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Plasma Lipoprotein Cholesterol in Rats Fed a Diet Enriched in Chitosan and Cholesterol

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Summary To investigate the effect of dietary chitosan on plasma lipoprotein cholesterol metabolism, male Sprague-Dawley (SD) rats fed a cholesterol-enriched diet containing cellulose (CE) or chitosan (CS) were studied for 2 wk. Lower plasma total cholesterol, low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol were observed in rats fed a diet containing chitosan. In addition, significantly higher high-density lipoprotein (HDL) cholesterol and HDL_2 cholesterol were observed in rats after 2 wk of chitosan feeding. Rats fed the chitosan diet had increased triacylglycerol percentages and decreased free cholesterol, cholesteryl ester and phospholipid percentages in VLDL lipid composition. Chitosan significantly decreased the surface lipid proportions and increased the core lipid proportions in VLDL particles. In addition, the ratios of surface lipids to core lipids of the VLDL particles in rats fed a diet containing chitosan were significantly decreased. A significantly lower plasma apolipoprotein B (Apo B) concentration was observed in rats fed the chitosan diet as compared to those fed the cellulose diet. No significant difference in plasma triacylglycerols or glucose levels was observed between the two dietary groups. Results from this study suggest that chitosan may alter the VLDL particle size and also play an important role in the regulation of lipoprotein metabolism in rats.

Key Words chitosan, rats, lipoprotein cholesterol

Chitosan is a polymer of glucosamine derived from shellfish chitin. The structure of chitosan is similar to that of cellulose. Chitosan is, however, the only amine polysaccharide in nature (*1*). In addition, chitosan is hard to hydrolyze by human digestive enzymes. Many investigators have demonstrated that the consumption of chitosan may result in a decrease in plasma cholesterol (*2–6*). Although the hypocholesterolemic action of chitosan has been explained by depressing cholesterol absorption and interfering with bile acid absorption (*5*, *6*), little information is known about the effect of chitosan on the plasma lipoprotein cholesterol metabolism. We have reported that the hypocholesterolemic effect of chitosan may be primarily related to a decrease in cholesterol carried in the VLDL fraction (*3*). However, the mechanism is still unclear.

On the other hand, the effects of dietary chitosan on HDL cholesterol and LDL cholesterol are often inconclusive. Some studies have shown that chitosan has an HDL cholesterol elevating-effect (*2*, *3*, *5*), while other investigators have found no significant change in HDL cholesterol (*7*, *8*), but a decrease in LDL cholesterol (*3*) due to chitosan supplementation. A major reason for this discrepancy seems to be due to the difference in the degree of deacetylation and the amount of dietary chitosan ingested. It is therefore of considerable importance to further elucidate the mechanism involved in the lipoprotein-lowering effect in rats fed chitosan.

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MATERIALS AND METHODS

Materials. Samples of chitosan, prepared from shrimp shell chitin, were generously supplied by Kiotek Corporation (Hsinchu, Taiwan), and deacetylation was about 90%.

Animals and treatment. Five-week-old male Sprague-Dawley (SD) rats were used as experimental animals. The feeding study consisted of two groups: (a) cellulose group (CE) and (b) chitosan group (CS). Rats were randomly divided into two groups of equal body weight (174.6±10.5 g for the CE group; 177.9±13.4 g for the CS group). The composition of the basal diet is shown in Table 1. Each group consisted of eight rats. They were housed in individual stainless-steel cages in a room kept at 23±1°C with a 12-h light/dark cycle (lighting from 8:00 a.m. to 8:00 p.m.). Fresh diet and drinking water were available ad libitum for 2 wk. This study was approved by the Animal House Management Committee of National Taiwan Ocean University. The animals were maintained in accordance with the guidelines of the Animal Center of the National Science Council for the Care and Use of Laboratory Animals.

Viscosity. The viscosity of 2% (w/v) chitosan in a volume of 0.1 M HCl was measured by a rotation viscometer (Haake CV 20, Germany) at 37°C.

Blood and tissue sampling. After feeding for 2 wk, rats were fasted for 14 h, and blood was collected from the abdominal aorta using a heparin anticoagulant

Table 1. Composition of the experimental diet (%).

