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Effects of dietary soybean lecithin on plasma lipid transport and hepatic cholesterol metabolism in rats

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Abstract

Dietary lecithin can stimulate bile formation and biliary lipid secretion, particularly cholesterol output in bile. Studies also suggested that the lecithin-rich diet might modify hepatic cholesterol homeostasis and lipoprotein metabolism. Therefore, we examined hepatic activities of 3-hydroxy-3 methylglutaryl coenzyme A reductase "HMG -CoA reductase", cholesterol 7α-hydroxylase and acyl-CoA: cholesterol acyltransferase "ACAT" as well as plasma lipids and lipoprotein composition in rats fed diets enriched with 20% of soybean lecithin during 14 days. We also evaluated the content of hepatic canalicular membrane proteins involved in lipid transport to the bile (all P-glycoproteins as detected by the C 219 antibody and the sister of P-glycoprotein "spgp" or bile acid export pump) by Western blotting.

As predicted, lecithin diet modified hepatic cholesterol homeostasis. The activity of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase was enhanced by 30 and 12% respectively, while microsomal ACAT activity showed a dramatic decrease of 75%. As previously reported from ACAT inhibition, the plasma level and size of very low-density lipoprotein (VLDL) were significantly decreased and bile acid pool size and biliary lipid output were significantly increased. The canalicular membrane content of lipid transporters was not significantly affected by dietary lecithin. The current data on inhibition of ACAT activity and related metabolic effects by lecithin mimic the previously reported effects following drug-induced inhibition of ACAT activity, suggesting potential beneficial effects of dietary lecithin supplementation in vascular disease. © 2003 Elsevier Science Inc. All rights reserved.

Keywords: Dietary lecithin; Plasma lipoproteins; Biliary cholesterol; Hepatic cholesterol; Regulatory enzymes

1. Introduction

The liver plays a major role in cholesterol homeostasis by regulating plasma lipoprotein metabolism and lipid output in bile [1,2]. The organ is the main site of formation of very low-density lipoprotein (VLDL) and takes up and degrades high-density lipoprotein (HDL) and other lipoprotein fractions [3,4]. Hepatic bile formation and secretion are essential in maintaining cholesterol balance since the sterol is excreted from the body almost exclusively via bile as such, or after its conversion into bile acids. Several hepatic microsomal enzymes are implicated in regulating cholesterol hepatic homeostasis: 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate limiting enzyme in cholesterol synthesis [5], cholesterol 7 α-hydroxylase the main route catalyzing the conversion of cholesterol into bile acids [6] and, acyl-CoA: cholesterol acyltransferase (ACAT) which is implicated in the acylation of cholesterol and in the assembly and secretion of VLDL particles. Thus, ACAT activity plays a major role in the VLDL secretion in blood and the subsequent cholesteryl ester accumulation and risk of vascular disease [7,8]. There have been studies indicating a correlation between inhibitions of ACAT activity, reduced hepatic cholesteryl ester content and reduced VLDL secretion. Drug induced ACAT inhibition in hepatocyte's cell culture was shown to enhance the pool of free cholesterol which served as substrate and inducer of cholesterol 7 α-hydroxylase [9-11]. This suggested that enhanced excretion of biliary bile acids may

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account for the reduction of VLDL secretion by the liver and could represent a major mechanism in the lipid lowering effect of ACAT inhibitors and their potential antiatherogenic action.

The nutritional effects of soybean lecithin on lipid homeostasis and secretion of lipids in bile has been the subject of several studies [12-15]. In rats, feeding diets enriched in soybean lecithin can stimulate bile formation and secretion rate of biliary bile acids, phospholipid and cholesterol [12-14].

In normolipidemic rats, lecithin-rich diets can modulate plasma total and HDL-cholesterol and it was suggested that the transport of unesterified cholesterol to the liver may be stimulated and directed towards bile to be secreted as such or as its metabolites [13]. In the model of hypercholesterolemic rabbit, lecithin also induces a decrease of beta-VLDL cholesterol and of beta-VLDL-triacylglycerol while enhancing biliary lipid output [15]. Thus, we hypothesized that lecithin- induced decrease of VLDL and stimulation of lipid secretion in bile may be linked to inhibition of ACAT activity. In the present study, we investigated in rats the effects of lecithin on regulatory enzymes of hepatic cholesterol metabolism, cholesterol compartmentalization in the liver and lipoprotein lipid composition. In addition, we measured the content of canalicular transporter proteins involved in biliary lipid secretion.

