한국영양식량학회지

Lecithin : Cholesterol Acyltransferase Activities in Rats Fed Cow's Milk with Different Levels of Cholesterol

Myung-Sook Choi

Dept. of Food and Nutrition Science, Kyungpook National University, Taegu 702-701, Korea

Abstract

The effect of milk in low and high cholesterol diets were investigated on serum cholesterol esterification. Weanling male Sprague-Dawley rats were divided into low (0.01% w/w) and high (1.01% w/w) cholesterol-diets groups. Both low and high cholesterol groups were consisted of three groups : control, LM (low heat milk), and HM (high heat milk) groups. After feeding these experimental diets for six weeks, serum cholesterol (free cholesterol and cholesteryl ester) concentration and activity of lecithin : cholesterol acyltransferase (LCAT) were measured, and serum lipoprotein profile was examined using gel column chromatography. According to the result, activity of LCAT was elevated independently by intakes of high cholesterol and milk, which resulted in the increase of daily turnover of serum cholesteryl ester. However, the turnover of HDL-cholesteryl ester increased only by milk. LCAT activity was moderately correlated with levels of total- and HDL-free cholesterol. It is concluded from the present study that milk had the cholesterol-lowering effect which partly appears to be mediated through facilitated reverse cholesterol transport.

Key words : LCAT, cholesterol esterification, reverse cholesterol transport, cholesterol-lowering effect

INTRODUCTION

Hypercholesterolemia is a major risk factor of coronary heart disease (CHD), the leading cause of death in the many countries. An increased concentration of high density lipoprotein (HDL) cholesterol exerts a protective role in the pathogenesis of atherosclerosis, whereas low density lipoprotein (LDL) cholesterol is associated with development of atherosclerotic lesions[1-4].

The reverse cholestrol transport which is instrumental in protecting the cardiovascular system against atherosclerosis in human and animal, comparises a series of reactions by which peripheral cell cholesterol is returned to the liver[5,6]. In this transport system HDL is the initial acceptor of cellular cholesterol and the enzyme LCAT contributes to reverse cholesterol transport by converting the free cholesterol (FC) on the surface of the circulating HDLs to cholesteryl ester (CE). In reverse cholesterol transport of rats, excessive cholesterol in peripheral tissue is removed mainly by HDL action in conjuction with LCAT, which provides the deriving force for net cholesterol removal[7]. Petersburg and Ellefson[8] showed that esterified lipoprotein cholesterol is cleared from rat plasma much faster than free cholesterol.

LCAT activities can be altered by various factors. Liu *et al.*[9] reported that plasma LCAT activities were significantly lower than normal in hyperlipidemic (type IIa) patients with coronary heart disease. An age related decrease was observed in the fractional rate of plasma cholesterol esterification[10]. Lacko *et al.*[11] reported that LCAT activities were associated with hypercholesterolemia and premature atherosclerosis and these activities also can be influenced by types of fats[12,13]. Yashiro and Kimura[14] showed an increase in LCAT activities with an increase in dietary protein and in exercise levels in rats. And disminished plasma LCAT activities were observed in rats fed animal protein[15] and iron-deficient rats[16]. Among dietary factors

which influence on lipid metabolism, dairy products have recently received much attention regarding their possible effects on plasma cholesterol metabolism. They contain lipids including saturated fatty acids and cholesterol which are able to change serum cholesterol levels. Feeding whole milk or skim milk to experimental animals reduced serum cholesterol[17-21] and activity of the rate-limiting enzyme, hydroxymethylglutaryl coenzyme A reductase[22]. The human studies have produced some conflicting results which have shown a decrease or no changes in plasma lipids with consumption of various milk products[23-25], but this can be partly due to different experimental conditions used. Although significant amounts of information have accumulated on these topics, the reasons for plasma cholesterol changes upon feeding milk are presently unknown. It is of our interest to investigate how the supplementation of cow's milk alters regulation of cholesterol metabolism. The objective of this study was to determine the effects of cow's milk on serum cholesterol metabolism via LCAT reactions in rats fed two different levels of cholesterol. Cow's milk used for this study was two types which are commercially processed at different temperatures.

