Egg Sphingomyelin Lowers the Lymphatic Absorption of Cholesterol and α-Tocopherol in Rats[1]

Sang K. Noh and Sung I. Koo[2,3]

Department of Human Nutrition, Kansas State University, Manhattan, KS 66506

ABSTRACT Evidence indicates that phosphatidylcholine (PC) inhibits the intestinal absorption of cholesterol (CH) in rats. This study was designed to determine whether sphingomyelin (SM), structurally similar to PC, also inhibits the lymphatic absorption of CH. Sprague-Dawley rats with lymph cannulae were infused at 3.0 mL/h for 8 h via a duodenal catheter with a lipid emulsion [33.3 kBq ^{14}C-CH, 20.7 μmol CH, 451.7 μmol triolein, 3.1 μmol α-tocopherol (αTP), 75.4 nmol retinol and 396.0 μmol sodium taurocholate in 24 mL of PBS (pH, 6.5)], without egg SM (SM0) as control, or with 5.0 μmol/h (SM5) or 10.0 μmol/h (SM10). Egg SM lowered the lymphatic absorption of ^{14}C-CH in a dose dependent manner. Likewise, SM lowered the lymphatic absorptions of αTP and fatty acid (oleic acid), whereas it had no effect on retinol absorption. SM at a high dose (SM10) lowered the lymphatic outputs of both PC and SM, whereas there was no such effect at a lower dose (SM5). These results indicate that luminal egg SM has an inhibitory effect on the intestinal absorption of CH and other lipids of relatively high hydrophobicity. Our findings suggest that SM, if ingested in sufficient amounts, may inhibit the intestinal absorption of dietary lipids including cholesterol and αTP. J. Nutr. 133: 3571–3576, 2003.

KEY WORDS: • *sphingomyelin* • *α-tocopherol* • *cholesterol* • *intestinal absorption* • *rats* • *retinol*

Sphingolipid is an important structural and bioactive lipid (1–3). It is found in eggs, milk, meat, fish and soybeans (4). The per capita consumption of sphingolipid in the United States is estimated to be 0.3–0.4 g/d (4).

Sphingomyelin (SM)[4] preferentially interacts with cholesterol (CH) in cell membranes and the cellular concentration of SM or perturbation of SM/cholesterol ratio alters CH synthesis, transport and balance (5). Studies have shown that plasma SM, as carried by lipoproteins, is elevated in familial hypercholesterolemia (6) and positively related to coronary artery disease (7). Despite the proposed relationship between plasma SM and atherosclerosis, little is known about whether dietary SM directly influences cholesterol metabolism and the risk for coronary heart disease in humans or in animal models.

Available evidence indicates that dietary sphingolipids lower the blood levels of CH in rats (8). The mechanism underlying this effect of sphingolipids is unknown. Recently, we showed that an enteral infusion of egg phosphatidylcholine (PC), structurally similar to SM, inhibits the intestinal absorption of CH in rats with lymph cannulae (9), whereas lysophosphatidylcholine (lysoPC) promotes its absorption (10). Also, studies using intestinal cells in vitro have shown that the presence of intact PC on the surface of lipid emulsions slows the hydrolysis of the core triacylglycerol (TG) by pancreatic lipase (11–13) and that initial hydrolysis of PC is required for TG hydrolysis and CH uptake (14,15).

SM is digested mainly by intestinal alkaline sphingomyelinase (SMase) in the lower part of the jejunum and colon. The hydrolysis of SM by the enzyme is a slower and less efficient process (16,17) than that of PC by pancreatic phospholipase A_2 (PLA_2). 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 support of this hypothesis, a recent study (18) showed that dietary SM increased fecal excretion of CH in mice fed a diet enriched with milk SM.

This study was conducted to examine whether intraduodenal infusion of egg SM affects the lymphatic absorption of CH and other lipids in rats under in vivo conditions.

MATERIALS AND METHODS

Animals and diet. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) aged 8 wk and weighing 256 ± 8 g were housed individually in plastic cages, subjected to a 12-h light/dark cycle with the light phase starting at 1530 h. Rats ate ad libitum a diet (Dyets, Bethlehem, PA) formulated according to the AIN-93G recommendations (19,20) (**Table 1**) and had free access to deionized water provided via a stainless steel watering system. All rats were cared for in an animal care facility at Kansas State University, accredited by the American Association for the Accreditation of Laboratory Animal Care. All protocols for animal care and use were

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[2] To whom correspondence should be addressed.
E-mail: skoo@canr.uconn.edu.