2. Materials and methods

2.1. Animals and diets

Adult male Sprague-Dawley rats, weighing 200-250 g were purchased from Charles River Breeding Laboratories (St-Constant, Quebec, Canada). Rats were allowed free access to water and food and were maintained at 22°C with a 12-hr light cycle. After 2 to 3 days of acclimatization we followed the dietary protocol we previously described [14]. The rats were randomly divided into two groups: one was assigned to an 16% polyunsaturated TG-enriched diet containing mainly sunflower seed oil, referred to as the control diet, and the other was a 20% semi-purified soybean lecithin diet. The percentage of TG in both diets was similar. Fatty acid composition did not differ significantly between diets with palmitic and linoleic acid contributing 25.1% and 54.6% in lecithin diet compared to 18.5% and 49.6% in control diet respectively. The diets composition is given in Table 1. Energy intake of control and lecithin-fed groups was 404 and 375 KJ/day respectively. At the end of the experiment, mean weight gain of the rats was comparable in both groups. Protocols were approved by the University of Montreal Animal Care Committee, in accordance with the Canadian Council on Animal Care guidelines. After 14 days on the control and experimental diets, rats were food-deprived overnight. Blood, bile and liver samples were collected as described below.

Table 1
Composition of diet (g/kg food)

Ingredient	Control diet	Lecithin-enriched diet
Casein	197	197
Methionine	3	3
Sucrose	185	172
Dextrose	186	172
Dextrine	185	172
Vitamin mixture	10	10
Mineral mixture	40	40
Alphacel	34	34
Triacylglycerols[a]	160	
Lecithin granules[b]		200

[a] Triacylglycerols: Becel margarine made from 87% sunflower oil and 13% vegetable oils.

[b] Lecithin granules: Sigma (St-Louis, MO) from soybean contained 22% total phospholipids, of which 11% was phosphatidylcholine and 78% triacylglycerols.

2.2. Blood collection and lipoprotein isolation

Rats were anesthetized by the intraperitoneal injection of pentobarbital (45 mg/kg body weight) after overnight fast. Blood was collected from the abdominal aorta into EDTA-containing tubes (1 mg/ml) and plasma separated immediately by low speed centrifugation (2800 rpm, 15 min, 4°C). A fraction of plasma was rapidly frozen and stored at -80°C for lipid analysis. Lipoproteins were isolated from fresh plasma by conventional discontinuous density gradient ultracentrifugation as previously described in detail [16]. Briefly, after preliminary centrifugation to remove chylomicrons, VLDL and low density lipoproteins (LDL) were isolated at a density of 1.006 and 1.063 g/ml, respectively, running at 40,000 rpm for 18 hr at 5°C. The separation of HDL2 and HDL3 was performed at 40,000 rpm for 48 hr at 5°C. Each fraction was dialyzed against phosphate buffered saline (PBS) with 0.001 mol/L EDTA, pH 7.0 at 4°C.

2.3. Plasma lipid and lipoprotein analysis

Plasma concentrations of total cholesterol, free cholesterol, HDL-cholesterol and TG were measured enzymatically using commercial kits (Boehringer Mannheim, Montreal, Canada). CE was calculated as the difference between total and free cholesterol × 1.6. The lipoprotein lipid composition was determined by the same methodology as above. Lipoprotein-protein was quantified according to Lowry et al [17]with bovine serum albumin as a standard. Phospholipids were determined by the Bartlett method [18].

2.4. Hepatic lipid determination

Rats were sacrificed after blood collection and a portion of the liver was taken, frozen in liquid nitrogen and kept at −80°C until assayed. Samples were homogenized in 0.9% NaCl and lipids extracted with chloroform-methanol (2:1,

vol/vol), followed by TG, total and free cholesterol, as well as phospholipid analysis as described before. Total liver lipid content was determined gravimetrically, after extraction with chloroform-methanol (2:1, vol/vol).

2.5. Bile sampling and bile analysis

Biliary lipid output and bile acid pool size was evaluated in additional groups of rats fed control and lecithin rich-diets. Animals were anesthetized with pentobarbital (45 mg/kg body weight i.p.), the abdomen was opened and the common bile duct was cannulated with a PE-10 catheter. The rats were then immediately placed in restraining cages with free access to water. Bile was collected on ice in pre-tared tubes for 17 h at 1 h intervals with an automatic fraction collector. The flow rate was determined by the weighing of the tubes after collection. The body temperature was maintained throughout at 37°C using a rectal probe and thermostatically controlled heat lamp. Biliary lipid secretion rate (cholesterol and phospholipid) was determined on samples collected during the first hour of sampling as described above for plasma. The bile acid pool was measured according to the 'washout' technique of Ericksson [19]. This method involves cannulating the bile duct and allowing the bile acid pool to wash out until a steady state, where secretion equals synthesis, is reached. Total bile acids in bile were assessed by using 3 α-hydroxysteroid dehydrogenase (Sigma, St-Louis, MO, USA) [14].

