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Effects of Egg Phospholipids on the Intestinal Absorption of Lipids

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ABSTRACT

This study was conducted to determine the effects of egg phospholipids [(phosphatidylcholine (PC) and sphingomyelin (SM)] on intestinal absorption of cholesterol and other lipids. Each rat with lymph cannula was infused via a duodenal catheter at 3.0 mL/h for 8 h with a lipid emulsion containing triolein, cholesterol and PC in 24 mL PBS. The PC in the lipid emulsion was egg PC (EPC), hydrogenated egg PC (HPC), or soy PC (SPC). The EPC in the lipid emulsion markedly lowered the lymphatic absorption of cholesterol, compared with SPC and a lipid emulsion containing no PC. The HPC further lowered the absorption of cholesterol. The phospholipid output was not affected by the source of PC infused. The total lymphatic output of oleic acid (18:1), the major fatty acid infused in the form of triolein, did not differ among the NPC, SPC and EPC groups, but was significantly lower in the HPC group. The findings provide the first evidence that EPC markedly lowers the lymphatic absorption of cholesterol under in vivo conditions. The inhibitory effect of EPC appears to be due to the higher degree of saturation of its acyl groups relative to SPC, suggesting that the intestinal absorption of egg cholesterol may be reduced by the presence of PC in egg yolk. Experiment 2 was designed to determine whether egg SM, structurally similar to PC, also inhibits the lymphatic absorption of cholesterol. Egg SM lowered the lymphatic absorption of cholesterol in a dose dependent manner. Likewise, SM lowered the lymphatic absorption of oleic acid, whereas it had no effect on retinol absorption. SM at a high dose lowered the lymphatic outputs of both PC and SM, whereas there was no such effect at a lower dose. These results also indicate that luminal egg SM has an inhibitory effect on the intestinal absorption of cholesterol and other lipids of relatively high hydrophobicity.

INTRODUCTION

Studies have shown that phosphatidylcholine (PC) profoundly influences the intestinal absorption of lipid by increasing micellar lipid solubility and providing the surface PC for the formation of chylomicrons (1). Although a sufficient supply of dietary or biliary PC is required to support normal fat absorption, it has been shown to inhibit cholesterol uptake by various absorptive cell systems (2-4). Recently, pancreatic phospholipase A_2 (PLA_2), when provided to micellar solutions, was shown to relieve the PC-induced inhibition of cholesterol uptake (5). Also, addition of PLA_2 to a lipid emulsion increased the triacylglycerol hydrolysis rates, along with cholesterol uptake (6-8). However, the mechanism underlying the inhibitory effect of PC on cholesterol absorption is yet to be elucidated. Despite efficient hydrolysis of PC by PLA_2, PC with unsaturated fatty acids is hydrolyzed more readily by the enzyme than that with saturated fatty acids (9,10). Therefore, saturation of the acyl groups may hinder the rate of PC hydrolysis by PLA_2 and then decrease the intestinal absorption of cholesterol.

Studies have shown that sphingolipid, structurally similar to PC, is an essential structural and bioactive lipid

(11), which is present in significant amounts in egg, milk, and soybean (12). Sphingomyelin (SM) prefers to interact with cholesterol in cell membranes. The cellular concentration of SM or its ratio to cholesterol is known to alter cholesterol homeostasis. Plasma SM is elevated in familial hypercholesterolemia, and positively linked to coronary artery disease (13). However, little is known about whether dietary SM directly affects cholesterol homeostasis in humans or in animal models. Limited evidence indicates that dietary SM significantly decreases the plasma levels of cholesterol in rats, although the mechanism underlying its effect is unknown.

The hydrolysis of SM by the enzyme is a slower and inefficient process than that of PC by pancreatic PLA_2 (14,15). The inefficient hydrolysis of SM in the upper intestinal tract in turn may slow the rates of hydrolysis, formation of mixed micelles, and/or uptake of other lipids by the enterocyte. In keeping with the hypothesis, a recent study showed that dietary SM increased fecal excretion of cholesterol in mice fed a chow enriched with milk SM (16).

Our data presented here provide the first direct evidence that an intraduodenal infusion of either egg PC or SM drastically affects the intestinal absorption of cholesterol and fats.

