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Effect of Cholesterol on Hepatic Phospholipid Metabolism in Rats Fed a Diet Containing Fish Oil and Beef Tallow

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Abstract

The influence of dietary cholesterol on phospholipid metabolism in rat liver microsomes was studied in rats fed a diet containing fish oil (FO) or beef tallow (BT). The hepatic phospholipid content decreased whereas hepatic triglyceride and cholesterol increased significantly in both groups after cholesterol supplementation. Plasma concentrations of phospholipid and triglyceride increased with cholesterol supplement in both groups while cholesterol decreased only moderately in the FO group. Dietary cholesterol affected microsomal phospholipids in liver; the proportion of phosphatidylcholine decreased in the FO group, and it also slightly decreased in the BT group at the expense of phosphatidylethanolamine. The activity of CTP:phosphocholine cytidylyltransferase, the rate-limiting enzyme of phosphatidylcholine synthesis, increased in hepatic microsomes whereas it decreased in hepatic cytosol of both groups by cholesterol supplementation. In conclusion, these results indicate that the dietary cholesterol profoundly influences phospholipid metabolism in the rat liver.

Key words: cholesterol, phospholipid, fish oil, beef tallow, CTP:cholinephosphate cytidylyltransferase

INTRODUCTION

The consumption of high cholesterol diet in rats increases hepatic cholesterol and triglyceride contents, and very-low-density lipoprotein (VLDL) secretion, which suggests cholesterol is required for VLDL formation (1-3). Inhibition of phospholipid synthesis decreases VLDL secretion which can cause accumulation of lipids in the liver (4). An alteration of phospholipid metabolism may play an important role in the formation of hepatic lipoproteins. The modulation of some membrane-associated enzyme activities and some membrane receptor functions could be influenced when cholesterol content or molecular composition of phospholipid is changed (5).

CTP:phosphocholine cytidylyltransferase which converts phosphorylcholine into CDP-choline is a rate limiting enzyme in phosphatidylcholine synthesis (6,7). Phosphatidylcholine synthesized via this pathway is appearently involved in the secretion of VLDL (6,8). Cholesterol stimulates the membrane-bound CTP:phosphocholine cytidylyltransferase activity in rat liver (2). Hepatocytes isolated from rats fed a choline-deficient diet reduced the secretion of VLDL but not that of high-density lipoprotein (9). It would be important to test whether dietary cholesterol can influence enzymes activities involed in the phospholipid metabolism, and distribution in the hepatic cell membranes upon distinct fat diets.

The ingestion of fish oil containing n-3 polyunsaturated fatty acids lowered plasma triglyceride levels in human subjects and experimental animals (10-13). Dietary lipids play a significant role in changes of the plasma concentrations of triglyceride and cholesterol (10,11,14); high concentration of plasma lipids is known to be atherogenic (15). Different sources of dietary fat affect the fatty acid composition of microsomal phospholipid and the synthesis of hepatic phospholipid (16,17). Many studies are related to plasma lipid concentration, while some studies have focused on the potential effect of cholesterol supplement to these diets on hepatic lipid metabolism (17, 18). The present study was undertaken to elucidate how dietary cholesterol affects phospholipid metabolism in rats fed different sources of fat.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (four-weeks-old) were purchased from Kyudo Experimental Animals (Tosu, Japan) and acclimated in a room maintained at 20~23°C with a 12-h light-dark cycle. Before the experiment, rats were allowed free access to commercial chow diet for one week. Then rats were randomly divided into four groups of six and assigned to different fat diets with or without cholesterol supplement. The diets were prepared according to recommendations of American Institute of Nutrition (19) and contained 20% casein, 15% corn starch, 10% fat, 1% vitamin mixture (AIN-76), 3.5% mineral mixture (AIN-76), 0.15% choline chloride, 4% cellulose powder, 3% DL-methionine, and sucrose. A supplement of 0.5% (w/w) cholesterol and 0.125% (w/w) cholic acid was added to cholesterol diets instead of sucrose. The fatty acid composition of dietary fats is shown in Table 1. The rats were fed experimental diets *ad libitum* for 14 days. At the end of the experimental period, they were killed by decapitation, and the liver was excised immediately.

