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Dietary soybean phosphatidylcholines lower lipidemia: Mechanisms at the levels of intestine, endothelial cell, and hepato-biliary axis

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The beneficial metabolic effects of dietary soybean lecithin on lipid metabolism are now more clearly established. The intestinal absorption of cholesterol is decreased by soybean phosphatidylcholine-enriched diet and results in a cholesterol-lowering effect. There is an enhancement of the cholesterol efflux by endothelial cells incubated with soybean phosphatidylcholines, and a stimulation of the reverse cholesterol transport by high density lipoprotein-phosphatidylcholines. As a result of all these processes, phosphatidylcholines provided by the soybean lecithin metabolism appear to be key molecules controlling the biodynamic exchanges of lipids. They regulate homeostasis of cholesterol and fatty acids by decreasing their synthesis and promoting cholesterol oxidation into bile salts. Finally, the outcome is the increase in bile secretion of these lipids and/or their metabolite forms. Such findings constitute promising goals in the field of nutritional effects of soybean lecithin in the treatment or prevention of hyperlipidemia and related atherosclerosis. (J. Nutr. Biochem. 11:461–466, 2000)

Keywords: soybean phosphatidylcholine; diet; lipids; HDLs

Introduction

In Western countries, dietary recommendations aim to reduce lipid consumption so as to reduce the risk of cardiovascular disease.[1,2] Fat-rich foods are also rich in phosphatidylcholines, the main components of lecithin. One unfavorable consequence of decreasing lipid consumption is a probable decrease in lecithin intake.[3] Thus, many studies were carried out with lecithin as a macronutrient in order to evaluate its effects, notably on dyslipidemia and related risk factors of atherosclerosis.

For instance, some discrepancies concern the cholesterol-lowering effect of dietary soybean lecithin.[4–6] However, we recently found that soybean phosphatidylcholines could be useful in the dietary treatment of mild hypercholesterolemia in humans.[7]

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Thus, the aim of this work was to research links between soybean lecithin feeding, lipid homeostasis, and secretion of these lipids or their metabolites into the bile. Experiments were carried out in animals in vivo in order to study the effect of dietary soybean lecithin in prevention or treatment of atherosclerosis in normolipidemic rats and hypercholesterolemic rabbits, respectively. Rabbits were chosen for their exacerbated sensitivity to dietary fat and cholesterol and the related hyperlipemia and atheroma deposition.[8] Mechanistic studies were carried out to determine the effect of lecithin on the atherosclerosis process, through cholesterol efflux by endothelial cells.

Materials and methods

Experiment in rabbits: Assays in the plasma, liver, and bile

Male New Zealand white rabbits (Dombe's breeding, Romans, France), 3 months old and weighing approximately 2.3 kg, were kept for 2 weeks in an animal room under controlled conditions (temper-

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ature: 18°C; light cycle: 7:00 AM–7:00 PM; humidity: 65%). Rabbits had free access to standardized diet UAR 112 (UAR, Epinay sur Orge, France) and tap water. Then, the rabbits were divided into four groups ($n = 6$ in each group) and fed one of the following diets for 10 weeks: (1) control low-fat diet containing 27 g fat/kg or high-fat diet containing 2 g cholesterol/kg, (2) 77 g fat/kg in saturated lard diet (50 g lard/kg), (3) polyunsaturated soybean triacylglycerol diet (50 g fat/kg), or (4) polyunsaturated soybean phosphatidylcholine diet (50 g purified lecithin/kg). Purified soybean lecithin contained 93% of pure soybean phosphatidylcholine. The three high-fat diets were prepared by UAR (Epinay sur Orge, France) by blending the standard UAR diet for rabbits with cholesterol and lard, soybean triacylglycerols, or soybean lecithin. Finally, the diets were pelleted. At the end of the 10-week period, mean weight gain of the rabbits was 1.10 ± 0.25 kg and there was no significant difference in weight gain between the four groups. The amount of food intake was similar in the four groups (130 ± 15 g/day/rabbit). The three high-fat diets had similar energy densities (10.7, 10.7, and 10.1 kJ/g, respectively, for lard, soybean triacylglycerols, and soybean lecithin groups). Thus, the effects of the three high-fat diets can be directly compared in terms of cholesterol, triacylglycerol, and phospholipid energy.