2.6. Preparation of liver microsomes

Livers were removed and placed in ice-cold buffer (pH 7.4) containing 250 mmol/L of sucrose, 20 mmol/L of tris-HCL, 1mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium fluoride, and 20 mM dithiolthreitol (DTT). After tissue homogenisation, microsomal fractions were prepared according to a previously described technique [20]. The washed microsomal pellets were quickly frozen and stored at −80°C for later use. Protein yields in microsomal membrane fractions were not affected by diet intervention.

2.7. Microsomal HMG-CoA reductase activity

Microsomal enzymatic activity was assayed as described previously [21]. Briefly, the reaction mixture contained 160 mM potassium phosphate (pH 7.4), 300 μg microsomal protein, 20 mM glucose-6-phosphatase, 2.5 mM DTT and 1.2 units glucose-6-phosphatase dehydrogenase. The reaction was initiated by the addition of [3-^{14}C] HMG-CoA (15,000 dpm/nmol). After incubation for 30 min at 37°C, the [^{14}C] mevalonate formed was converted into lactone, isolated by thin-layer chromatography, and counted using an internal standard to correct for incomplete recovery. HMG-CoA reductase activity was expressed as nanomoles of mevalonate synthesized per milligram of protein per minute.

2.8. Microsomal cholesterol 7 α-hydroxylase activity

Microsomal cholesterol 7 α-hydroxylase activity was measured according to Hylemon et al. [22] after modification by Brunet et al [23]. Briefly, 1 mg of microsomal protein was diluted in a potassium phosphate buffer, pH 7.4, containing 50 mM NaF, 5 mM DTT, 1 mM EDTA, 20% glycerol, 0.015% CHAPS and 0.25 mM desferal. The reaction was started by adding an NADPH regenerating system containing 1 mM NADP, 10 nM glucose-6-phosphate and 0.15 UI glucose-6 phosphate dehydrogenase. Following 20 min reaction at 37°C, the endogenous 7 α-hydroxylcholesterol form was derivatized with a 0.1% cholesterol oxidase solution. Dried petroleum ether extract was resuspended in 70% acetonitrile/30% methanol mobile phase, prior to HPLC chromatography, with UV detection at 240 nm, using 7 β-hydroxycholesterol as an internal standard. The 7 α-hydroxycholesterol product was expressed as pmoles/min/mg protein.

2.9. Microsomal ACAT Activity

The determination of ACAT activity was based on an assay described previously [20]. A sample containing 75 μg microsomal protein was mixed with 5 μmol [^{14}C] oleoyl coenzyme A (specific activity ~ 10,000 dpm/μmol) to initiate the reaction in a buffer solution (pH 7.5) consisting of 0.1 M Tris-HCl, 0.25 M sucrose, and 1 mM EDTA. After incubation for 10 min at 37°C, the reaction was stopped by adding chloroform/methanol (2:1, vol/vol) and this was followed by addition of [^{3}H] cholesteryl oleate as an internal standard to estimate recovery.

2.10. Analysis of microsome lipid composition and fluidity measurement

The lipids in microsomal membranes were extracted by the method of Bligh and Dyer [24]. Total phospholipid and cholesterol content were determined as described above. Fluidity of microsomal membranes was estimated by the incorporation of a fluorescent probe (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluene-sulfonate) (TMA-DPH) and measurement of polarization [25]. To membrane samples containing 500 μg microsomal protein in potassium phosphate buffer solution (pH 7.2) were added 6 μl TMA-DPH (1 mmol/L in acetonitrile) by vortexing for 10 s. Following an incubation of 30 min at 37°C and evaporation of acetonitrile, fluorescence polarization was measured in a spectrofluorophotometer at 22°C with polarization filters parallel and perpendicular to the excitation beam. The excitation wavelength was at 360 nm and emission wavelength was 430 nm. The polarization parameter was calculated from the ratio of fluorescence intensities