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Table 1. Fatty acid composition of dietary fats used in experimental diet (weight %)

Fatty acid	Beef tallow	Fish oil
14 : 0	4.3	6.9
16 : 0	32.0	8.1
16 : 1	4.1	10.2
18 : 0	19.1	2.1
18 : 1	36.1	11.8
18 : 2 (n-6)	2.3	6.6
18 : 3 (n-3)	0.0	2.9
20 : 5 (n-3)	0.0	28.1
22 : 6 (n-3)	0.0	11.4
Saturated fatty acid	55.4	26.8
Monounsaturated fatty acid	40.2	22.0
Polyunsaturated fatty acid (n-6)	2.3	6.6
(n-3)	0.0	42.4

Lipid analysis

Total hepatic lipids were extracted by the method of Folch et al. (20). The concentrations of hepatic cholesterol and triglyceride were measured by the methods of Sperry and Webb (21), and Fletcher (22), respectively. Phospholipid as quantified by phosphorus content was measured by the method of Rouser et al. (23). Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine plus phosphatidylinositol, lysophosphatidylcholine, sphingomyelin, and phosphatidic acid were separated by thin layer chromatography using chloroform/methanol/water/acetic acid (25/15/4/2, v/v) as a developing solvent system (24). The distribution of phospholipid classes was determined by their phosphorus contents (23). The fatty acid compositions of hepatic microsomal phosphatidylcholine and phosphatidylethanolamine were determined by gas chromatography (Shimadzu GC-14 equiped with flame ion detector and capillary column Omega Wax, 0.25 mm × 30 m, Supelco, USA) after transmethylation with HCl-methanol (25). The concentrations of serum cholesterol, triglyceride and phospholipid were assayed enzymatically with commercial kits (Wako Pure Chemical Ind., Osaka, Japan).

Analytical methods

Hepatic microsomal and cytosolic fractions were prepared as described previously (24) and stored at -80°C. Protein was assayed by the method of Lowry et al. (26) using bovine serum albumin as a standard. The activity of CTP:phosphocholine cytidylyltransferase was measured by the procedure described by Wright et al. (27) using phospho [methyl-^{14}C] choline as a substrate; 1 mM of phosphatidylcholine-oleate was added to the assay medium to measure the activity of cytosolic CTP:phosphocholine cytidylyltransferase. The activity of choline kinase in hepatic cytosol was measured using [methyl-^{14}C] choline as a substrate by a procedure described previously (24,28).

Statistical analysis

Data were analyzed by ANOVA and Duncan's new multiple-range test (29).

RESULTS

Hepatic and plasma lipids

There was no significant difference in hepatic contents of triglyceride and cholesterol between the BT and the FO groups, while hepatic phospholipid content was higher in the FO group than in the BT group (Fig. 1). The cholesterol supplement lowered hepatic phospholipid content and increased hepatic triglyceride and cholesterol contents significantly in both groups. In the cholesterol-free diet groups, plasma concentrations of phospholipid, triglyceride, and cholesterol were lower in the FO group than in the BT group. Dietary cholesterol supplement resulted in an elevated plasma concentrations of phospholipid and cholesterol, and decreased plasma triglyceride in the BT group ($p<0.05$) (Fig. 2). In contrast, the concentration of plasma phospholipid increased while that of cholesterol decreased moderately in the FO group.

Distribution of hepatic microsomal phospholipid

Phospholipid distribution of hepatic microsomal membranes was altered by cholesterol supplement in both groups (Table 2). In the BT group, the proportion of phosphatidylcholine decreased slightly while that of phosphatidylethanolamine increased modestly by cholesterol supplement and the propor-

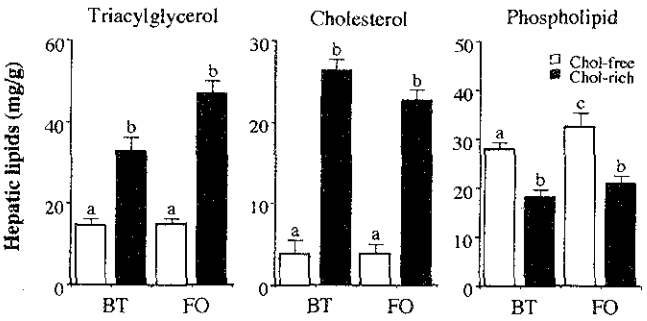

Fig. 1. The hepatic contents of triglyceride, cholesterol, and phospholipid in rats fed a beef tallow (BT) or fish oil (FO) diet with or without cholesterol supplement. Values are given as the means ±SE of 6 rats in each group. Values with different letters are significantly different among groups at $p<0.05$.