[3] Current address: Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269-4017.

[4] Abbreviations used: ABCA, ATP-binding cassette transporter; αTP, α-tocopherol; ^{14}C-CH, ^{14}C-cholesterol; CH, cholesterol; lysoPC, lysophosphatidylcholine; PBS, phosphate buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipid; PLA_2, phospholipase A_2; PS, phosphatidylserine; SM, sphingomyelin; SMase, sphingomyelinase; TG, triacylglycerol.

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TABLE 1

Composition of diet Sprague-Dawley rats ate ad libitum for 8 wk[1]

Ingredient	Amount
	g/kg
Egg white	200.0
Cornstarch	396.5
Dextrinized cornstarch	132.0
Dextrose	100.0
Cellulose	50.0
Soybean oil[2]	70.0
Mineral mix	35.0
Vitamin mix	10.0
Biotin (1 mg/g biotin sucrose mix)	4.0
Choline bitartrate	2.5

[1] Formulated and supplied from Dyets, Bethlehem, PA, according to the recommendations of the American Institute of Nutrition (19, 20).
[2] Contained 0.02% tert-butylhydroquinone.

approved by the Kansas State University Institutional Animal Care and Use Committee.

Mesenteric lymph duct cannulation. At 8 wk of feeding, rats were starved for 16 h (overnight) but drank water ad libitum. Cannulation of the mesenteric lymph duct and insertion of an intraduodenal infusion catheter was performed under halothane anesthesia as described previously (21). Postoperatively, the rats were placed in restraining cages in a recovery chamber (30°C) for 22–24 h. To ensure adequate hydration of the rats, they were infused continuously via the infusion catheter with PBS solution containing 5% glucose (in mmol/L: 277 glucose, 6.75 Na_2HPO_2, 16.5 NaH_2PO_2, 115 NaCl and 5 KCl; pH 6.5) at 3.0 mL/h by a syringe pump (Model 935; Harvard Apparatus, South Natick, MA).

Lymphatic ^{14}C-CH absorption. After postoperative recovery, each rat was infused with a lipid emulsion containing egg SM (purity > 99%; Avanti Polar Lipids, Alabaster, AL) at 3 mL/h for 8 h via the duodenal catheter in subdued light. The lipid emulsion consisted of 451.8 μmol triolein (95%; Sigma Chemical, St. Louis, MO), 33.3 kBq [4-^{14}C]-CH (specific activity, 1.9 GBq/mmol; DuPont NEN, Boston, MA), 20.7 μmol CH, 3.1 μmol α-tocopherol (all-rac-dl-α-tocopherol, 97%; Aldrich Chemical, Milwaukee, WI), 75.4 nmol retinol (all-*trans*-retinol, 95%; Sigma Chemical), and 396.0 μmol sodium taurocholate (Sigma Chemical) in 24 mL of PBS buffer without SM (SM0), or with 5.0 (SM5) or 10.0 μmol/h (SM10). The amount of triolein provided for 8 h was ~29% of the daily fat intake of a rat consuming 20.0 g/d of the AIN-93G diet that contains 7.0% fat (19), representing a moderate fat intake, whereas the amount of CH represented a moderately high intake in proportion to the total amount of fat given. The amount of αTP was set at 100% of the daily intake of the vitamin as recommended by the AIN-93G (19,20). Egg SM contained entirely saturated fatty acids, consisting of 16:0 (83.9 g/100g), 18:0 (6.3 g/100 g), 24:0 (4.2 g/100g), 22:0 (3.8 g/100g) and 20:0 (1.8 g/100g). Lymph samples were collected hourly in preweighed ice-chilled centrifuge tubes containing 4 mg of Na_2-EDTA and 30 μg of n-propyl gallate (Sigma Chemical). The hourly lymph samples (100 μL) were mixed with scintillation liquid (ScintiVerse; Fisher Scientific, Fair Lawn, NJ) and counted by scintillation spectrometry (Beckman LS-6500; Beckman Instruments, Fullerton, CA). The total ^{14}C-radioactivity appearing in the lymph collected hourly was used to determine the amount of ^{14}C-CH absorbed. The hourly rate of ^{14}C-CH absorption was expressed as percent (%) of the total dose of ^{14}C-radioactivity infused. All samples were ice chilled and handled in subdued light.