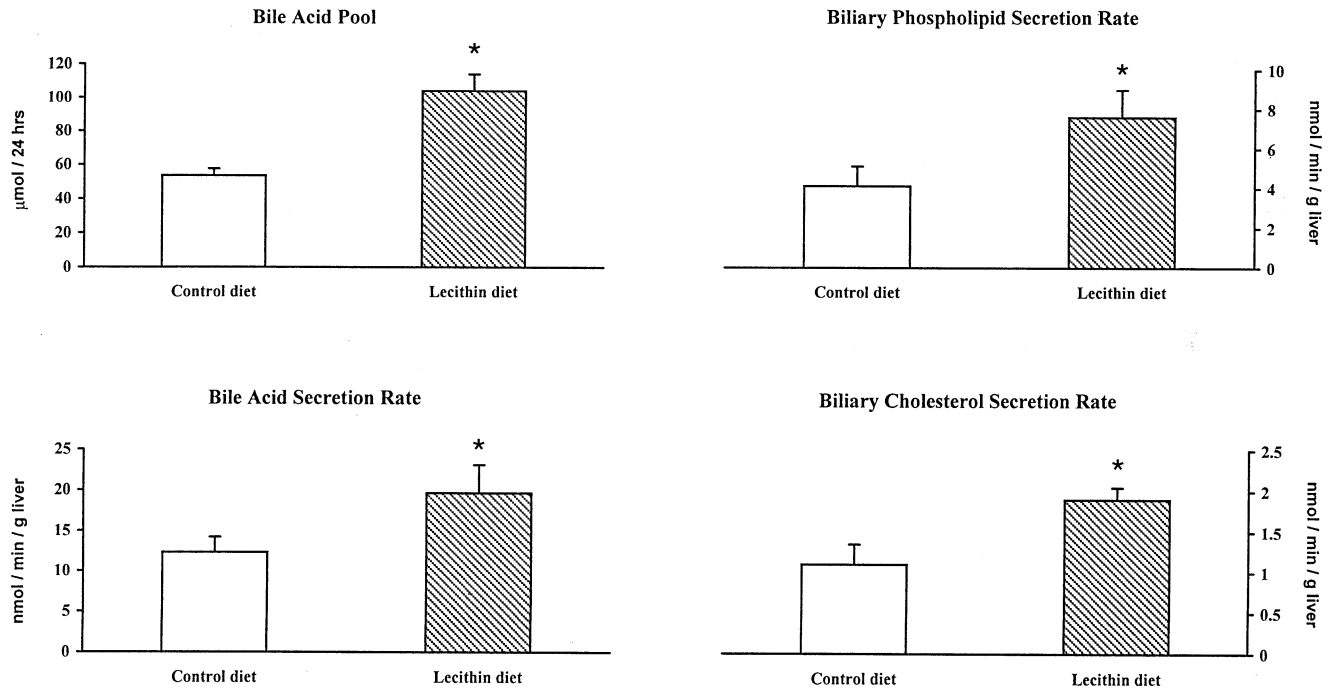

Fig. 1. Values of biliary bile acid pool, biliary bile acid secretion, biliary phospholipid secretion and biliary cholesterol secretion in rats fed control diet and lecithin diet.Values represent the mean ± SEM of 4–5 animals per group.*$P < 0.05$ when compared to Control diet.

in different directions of the excitation beam. Membrane fluidity was inversely related to polarization value.

2.11. Preparation of liver plasma membranes enriched in bile canalicular complexes and Western blotting

Additional groups of rats fed control and lecithin rich diets for 14 days were used to prepare bile canalicular enriched membrane fractions and to asses lipid transport proteins.

Plasma membranes were isolated from liver homogenates by a procedure described by this laboratory [26] with the addition of protease inhibitor cocktail as described [27]. Protein concentrations in membrane fractions were determined with the Micro BCA protein assay reagent kit from Pierce and the degree of purification was determined by the relative enrichment of marker enzymes [26]. Lecithin feeding did not modify marker enzymes enrichment.

Western blot analysis was carried out as published [27]. Briefly, 30 μg protein normalized for enrichment in the membrane marker leucine aminopeptidase was separated using SDS gel electrophoresis and subsequently blotted onto nitrocellulose and probed with the mouse monoclonal C 219 antibody (Signet Laboratories Inc. Dedham. MA) which detects all p-glycoproteins including mdr 2 and with a rabbit polyclonal anti-spgp antibody [28] (kindly provided by Dr V Ling, British Columbia Cancer Res. Center, Vancouver, BC, Canada) at a 1: 5000 dilutions. Protein density was determined by image analysis of the digitized gel blots using Image J, a public domain software originally written at the National Institutes of Health (USA).

2.12. Statistical Analysis

All values were expressed as the mean ± SEM. Statistical differences were assessed by Student's two-tailed *t*test.

