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Hypocholesterolemic Effects of Soybean Lecithin in Cholesterol-Fed Rats*

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The present study was performed to elucidate the hypocholesterolemic action of lecithin on the diet-induced hypercholesterolemia in rats. Male Sprague-Dawley rats (n=24) were fed lecithin-free (control) diet or diets containing 2% or 5% lecithin for 4 weeks. Hypercholesterolemia was induced by adding 1% cholesterol and 0.5% cholic acid to all diets. No difference was found in food intake and body weight gain among groups. The lecithin treated groups showed significant improvement in the plasma levels of total cholesterol and LDL-cholesterol ($p<0.05$) compared to the control group, while the plasma triacylglyceride was not significantly affected. The atherogenic index and HDL-cholesterol level were decreased in the lecithin groups. The diets with 2% or 5% lecithin significantly decreased the activity of cholesteryl ester transfer protein (CETP) by 14% or 17%, respectively. Also, lecithin diets increased the activity of lecithin: cholesterol acyltransferase (LCAT). These results suggest that lecithin accounts for the hypocholesterolemic effect due to the decreased CETP activity and increased LCAT activity.

Key words: Lecithin, LCAT, CETP, Hypocholesterolemic, Rat

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INTRODUCTION

Heart disease is the third leading cause of death in Korea (mortality rates: 72.9 persons per 100,000 persons), as reported by 2004 yearbook of health and welfare statistics.[1)] Risk factors for coronary heart disease (CHD) include aging, hypercholesterolemia, hypertension and hyperlipidemia.[2)] A high blood cholesterol level is ranked as one of the greatest risk factors contributing to the prevalence and severity of CHD.[3,4)] The blood cholesterol level is determined by the balance among the dietary cholesterol, cholesterol biosynthesis, conversion of cholesterol to bile acids in the liver, and cholesterol absorption and reuptake by the small intestine.[5)] Consequently, the awareness of the need to reduce plasma lipids has been increased and the natural compounds as regulators for cholesterol metabolism are recognized for therapeutic importance.[6)]

Cholesterol-lowering action can be activated through cellular cholesterol homeostasis that is the balance between cholesterol synthesis, cholesterol uptake from lipoproteins, and cholesterol efflux.[7)] Among them, the reverse cholesterol transport (RCT) is the pathway by which peripheral cell cholesterol and cholesterol esters move into the liver, where endocytosis and lysosomal digestion occur, and is a major function of HDL. The HDL obtains cholesterol from other lipoproteins and from cell membranes and converts it to cholesterol esters by lecithin: cholesterol acyltransferase (LCAT) reaction.[8)] Also, the plasma cholesteryl ester transfer protein (CETP) plays a key role in the RCT system that transports cholesterol from peripheral tissues to the liver.[9)] In recent years, accumulating evidence points to a role for certain dietary components in the prevention of rising blood cholesterol levels.[10)] Among them, we were especially interested in soybean lecithin as a protective agent against CHD. Previous studies showed that soybean lecithin had a potent plasma lipid lowering effect in the rat,[11)] pig[12)] and human.[13)] On the other hand, Wong *et al.*[14)] suggested that soybean lecithin have no influence on LCAT activity in hyperlipemic monkeys. The mechanism of cholesterol-lowering action of dietary lecithin is still controversial because of differences in the animal species and in the type of diet used.

The objective of this study was to elucidate the precise mechanism for the hypocholesterolemic action of soybean

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lecithin. The extent which can be attributed to lecithin action was analyzed by measuring the activity levels of CETP and LCAT in rats fed with diets containing 2% or 5% soybean lecithin.

MATERIALS AND METHODS

1. Experimental Animals and Diets

The male Sprague-Dawley rats (initial weights, 200±1 g, SLC, Japan) were housed individually in a temperature (22±2℃), relative humidity (55±5%) and light (dark, 06:00-18:00 hour) controlled room. Rats were given free access to non-purified diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO) and tap water for a week as acclimatization before the experiment. After one week of acclimatization, rats were randomly divided into three groups (n=8) and were assigned to different dietary treatments.

The compositions of the experimental diets are shown in Table 1. Mineral (AIN-76) and vitamin (AIN-76) mixes were purchased from Dyets (Bethlehem, PA).[15] Rats were fed soybean lecithin-free diet (control), diets with 2% or 5% soybean lecithin and tap water *ad libitum* for 4 weeks. Soybean lecithin was obtained from Central Soya Company Inc. (Indiana, U.S.A). All diets contained 1% cholesterol and 0.5% cholic acid in order to induce hypercholesterolemia. Food intake was measured three times per week and body weight gain was measured weekly.

