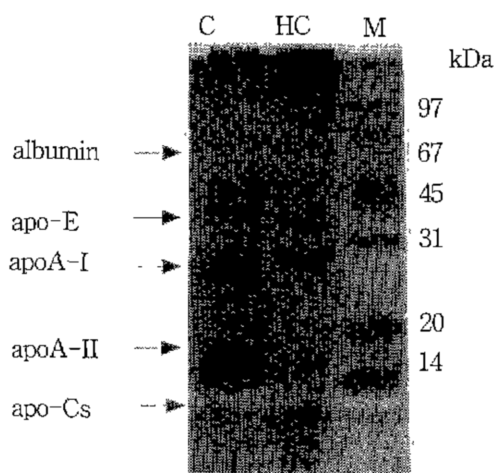

Fig. 4. SDS-PAGE analysis of the apolipoproteins of rabbits fed control and high cholesterol diet.
C: Control group, HC: High-cholesterol group

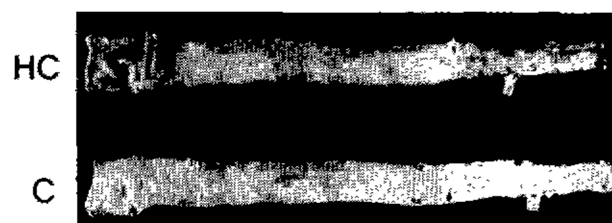

Fig. 5. Lipid-stained aortas of rabbits fed control and high cholesterol diet.
C: Control group, HC: High-cholesterol group

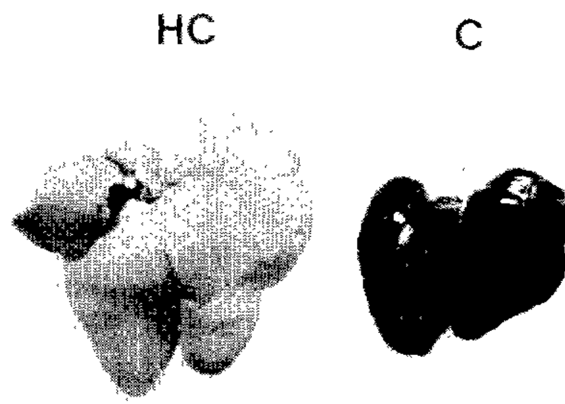

Fig. 6. Comparison of liver appearance of rabbits fed control and high-cholesterol diet.
C: Control group, HC: High-cholesterol group

HDL-cholesterol and decreases HDL-cholesterol/total cholesterol ratio(8).

Plasma HDL level can also be regulated by CETP activities. Reverse cholesterol transport which is mediated by HDL via action of LCAT and CETP is important for removal of blood cholesterol. For the protective role of HDL to atherosclerosis, several lines of evidence have suggested a role for HDL in the protection of LDL against oxidative modification. LCAT reaction can be inhibited by the presence of oxidized LDL in plasma, which impairs HDL-mediated reverse cholesterol transport(25). HDL also has been shown to be an effective scavenger of superoxide *in vitro*(26), possibly indicating a direct effect of HDL as a reactive oxygen scavenger. Recent experimental evidences suggest that products of LDL oxidation can transfer from minimally oxidized LDL to HDL(27,28). Thus, it has been proposed that by accepting and transporting such oxidation product, HDL may protect against some of the atherogenic effects of oxidized LDL. Other workers have reported that HDL is the principal carrier of lipid hydroperoxides, and oxidized cholesteryl esters in plasma HDL are taken up to a greater extent by liver cells than unoxidized cholesteryl esters(29).

There are more increasing evidences that the initiation of atherosclerosis is related to the lipid peroxidation and oxidative modification of LDL(30-33). The propagated oxidation process includes breakdown of essential membrane lipids, and also peroxidation of unsaturated fatty acids derived from triglycerides, phospholipids and cholesterol in lipoproteins(33). The resistance of lipoproteins to oxidation is partly determined by their content of antioxidants. Alpha-tocopherol is the most active compound of vitamin E(34). Plasma level of vitamin E is the strongest predictive factor for cross- cultural mortality from ischemic heart disease among all essential antioxidants, including vitamin A, C, E, carotenoid and selenium(35). Vitamin E also is the major antioxidant in LDL and is among the first antioxidant to be consumed during LDL-oxidation(36,37). *In vitro*, oxidation of isolated whole lipoproteins antioxidants within LDL become rapidly exhausted, allowing lipid peroxidation to proceed. This may also be the case *in vivo* in case of high oxidative stress or where antioxidants are deficient. Plasma vitamin E level was lower in cholesterol-fed rabbits than control rabbits in our study, which suggests more oxidative stress was given to this group than control group. This can be supported by Matz et al's finding(38) in which reported that the susceptibility of rabbit β-VLDL to oxidation was markedly decreased in the vitamin E treated animals as assessed by the formation of conjugated dienes. Alpha-tocopherol was shown to be an important antioxidant preventing the *in vitro* oxidation of VLDL and LDL fractions even in non-supplemented human subjects(39). The effect of vitamin E supplementation also changes the plasma CETP activities. In recent findings on roles of vitamin E in lipoprotein metabolism(40), it has been suggested that the vitamin E supplementation may help to ameliorate the dyslipidemia caused by a diet high in cholesterol and saturated fat through its inhibitory influence on plasma CETP activities and CETP production in adipose tissue. Therefore vitamin E may not only protect the oxidation of plasma LDL but improve the reverse cholesterol transport through the inhibition of CETP in the pathway of lipoprotein metabolism.