MATERIALS AND METHODS

Cannulation of the mesenteric lymph duct

Cannulation of the mesenteric lymph duct and insertion of an intraduodenal infusion catheter was performed under halothane anesthesia, as described previously (17). Postoperatively, rats were placed in restraining cages in a recovery chamber (30°C) for 22~24 h. In order to ensure adequate hydration of the rats, they were infused continuously via the infusion catheter with PBS solution containing 5% glucose.

Measurement of the lymphatic absorption of ^{14}C-cholesterol

After postoperative recovery, each rat was infused via the duodenal catheter at 3 mL/h for 8 h with a lipid emulsion consisting of 452 μmol triolein, 27.8 kBq [4-^{14}C]-cholesterol, 20.7 μmol cholesterol, 3.6 μmol α-tocopherol, and 396.0 μmol sodium taurocholate with 100 μmol PC or without PC in 24 mL of PBS. The PC included in the lipid emulsion was soy PC (SPC; >99%), egg yolk PC (EPC; >99%), or hydrogenated egg yolk PC (HPC; >99%). During lipid infusion, lymph samples were collected hourly under subdued light in preweighed ice-cold centrifuge tubes. The hourly lymph samples were mixed with scintillation liquid and counted to determine ^{14}C-radioactivity appearing in the lymph. The distributions of the lymph ^{14}C-radioactivity in free and esterified cholesterol were determined by digitonin precipitation.

For experiment 2, each rat was infused with a lipid emulsion containing egg SM at 3 mL/h for 8 h under the same conditions as in experiment 1. The lipid emulsion contained 452 μmol triolein, 33.3 kBq [4-^{14}C]-cholesterol, 20.7 μmol cholesterol, 3.1 μmol α-tocopherol, 75.4 nmol retinol, and 396.0 μmol sodium taurocholate in 24 mL of PBS without SM (SM0), or with 5.0 μmol/h (SM5) or 10.0 μmol/h (SM10). Egg SM contained entirely saturated fatty acids, consisting of 16:0 (83.9%, as wt %), 18:0 (6.3%), 24:0 (4.2%), 22:0 (3.8%), and 20:0 (1.8%). Lymph samples were collected hourly in preweighed ice-chilled centrifuge tubes. The hourly rate of ^{14}C-cholesterol absorption was expressed as percent (%) of the total dose of ^{14}C-radioactivity infused.

Analyses of α-tocopherol, retinol, phospholipids, and fatty acids

α-Tocopherol and retinol were extracted from lymph by a modification of the method of Ross (18). α-Tocopherol was monitored at 292 nm and retinol at 325 nm. Under the conditions, retinol, tocol, and α-tocopherol were eluted at 1.6, 2.6, and 4.2 min, respectively. The total amounts of α-tocopherol and retinol absorbed into the

lymph were determined based on the concentrations of the vitamins in 100-μL aliquots of hourly lymph samples. For individual phospholipid class analysis, phosphatidylserine (PS), phosphatidylethanolamine (PE), PC, lysoPC, and SM were analyzed simultaneously from 200-μL aliquots of lymph samples with a slight modification of the HPLC described by Kaduce et al. and Patton et al. The typical elution times (in min) were: 8.7 for PS, 10.4 for PE, 13.6 for PC, 19.5 for lysoPC, and 22.3 for SM. For fatty acid analysis, total lipids from 100-μL lymph samples were extracted and hydrolyzed with 1 mL of 0.5 N methanolic NaOH in boiling water for 15 min, following addition of 17:0. Fatty acids were saponified and methylated with 2 mL of 14% methanolic BF_3.

RESULTS AND DISCUSSION

The present study using rats with lymph cannula has shown the following findings obtained under in vivo conditions: 1) egg PC (EPC) significantly decreases the lymphatic absorption of cholesterol compared with soy PC (SPC) and a no PC (NPC) control as shown in Table 1; 2) the inhibitory effect of EPC on the intestinal absorption of cholesterol is further decreased when the egg PC is hydrogenated; 3) SPC, which is highly unsaturated, does not interfere with cholesterol absorption compared with the NPC control; and 4) Egg SM significantly lowers the intestinal absorption of cholesterol in rats in a dose-dependent manner, along with parallel decreases in the intestinal absorption of other lipids such as α-tocopherol and fatty acids (fat) as shown in Table 2.