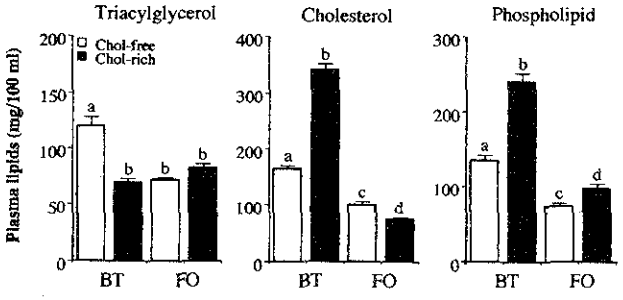

Fig. 2. The plasma contents of triglyceride, cholesterol, and phospholipid in rats fed a beef tallow (BT) or fish oil (FO) diet with or without cholesterol supplement. Values are given as the means ±SE of 6 rats in each group. Values with different letters are significantly different among groups at $p<0.05$.

tions of sphingomyelin and phosphatidic acid decreased. In the FO group, the proportion of phosphatidylcholine decreased upon cholesterol supplement while that of phosphatidylethanolamine showed no variation. In addition, the proportions of sphingomyelin, lysophosphatidylcholine and phosphatidic acid increased upon cholesterol supplement; while the acidic phospholipids, phosphatidylserine and phoshatidylinositol, remained unchanged.

Hepatic enzyme activities relating to phospholipid synthesis

The cytosolic activity of choline kinase, the first enzyme that catalyzes phosphatidylcholine formation, was reduced sligthly by cholesterol supplement in both groups (Fig. 3). However, the activity of CTP:phosphocholine cytidylyltransferase, which catalyzes CDP-choline formation from phosphocholine, increased in microsomes and decreased in cytosol by cholesterol supplement in both groups. These coordinate translocation of CTP:phosphocholine cytidylyltransferase activities from cytosol to microsome was shown in both groups after adding cholesterol to the diets. The increments of microsomal CT activity were correlated with the increased activity in liver homogenates. However, the increase in CTP:phosphocholine cytidylyltransferase activity was larger in the FO group (60%) than in the BT group (34%).

Table 2. Compositions of hepatic microsomal phospholipid in rats fed different fats with or without cholesterol supplement

	Beef tallow diet		Fish oil diet	
	Cholesterol-free	Cholesterol-rich	Cholesterol-free	Cholesterol-rich
	(% of total phospholipid)			
Lyso-PC	2.24±0.1	2.44±0.2	2.33±0.1	4.87±1.6
SPM	3.54±0.3	2.87±0.5	3.59±0.3	4.45±0.8
PC	65.1 ±1.7^{a}	61.9 ±2.2^{ab}	60.7 ±2.3^{ab}	53.6 ±1.9^{b}
PS+PI	13.3 ±0.7	14.3 ±0.9	13.3 ±0.3	14.3 ±1.4
PE	14.7 ±0.3^{a}	17.5 ±0.4^{b}	19.4 ±0.1^{b}	18.8 ±2.8^{b}
PA	1.17±0.2	0.97±0.2	1.37±0.7	2.25±0.2
PC/PE	4.42	3.53	3.12	2.85

Rats were fed semipurified diets containing beef tallow or fish oil with or without cholesterol supplement for 2 weeks. Values are given as the means±SE of 6 rats in each group. Values with different letters are significantly different among groups at $p<0.05$. Abbreviations: Lyso-PC, lysophosphatidylcholine; SPM, sphingomyelin; PC, phosphatidylcholine; PS+PI, phosphatidylserine+phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid.

Fatty acid composition of liver microsomal phospholipids

Cholesterol supplement affected the distribution of fatty acids in phosphatidylcholine and phosphatidylethanolamine molecules (Table 3). Major changes occured in arachidonic

Table 3. Compositions of hepatic microsomal phosphatidylcholine and phosphatidylethanolamine in rats fed different fats with or without cholesterol supplement