Ingredient	CE[1]	CS
Casein	20	20
Lard	10	10
Vitamin mixture[2]	1	1
Mineral mixture[3]	4	4
Cholesterol	0.5	0.5
Cholic acid	0.3	0.3
Choline chloride	0.2	0.2
Cellulose	7	
Chitosan[4]		7
Corn starch	57	57

[1] CE: cellulose group; CS: chitosan group.
[2] AIN 76 vitamin mixture.
[3] AIN 76 mineral mixture.
[4] Chitosan: The viscosity of chitosan was about 266 cps (measured using a 2% solution of dried chitosan sample in 0.1 M HCl at 37°C with a rotary viscometer). The degree of deacetylation was about 90%.

while under diethyl ether anesthesia. After the blood sampling, the liver and abdominal adipose tissue were isolated, weighed and immediately frozen and stored at −80°C until needed for analysis.

Determination of plasma lipids, glucose and apolipoprotein B (Apo B). Plasma total cholesterol, triacylglycerols and phospholipids were determined by enzymatic methods using kits purchased from Kyokuto Pharmaceutical Industrial Co. (Tokyo, Japan). Free cholesterol was measured by a kit purchased from Merck-Biotol (Chenneviers, France). The free fatty acid and glucose in the plasma were determined using kits purchased from Wako Pure Chemical Company (Osaka, Japan). HDL, HDL_3, LDL and VLDL in the plasma were determined by ultracentrifugation (194,000×*g* for 3 h at 10°C) (*9*). Plasma Apo B was determined with a immunoturbidimetric method using kits purchased from Sigma Chemical Co. (MO, USA).

Determination of liver lipids. Total cholesterol, free cholesterol and triacylglycerol in the liver were assayed (*10*) from the total lipids extracted using the Folch method (*11*).

Determination of fatty acid synthetase activity. The livers were homogenized with two volumes of 0.1 M potassium phosphate, pH 7.0–1 mM DTT–0.1 mM EDTA. The resulting homogenate was centrifuged at 31,000×*g* for 20 min. The supernatant was then centrifuged again at 100,000×*g* for 1 h. The resulting supernatant was assayed using the spectrophotometric method. Fatty acid synthetase activity was determined by the rate of malonyl CoA-dependent NADPH oxidation (*12*). The activity was expressed as nmol/min/mg of protein and nmol/min/g of liver.

Statistical evaluation. Results are given as the mean±standard deviation (SD). Statistical analyses were performed using Student's *t*-test, the level of significance being accepted as $p<0.05$.

Table 2. Plasma parameters.[1]

Diet	CE[2]	CS
Total cholesterol (mg/dL)	150.4±58.7[a]	70.0±14.0[b]
Free cholesterol (mg/dL)	45.8±22.3[a]	20.1±9.6[b]
HDL cholesterol[3] (mg/dL)		
Total	22.7±2.6[b]	27.8±5.0[a]
Free	3.0±0.9	4.6±1.8
HDL_2 cholesterol (mg/dL)	16.5±2.7[b]	21.8±4.6[a]
HDL_3 cholesterol (mg/dL)	6.3±1.3	6.0±1.5
VLDL cholesterol[4] (mg/dL)	92.7±37.9[a]	27.2±10.2[b]
LDL cholesterol[5] (mg/dL)	40.8±21.8[a]	17.1±7.1[b]
LDL cholesterol/ HDL cholesterol	1.80±0.91[a]	0.62±0.25[b]
Apo B[6] (mg/dL)	60.2±31.7[a]	17.8±11.0[b]
Triacylglycerol (mg/dL)	77.1±16.6	73.3±20.0
Glucose (mg/dL)	142.5±25.4	127.1±18.7
Free fatty acid (mEq/L)	0.8±0.1	0.8±0.1

[1] Each value is expressed as mean±SD for 8 rats per dietary group. Values in the same row with different superscript letters are significantly different ($p<0.05$).
[2] CE: cellulose group; CS: chitosan group.
[3] HDL cholesterol: high-density lipoprotein cholesterol,
[4] VLDL cholesterol: very-low-density lipoprotein cholesterol, [5] LDL cholesterol: low-density lipoprotein cholesterol, [6] Apo B: apolipoprotein B.