At the end of the experiment, rats were deprived of food for 16 hours and then anesthetized using diethyl ether. A central longitudinal incision was made into the abdominal wall and blood samples were collected by cardiac puncture with syringes. Blood samples were centrifuged at 1500×g for 30 minutes at 4 ℃ and the plasma was separated and stored at -20 ℃ until analyzed. Liver samples were excised, immediately frozen in liquid nitrogen and stored at -70 ℃ until analyzed. All animal procedures described conformed to NIH guidelines.[16]

Table 1. Composition of experimental diets[1]

(g/100 g diet)

Component	Control	Lecithin 2%	Lecithin 5%
Casein	20	20	20
D,L-methionine	0.3	0.5	0.3
Corn starch	1.5	15	15
Sucrose	48.5	46.5	45
Cellulose	5	5	5
Corn oil	5	5	3.5
Mineral mix(AIN-76)[2]	3.5	3.5	3.5
Vitamin mix(AIN-76)[3]	1	1	1
Choline bitartarate	0.2	0.2	0.2
Cholesterol	1	1	1
Cholic acid	0.5	0.5	0.5
Lecithin	-	2	5
Total	100	100	100

1) Diets were AIN-76 semipurified, and given in powdered form.
2) AIN-76 mineral mixture.
3) AIN-76 vitamin mixture.

2. Determination of Cholesterol and Triacylglyceride Concentrations

The plasma total cholesterol, HDL-cholesterol, and triacylglyceride levels were determined by enzymatic colorimetric methods using commercial kits (Asan pharmaceutical, Korea) without extraction.[17] LDL cholesterol was calculated using the formula of Friedewald *et al.*.[18] Friedewald Calculation: (LDL-C)=(Total Cholesterol)-(HDL)-(0.2×Triacylglycerides). Liver cholesterol and triacylglyceride concentrations were determined by enzymatic colorimetric methods after extraction with chloroform/methanol (2:1, v/v).[19]

3. Assay of Plasma CETP and LCAT Activities

CETP activity was analyzed by using commercial kits (BioVision, U.S.A). LCAT activity was performed with the procedure of Stokke and Norum[20] as modified by An *et al.*.[21] The free cholesterol content in plasma was measured by using the cholesterol/cholesteryl ester quantitation kit (BioVision, U.S.A). The substrate and enzyme were from the same plasma whose LCAT was inactivated prior to plasma separation. The emulsion of [7(n)-^{3}H] cholesterol-albumin was prepared as described following. Briefly 250 μl Ci of [7(n)-^{3}H] cholesterol in acetone was slowly added to 5 mL of bovine serum albumin (BSA) solution (250 mg of BSA dissolved in 0.2 M phosphate buffer, pH 7.1) with nitrogen pudding. This solution was then placed under nitrogen until it was free of acetone. The plasma, BSA- [7(n)-^{3}H] cholesterol-emulsion, and 10.4 mM 5,5-dithiobis-2-nitrobenzoic acid were prepared and preincubated at 37 ℃ for 4 hours. The enzyme reaction was initiated by the addition of 100 mM 2-mercaptoethanol and then incubation at 37 ℃ for 1 hour. The reaction was stopped by the addition of methanol. The lipid residue was extracted with 3 mL of hexane: chloroform (4:1) for 15 minutes by oscillation and agitation. Then the reaction mixture was centrifuged at 2,500×g for 10 minutes. The supernatant was filtered under vacuum and evaporated. It was then transferred to thin layer plates of silica gels (Sigma, U.S.A) in 50 μl of ether. The plates were developed in petroleum hexane: diethyl ether: formic acid (70:30:1, v/v/v) and identified

in iodine vapor. The areas containing free cholesterol and cholesteryl ester were scraped into liquid scintillation counting vials and counted using liquid scintillation cocktail (Amersham, U.S.A). Total activity was 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^{-1}/hr) was also calculated.

4. Statistical Analysis

Data were expressed mean±SD. Data for the control or lecithin groups were analyzed by one-way ANOVA; $P \geq 0.05$ was taken as indicating no significant difference. Where ANOVA showed significance, differences among groups were evaluated by Duncan's multiple range test.[22]

RESULTS

1. Body Weight Gain, Food Intake and Food Efficiency

Rats assigned randomly to three experimental groups resulted in initial body weights that were not different (Table 2). The food intake and body weight gain did not differ among the rats fed control, 2% or 5% lecithin diets but there were significant differences in food efficiency ($P<0.05$). Both 2% and 5% lecithin diets increased food efficiency in comparison to the control.