MATERIALS AND METHODS

Animal experiment

Whole milk was fed to rats using liquid diet bottles. The two different whole milk used were sterilized at 65°C for 30min and 140°C for 2 min respectively. Experimental diets used were three low cholesterol diets (0.01% cholesterol) and three high cholesterol diets (1.01% cholesterol). In each level of cholesterol a control diet was given without milk supplementation as shown in Table 1. Low cholesterol diets (LC) were a cholesterol added (0.01%) basal diet (LC-Control) with water, a basal diet with high heated milk (LC-HM) and a basal diet with low heated milk (LC-LM). High cholesterol diets (HC) were a cholesterol added (1.01%) basal diet with water (HC-Control), a cholesterol added(1.0%) basal diet with high heated milk (HC-HM) and a cholesterol added (1.0%) basal diet with low heated milk (HC-LM). Cholesterol and Na-taurocholate were added to the high cholesterol diet to elevate serum cholesterol sufficient to induce experimental hypercholesterolemia[26]. The compositions of six experimental diets are shown in Table 2. Each group of Sprague Dawley rats (animal breeding labs, Taejon, Korea) was fed one of experimental diets for 6wk following 5 days of adjusting period. Animals were housed in temperature (21~23°C) and light controlled rooms with a 12-h light-dark cycle beginning at 0800h and at ambient humidity (30 ~60 %). Prior to sacrificing animals, food was removed from their cages for about 16h. Each rat was anesthetized with ether, and blood was removed from inferior vena cave puncture into syringes containing a final concentration of 4mM DTNB (5,5-dithiobis-2-benzoic acid) to inactivate LCAT.

Table 1. Six dietary groups with two levels of cholesterol and two types of milk in diets

Milk types	Cholesterol levels	
	LC (Low cholestrol[a])	HC (High cholestrol[b])
Control	Basal diet[c] +0.01% Chol. +Water (n=8)	Basal diet +1.01% Chol.+0.3% Cholate +Water (n=7)
HM (High heat milk)	Basal diet +Milk (n=7)	Basal diet +1% Chol.+0.3% Cholate +Milk (n=8)
LM (Low Heat Milk)	Basal diet +Milk (n=8)	Basal diet +1% Chol.+0.3% Cholate +Milk (n=8)

[a] In the preliminary experiment, cholesterol intake by milk supplementation was about 0.01% (g/g diet) in milk fed rats Therefore, the same amount of cholesterol was added to the basal diet in control diet of LC groups
[b] Additional 1% cholesterol (g/g diet) was inclulded in the solid diets. Control diet for HC include 0.01% (g/g diet) more cholesterol than milk groups due to the same reason described above^
[c] AIN-76 semipurified diet

Serum cholesterol measurements and lipoprotein profile

The blood was centrifuged at $1,200 \times g$ for 20min at 4°C to separate serum. A sample of serum was immediately frozen until analyzed for LCAT activity and cholesterol concentrations. HDL was separated using heparin–MnCl2 precipitation method[27] and total serum– and HDL–cholesterol were measured for total and free cholesterol using Asanset cholesterol kit. The concentration of esterified cholesterol were calculated as the difference of free cholesterol concentration from total cholesterol concentration. For isolation of serum lipoproteins, densities of pooled serum (7ml) which obtained from each diet group were 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 capacity)[28]. Ultracentrifugation was carried out by using a Ti 70.1 rotor at 38,000rpm for 40 hours at 10°C in a Beckman L5–50 ultracentrifuge (Beckman, Fullerton, CA, USA). Lipoproteins isolated in the $d < 1.225g/ml$ supernatant (the upper 1~2ml of the tubes) were obtained by pipette with careful handling. Aliquots of the concentrated lipoprotein samples for each group were incubated for 30 minutes at room temperature with a trace amount of [³H]cholesterol as a marker. A column (1.5 ×100cm) packed with Bio–Gel A-5M (Bio–Rad Laboratories, Richmond, CA, USA) was used to separate the serum lipoproteins as described previously[29]. The system was operated under gravity with a flow rate of 10ml/h. The column was equilibrated with 0.15M NaCl, 0.01% EDTA, pH 7.4 for 24 hours before use.