3. Results

3.1. Biliary lipid secretion, bile acid pool and expression of canalicular transporters

As shown in Fig. 1 and in accordance with previous results [14], lecithin-rich diet stimulated biliary bile acid, phospholipid and cholesterol secretion rates. Total daily biliary bile acid secretion for the lecithin-fed rats was also elevated when compared to triglyceride fed rats (442.84 ± 57.65 versus 282.89 ± 16.17 μmol/24 hrs, $P < 0.05$). The calculation of the bile acid pool size, following a 17 hr biliary washout revealed a marked increase in the lecithin group (Fig. 1). The similar degree of elevation of bile acid secretory rate and pool are indicative of unchanged recycling frequency of bile acids.

Representative Western blot showing membranes preparation from rats fed control and lecithin-rich diets is presented in Fig. 2. C219 and spgp levels revealed no significant differences between the control and lecithin groups. Relative areas for C219 and spgp protein expression did not differ between groups. Levels of p-gps and spgp protein in liver homogenates were not different between control and lecithin fed rats (results not shown).

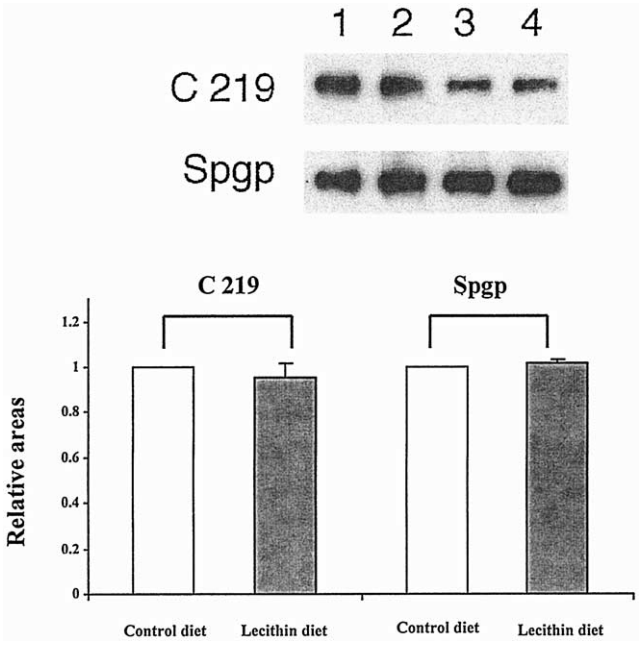

Fig. 2. P-gps and spgp protein levels in membranes fractions of control rat liver and lecithin diet rat liver. Lanes 1 and 2, control; lanes 3 and 4, lecithin group. Immunoblotting analysis was performed using the primary antibodies C219 and spgp, recognizing all Pgps and spgp respectively. Each band represents the results of a single animal.

3.2. Plasma lipids, lipoprotein profile and composition

Table 2 illustrates the influence of feeding lecithin diet on plasma lipids. The dietary intervention significantly increased total cholesterol, contributed by both higher free and cholesterol ester levels. However, the lecithin fed rats exhibited a decreased plasma TG and phospholipid levels although reaching statistically significant difference only for phospholipids.

Feeding the lecithin diet did not affect significantly IDL and HDL lipoprotein fractions but produced a marked decrease of VLDL (54%, $P < 0.05$ compared to control group). By contrast, LDL class increased (Table 2).

Table 2
Plasma lipids and plasma lipoprotein fractions

Plasma lipids mmol/L	Control diet	Lecithin diet
Triglycerides	0.43 ± 0.07	0.33 ± 0.02
Phospholipids	1.42 ± 0.04	1.17 ± 0.06*
Total cholesterol	1.54 ± 0.04	1.87 ± 0.09*
Free cholesterol	0.207 ± 0.002	0.29 ± 0.03*
Cholesterol ester	1.32 ± 0.05	1.55 ± 0.08*
Lipoprotein fractions mg/ml		
VLDL	0.50 ± 0.08	0.23 ± 0.04*
IDL	0.04 ± 0.03	0.07 ± 0.03
LDL	0.08 ± 0.01	0.13 ± 0.02*
HDL_2	0.42 ± 0.06	0.53 ± 0.06
HDL_3	0.58 ± 0.06	0.39 ± 0.10

Values represent the means of ±SEM of 5–6 animals per group.
* $P < 0.05$ compared to control diet.

The relative lipid content of plasma lipoproteins, isolated by sequential ultra-centrifugation, is shown in Table 3. VLDL (1.006g/ml) particles from lecithin fed rats demonstrated a significant decrease of the proportion of TG. This change resulted in a lower ratio of TG+CE/FC+PL+protein, indicative of smaller VLDL particles in the experimental group.