Composition of fatty acids was different in the high-fat diets. Contents in linolenic acid (18:3, n-6) were 5.6% and 4.8% more in soybean triacylglycerol- and soybean lecithin-enriched diets, respectively, than in the lard-enriched diet. Contents in total polyunsaturated fatty acids were 27.8% and 26.3% more in soybean triacylglycerol and lecithin diets, respectively, than in the lard diet. Iwata et al.[9] showed that the cholesterol-lowering effect of vegetable lecithins such as safflower seed or soybean lecithins was not due to an increase in polyunsaturated (n-6) fatty acid level. The supply, then, of fatty acids in the form of triacylglycerols or phosphatidylcholines seems more involved in cholesterolemia changes than in differences in composition of the (n-6) fatty acids. Therefore, results of the lecithin group can be compared with those of the lard and soybean triacylglycerol groups.

After 10 weeks of consuming diets, rabbits were food-deprived overnight. At 9:00 AM they received, by an intramuscular injection, a mixture of xylazine (20 mg Rompun/kg body weight) and ketamine (15 mg Imalgène/kg body weight). Gallbladder bile was then recovered, and rabbits were killed by bleeding through abdominal aorta. Livers were removed, homogenized with a Polytron at 4°C in a 1 mmol/L $NaHCO_3$ buffer, pH 7.4, and stored at −80°C.

Lipoprotein fractions were separated on a KBr gradient in a Beckman SW 41 rotor (Beckman, Gagny, France) using the technique of Lacombe and Abadie.[10]

Total phospholipids were analyzed by measuring inorganic phosphate as described by Amic et al.[11] after digestion in perchloric acid. Total and unesterified cholesterol in plasma, lipoprotein fractions, and liver were respectively measured by the enzymatic methods of Siedel et al.[12] and Stähler et al.[13] Triacylglycerol concentration was assayed by the enzymatic method of Fossati and Prencipe.[14] Protein was determined by the method of Lowry et al.[15] Bile acids in bile were enzymatically determined using the 3α-hydroxysteroid dehydrogenase (Sigma, St. Quentin Fallavier, France).[16] Bile cholesterol was measured by the enzymatic and kinetic method of Deeg and Ziegenhorn[17] in order to eliminate interference due to bile pigments.

Experiment in rats: Assays of mitochondrial fatty acid oxidation

Male Wistar rats (255 ± 10 g) were given high- or low-fat diets for 3 weeks. All diets contained 24 g % casein, 4 g % cellulose, 1 g % vitamin mixture, and 4.7 g % minerals. The low-fat control diet contained 4 g % low-fat, 24 g % starch, and 18.3 g % sucrose. The composition of high-fat diets was as follows: 14 g % starch, 12.3 g % sucrose, and either 20 g % soybean triacylglycerols or 20 g % soybean lecithin. The lecithin contained 93% of pure soybean phosphatidylcholine. The diets were isoenergetic (390 kJ/day with high-fat diets, 380 kJ/day with standard low-fat diet). After this 3-week period, rats were anesthetized by an intramuscular injection of a mixture of 20 mg/kg xylazine (Rompun, Bayer, Puteaux, France) and 15 mg/kg ketamine (Imalgène 1000, Rhône Mérieux, Lyon, France). Rats were killed by bleeding through abdominal aorta. Livers were removed, and homogenized with a Polytron at 4°C in a 0.25 M sucrose buffer for the preparation of the mitochondria, using the technique described by Ide et al.[18] After centrifugation for 10 min at 500 × *g*, peroxisomes were discarded and the supernatant was centrifuged for 10 min at 9,000 × *g* at 4°C in a Beckman J 25 centrifuge equipped with a fixed angle rotor type JA 25–50. The pellet was rinsed in a 0.25 M sucrose/1 mM EDTA/3 mM Tris-HCl (pH 7.0) buffer and stored in this buffer at −80°C.