Radioactivities of fractions containing lipoproteins were counted with 20µl aliquots in scintillation vials and plotted in a chromatogram.

LCAT enzyme assay

LCAT activity was assayed essentially by the method of Stokke and Norum[30] as modified by Lacko et al.[31] wherein the substrate and enzyme were from the same serum whose LCAT was inactivated prior to serum separation. 7-[³H]cholesterol (specific activity 23. 0µCi/mmol) was obtained from New England Nuclear Corp. (Boston, MA, USA) and diluted with acetone to a final concentration of 100µCi/ml. The emulsion of [³H]cholesterol–albumin was prepared as described following. Briefly 250µCi of [³H]cholesterol in acetone was slowly added to 5ml of human albumin solution (250mg of human albumin dissolved in 0.2M phosphate buffer, pH 7.4) with nitrogen pudding. This solution was then placed under nitrogen until it was free of acetone. The emulsion was found to be stable for 4 weeks at 4°C. Rat serum (420µl) were incubated with 105µl of [³H]cholestrol–albumin emulsion (5µ–Ci/ml), the enzyme was then reactivated with 70µl of 0.1M mercaptoethanol and further incubated. When the activation of LCAT occurred by adding mercaptoethanol, aliquots (123 µl) of samples were removed at 5 minute intervals over a 20 minute period and the reaction was stopped by the addition of chloroform/methanol (2 : 1, v/v) immediately after removal from incubation. The lipid residue was extracted with 6ml of chloroform/methanol (2 : 1, v/v), filtered under vacuo, and evaporated to dryness. It was then transferred to thin layer plates of silical gels (Whatman, Inc., Clifton, NJ, USA) in a small volume of hexane. The plates were developed in petroleum ether/diethyl ether/acetic acid (90 : 10 : 1, v/v/v) and identif-

Table 2. Compositions of solid diet for six dietary groups

Diets	LC		HC	
Ingredients	Control	HM, LM	Control	HM, LM
	— g/100g diet —			
Casein	20.0	20.0	20.0	20.0
D,L–methionine	0.3	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0	15.0
Sucrose	50.0	50.0	48.7	48.0
Cellulose powder	5.0	5.0	5.0	5.0
Mineral mixture[a]	3.5	3.5	3.5	3.5
Vitamin mixture[b]	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2
Corn oil	4.99	5.0	4.99	5.0
Cholesterol	0.01	0.0	1.01	1.0
Na–taurocholate	0.0	0.0	0.3	0.3