RESULTS

Body weight and tissue weight

Rats that consumed the CS diet had lower body weight, as compared to the rats fed the control diet, at the end of the experimental period (303.1±24.3 g for CE group; 248.5±13.4 g for CS group). Lower food intake was observed in the rats fed the chitosan diet as compared to those fed the control diet (30.2±2.5 g for CE group; 25.0±2.6 g for CS group). In addition, chitosan induced a decrease in relative liver and adipose weights in the rats. The relative liver weight for the rats on the chitosan diet (3.3±0.2 g/100 g B.W.) was significantly lower than that of the animals that received the cellulose diet (5.2±0.4 g/100 g B.W.). The relative adipose (perirenal+epididymal fat) weight in rats fed the diet containing chitosan was significantly decreased (1.4±0.5 g/100 g B.W.) as compared to that of the rats on the cellulose diet (2.4±0.4 g/100 g B.W.).

Plasma lipids and Apo B concentration

A statistical analysis of plasma lipoprotein cholesterol and Apo B revealed a significant difference between the dietary groups (Table 2). At the end of the experimental period, rats fed the diet containing chitosan showed a significant decrease in total cholesterol, free cholesterol, VLDL cholesterol, LDL cholesterol, LDL cholesterol-to-HDL cholesterol ratio and Apo B level in the plasma. In addition, a significant increase in HDL cholesterol and HDL_2 cholesterol was observed in rats fed the chitosan diet. There was no difference in plasma HDL_3 cholesterol concentration between the two dietary groups. No significant change in plasma glucose, triacylglycerol or free fatty acid levels was observed between the two di-

etary groups.

VLDL lipid composition and lipid percentage ratios are shown in Table 3. Although the CS group had a lower cholesteryl ester (EC) ratio, lower free cholesterol (FC) ratio and lower phospholipid (PL) ratio for the VLDL lipids, a higher triacylglycerol (TG) ratio was observed in rats fed the CS diet as compared to those fed the cellulose diet. The ratio of surface lipids (PL+FC) to core lipids (TG+EC) was significantly decreased in the CS group.

Liver lipid levels and fatty acid synthetase activity

The liver lipid contents of rats are shown in Table 4. Significant decreases in liver total cholesterol, FC, EC and triacylglycerol contents were observed in rats fed a diet after chitosan treatment. The activity of fatty acid synthetase in the liver from rats fed the experimental diets for 2 wk is shown in Fig. 1. Rats fed a diet containing chitosan showed an increase in hepatic fatty acid synthetase activity.

DISCUSSION

The present study demonstrates the chitosan influence on cholesterol distribution among various pools of lipoprotein in rats. The results of this study show that chitosan supplement decreased plasma VLDL cholesterol and LDL cholesterol, and increased HDL cholesterol. An analysis of the VLDL lipid composition showed that rats fed a chitosan diet had increased core lipid proportions and decreased surface lipid proportions, which might result in a lower ratio of surface lipids to core lipids in VLDL particles. On the basis of the information obtained from the ratios of the surface lipids to core lipids in VLDL particles, it is possible that VLDL particle size might be altered by chitosan treatment. Fisher et al. (*13*) reported that increasing the size and triacylglycerol contents of a lipoprotein particle increased its susceptibility to hydrolysis by lipoprotein lipase (LPL), indicating that the larger particle was a better substrate for

Table 3. VLDL lipid composition and percentage of core lipid and surface lipid ratio.[1]

Diet	VLDL[2]	
	CE[3]	CS
	(wt%)	
Triacylglycerol (TG)	26.5±4.4[b]	55.0±7.5[a]
Cholesteryl ester (EC)	37.1±7.2[a]	18.8±5.2[b]
Free cholesterol (FC)	12.4±4.9[a]	5.2±2.1[b]
Phospholipid (PL)	24.2±2.7[a]	21.4±2.5[b]
Surface lipid[4]	36.6±3.3[a]	26.6±2.9[b]
FC/PL	0.51±0.09[a]	0.24±0.09[b]
Core lipid[5]	63.5±3.3[b]	73.8±2.9[a]
EC/TG	1.4±0.4[a]	0.34±0.15[b]
Surface/Core[6]	0.58±0.08[a]	0.36±0.05[b]

[1] Values are percent of VLDL lipid. Each value is expressed as mean±SD for 8 rats per dietary group. Values in the same row with different superscript letters are significantly different ($p<0.05$).
[2] VLDL indicates very-low-density lipoprotein.
[3] CE: cellulose group; CS: chitosan group.
[4] Surface lipids are expressed as weight percent of phospholipid (PL)+free cholesterol (FC).
[5] Core lipids are expressed as weight percent of triacylglycerol (TG)+cholesteryl ester (EC).
[6] Surface/Core is the weight percent ratio of phospholipids (PL)+free cholesterol (FC)/triacylglycerol (TG)+cholesteryl ester (EC).