2. Effect on Plasma and Liver Lipid Levels

Rats treated with 2% or 5% lecithin had significantly lowered plasma total cholesterol concentrations as 29% and 58%, respectively ($P<0.05$) (Table 3) than that of control group. The 2% or 5% lecithin diets reduced the plasma LDL cholesterol concentrations about 20% and 53%, respectively ($P<0.05$). Although there were no significant effects of lecithin on the plasma HDL-cholesterol and triacylglyceride concentrations, the atherogenic index was decreased in lecithin groups. The order of effect of lecithin consumption on plasma lipid levels was 5% lecithin > 2% lecithin > control. Lecithin treatments increased the liver total lipid, total cholesterol and triacylglyceride in comparison to the control diet (Table 4).

Table 2. Food intake, body weight gain and food efficiency of rats fed experimental diets.[1,2]

(g/4week)

Group	Initial body weight	Weight gain	Food intake	Food efficiency[3]
Control	200.18±11.62^{NS}	157.88±15.47^{NS}	24.04±2.24^{NS}	0.25±0.02^{b}
Lecithin 2%	200.38±11.39	174.60±35.70	23.29±1.97	0.28±0.04^{a}
Lecithin 5%	200.53±10.48	158.35±13.69	21.84±1.65	0.27±0.02^{a}

1) Valuesare expressed as mean±SD, n=8.
2) Valuesin a column with different superscripts are significantly different, $p<0.05$.
3) Food efficiency Ratio=Body weight gain (g) /Food intake (g).
NS is not significant.

Table 3. Plasma total cholesterol, HDL-cholesterol and triacylglyceride concentrations in rats fed experimental diets.[1,2]

(mg/dl)

Group	Total cholesterol	HDL cholesterol	LDL cholesterol[3]	Triacylglyceride	AI[4]
Control	190.59±31.59^{a}	41.60±8.09^{NS}	126.74±32.84^{a}	111.23±36.29^{NS}	3.32±0.87^{NS}
Lecithin 2%	135.86±39.76^{b}	33.65±5.12	76.44±35.56^{b}	128.82±37.03	2.77±1.14
Lecithin 5%	112.60±29.53^{b}	33.25±12.69	58.48±19.45^{b}	104.37±36.21	2.60±0.94

1) Valuesare expressed as mean±SD, n=8.
2) Valuesin a column with different superscripts are significantly different, $p<0.05$.
3) LDL cholesterol was calculated by the method of Friedewald WT formula.
4) AI (Atherogenic Index) = (Total Cholesterol - HDL-Cholesterol) / HDL-Cholesterol
NS is not significant.

Table 4. Liver total lipid, total cholesterol and triacylglyceride concentrations in rats fed experimental diets.[1,2]

mg/g (wet weight)

Groups	Total lipid	Total Cholesterol	Triacylglyceride
Control	20.14±15.14^{b}	8.23±3.79^{NS}	0.61±0.34^{b}
Lecithin 2%	38.09±26.25^{b}	9.77±2.77	0.90±0.30^{b}
Lecithin 5%	98.43±24.18^{a}	10.17±1.42	2.40±1.52^{a}

1) Valuesare expressed as mean±SD, n=8.
2) Valuesin a column with different superscripts are significantly different, $p<0.05$.
NS is not significant.

3. Effect of Lecithin on the Plasma CETP and LCAT Activities

Effects of lecithin on the plasma CETP (Fig. 1, A) and LCAT (Fig. 1, B) activities were measured. The 2% or 5% lecithin diet significantly decreased the activity of CETP by 14% or 17%, respectively ($P<0.05$). Also, 2% or 5% lecithin diet increased the activity of LCAT by 7% or 60%, respectively, after the dietary intervention.