	Beef tallow diet		Fish oil diet	
	Cholesterol-free	Cholesterol-rich	Cholesterol-free	Cholesterol-rich
	(% of total fatty acids)			
Phosphatidylcholine				
16 : 0	27.3±1.2	22.5±0.4	38.2±0.1	27.8±1.6
16 : 1	3.9±0.4	4.5±0.1	5.5±0.1	5.9±0.2
18 : 0	23.1±1.1^{a}	19.9±0.1^{a}	18.0±0.2^{a}	10.5±0.9^{b}
18 : 1	20.8±1.1^{a}	23.8±0.8^{a}	12.9±0.4^{a}	15.7±0.7^{a}
18 : 2 n-6	5.9±0.1^{a}	9.3±1.1^{b}	1.9±0.1^{c}	3.1±0.3^{c}
20 : 4 n-6	8.4±1.7^{ab}	13.3±3.3^{b}	5.6±0.3^{a}	8.0±0.4^{ab}
20 : 5 n-3	n.d.	n.d.	5.3±0.2	9.4±0.4
22 : 6 n-3	3.5±1.3^{a}	3.9±0.1^{a}	6.4±0.7^{b}	8.8±1.3^{b}
Phosphatidylethanolamine				
16 : 0	20.1±0.5	19.6±1.8	25.5±1.8	21.4±2.2
16 : 1	1.5±0.1	0.7±0.1	1.3±0.1	0.7±0.2
18 : 0	24.7±0.5	25.9±1.8	23.5±0.9	22.9±2.2
18 : 1	9.6±0.6^{ab}	13.1±0.3^{a}	4.6±0.3^{b}	9.2±0.2^{ab}
18 : 2 n-6	2.0±0.2	n.d.	2.7±0.2	1.0±0.2
20 : 4 n-6	18.9±0.8^{a}	16.9±1.9^{a}	6.3±0.8^{b}	6.8±0.8^{b}
20 : 5 n-3	n.d.	n.d.	n.d.	7.7±0.7
22 : 6 n-3	13.9±1.8^{ab}	8.4±1.6^{a}	23.2±1.9^{b}	18.2±1.1^{b}

Rats were fed semipurified diets containing beef tallow or fish oil with or without cholesterol supplement for 2 weeks. Values are given as the means±SE of 6 rats in each group. Values with different letters are significantly different among groups at $p<0.05$. n.d.: not detected

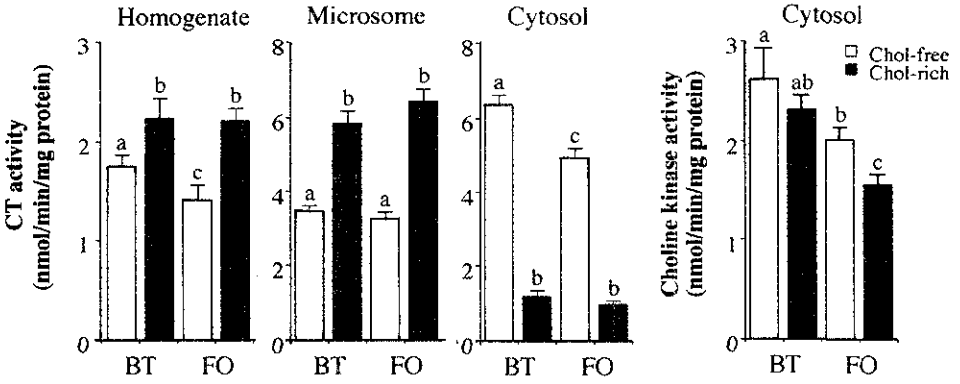

Fig. 3. The activities hepatic CTP:phosphorylcholine cytidylyltransferase and choline kinase in rats fed a beef tallow (BT) or fish oil (FO) diet with or without cholesterol supplement. Values are given as the means±SE of 6 rats in each group. Values with different letters are significantly different among groups at $p<0.05$.

acid, docosahexaenoic acid, and stearic acid. The distribution of saturated fatty acid in phosphatidylcholine decreased moderately by cholesterol supplement in both groups, while that in phosphatidylethanolamine decreased in the FO group only upon cholesterol supplement. The distribution of oleic acid in phosphatidylcholine and phosphatidylethanolamine increased upon cholesterol supplement in both the BT and the FO groups, but the effect was more profound in the FO group. The arachidonic acid distribution greatly increased in phosphatidylcholine fraction in both the BT and the FO groups. In phosphatidylethanolamine fraction, arachidonic acid proportion decreased in the BT group while it increased in the FO group. The distribution of docosahexaenoic acid in phosphatidylcholine increased moderately in both the BT and the FO groups. In phosphatidylethanolamine fraction, the distribution of docosahexaenoic acid decreased.

DISCUSSION

The present study investigated the effect of cholesterol supplement to fish oil or beef tallow diets on phospholipid metabolism in hepatic microsomes of rats. Cholesterol-free diet did not influence hepatic lipid contents in both types of fat diets. However, cholesterol supplement decreased hepatic phospholipid content while it increased the hepatic triglyceride and cholesterol contents especially in the FO group (Fig. 1). Fungwe et al. (3) and Lie et al. (30) have reported that dietary cholesterol increased the synthesis and the mass of triglyceride and cholesteryl ester in the liver, concomitantly with an increase in the secretion of VLDL-lipids. They also observed that cholesterol feeding reduced the activity of carnitine palmitoyltransferase which may lead to cholesterol and triglyceride accumulation by increasing substrate pool. However, hepatic phospholipid content decreased in the cholesterol-fed rats (30), which may impair the membrane functions in the liver.