Control : water, HM : high heat milk, LM : low heat milk
[a] AIN–76 mix used at this level in 100mg diet provided the following amounts of each mineral : Ca, 520mg ; P, 400mg ; K, 360mg ; Na, 102mg ; Cl, 157mg ; S, 337mg ; I, 0.02mg ; Fe, 3.5mg ; Mg, 50.6mg ; Zn, 3mg ; Cu, 0.6mg ; Mn, 5.9mg
[b] AIN–76 mix used at this level in 100mg diet provided the following amounts of each vitamins : thiamin, 0.53mg ; riboflavin, 0.53mg ; pyridoxine HCl, 0.7mg ; niacin, 3.0mg ; calcium pantothenate, 1.6mg ; folic acid, 0.02mg ; vitamin B12, 1.0mg ; vitamin A, 120R.E. ; vitamin E, 3.9T.E. ; vitamin D3, 100U ; menadolin sodium bisulfite, 0.15mg
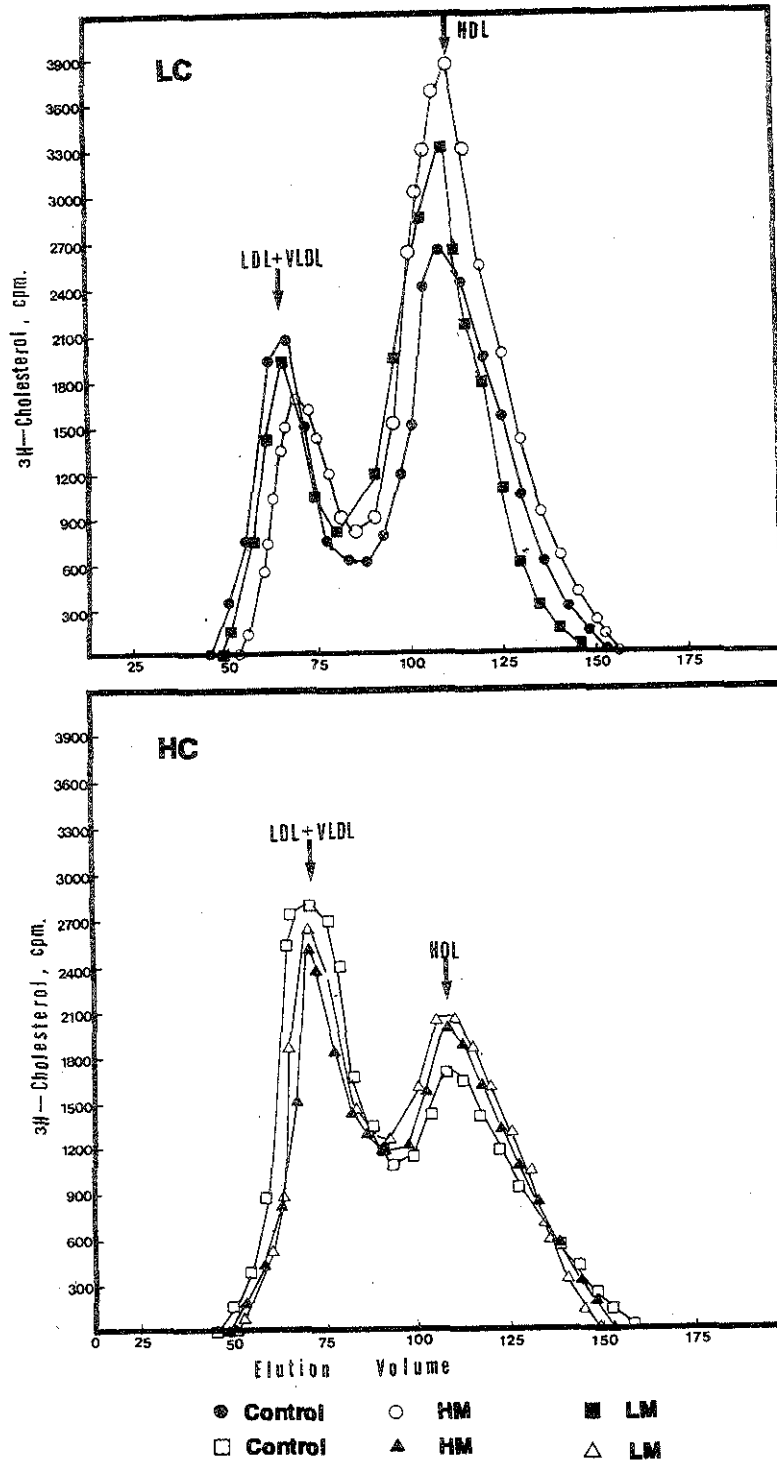
Fig. 1. **Comparison of the distribution of radiolabeled cholesterol among serum lipoproteins in rats fed six experimental diets.**
Serum lipoprotein from LC and HC groups were labeled with [³H]cholesterol and chromatographed on a Bio–Gel A 5M column (1.5 × 100cm). Fractions (2ml) were collected and analyzed for radioactivity as described under methods. The elution volumes of standard rat lipoprotein are indicated by arrows. Distribution of HDL–cholesterol and (LDL + VLDL) cholesterol among fractionated lipoproteins are shown for LC (upper panel) and HC (lower panel) groups.