3.3. Sterol enzymes

The activity of HMG-CoA reductase and cholesterol 7 alpha-hydroxylase in liver microsomes showed a tendency to be increased by 30% and 12% respectively after 14 days of dietary intervention (Fig. 3). In contrast, ACAT activity was profoundly inhibited (75%) by lecithin diet when compared to rats fed control diet.

3.4. Hepatic lipid content

As shown in Table 4, the total lipid content in liver homogenates from lecithin fed rats was significantly decreased compared to control rats. This was attributed to lower TG and total cholesterol contents. Both free and esterified cholesterol contributed to the decline (although not reaching statistically significant differences for the latter).

3.5. Phospholipid and cholesterol content, fluidity in microsomes

Analysis of the lipid content in isolated microsomes (Table 5) reveals that total cholesterol was decreased by 30% in lecithin group ($P < 0.01$) whereas the PL content was not significantly different between two groups. Consequently, the cholesterol/PL ratio was decreased in the experimental group and a significant increase in membrane fluidity was observed. Since the protein yield in microsomes was comparable between both groups, the membrane fluidity was not influenced by the protein content but rather by the cholesterol/phospholipids ratio.

4. Discussion

This study establishes that feeding rats diets enriched in soybean lecithin results in remarkable changes in hepatic cholesterol homeostasis. The major finding is a decrease of ACAT activity and an altered cholesterol compartmentalization in the liver. Previous work indicated that this enzyme plays a pivotal role in cholesterol distribution in the hepatocyte, specifically, whether it is diverted to lipoprotein or biliary pathways or to the cholesterol ester storage compartment [11,29,30]. Under the present experimental conditions, lower ACAT activity was associated with lower free and cholesterol ester content. It is likely that the free cholesterol available after ACAT inhibition in the hepatocyte

Table 3
Chemical composition of plasma lipoproteins

Lipoproteins	Composition					Weight Ratios		
	TG	FC	CE	PL	PR	TG/PR	CE/PR	(TG+CE)/(FC+PL+PR)
VLDL (1.006 g/ml)								
Control	60.23 ± 2.69	2.41 ± 0.46	5.34 ± 0.63	17.23 ± 1.58	14.79 ± 3.91	4.42 ± 1.14	0.78 ± 0.41	2.00 ± 0.23
Lecithin	51.53 ± 1.82*	2.32 ± 0.32	5.00 ± 0.24	17.49 ± 0.48	23.69 ± 2.49	2.4 ± 0.46	0.23 ± 0.04	1.32 ± 0.11*
LDL (1.063 g/ml)								
Control	3.33 ± 1.14	2.73 ± 0.51	26.64 ± 1.67	29.98 ± 5.90	37.32 ± 7.69	0.09 ± 0.04	0.57 ± 0.04	0.44 ± 0.04
Lecithin	2.88 ± 0.32	3.44 ± 0.32	32.35 ± 3.00	22.37 ± 2.59	39.03 ± 2.28	0.08 ± 0.02	0.86 ± 0.12	0.56 ± 0.07
HDL2 (1.21 g/ml)								
Control	0.26 ± 0.17	2.05 ± 0.44	36.42 ± 5.66	27.53 ± 3.34	30.75 ± 7.38	0.03 ± 0.01	0.93 ± 0.17	0.68 ± 0.19
Lecithin	0.40 ± 0.25	2.52 ± 0.36	34.02 ± 2.35	26.79 ± 1.10	36.32 ± 3.01	0.04 ± 0.01	0.99 ± 0.14	0.54 ± 0.06
HDL3 (1.21 g/ml)								
Control	0.24 ± 0.15	1.17 ± 0.13	32.67 ± 3.98	28.60 ± 4.08	34.70 ± 47.62	0.02 ± 0.001	0.74 ± 0.11	0.53 ± 0.09
Lecithin	0.22 ± 0.14	0.94 ± 0.12	29.48 ± 3.10	21.75 ± 3.39	47.62 ± 5.54	0.02 ± 0.002	0.68 ± 0.12	0.44 ± 0.06