Table 4. Effect of dietary chitosan on liver lipid contents in rats.[1]

Diet	CE[2]	CS
Total cholesterol		
(mg/g liver)	71.5±6.2[a]	22.5±9.3[b]
(mg/liver)	1126.7±152.6[a]	193.7±103.2[b]
Free cholesterol		
(mg/g liver)	6.4±1.2[a]	4.9±0.6[b]
(mg/liver)	102.6±31.2[a]	40.9±10.0[b]
Cholesteryl ester		
(mg/g liver)	65.0±7.3[a]	17.6±8.8[b]
(mg/liver)	1024.0±140.9[a]	152.8±94.2[b]
Triacylglycerol		
(mg/g liver)	47.4±9.2[a]	25.4±5.6[b]
(mg/liver)	749.3±215.3[a]	207.1±70.3[b]

[1] Each value is expressed as mean±SD for 8 rats per dietary group. Values in the same row with different superscript letters are significantly different ($p<0.05$).
[2] CE: cellulose group; CS: chitosan group.

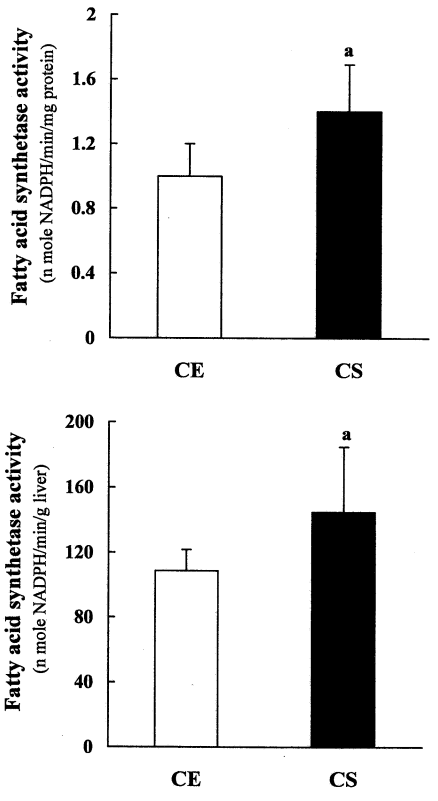

Fig. 1. Effect of dietary chitosan on fatty acid synthetase activity in the rat liver. Results are expressed as mean±SD for 8 rats in each dietary group. CE: cellulose group; CS: chitosan group. [a] Significant difference from the CE group at $p<0.05$.

LPL than the smaller particle. Thus, rats fed a chitosan diet might have larger VLDL particles, which is a preferential substrate for LPL, and this might lead to quicker VLDL clearance from the plasma. Lower acyl coenzyme A: cholesterol acyltransferase (ACAT) activity might make less cholesteryl ester available for VLDL packing, and this may induce a reduction in VLDL secretion from the liver (*14*). Since lower hepatic cholesteryl esters in rats fed the chitosan diet may have been caused by the lower hepatic ACAT activity (*3*), it is possible that chitosan induced a marked decrease in VLDL cholesterol, which might be related to the decreased secretion of VLDL from the liver. In addition, results from the present study show that lower plasma Apo B concentration associated with the decreased liver cholesteryl ester contents was observed in rats fed the chitosan diet. Since Apo B secretion is positively correlated with the cellular cholesteryl ester contents (*15*), it is possible that the lower plasma Apo B concentration might be due to the reduction in Apo B secretion. Therefore, the decreased content of VLDL in rats fed the chitosan diet might be related to the increased clearance of VLDL from the plasma and decreased secretion from the liver.

The hypolipidemic properties of chitosan are well known. Many investigators have focused on the effect of chitosan on plasma total cholesterol and HDL cholesterol. However, little information is known about LDL cholesterol. Results from this study show that significantly decreased LDL cholesterol was observed in rats fed the chitosan diet. In addition, chitosan significantly reduced hepatic FC and EC contents. Although chitosan decreased fat digestion and cholesterol absorption (*4*, *6*), Lehoux and Grondin demonstrated that no significant difference in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activities was observed in rats after a 7.5% chitosan treatment (*7*). Fukada et al. showed that chitosan consumption did not increase fecal excretion of bile acids, but caused a marked change in fecal bile acid composition (*6*). It is therefore suggested that the hepatic cholesterol pool may be reduced by chitosan. Since VLDL is the precursor of LDL, it is possible that the decrease in LDL cholesterol may be the result of decreased VLDL cholesterol in rats fed the chitosan diet and the hepatic LDL receptor may not be affected by chitosan treatment.