PC from bile or diets serves as the major source of PC for the formation of surface PC of chylomicrons, which facilitates intestinal absorption of fat. In view of the overall stimulatory effect of PC on fat absorption, the precise mechanism underlying the PC-mediated inhibition of cholesterol absorption remains unclear. However, an earlier in-vitro evidence (7) suggested that PC increases the size of bile salt micelles luminally, thus slowing their passage across the unstirred water layer to the absorptive cells. As the concentration of PC increases in a micellar matrix, the rate of micellar diffusion through the unstirred layer slows, decreasing cholesterol uptake by the intestinal cells (3). In contrast, lysoPC-added micelles are much smaller than PC-added micelles, resulting in

Table 1. Total lymphatic absorption of ^{14}C-cholesterol (^{14}C-CH), phospholipid (PL) output and lymph flow in rats not infused with NPC or SPC or HPC for 8 h

Lipids	NPC	SPC	EPC	HPC
^{14}C-CH, % dose	30.8±2.0[1)b2)]	34.9±1.2[a]	24.7±2.5[c]	21.1±1.4[d]
PL, μmol	27.2±1.3[b]	32.9±1.8[a]	32.2±1.7[a]	31.8±1.6[a]
Lymph, mL	21.0±3.2[a]	17.2±1.8[b]	18.7±3.0[ab]	20.7±2.4[a]

[1)]Means±SD, n = 5. [2)]Values in a row not sharing a superscript differ ($p<0.05$).

Table 2. Cumulative lymphatic absorptions of ^{14}C-cholesterol (^{14}C-CH), α-tocopherol (TP), and retinol and outputs of phospholipids (PL) and sphingomyelin (SM) in rats during duodenal infusion of a lipid emulsion with SM at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0)

Lipids	SM0	SM5	SM10
^{14}C-CH, % dose/8 h	38.8±1.8[1)a2)]	32.4±1.2[b]	20.4±2.3[c]
aTP, nmol/8 h	931.8±82.9[a]	817.3±57.9[b]	554.1±48.3[c]
Retinol, nmol/8 h	14.6±2.0	14.0±1.0	13.1±1.8
PL, μmol/8 h	46.3±1.3[a]	43.3±1.5[a]	33.6±4.2[b]
SM, μmol/8 h	0.38±0.07[a]	0.39±0.09[a]	0.17±0.10[b]
Lymph, mL/8 h	19.2±3.9	19.9±1.2	18.2±4.7

[1)]Means±SD, n = 5. [2)]Values in the same row not sharing a common superscript are significantly different ($p<0.05$).

higher cholesterol uptake from the lysoPC-added micelles (3).

A study demonstrated EPC decreased the core triacylglycerol hydrolysis in a lipid emulsion and that initial hydrolysis of the surface PC by PLA_2 facilitated the binding of pancreatic lipase and colipase to the substrate, increasing the core triacylglycerol hydrolysis. A recent in-vitro study (6) also showed that when the molar ratio of PC to triacylglycerol in a lipid emulsion is >0.3, PLA_2-mediated hydrolysis of the surface coat PC is critical for the effective hydrolysis of triacylglycerol in the core by pancreatic lipase/colipase and also for stimulation of cholesterol uptake by the intestinal cells. Other studies using Caco-2 cells also demonstrated that addition of PLA_2 or replacement of lysoPC for PC in mixed micelles abolishes the PC-induced inhibition of cholesterol uptake (1,5).