ied in iodine vapor. The areas containing free cholesterol and cholesteryl ester were scraped into liquid scintillation counting vials and counted using 7ml of PC-S liquid scintillation fluid (Amersham/Searle, Arlington Height, IL, USA). Total activity is expressed as fractional rate of esterification (FR, % cholesterol esterified/h) which was calculated from the slope of the line by least square analysis, and molar rate of esterification (nmoles/ml serum/h) was also calculated. The net turnover rate (NR) of serum CE was calculated as a product of the serum FC pool (mg) and the FR. The serum FC pool was obtained as the product of total serum cholesterol concentration (mg/ml) and the serum volume (ml), which was assumed to be 4% of the body weight[32].

Statistical analyses

Mean and standard error were computed by SPSS statistical package. Comparison among diet groups was made using one-way analysis of variance, and effects of cholesterol and milk were analyzed by two-way analysis of variance. Correlation coefficients were computed to find any correlation between dietary treatment and LCAT activities.

RESULTS

Serum cholesterol and lipoprotein profile

All diet groups showed similar nutrient consumption patterns and weight gains (data not shown here). Daily milk intakes were 21.1 ± 0.3g, and 22.1 ± 0.7g for HM- and LM-group of low cholesterol-fed animals, and 26.1 ± 0.9g and 24.7 ± 1.4g for HM- and LM-group in high cholesterol-fed groups, respectively. Serum lipoprotein profile which was obtained from the distribution of radioactivity on gel column chromatography clearly shows only 2 major peaks, HDL and apo-B containing lipoproteins, as shown Fig. 1. Effects of cholesterol and milk intake were observed in the profile of this chromatogram showing that the radioactivities of HDL-cholesterol decreased by high cholesterol feeding and those of lower density lipoprotein cholesterol by milk supplementation. As shown Fig. 1, the radioactivities of HDL in LC groups were higher than those of HC groups. Milk fed groups in both LC and HC groups showed the radioactivities of HDL-cholesterol higher than that of their control (LC -Control or HC-Control). Data pertaining to the effects of dietary cholesterol and milk in serum and lipoprotein cholesterol are shown in Table 3 where cholesterol concentrations are expressed as free and esterified content respectively. In HC groups, both types of cholesterol were significantly lower ($p < 0.001$) in HDL fractions but higher ($p < 0.001$) in serum and lower density lipoprotein than those of LC groups (cholesterol effect). Milk effects appeared in serum

Table 3. Contents of serum free and esterified cholesterol in rats fed two types of milk and different level of cholesterol in diets for six weeks[1]