Analysis of α-tocopherol and retinol. α-Tocopherol (αTP) and retinol were extracted from lymph by a modification of the method of Ross (22). Briefly, a 100-μL lymph sample was saponified in 10 volumes of 95% ethanol and 1% KOH solution containing 1% pyrogallol (99%; Acros Organics, Pittsburgh, PA) as an antioxidant at 60°C for 10 min. After cooling, the contents were mixed vigorously with 20 volumes of hexane for 7 min and then 10 volumes of water. After centrifugation at 1000 × *g* for 10 min, the upper phase was transferred into a vial, dried under N_2 and dissolved in chloroform:methanol (1:3, v/v). Tocol (a gift from Hoffmann-La Roche, Basel, Switzerland) was used as an internal standard to monitor extraction efficiency and recovery, which exceeded 95%. αTP and retinol were simultaneously analyzed by using a Beckman HPLC system with a photodiode-array detector and an autosampler (Beckman Instruments) equipped with a C-18 reversed-phase column (Alltima C18, 5 μm, 4.6 × 150 mm; Alltech Associates, Deerfield, IL). Methanol was used as the mobile phase at 2 mL/min (23). αTP was monitored at 292 nm and retinol at 325 nm (UV detector, Module 168; Beckman Instruments). Under the conditions, retinol, tocol and αTP were eluted at 1.6, 2.6 and 4.2 min, respectively. The total amounts of αTP and retinol absorbed into the lymph were determined based on the concentrations of the vitamins in 100-μL aliquots of hourly lymph samples.

Analysis of phospholipids. From 200-μL aliquots of lymph samples, phosphatidylserine (PS), phosphatidylethanolamine (PE), PC, lysoPC and SM were analyzed simultaneously with a slight modification of the HPLC method described by Kaduce et al. (24) and Patton et al. (25). Total lipids were extracted by the method of Folch et al. (26), dried under N_2, dissolved in chloroform:methanol (5:1, v/v) and injected by an automatic injection system (Beckman Instruments, Fullerton, CA). Individual phospholipid (PL) classes were separated with a Beckman HPLC system (Beckman Instruments) equipped with a 4.6 × 250-mm silica column (Adsorbosphere HS Silica, 5 μm; Alltech Associates). The mobile phase consisted of acetonitrile: methanol:phosphoric acid:sulfuric acid (100:4.8:0.864:0.026, v/v/v/v) and the flow rate was 0.7 mL/min. Detection was monitored at 202 nm (Module 168; Beckman Instruments). 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.

Analysis of fatty acids. Total lipids from 100-μL lymph samples were extracted (26) and hydrolyzed with 1 mL of 0.5 N methanolic NaOH in boiling water for 15 min, following addition of 17:0 (Nu-Chek-Prep, Elysian, MN) as an internal standard. Fatty acids were saponified and methylated with 2 mL of 14% methanolic BF_3, as described by Slover and Lanza (27). Fatty acid methyl esters (FAME) were redissolved in 100 μL of petroleum ether. FAME were quantitated using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) with a flame-ionization detector and a 15.0 m × 0.25-mm (i.d.) Stabilwax fused-silica capillary column (Restek, Bellefonte, PA). Nu-Chek-Prep fatty acid standards were used for analysis.

Statistics. All statistical analyses were performed using PC SAS (SAS Institute, Cary, NC). Repeated measures ANOVA and the least significance difference test were used to compare multiple group means and time-dependent changes within groups. Values are means ± SD. Differences were considered significant at $P < 0.05$.

RESULTS

Lymph flow. The rate of lymph flow increased in response to lipid infusion in all groups. The flow rates were 2.4 ± 0.5 in the SM0, 2.5 ± 0.1 in the SM5 and 2.3 ± 0.6 mL/h in the SM10 group, with no differences among groups. Also, groups did not differ in total lymph volume collected for 8 h (**Table 2**).