Assay of mitochondrial carnitine palmitoyl transferase (the key enzyme of the fatty acid oxidation) was achieved by the methods of Bremer et al.[19] and Ide et al.,[18] using [^{14}C] carnitine as substrate. Radioactivity was finally counted in the fatty acid-carnitine complex. Protein was determined by the method of Lowry et al.[15]

Incubation of ECV cells in culture with soybean phosphatidylcholine

ECV304 cells, a human endothelial cell line, were generously provided by Dr. Bongrand (Marseille, France). The cells were grown until confluent in 24-well plates (Nunc, Denmark). They were cultured in M199 medium containing 20% heat-inactivated fetal calf serum, 2 mM glutamin, 100 U/mL penicillin G, and 100 μg/mL streptomycin (Gibco Life Technologies, Cergy Pontoise, France). The culture was maintained at 37°C in a humidified atmosphere containing 95% oxygen and 5% carbon dioxide. The culture medium was deprived from 20% to 5% fetal calf serum when cells were confluent 24 hr before addition of human low density lipoprotein (LDL) and for the duration of the experiment. Polymixin was added (Sigma, St. Quentin Fallavier, France) at the concentration of 7 μg/mL. Cells were then incubated for 48 hr with a LDL load (50 μg ApoB/well) in the presence of [4-^{14}C] cholesterol. After washes, cells were incubated for 1 hr either without added lipids (control) or with human high density lipoproteins (HDLs; 150 μg apoA-I), soybean phosphatidylcholines (430 μg), dimyristoylphosphatidylcholine (430 μg), apoA-I (150 μg), or reassociations of phospholipids (430 μg) with 150 μg apoA-I.

Statistical analyses

Results are expressed as the arithmetical means of each group with their standard errors. We previously verified the homogeneity of the data in all conditions. Significance was analyzed by one-way analysis of variance and the differences between groups were determined by Fisher's test ($P < 0.05$). All calculations were performed using Stat View™ SE + graphics (Abacus Concepts, Inc., CA USA) on a Macintosh computer.

Results

Lipoprotein composition, hepatic content, and bile lipid content in rabbits

The high-fat diets induced hyperlipidemia. However, after 10 weeks of dietary treatment with soybean lecithin, the level of the total and esterified cholesterol in the β-very low density lipoprotein (β-VLDL) significantly decreased in hypercholesterolemic rabbits compared to those fed soy-