In conclusion, hypercholesterolemia due to cholesterol supplementation induced fatty streaks in the rabbit arterial wall, retardation of reverse cholesterol transport due to the decrease of CE transfer, and a marked increase of the copper-mediated oxidation of rabbit lipoprotein *in vitro*. These data also suggest that supplementation of dietary vitamin E could be beneficial in reducing the oxidative stress from the retarded process of reverse cholesterol transport associated with hypercholesterolemia.

ACKNOWLEDGEMENTS

This research was partly supported by the 1994 Korea Science and Engineering Foundation(KOSEF) grant (No. 94-0403-16-01-3).

REFERENCES

1. Gorden, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. and Dawber, T. R. : High density lipoprotein as a protective factor against coronary heart disease : the Framingham study. *Am. J. Med.*, **62**, 707(1977)
2. Miller, N. E., Forde, O. H., Theille, D. S. and Mjos, O. D. : The Tromso heart-study. High density lipoprotein and coronary heart-disease : a prospective case-control study. *Lancet*, **1**, 965(1977)
3. Gordon, D. : Where should the clinician stand? In "*HDL*" Durington, P. N.(ed.), Mark Allen Publishing, London, p. 17(1992)
4. Klimov, A. N., Kkozhemyakin, L. A., Pleskov, V. M. and

Andreeva, L. I. : Antioxidative effect of high density lipoproteins in the oxidation of low density lipoproteins. *Bull. Exp. Biol. Med.(Russ)*, **103**, 550(1987)