Lymphatic absorption of ^{14}C-CH. The hourly rates of ^{14}C-CH absorption were lower in the SM5 and SM10 groups than in the SM0 group at 3 h and thereafter (**Fig. 1**; $P < 0.05$). The rates of ^{14}C-CH absorption over the 8-h period were 4.84 ± 0.23 in SM0, 4.05 ± 0.15 in SM5 and 2.55 ± 0.29% dose/h in SM10 group. SM lowered the rate and amount of lymphatic ^{14}C-CH absorption in a dose-dependent manner (Table 2).

Lymphatic absorption of αTP and retinol. The infusion of SM lowered the hourly rates of αTP absorption (**Fig. 2**; $P < 0.05$). The average rates of αTP absorption in SM0, SM5 and SM10 groups were 116.5 ± 10.4, 102.2 ± 7.2 and 69.3 ± 6.0 nmol/h, respectively, with differences among all three

TABLE 2

Cumulative lymphatic absorptions of ^{14}C-cholesterol (^{14}C-CH), α-tocopherol (αTP) 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)[1]

Lipids	SM0	SM5	SM10
^{14}C-CH, % dose/8 h	38.8 ± 1.8[a]	32.4 ± 1.2[b]	20.4 ± 2.3[c]
αTP, nmol/8 h	931.8 ± 82.9[a]	817.3 ± 57.9[b]	554.1 ± 48.3[c]
% dose/8 h	29.7 ± 2.6[a]	26.1 ± 1.9[b]	17.7 ± 1.5[c]
Retinol, nmol/8 h	14.6 ± 2.0	14.0 ± 1.0	13.1 ± 1.8
% dose/8 h	19.2 ± 2.7	18.5 ± 1.3	17.4 ± 2.4
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] Values are means ± SD, $n = 5$. [a,b,c] Values in a row without a common superscript differ ($P < 0.05$).

groups. The cumulative absorption of αTP also was decreased by SM in a dose-dependent manner (Table 2; $P < 0.05$). The total amounts of αTP absorbed in the SM5 and SM10 groups represented 87.7 and 59.5%, respectively, of the control (SM0). By contrast, the rates and the total amounts of retinol absorption were not affected by SM (Table 2, **Fig. 3**). The rates of retinol absorption in SM0, SM5 and SM10 groups were 1.8 ± 0.2, 1.7 ± 0.1 and 1.6 ± 0.2 nmol/h, respectively (Table 2)

Lymphatic outputs of PL classes. Total PL output did not differ between the SM0 and SM5 groups (**Table 3**). However, the PL output in the SM10 group was lower than in the SM0 and SM5 groups at 4 h and thereafter. Likewise, the lymphatic output of PC, which is the major PL secreted into the lymph, was unaffected in the SM5 group, but was lower in the SM10 group at 3 h and thereafter compared with the SM0 group (Table 3, **Fig. 4**). The outputs of other PL classes such as PI and PE generally reflected the output of PC. The lymphatic output of SM did not change in SM5 group, but was lower in the SM10 than in the SM0 and SM5 groups.

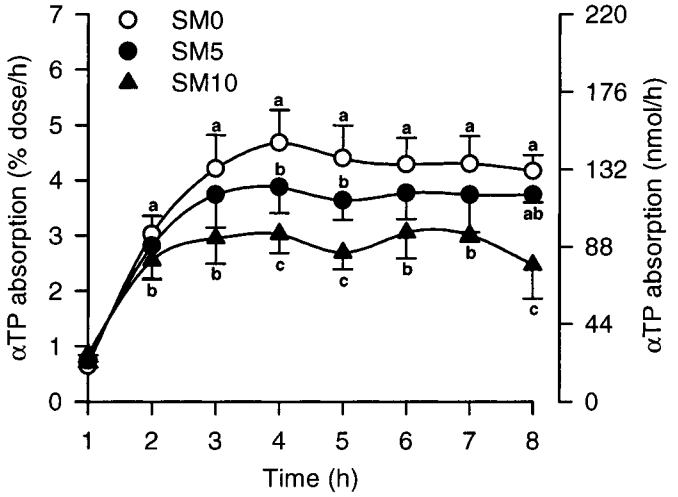

FIGURE 2 Hourly rates of lymphatic absorption of α-tocopherol (αTP) in rats during intraduodenal infusion of a lipid emulsion containing egg sphingomyelin (SM) at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0). All values are means ± SD, $n = 5$. Means with different superscript letters at each time differ, $P < 0.05$.