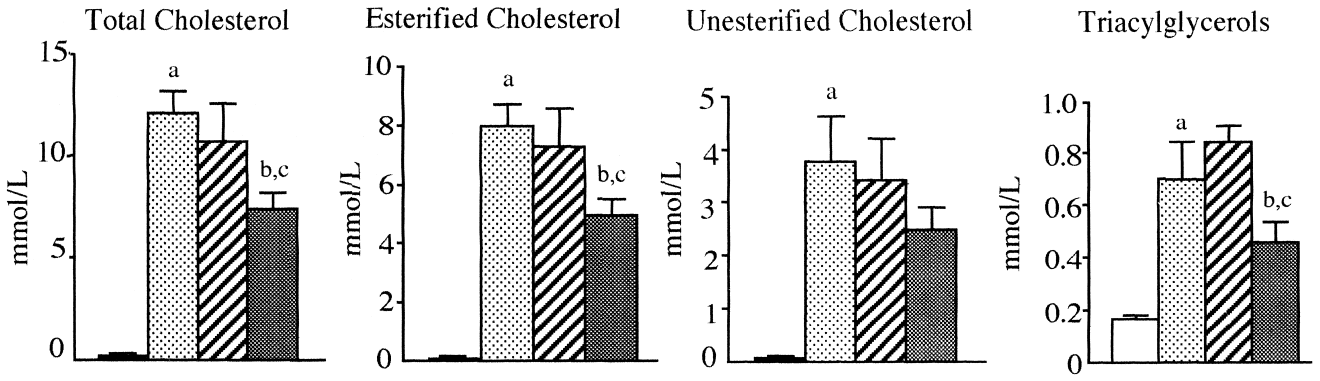

Figure 1 Lipids in the β-VLDL fraction of rabbits after 10 weeks of feeding either a control low-fat diet □ containing 27g fat/kg or high-fat diets (77g fat/kg). These diets contained 2g cholesterol/kg and 50g saturated triacylglycerol (lard)/kg ⬚, 50g polyunsaturated soybean triacylglycerol/kg ▨, or 50g polyunsaturated soybean phosphatidylcholine/kg (purified lecithin) ■. Values are means with their standard errors ($n = 6$ for each group). Significance was analyzed by one-way analysis of variance and the differences between groups were determined by Fisher's test ($P < 0.05$). a: control vs. lard; b: lard vs. soybean phosphatidylcholines; c: soybean triacylglycerols vs. soybean phosphatidylcholines.

bean triacylglycerols and lard. There was also a significant decrease by lecithin in the level of β-VLDL triacylglycerol level (*Figure 1*). The level of β-VLDL phospholipids was unchanged. Lipid composition of LDL, HDL_2, and HDL_3 was unchanged in the three high-fat groups (results given only in the text).

In bile, the levels of bile salt, phospholipid, and cholesterol were significantly enhanced by dietary treatment with soybean lecithin in comparison with soybean triacylglycerol (*Table 1*).

In liver, the concentration of total, esterified, and unesterified cholesterol was significantly decreased at comparable levels in both soybean lecithin and triacylglycerol groups compared to the lard group (47, 44, and 75.5 μmol total cholesterol/g liver, respectively). Triacylgerol content was significantly decreased in the lecithin group compared to triacylglycerol and lard groups (19, 26, and 26 μmol/g liver, respectively). The level of phospholipids remained unchanged (about 93 μmol/g liver in the three groups; results given only in the text).

Mitochondrial fatty acid oxidation in rats

Activity of carnitine palmitoyl transferase in hepatic mitochondria was comparable in rats fed soybean lecithin to that of control rats. Soybean triacylglycerols led to a nonsignificant enhancement of this enzyme activity (*Table 2*).

Cholesterol cellular efflux from ECV cells by soybean phosphatidylcholines

There was a significant rise in cholesterol efflux by endothelial ECV304 cells incubated with soybean phosphatidylcholines in comparison with saturated dimyristoylphosphatidylcholine or control without phospholipids (*Figure 2*). This efflux is comparable to that obtained with native HDLs and it is noticeable that this effect is more due to the phospholipid component than to the apoprotein AI, the main component of HDLs.

Discussion

Our objective was to study the effect of dietary soybean lecithin on lipidemia level in the cases of normolipidemia and dyslipidemia related to atherosclerosis development. There are two main sources of plasma cholesterol: dietary cholesterol resulting in intestinal absorption, and the cholesterol provided by cholesterol efflux from peripheral cells such as endothelial cells. We observed that these two cholesterol sources are regulated by phosphatidylcholine intake and we focused on the consequences of such changes on lipoproteins and related bile lipids.

Dietary lecithin was previously described to decrease intestinal absorption of cholesterol.[20,21] One explanation is

Table 1 Lipids in rabbit gallbladder bile (μmol/mL)

	Control	Lard	Soybean triacylglycerols	Soybean phosphatidylcholines
Bile salts	452 ± 35	291 ± 23[a]	350 ± 28	386 ± 52[b]
Cholesterol	1.37 ± 0.11	3.63 ± 0.37[a]	3.01 ± 0.36	4.62 ± 0.43[b,c]
Phospholipids	2.68 ± 0.11	8.09 ± 0.98[a]	7.29 ± 0.94	10.76 ± 0.88[b,c]

Rabbits were fed for 10 weeks either a control low-fat diet containing 27g fat/kg, or high-fat diets (77g fat/kg) containing 2g cholesterol/kg and 50g saturated triacylglycerol (lard)/kg, 50g polyunsaturated soybean triacylglycerol/kg, or 50g polyunsaturated soybean phosphatidylcholine/kg (purified lecithin).
Values are means with their standard errors ($n = 6$ in each group). Significance was analyzed by one-way analysis of variance and the differences between groups were determined by Fisher's test ($P < 0.05$) [a]control vs. lard; [b]lard vs. soybean phosphatidylcholine; [c]soybean triacylglycerol vs. soybean phosphatidylcholine.