Cholesterol level	Milk types	Total cholesterol		HDL cholesterol		LDL + VLDL	
		FC	CE	FC	CE	FC	CE
		—— mg/dl ——					
LC	Control	24.1 ± 2.0^{NS}	69.8 ± 2.0^{a}	13.1 ± 1.0^{NS}	50.8 ± 1.6^{NS}	11.1 ± 0.8^{a}	18.9 ± 2.6^{a}
	HM	19.2 ± 0.9	61.1 ± 1.6^{ab}	11.5 ± 1.0	52.5 ± 1.8	7.7 ± 0.5^{b}	8.8 ± 1.2^{b}
	LM	21.4 ± 0.8	56.9 ± 3.2^{b}	13.1 ± 0.6	49.6 ± 3.2	8.3 ± 1.4^{ab}	7.4 ± 0.7^{b}
HC	Control	31.2 ± 1.8^{NS}	86.3 ± 4.7^{NS}	7.5 ± 1.7^{NS}	27.4 ± 1.7^{NS}	22.0 ± 1.2^{NS}	62.4 ± 5.5^{a}
	HM	30.4 ± 2.2	77.1 ± 1.8	8.2 ± 0.8	28.9 ± 3.2	22.1 ± 2.2	48.3 ± 3.3^{b}
	LM	33.1 ± 1.2	86.3 ± 2.8	8.0 ± 0.7	32.9 ± 2.0	24.3 ± 1.2	53.4 ± 2.5^{ab}
Cholesterol effect*		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Milk effect*		$p < 0.01$	$p < 0.01$	NS	NS	$p < 0.001$	$p < 0.001$

Control ; water, HM ; high heat milk, LM ; low heat milk
[1] Values without common superscript letters denote significant differences between diets within same cholesterol level at $p < 0.05$
*Denotes significant differences between three low and three high cholesterol levels or between four milk fed groups and two control groups
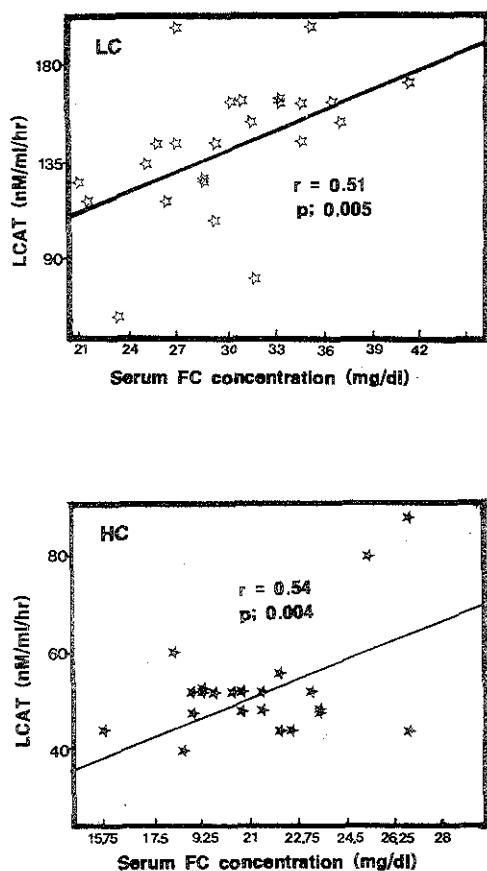NS Not significant

Fig. 2. Linear regression of LCAT activity with serum free cholesterol.
Upper and lower panel represent low cholesterol (LC) and high cholesterol (HC) groups, respectively.

total (p<0.01) and lower density lipoprotein fractions (p<0.001) showing a decrease of FC and CE by milk supplementation, and there was no significant differences between HM and LM group in both LC and HC groups. Within LC, the level of CE in lower density lipoprotein was higher (p<0.05) in control group than milk fed groups while the level of CE in same lipoprotein fraction was higher in control group than HM group within HC groups.

LCAT activities and cholesterol turnover

When LCAT activity was expressed as nmole/ml/h, these activities were positively correlated with serum FC concentrations in both LC (r=0.51, p<0.005) and HC (r=0.54, p<0.004) groups (Fig. 2) and with HDL-FC concentrations (data not shown here, LC : r=0.47, p<0.012, HC : r=0.36, p<0.042). The data regarding the FR of LCAT activity and the NR of total (serum)- and HDL-CE as well as that regarding the FC pool size are given Table 4. FR and NR of total serum-CE in HC groups were significantly higher (p<0.001) than those of LC groups, but not for HDL-CE. The milk supplementation significantly increased the NR of total-CE and HDL-CE at p<0.05 and p< 0.005 respectively, as well as FR at p<0.001. When comparisons are made within HC or LC groups, the NR of HDL-CE in LC groups was higher in LM group than the other two groups and the NR of total-CE in HC groups was higher in HM group than control gro-