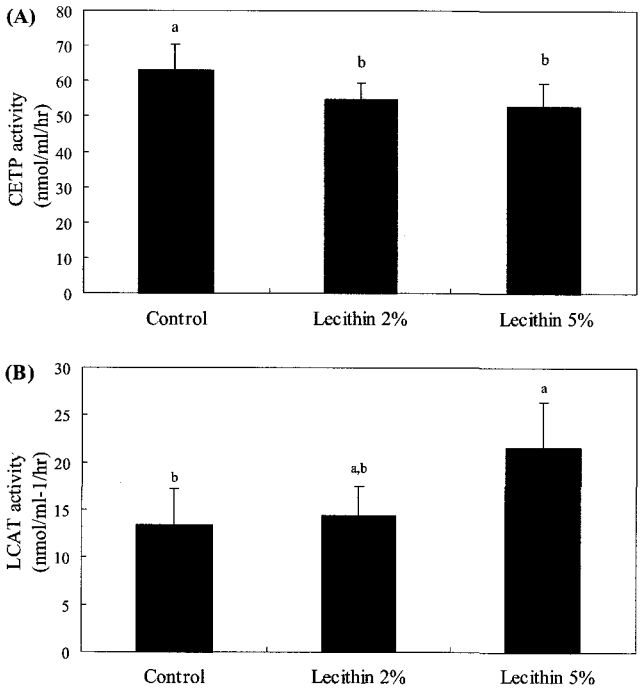

Fig. 1 Levels of plasma CETP (A) and LCAT (B) activities in rats fed experimental diets.

Values are expressed as mean±SD, n=8. Different superscripts are significantly different ($p<0.05$).

DISCUSSION

Our study was carried out in order to clarify how soybean lecithin affects the lipid metabolism in hypercholesterolemic rats. The results showed that concentrations of plasma cholesterol in rats fed soybean lecithin were reduced. In this study, we added 2% or 5% soybean lecithin to diets containing 1% cholesterol and 0.5% cholic acid and treated rats for 4 weeks. Body weight gain and food intake were not significantly different among the groups, but the diet of lecithin treatments increased food efficiency compared to the control. Similar to our result, Imazumi *et al.*[23] reported that intake of lecithin did not show any significant difference in the body weight gain and food intake compared to control. Takashi *et al.*[24] treated rats for 18 days with 0.5% soybean oil, 10% soybean oil, 10% egg yolk phospholipids and 10% soybean phospholipids. There was no difference in the food intake, body weight gain and liver weight by dietary treatments. These results showed that lecithin did not affect the food intake or body weight gain in animals.

After 4 weeks of experimental diets, lecithin diets reduced plasma total cholesterol, LDL cholesterol and atherogenic index in diet-induced hypercholesterolemic rats. The effect of lecithin on the hypocholesterolemic action was shown in the following order: 5% lecithin > 2% lecithin > control. This finding indicates that lecithin may be one of the major active components or contributes of the hypocholesterolemic activity. A decrease of circulating LDL cholesterol level prevents cardiovascular disease. This study showed that feeding rats diets enriched in soybean lecithin resulted in the remarkable changes in cholesterol homeostasis. Many other studies also have reported the inverse association between the consumption of lecithin and the plasma concentration of cholesterol.[11-13,25] Lecithin treatments increased the liver total lipid, total cholesterol and triacylglyceride in comparison to the control diet. Similar to our result, O'Brien *et al.*[26] reported that intake of 7.5% soybean lecithin and 7.5% egg lecithin increased the liver total lipid and cholesterol compared to control.

The plasma CETP activity was significantly decreased in the lecithin groups compared to the control group. The CETP transports cholesteryl ester made by LCAT to other lipoproteins, particularly triacylglyceride-rich lipoproteins and LDL cholesterol.[27] Rats consuming 5% lecithin showed a significant rise in LCAT activity. This result showed the similar findings as in the result of Iwata *et al.*[28] that safflower phospholipids diet increased the LCAT activities. Rosseneu *et al.*[29] reported that diet with polyunsaturated lecithin increased the cholesterol esters and lysolecithin content in HDL_3 by the activation of LCAT in hypercholesterolemic chimpanzees. Our finding indicated that lecithin possess hypocholesterolemic actions, which operate in a manner dependent of CETP and LCAT activities.

In conclusion, the addition of lecithin to rat diets had a beneficial effect on the cholesterol metabolism which has been shown to be hypocholesterolemic action based on the mechanism through increased RCT. Lecithin was associated with lowering plasma total cholesterol, LDL-cholesterol and the CETP activity and increasing their LCAT activities.

Therefore, the regulations of LCAT and CETP activities may play major roles in the hypocholesterolemic actions of

lecithin *in vivo*. Further research is required to fully delineate the mechanisms that contribute to the hypocholesterolemic effects of lecithin and its constituents.

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