Table 2 Mitochondrial carnitine palmitoyl transferase activity in rat liver

Diet	*n*	nmol carnitine/min/g protein
Control	4	27.7 ± 9.4
Soybean triacylglycerols	10	35.6 ± 4.3
Soybean phosphatidylcholines	11	25.9 ± 4.1

Rats were fed for 3 weeks control diet containing 4 g % low-fat or high-fat diets containing either 20 g % soybean triacylglycerols or 20 g % soybean phosphatidylcholines.
Values are means with their standard errors. Significance was analyzed by one-way analysis of variance and the differences between groups were determined by Fisher's test ($P < 0.05$).

the regulation of dietary cholesterol absorption by bile cholesterol.[22] Thus, an increase in bile cholesterol of animals fed soybean lecithin-enriched diets[23,24] could contribute to drastically lower absorption of dietary cholesterol. A consequence is a decrease in the cholesterolemia level. Such a decrease was previously observed in normolipidemic rats fed soybean lecithin.[23] In hypercholesterolemic rabbits fed for 10 weeks a diet enriched with soybean phosphatidylcholine, we found a significant decrease in the cholesterol level of β-VLDL, the main lipoprotein triggering the cholesterol and resulting from the postprandial lipoproteins, chylomicrons, and chylomicron remnants.[24] Such particles are directly related to hyperlipidemia and atheroma deposition in rabbits.[8] Thus, these findings constitute a first series of results proving the efficacy of soybean lecithin in prevention or treatment of dyslipidemia related to atherosclerosis.

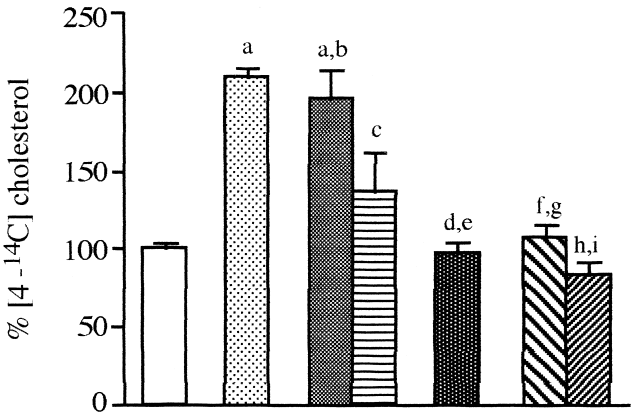

Figure 2 Cellular cholesterol efflux by ECV304 cells in culture. Cholesterol efflux is expressed by percentage of the [4-^{14}C] cholesterol cellular load, calculated on a basis of control (C) value of 100%. Cell cultures were performed after a 48-hr load with LDL (50 μg ApoB/well) in presence of [4-^{14}C] cholesterol. After washes, cells were incubated for 1 hr either without added lipids (C) or with human HDLs (150 μg apoA-I), soybean phosphatidylcholines (SPC; 430 μg), dimyristoylphosphatidylcholine (DMPC; 430 μg), apoA-I (150 μg), or reassociations of phospholipids (430 μg) with 150 μg apoA-I. □ C; ▨ human HDLs; ■ SPC; ▤ DMPC; ■ apoA-I; ▧ apoA-I-SPC; ▨ apoA-I-DMPC. Values are means with their standard errors ($n = 6$ for each group). Significance was analyzed by one-way analysis of variance and the differences between groups were determined by Fisher's test ($P < 0.05$). a: SPC or HDLs vs. C; b: SPC vs. DMPC; c: DMPC vs. HDLs; d: apoA-I vs. SPC; e: apoA-I vs. HDLs; f: HDLs vs. apoA-I-SPC; g: SPC vs. apoA-I-SPC; h: DMPC vs. apoA-I-DMPC; i: HDLs vs. apoA-I-DMPC.