5. Reichl, D. and Miller, N. E. : Pathophysiology of reverse cholesterol transport : insights from inherited disorders of lipoprotein metabolism. *Arteriosclerosis*, **9**, 785(1989)
6. Swenson, T. L. : The role of the cholesteryl ester transfer protein in lipoprotein metabolism. *Diabetes/Metabolism Reviews*, **7**, 139(1991)
7. Schwartz, C. C., Halloran, L. G., Vlahcevic, Z. R., Gregory, D H. and Swell, L. : High and low density lipoprotein metabolism in man : Preferential utilization of HDL free cholesterol by the liver for biliary cholesterol secretion. *Science*, **200**, 62(1978)
8. Thomas, M. A., Mueller, S. B., Mazur, M. J., Uhlendor, P. D., Brown, E. Q. and Kieft, K. A. : The relationship between the degree of dietary-induced hypercholesterolemia in the rabbit and atherosclerotic lesion formation. *Atherosclerosis*, **102**, 9(1993)
9. Fielding, C. J. and Fielding, P. E. : Molecular physiology of reverse cholesterol transport. *J. Lipid Res.*, **36**, 211(1995)
10. Choi, M. S., Cho, S. H. and Yoon, H. S. : Effect of milk on cholesterol metabolism of rats with different levels of dietary cholesterol. *Korean J. Nutr.*, **27**, 127(1994)
11. Roth, R. I., Gaubatz, J. W. and Gotto, Jr., A. M. : Effect of cholesterol feeding on the distribution of plasma lipoproteins and on the metabolism of apolipoprotein E in the rabbit. *J. Lipid Res.*, **24**, 1(1983)
12. Russel Warnick, G. and Albers, J. : A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J. Lipid Res.*, **19**, 65(1978)
13. Desai, I. D. : A comprehensive treatise. In "*Vitamin E*" Marchim. L J.(ed.), Dekker, New York Publishers, p. 67(1980)
14. Chapman, M. J., Goldstein, S., Lagrange, D. and Laplaud, P. M. : A density gradient ultracentrifuge procedure for the isolation of the major lipoprotein classes from human serum. *J. Lipid Res.*, **22**, 339(1981)
15. Laemmli, U. K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680(1970)
16. El-Saadani, M., Esterbaucer, H., El-Sayed, M., Goher, M., Nassar, A. Y. and Jurgens, G. : A spectrophotometric assay for lipid peroxides in serum lipoprotein using a commerically available reagent. *J. Lipid Res.*, **30**, 627 (1989)
17. Hulcher, F. H. and Oleson, W. H. : Simplified spectrophotometric assay for microsomal HMG-CoA reductase measurement of coenzyme A. *J. Lipid Res.*, **14**, 625 (1973)
18. Park, Y. B., Jeoung, N. H., Kim, H. S. and Choi, M. S. : Reaction of HDL-bound choleseryl ester transfer protein and a new method for its activity measurement. *Kor. Biochem. J.*, **25**, 409(1992)
19. Steel, R. G. D. and Torrie, J. H. : In "*Principles and procedures of statistics*" London : McGrow-Hill Book Company, N.Y., p. 481(1960)
20. Grundy, S. M. and Vega, G. L. : Hypertriglyceridemia: Causes and relation to coronary heart disease. Semin. *Thromb. Hemost.*, **14**, 149(1988)
21. Sirikci, O., Ozer, N. K. and Azzi, A. : Dietary cholesterol-induced changes of protein kinase C and the effect of vitamin E in rabbit aortic smooth muscle cells. *Atherosclerosis*, **126**, 253(1996)
22. Tall, A. R. : Plasma cholesteryl ester transfer protein. *J. Lipid Res.*, **34**, 1255(1993)
23. Stein, Y., Dabach, Y., Hollander, G. and Stein, O. : Cholesteryl ester transfer protein activity in hamster plasma increased by fat and cholesterol rich diets. *Biochimica et Biophysica Acta*, **1042**, 138(1990)
24. Martin, L. J., Connelly, P. W., Nancoo, D., Wood, N., Zhang, Z. J., Maguire, G., Quinet, E., Tall, A. R., Marcel, Y. L. and McPherson, R. : Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. *J. Lipid Res.*, **34**, 437(1993)
25. Bjielicki, J. K., Forte, T. M. and McCall, M. R. : Minimally oxidized LDL is a potent inhibitor of lecithin: cholesterol actyltrasferase activity. *J. Lipid Res.*, **37**, 1012(1996)
26. Chander, R. and Kapoor, N. K. : High density lipoprotein is a scavanger of superoxide anions. *Biochem. Pharmacol.*, **40**, 1663(1990)
27. Watson, A. D., Navab, M., Hama, S. Y., Sevanian, A., Precott, S. M., Stafforini, D. M., McIntyre, T. M., La Du, B. N., Fogelman, A. M. and Berliner, J A. : Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. *J. Clin. Invest.*, **95**, 774(1995)
28. Mastsuda, Y., Hirata, K., Inoue, N., Suematsu, M., Kawashima, S., Akita, H. and Yokoyama, M. : High density lipoprotein on endothelium-dependent arterial relaxation. *Circulation Research*, **72**, 1103(1993)
29. Bowry, V. W., Stanley, K. K. and Stocker, R. : High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. *Proc. Natl. Acad. Sci.*, **89**, 10316(1992)
30. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W B. and Dawber, T. R : High density lipoprotein as a protective factor against coronary heart disease: The Framingham Study. *Am. J. Med.*, **62**, 707(1977)
31. Rifkind, B. M. : High density lipoprotein cholesterol and coronary artery disease: survey of the evidence. *Am. J. Cardiol.*, **66**, 3A(1990)
32. Wilson, P. W. F. : High density lipoprotein, low density lipoprotein and coronary artery disease. *Am. J. Cardiol.* **66**, 7A(1990)
33. Durrington, P. N. : Hyperlipidemia, diagnosis and management. Oxford, Butterworth Heinemann(1994)
34. Burton, G. W. and Ingold, K. U. : Vitamin E as an *in vitro* and *in vivo* antioxidant. *Ann. NY Acad. Sci.*, **570**, 7(1989)
35. Gey, K. F., Puska, P., Jordan, P. and Moser, U. : Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross culture epidemiology. *Am. J. Clin. Nutr.*, **53**, 326(1987)
36. Esterbauer, H., Gebicki, J., Puhl, H and Jurgens, G. :

The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.*, **13**, 341d(1992)

37. Esterbauer, H., Rotheneder, M. D., Striegl, G. and Waeg, G. : Role of vitamin E in preventing the oxidation of LDL. *Am. J. Clin. Nutr.*, **53**, 314S(1991)
38. Matz, J., Andersson. T. M. G., Ferns, G A. A. and Anggard, E. K. : Dietary vitamin E increases the resistance to lipoprotein oxidation and attenuates endothelial dysfunction in the cholesterol-fed rabbits. *Atherosclerosis*, **110**, 241(1994)
39. Porkkala-Sarataho, E., Nyyssonen, K. and Salonen, J. T. : Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in non-vitamin E supplemented men. *Atherosclerosis*, **124**, 83 (1996)
40. Shen, G. X., Novak, C. and Angel, A. : Effect of dietary vitamin E supplements on cholestryl ester transfer activity in hamster adipose tissue. *Atherosclerosis*, **124**, 211(1996)

(Received February 1, 1997)