This study is the first to measure the effects of egg and soy PC on the intestinal absorption of cholesterol in rats with lymph cannula. An important new finding is that not all PC inhibit the intestinal absorption of cholesterol and that the PC-induced inhibition of the intestinal absorption of cholesterol depends on the degree of saturation of its acyl moiety. Among the PC used here, the degree of fatty acid saturation increased in the order of SPC < EPC < HPC. SPC fatty acids were mainly unsaturated, with 18:2 accounting for 61% of the total fatty acid content. In contrast, EPC contains mostly saturated and monounsaturated fatty acid (18:1, 32%), and 18:2. HPC contains mostly saturated fatty acids. The lymphatic absorption of cholesterol decreased with increasing saturation of the PC infused. The lower lymphatic outputs of fatty acids with relatively more saturated PC may be attributable to slower hydrolysis of the core triacylglycerol in the lipid emulsions coated with saturated PC. Because saturated PC are poor substrates for PLA_2 and not hydrolyzed readily (10), their presence would hinder pancreatic lipase from accessing the core triacylglycerol of the emulsion particle.

The significant decrease in cholesterol absorption by EPC is of particular interest in view of the high concentration of PC in egg yolk. A fresh egg yolk weighing approximately 20 g based on a 70-g whole egg is measured to contain 1.3 g PC and 260 mg cholesterol (19). At present, no data are available to show that the PC in egg yolk affects the intestinal absorption of cholesterol in humans consuming eggs. Evidence from numerous human studies has shown that although egg contains high cholesterol, consumption of one or two eggs per day has little effect on blood cholesterol levels and coronary heart disease risk.

In experiment 2, enteral infusion of egg SM significantly lowers the intestinal absorption of cholesterol in rats in a dose-dependent manner, along with simultaneous decreases in the intestinal absorption of other lipids such as α-tocopherol and fatty acids (fats). Although the exact mechanism by which enteral SM influences the intestinal absorption of lipids still remains unclear, evidence from in vitro studies suggests that the inhibitory effect of SM on lipid absorption may be mostly mediated intraluminally. SM is hydrolyzed more slowly and incompletely in the intestinal lumen (20-22). Alkaline sphingomyelinase, having a pH optimum at 9.0, is virtually inactive under the conditions of the gastric and duodenal lumen and its activity, localized in the brush border membrane, is maximal in the distal jejunum and lower in the ileum and colon (22). Thus, the slow and incomplete hydrolysis of SM in the upper segment of the intestine, where much of lipid hydrolysis occurs, may allow for interactions between intact SM and other lipids in the luminal environment, influencing the rates of hydrolysis, micellar solubilization, and transfer of lipids from mixed micelles to the enterocyte. Evidence from an in vitro study (1) shows that the presence of intact PC in mixed micelles slows the desorption of more hydrophobic lipids such as cholesterol and α-tocopherol from the micellar matrix, whereas it does not influence the transfer of other less hydrophobic lipids such as retinol. Although no direct evidence is available for such interactions between SM and other lipids in micelles, studies with lipid vesicles and membrane systems indicate that SM interacts more tightly with cholesterol than PC does with cholesterol (23,24) and that the tighter molecular packing, as produced

via interaction of SM with cholesterol, results in slower transfer (or desorption) of cholesterol from SM vesicles or membranes than from those PC with matching acyl groups. Therefore, it was possible that SM decreases micellar solubilization and hence decreases concentrations of cholesterol monomers available for uptake. In our recent study, we also found that milk SM, composed mainly of saturated longer chain (22-24 carbon) fatty acids, is more effective in inhibiting cholesterol absorption than egg SM containing 18:1, 18:2, and 16:0 as major fatty acids, which is in agreement with the findings of experiment 1 that the degree of saturation and chain length of PC fatty acids is an important determinant of cholesterol absorption.

In summary, the results of this is the first to show that under in vivo conditions, enteral infusion of egg PC or SM lowers the intestinal absorption of cholesterol and fat. In addition, our observations indicate that egg PC and egg SM decrease the outputs of α-tocopherol and fat into the mesenteric lymph. This effect of egg phospholipid may be associated with the slow hydrolysis of phospholipid in the intestine, which in turn decreases the rates of hydrolysis of phospholipid and triacylglycerol, micelle formation, and transfer of lipids to the enterocyte. Further studies are warranted to determine whether phospholipid, present in a variety of foods including eggs, affects the intestinal absorption of cholesterol in humans. Also, our data raise a new question as to whether chronic egg phospholipid intake affects the absorption of other lipids including fat and fat-soluble nutrients.

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