The increase of hepatic cholesterol upon cholesterol supplement may modify membrane fluidity. The alteration in the activities of membrane-bound enzymes may influence the phospholipid metabolism in the liver. Phosphatidylcholine is a major component of phospholipids in biological membranes and VLDL (6,8,31). Phosphatidylcholine is synthesized mainly through CDP-choline pathway, catalyzed by choline kinase, CTP:cholinephosphate cytidylyltransferase, and cholinephosphate transferase (6,7,32). Newly synthesized phosphatidylcholine is required for the synthesis and secretion of VLDL in hepatocytes (6,8). Thus alteration of phosphatidylcholine biosynthesis may influence lipoprotein metabolism. Since choline kinase and CTP:phosphocholine cytidylyltransferase are rate-limiting enzymes for phosphatidylcholine biosynthesis (6,7). Our study that evaluated the effect of cholesterol-feeding on these enzyme activities showed that the activity of choline kinase in cytosol decreased slightly upon cholesterol supplement in both groups (Fig. 3). The activity of CTP:phosphocholine cytidylyltransferase increased in microsomes and decreased in cytosol by cholesterol supplement in both groups (Fig. 3). Lim et al. reported that rats fed a cholesterol and cholate rich diet displayed 2-fold translocation of cytosol CTP:phosphocholine cytidylyltransferase to microsomes compared to the control animals (33). This tendency suggests an altered distribution of CTP:phosphocholine cytidylyltransferase in subcellular fraction in rats fed a cholesterol supplemented diet. There is a consensus that CTP: phosphocholine cytidylyltransferase activity is regulated by translocation from the cytosolic inactive form to the microsomal active form (34-36). Whether this alteration of the subcelluar distribution of CTP:phosphocholine cytidylyltransferase is due to a decrease in the level of phosphatidylcholine level in the microsomal membranes should be tested. Other potential mechanism for CTP:phosphocholine cytidylyltransferase translocation such as changes in the modification of dietary fatty acids may also be considered.

The results of the present study showed that the contents of hepatic phospholipid decreased and that of cholesterol increased in rats by cholesterol supplement in both types of fat diets (Fig. 1), resulting in an increase of the cholesterol/phospholipid ratio. The phospholipid compositions (Table 2) and the fatty acid moiety of phosphatidylcholine and phosphatidylethanolamine (Table 3) in hepatic microsomal preparations were also observed. When rats were fed cholesterol-free diets, the BT group exhibited more phosphatidylcholine and less phosphatidylethanolamine compared to the FO group. The proportion of phosphatidylcholine decreased by cholesterol supplement in both groups while phosphatidylethanolamine in the BT group increased and phosphatidylethanolamine in the FO group decreased, resulting in the decreased phosphatidylcholine/phosphatidylethanolamine ratio upon cholesterol supplement. The microsomal CTP:phosphocholine cytidylyltransferase activity and phosphatidylcholine content were not changed. The result suggests that the catabolism of phosphatidylcholine may be active upon cholesterol supplement in the FO group only.

The main component of highly unsaturated fatty acids in hepatic microsomes of the FO group was docosahexaenoic acid in phosphatidylcholine and phosphatidylethanolamine, while it was arachidonic acid in phosphatidylcholine and phosphatidylethanolamine in the BT group. Supplementation of the BT or the FO with cholesterol resulted in an increase in the proportion of arachidonic acid and a decrease in the proportion of stearic acid in phosphatidylcholine. By contrast, the same dietary supplementation resulted in a decrease in docosahexaenoic acid in phosphatidylethanolamine. Thus, the effect of dietary cholesterol on the fatty acid compositions of phospholipid is different according to the type of dietary fats.

The secretion of phospholipid increased significantly by cholesterol supplement in both groups (Fig. 2), which is in agreement with the previous reports (2,18,37). Ohtani et al. reported that VLDL secretion was stimulated in cultured hepatocytes in cholesterol-fed hamsters when fat was added to the diet (9). Our data and previous reports (1,9) suggest that dietary cholesterol stimulates the secretion of VLDL. A positive correlation between cholesterol and phospholipid levels in plasma was noted, which is in agreement with the

previous reports (3,30). The decrease in plasma lipid concentrations in rats fed fish oil may be due to the inhibition of synthesis/secretion of VLDL in the liver (10-13). In conclusion, effect of dietary cholesterol on phospholipid metabolism is varied according to the sources of dietary fats in rats.

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