Values are means ± SE of total plasma lipoproteins for n = 6 in control and lecithin diets. TG, triglyceride; FC, free cholesterol; CE: cholesteryl ester, PL, phospholipid; PR, protein.
* : $p < 0.005$

was very rapidly directed for elimination in bile either directly or after conversion to bile acids. The increased diversion of cholesterol and its metabolites into bile by the inhibition of hepatic ACAT resulted in a decline of cholesteryl ester secretion by the cells accompanied by lower plasma VLDL levels [31,32]. In rats, lecithin supplements have been shown to reduce availability of fatty acids for triglyceride synthesis by decreasing the rate of fatty acid synthesis [33,34]. A reduced rate of triglyceride and/or cholesteryl ester synthesis may be an additional mechanism contributing to the diminished plasma VLDL. The decrease in plasma VLDL lipid appeared to be mostly due to a reduction in particle size, rather than in particle numbers as indicated by the ratio of surface lipids to core lipids. Since the LDL concentration can be influenced by the level of apo B100 particles secreted by the liver [35,36], VLDL could contribute to the increase in plasma LDL in this group of animals. The modification in lipid content of these VLDL

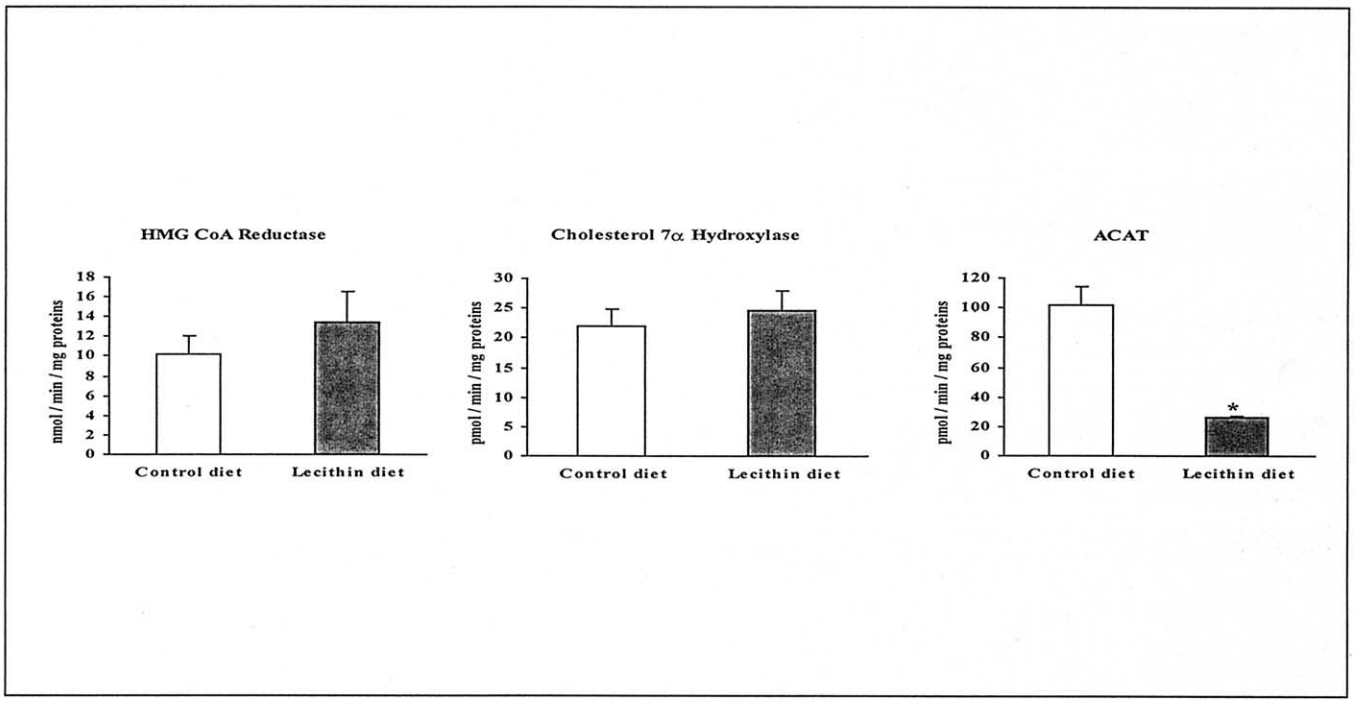

Fig. 3. Activity of liver hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, cholesterol 7 alpha-hydroxylase and acyl-coenzyme-A:cholesterol acyltransferase (ACAT). Rat liver microsomes were isolated and assayed for the three key regulatory enzymes. Values represent the mean of ± SEM of 6 rats per group.$P < 0.05$ compared to Control diet.