Elevated LDL cholesterol and decreased HDL cholesterol levels have been associated with an increased risk of premature coronary heart disease (CHD) (*16*). Several studies have shown that 5% chitosan in the diet has an HDL cholesterol-elevating effect (*3*, *17*). Other investigators found that there was no significant increase in plasma HDL cholesterol levels in rats fed 7.5–8.0% chitosan (*7*, *8*). Our results, however, found that rats fed a 7% chitosan diet had higher HDL and HDL_2 cholesterol levels. HDL cholesterol content is, in part, dependent on the activity of lecithin cholesterol acyltransferase (LCAT), and the HDL is reshaped due to the action of LCAT (*18*). Furthermore, HDL_2 acted as a carrier of cholesterol from peripheral tissue to the liver (*19*). Thus, it is possible that rats fed a chitosan diet might have higher LCAT activity. Because the action of LCAT is thought to prevent the accumulation of unesterified cholesterol derived from the surface of VLDL in the plasma (*20*), it is possible that lower VLDL cholesterol and LDL cholesterol associated with the higher HDL cholesterol observed in rats fed the chitosan diet may reflect acceleration of the lipoprotein metabolism. This may suggest that the lipoprotein metabolism is promoted by chitosan.

Many studies have shown that 5% chitosan feeding induced a reduction in liver weight as the result of the decrease in liver cholesterol contents (*3*, *21*), but no significant effect on body weight was observed (*3*, *6*). In the present study, lower body weight, liver weight and adipose tissue weight were observed in rats fed the 7% chitosan diet. In addition, chitosan significantly decreased the food intake of the rats. Gallaher et al. demonstrated that lower body weight and food intake were observed in rats fed a diet containing 7.5% chitosan (*22*). Although a reduction in adipose tissue and liver weight does not appear to explain the reduction in body weight, it is possible that the reduced food intake and adipose tissue weight of rats fed the 7% chitosan diet likely contributed to the lower body weight. Parrish et al. demonstrated that the decreased adipose tissue was possibly related to an increase in lipolysis (*23*). Chitosan feeding induced a reduction in VLDL cholesterol as the result of the decreased secretion of VLDL from the liver. In addition, lower fat digestion was observed in rats after chitosan ingestion (*4*). We previously reported that no significant effect of chitosan on plasma lipid peroxidation was observed in rats (*3*). It is therefore suggested that the decreased adipose tissue in rats after chitosan treatment might be the result of a decrease in lipogenesis rather than an increase in lipolysis, since chitosan did not affect the plasma free fatty acid concentration. Studies on the effects of chitosan on liver lipids have shown that chitosan decreased liver triacylglycerol contents (*3*, *17*). Although chitosan feeding decreased the hepatic triacylglycerol contents, increased hepatic fatty acid synthetase activity was observed in rats fed the chitosan diet. Fatty acid synthetase activity in the liver has been shown to be sensitive to dietary fat content (*24*). Middleton and Schneeman demonstrated that the hepatic fatty acid synthetase was significantly higher in rats fed a low-fat diet, and no increase in activity due to feeding was observed (*25*). Studies on the relationship between chitosan and fat digestion have shown that chitosan induced an inhibitory effect on fat digestion (*4*). It is therefore suggested that the stimulation of hepatic fatty acid synthetase caused by chitosan feeding resulted from the decreased absorption of dietary fat and hepatic triacylglycerol contents. Therefore, the decrease in adipose tissue and increase in hepatic fatty acid synthetase activity might be related to the decreased absorption of dietary fat in rats after chitosan treatment.

The present study indicates that both the daily intake of cholesterol and body weight gain in rats fed chitosan were lower than those fed cellulose because the food in-

take during chitosan-feeding was lower than that of cellulose. Therefore, the difference in lipid metabolism between chitosan and cellulose is due partly to the differences in both the daily intake of cholesterol and body weight gain. The exact mechanism for the regulation of lipoprotein metabolism by dietary chitosan has not yet been completely elucidated and further investigations are needed. Our results showed that chitosan treatment altered the VLDL particle size and increased hepatic fatty acid synthetase activity. These changes might have originated at the hepatocyte level via the effects that core lipid compositions have on the metabolism of plasma VLDL and its remnants.

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