Table 4. Effects of dietary cholesterol and milk supplementation on serum cholestryl ester turnover determined *in vitro*

Cholesterol level	Milk types	Final weight	FC-pool		LCAT activity	Net turnover rate	
			Total	LDL	Fractional rate	Total-CE	LDL-CE
		— g —	— mg —		— %/h —	— mg/day —	
LC	Control	316± 6[NS]	3.04±0.11[NS]	1.64±0.12[NS]	8.4±0.7[NS]	6.0±0.6[NS]	3.28±0.34[b]
	HM	342±15	2.65±0.20	1.59±0.19	9.5±0.4	6.0±0.5	3.58±0.41[b]
	LM	366±11	3.14±0.20	1.93±0.13	9.5±0.4	7.1±0.8	4.37±0.45[a]
HC	Control	325±12[NS]	4.05±0.27[NS]	0.97±0.06[NS]	14.3±1.8[NS]	14.0±1.8[b]	3.43±0.50[b]
	HM	356±11	4.35±0.39	1.15±0.12	18.8±0.8	19.5±1.8[a]	5.20±0.62[a]
	LM	331±17	4.41±0.32	1.07±0.12	18.3±0.4	18.3±1.4[ab]	4.48±0.58[a]
Cholesterol effect*		NS	p<0.001	p<0.001	p<0.001	p<0.001	NS
Milk effect*		NS	NS	p<0.05	p<0.001	p<0.05	p<0.005

Control ; water, HM ; high heat milk, LM ; low heat milk
[1] Values without common superscript letters denote significant differences between diets within same cholesterol level at p<0.05
*Denotes significant differences between three low and three high cholesterol levels or between four milk fed groups and two control groups
[NS] Not significant

up. No significant differences were existed between two types milks in NR or CE concentrations.

DISCUSSION

These studies were designed to study how cow's milk alters serum cholesterol metabolism. There are many dietary factors which influence the concentration of serum cholesterol. A good correlation is known to exist between dietary saturated fat or cholesterol intake and serum cholesterol levels[33]. And plant proteins are generally known to be less atherogenic than animal protein[34]. But whole milk is usually restricted to hypercholesterolemic patient since it contains 110mg of cholesterol per liter and about 3.5% fat. In 1974, however, Mann and Spoerry[35] discovered that large consumption of fermented milk actually lowered the serum choleserol level of Masai tribesmen. Since then many investigators have reported that whole milk, skim milk, yogurt, or other milk products have a hypocholesterolemic effect in man and animals[17-21, 23,24]. Thakur and Jha[20] suggested the possibility that yogurt prevented the development of atherosclerosis in cholesterol-fed rats. In our results the increased levels of serum cholesterol in cholesterol fed animals are predominantly due to the increased levels in VLDL and LDL fractions. Milk effect was clear in total serum- and (LDL+VLDL)-fractions as shown in Table 3. These data are in agreement with the suggestion that the supplementation of cow's milk are normocholesterolemic or hypocholesterolemic[17,20,21].

The effectiveness of LCAT in the serum has been assessed by either measuring its ability to esterify endogenous serum cholesterol[11,30,31] or by the esterification of exogenous cholesterol in artifically prepared substrate[36]. Although the levels of circulating LCAT activity measured with an exogenous substrate have been generally found to be normal, lower and higher rates of endogenous cholesterol esterification have been reported for a number of pathological states[11,16]. Our findings regarding the molar rate of cholesterol esterification are also in agreement with earlier studies in which LCAT activity was positively correlated with its substrate concentration in serum[11]. The *in vitro* NR of esterification of total serum–FC