In addition, our data show that soybean phosphatidylcholines promote cholesterol efflux by endothelial ECV cells. Enhancement of this cholesterol efflux is explained by a specific role of HDLs and a direct interaction of HDL-apoA-I and HDL-phospholipid with the cell membrane.[25,26] The resulting HDLs contain unesterified cholesterol extracted from the cells.[27] Thus, we advance the hypothesis of inhibition by phosphatidylcholine-enriched HDLs of the transformation of vascular endothelial macrophages to foam cells, origin of the atheromatous plaque.[28] Such speculation is relevant, seeing (1) a reinforcement of the HDL system in humans fed soybean lecithin[7] and (2) a significant reduction in early atherosclerosis in hamsters fed soybean lecithin.[29] Thus, our findings *in vitro* constitute a second series of results proving the efficacy of soybean lecithin for inhibition of atherosclerosis development. The reinforcement by lecithin feeding of the HDLs[7,30] consisted in an increase in the level of the apoA-I, the main apoprotein of HDLs[7] and in the level of unesterified plasma cholesterol. We recently advanced the concept of the enhancement by dietary soybean lecithin of the reverse cholesterol transport by HDLs.[7] This concept is also supported by previous findings of Martins et al.[31] and Williams et al.,[5] showing that dietary lecithin promoted the transfer of phosphatidylcholines resulting from the absorbed lecithin to plasma HDLs. However, soybean lecithin did not change activity of lecithin-cholesterol acyl transferase[7] involved in remodeling apoA-I HDLs into apoA-I-A-II HDLs.[32] Finally, dietary lecithin stimulates hepatic uptake of HDL-lipids, as observed in rats,[23] notably lipids from apoA-I-rich HDLs that enhance bile secretion.[7]

In animals, feeding diets enriched with soybean phosphatidylcholines resulted also in a triacylglycerol-lowering effect, particularly in the model of hypercholesterolemic rabbits.[24] Such reduction is to be related to the absence of triacylglycerols in the diet and to the 33% decrease of fatty acids provided by dietary soybean phosphatidylcholine compared with dietary triacylglycerols. The consequence was a decrease in the level of β-VLDL-triacylglycerol. We have previously observed a reduction in cholesterol of these lipoproteins. Thus, we can expect a likely decrease of exchange between the lipid components of the aforesaid lipoproteins and those of HDLs by cholesterol ester transfer protein and phospholipid transfer protein.[33–35] Regression of triacylglycerol-rich particles after lecithin feeding and relation between these particles with HDLs remain to be clarified. Nevertheless, our results constitute a promising effect of dietary soybean lecithin by reinforcement of the anti-atherogenic HDL lipoproteins and by a nonincrease or a decrease in atherogenic particles such as VLDL and LDL.

The hypolipidemic properties of soybean lecithin may be also related to a decrease in cholesterol and fatty acid synthesis. In rabbits, activity of hydroxy methyl glutaryl coenzyme A (CoA) reductase, the key enzyme of cholesterol synthesis, was not induced by dietary soybean lecithin compared to soybean triacylglycerols.[24] Ide et al.[36] showed that availability of fatty acids for triacylglycerol synthesis was reduced in rat liver through the depression in the rate of fatty acid synthesis by a diet enriched with soybean phos-