Table 4
Hepatic lipid content from control and lecithin groups

	Total lipids μg/mg protein	nmol/mg protein				
		TG	TC	FC	CE	PL
Control	227.5 ± 5.1	33.53 ± 1.82	23.1 ± 0.5	11.60 ± 0.80	11.5 ± 0.9	343.9 ± 10.5
Lecithin	169.5 ± 2.5*	26.49 ± 2.10*	18.3 ± 0.7*	8.74 ± 0.63*	9.5 ± 1.1	373.2 ± 7.3

Lipids were extracted from liver homogenates and quantified by enzymatic techniques.
Values are means ± SEM for n = 6 / group.
Abbreviations: TG, triglycerides; TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; PL, hospholipids
* $p < 0.05$

could increase their catabolism and produce higher levels of LDL. It has indeed been demonstrated that the appearance of TG-depleted VLDL leads to an increase in LDL production [37]. However, in our study, the hepatic free cholesterol was decreased, an effect which could have been expected to increase LDL receptor activity and therefore reduce LDL levels. Therefore, the exact mechanism by which lecithin affects lipoprotein metabolism needs more detailed investigation.

We further evaluated the HMG-CoA reductase and cholesterol alpha hydroxylase activity (the primary route to bile acid synthesis). We observed an increase in HMG-CoA activity which likely compensates for the reduction of cellular cholesterol pool. Several studies have shown incorporation of cholesterol, available from ACAT inhibition, into the bile acid synthetic pathway [9,38]. This is corroborated in the present study as lecithin-fed rats also showed greater bile acid pool size and an increase, although not statistically significant, of the 7 alpha-hydroxylase activity. Several factors may explain why this enzyme activity did not increase to the same extent as the bile acid pool size. Under the present conditions lecithin was fed for 2 weeks and it may be that enzyme activity was enhanced in earlier stages of feeding and decreased afterwards through feedback inhibition pathway. Another factor may be altered bile acid pool composition. It is known that the 7 alpha hydroxylase is sensitive to bile acid hydrophobicity and that in lecithin fed rats there is a more hydrophobic pool than in control rats which may have inhibited enzyme activity [39,40,14].

In this study, we corroborate that lecithin induces a substantial increase in biliary output of bile acid, cholesterol and phospholipid. The hepatic availability of substrates for biliary excretion is a significant factor in bile secretion. However, it is well established that the transport across the hepatic canalicular membrane is the rate limiting step for biliary secretion of most bile components and for hepatic bile formation [41]. This transport is driven mainly by ATP-dependent export pumps [42]. This raised the question whether increased availability of biliary lipid after lecithin-rich diet was also associated with greater content of membrane proteins involved in biliary lipid secretion. The canalicular secretion of phospholipid is mediated by and ATP-dependent mdr2 glycoprotein which transfers phospholipid from the inner to the outer canalicular membrane [42,43]. Mice with disrupted mdr2 protein do not secrete phospholipid and the cholesterol output in bile is markedly impaired [44]. The canalicular secretion of monovalent bile acids (e.g. taurocholate) is also mediated by an ATP-dependent glycoprotein, the spgp or bile acid export pump [28,45]and in mice inactivation of spgp, leads to failure to secrete monovalent bile acids [46]. In the present study, membrane canalicular content of spgp and of C 219 reactive proteins which includes mdr2 and spgp was evaluated and results indicated no significant modification by lecithin feeding. Since in normal rodent livers these transporters are present in high levels it may be that they are not functioning at maximal capacity. Thus, increased output of biliary lipid following lecithin diet could occur without modulation of the transport proteins.

In summary, dietary lecithin increases bile acid pool and stimulates biliary lipid secretion and bile which is associated with significant changes in hepatic cholesterol compartmentalization favoring a greater mobilization of cholesterol to bile. Diet intervention also drastically reduced ACAT activity and plasma VLDL as well as their triglyceride and cholesteryl ester content. Thus, lecithin feeding mimics the effect of drug-induced inhibition of ACAT activity which corroborates the potential antiatherogenic properties of lecithin supplementation.

Table 5
Cholesterol and phospholipid content, cholesterol/phospholipids ratio and fluidity of microsomes in rats fed control diet and lecithin diet

Parameter	Control diet	Lecithin diet
Cholesterol nmol/mg protein	43.48 ± 2.45	30.86 ± 3.13*
Phospholipid nmol/mg protein	359.72 ± 31.07	405.56 ± 17.98
Cholesterol/phospholipid ratio	0.127 ± 0.016	0.077 ± 0.009*
Fluidity (1/Polarization)	3.77 ± 0.11	4.14 ± 0.08*

Values are means ±SEM for 6 animals per group.
* $P < 0.05$ compared to control diet.

Acknowledgments

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