was higher ($p < 0.001$) in cholesterol fed animals due to an increase ($p < 0.001$) in the total FC pool size (Table 4). But the increase in NR was abolished in HDL fractions since the decrease of HDL–FC pool was accompanied with an increase of the fractional LCAT activity in high cholesterol fed groups. Milk supplementation increased not only HDL–FC pool size but also NR of total– and HDL–CE in both LC and HC. In other studies it has been suggested that the NR when estimated by measuring plasma LCAT activity *in vitro* may be indicative of the turnover of CE in HDL or the input of free cholesterol in HDL, since the values for NR obtained by the *in vitro* method were similar to those obtained for HDL–CE turnover by *in vivo* methods[37,38]. Our data on NR of CE would then suggest that the milk supplementation does affect the input of FC in serum HDL and its conversion to HDL–CE whereas the high cholesterol feeding increased total serum– and HDL–FC pool and total–CE turnover, but not HDL–CE turnover. There were no differences between HM and LM group in both low– and high–cholesterol fed animals throughout result which may suggests that heat treatment at 60°C or 140°C does not affect the cholesterol–lowering factors in cow's milk.

The increased LCAT activities in high cholesterol fed rats contribute to esterify FC on HDL more rapidly than normal rate, but may not be effective for FC on lower density lipoproteins in serum. It can be supposed that this consequence may result in accumulation of LDL– and VLDL–cholesterol due to their delayed clearence in serum (Table 3). Milk supplementation seemed to be effective not only for increasing LCAT activity but for increasing HDL–cholesterol levels which is one of protective factor for CHD.

Further investigation involving the quantitative relationship between the rate of hepatic and extrahepatic synthesis of cholesterol, the excretion and reabsorption, and the tissue lipoprotein receptor activity needs to be performed in order to gain a clearer understanding of the mechanism of milk induced or dietary cholesterol related changes in serum and tissue cholesterol levels.

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식이 콜레스테롤의 섭취량에 따라 우유가 흰쥐의 Lecithin : Cholesterol Acyltransferase 활성에 미치는 영향

최 명 숙

경북대학교 식품영양학과

요 약

고콜레스테롤과 저콜레스테롤 식이시 우유가 쥐의 혈청 콜레스테롤 저하 및 콜레스테롤 에스테르화에 미치는 영향을 조사하기 위하여 실험식이를 기본적으로 LC(0.01% 콜레스테롤)군과 HC(1.01% 콜레스테롤)군으로 대분하고 각 고체식이를 액체식이인 물(control), 고온 살균우유(HM), 저온 살균우유(LM)와 함께 공급하는 총 6식이군으로 하였다. 생후 한달 되는 수컷 흰쥐를 각 실험식이로 약 6주간 사육하여 혈청 콜레스테롤 농도를 유리와 에스테르화된 형태로 나누어 비교하고, 혈청 지단백질 분포는 gel column chromatography로 관찰하였으며 LCAT 활성도는 내인성 기질인 [³H] cholesterol을 사용하여 측정하였다. 그 결과 지단백질 분포에서 고콜레스테롤군과 저콜레스테롤군에 속한 우유군은 각 대조군에 비해 HDL peak의 radioactivity가 상대적으로 증가되었으며 이는 혈청 지단백질내의 콜레스테롤 수준 변화와 일치하였다. 효소 LCAT의 활성도(%/h)는 고콜레스테롤 식이와 우유섭취에 의하여 증가하여 체내 1일 총 cholesterol ester turnover도 증가되었으나 HDL-cholesteryl ester의 turnover는 우유섭취에 의해서만 증가되었다. 또한 혈청 유리 콜레스테롤 농도와 LCAT 활성도간에는 유의적수준의 양의 상관관계가 있었다. 이 실험에서 우유섭취는 LCAT의 활성을 증가시켜 HDL의 cholestryl ester의 turnover를 촉진시킴으로써 콜레스테롤 역수송에 기여함을 알 수 있었고, 우유의 콜레스테롤 저하효과도 재확인 되었다.