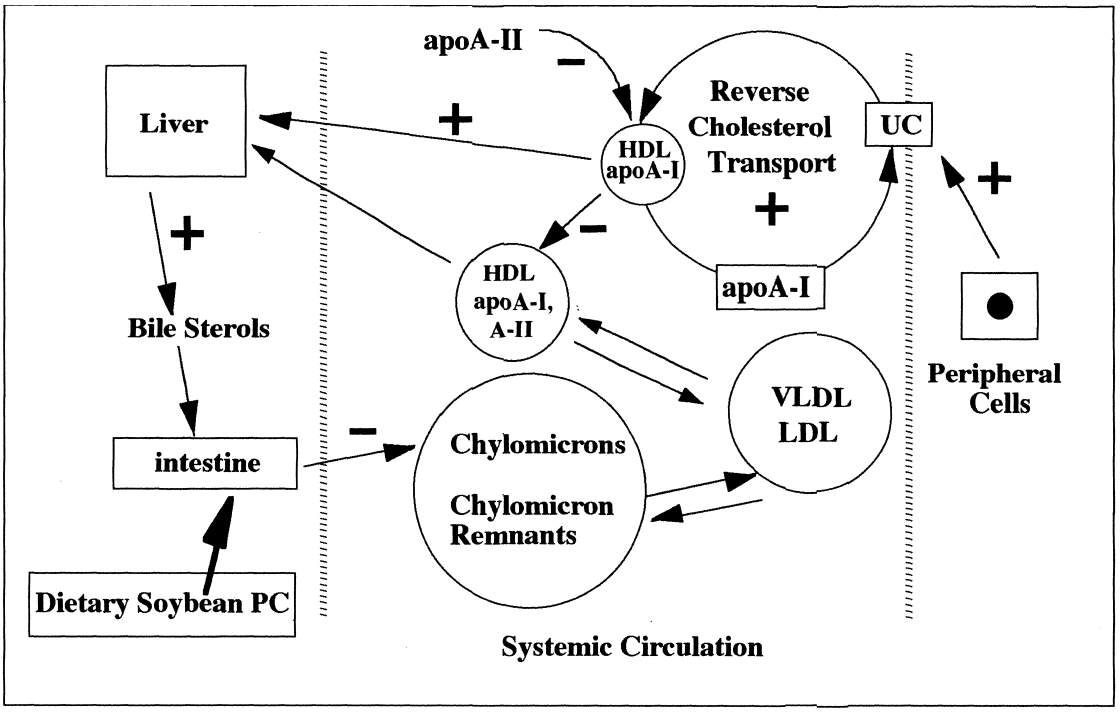

Figure 3 The beneficial effects of dietary soybean phosphatidylcholines on lipid homeostasis and interrelationships between lipoproteins. UC: unesterified cholesterol; PC: phosphatidylcholine; −: inhibition by PC; +: stimulation by PC; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein.

pholipids. A consequence of these two hypolipidemic effects of dietary lecithin is a significant regression of the steatosis previously described in rats or rabbits fed high-fat diets.[23,24] Such regression may be also linked to the processes of lipid oxidation in the liver. Cholesterol oxidation into bile salts was enhanced in rats[23] and in rabbits fed soybean phosphatidylcholines. Such findings are mostly due to an increase in 7α-hydroxylase activity in rats, but probably also to that of the 27-hydroxylase (this enzyme being the sole enzyme active in rabbits).[37] Another main consequence of lecithin feeding is a nonincrease in fatty acid oxidation. Kabir and Ide[38] showed that fatty acid oxidation in rat liver, assessed by the activities of carnitine palmitoyl transferase, acyl-CoA dehydrogenase, and acyl-CoA oxidase, was unchanged by dietary soybean phospholipids compared with dietary soybean oil. Our results on hepatic carnitine palmitoyl transferase in rats corroborate these previous results, our experiment being performed with higher percentages of lecithin in the diet. We deduce that both oxidations of fatty acids and cholesterol could be conversely reversed by dietary lecithin in comparison with dietary triacylglycerol. Thus, soybean lecithin intake promotes fatty acid metabolism by ways other than synthesis of triacylglycerol-rich lipoproteins or fatty acid oxidation; it acts by stimulation of fatty acid incorporation into phosphatidylcholines. These phospholipids are key molecules controlling the biodynamic exchanges of lipids and lipoproteins. They stimulate the transport of unesterified cholesterol from HDL to the liver, are ultimately secreted into bile in higher quantities,[23,24,39,40] and promote enhanced biliary secretion of cholesterol and its metabolites (*Figure 3*).

In conclusion, dietary soybean phosphatidylcholines exhibit beneficial effects charaterized by (1) a cholesterol and triacylglycerol decrease, (2) a reinforcement of the HDL system through enhancements of cholesterol efflux by endothelial cells and reverse cholesterol transport, (3) a restriction in fatty acid synthesis and oxidation, and (4) a rise in cholesterol oxidation into bile salts, resulting in an enhancement of bile sterol secretion. All these findings constitute promising goals in the field of nutritional effects of soybean phosphatidylcholines in the treatment or prevention of hyperlipidemia and related atherosclerosis.

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