Lymphatic fatty acid outputs. The lymphatic output of total fatty acids, as determined at hourly intervals, did not differ between rats infused with SM0 and SM5, but was significantly lower in the SM10 than in the SM0 and SM5 groups (**Table 4**). The cumulative lymphatic output of 18:1, which was the major fatty acid infused in the form of triolein, was decreased dose-dependently in the rats infused with SM. The output of 16:0, which was the major fatty acid (83.9 mol%) in infused egg SM, was highest in SM5 group, but only moderately increased in SM10, compared with SM0 group. No differences were noted between SM0 and SM5 groups in 18:0, 18:2 and 22:6 outputs. However, the outputs of these fatty acids in the SM10 group were lower than in SM0 and SM5

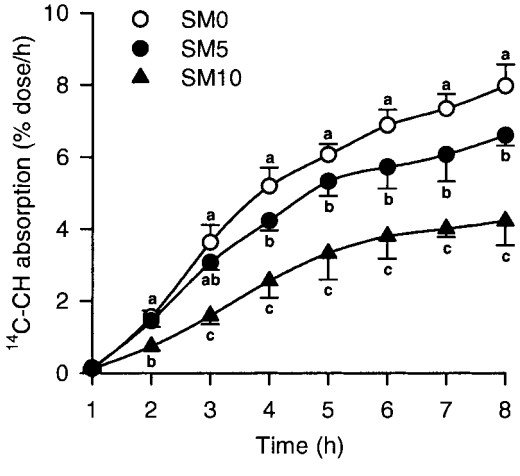

FIGURE 1 Hourly rates of lymphatic absorption of ^{14}C-labeled cholesterol (^{14}C-CH) in rats during intraduodenal infusion of a lipid emulsion containing egg sphingomyelin (SM) at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0). All values are expressed as means ± SD, $n = 5$. Means with different superscript letters at each time differ at $P < 0.05$.

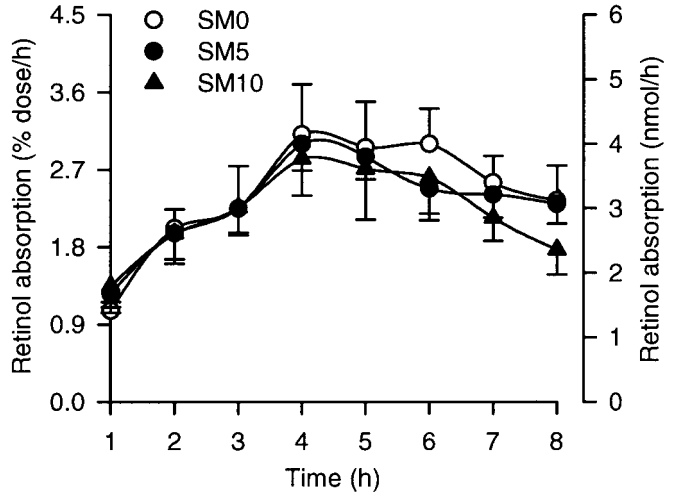

FIGURE 3 Hourly rates of lymphatic absorption of retinol in rats during intraduodenal infusion of a lipid emulsion containing egg sphingomyelin (SM) at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0). All values are means ± SD, $n = 5$. Means with different superscript letters at each time differ, $P < 0.05$.

TABLE 3

Cumulative lymphatic outputs of phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysoPC and sphingomyelin (SM) for 8 h during duodenal infusion of a lipid emulsion with SM at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0) in rats[1]

Phospholipids	SM0	SM5	SM10
		μmol/8 h	
PI	2.47 ± 0.18[a]	2.53 ± 0.20[a]	2.12 ± 0.22[b]
PS	0.50 ± 0.07[a]	0.43 ± 0.04[ab]	0.37 ± 0.09[bc]
PE	7.96 ± 0.33[a]	6.86 ± 0.28[b]	5.88 ± 0.63[c]
PC	30.01 ± 1.13[a]	28.16 ± 1.44[a]	20.69 ± 2.87[b]
lysoPC	4.98 ± 0.17	4.96 ± 0.45	4.40 ± 0.86
SM	0.38 ± 0.07[a]	0.39 ± 0.09[a]	0.17 ± 0.10[b]
Total	46.29 ± 1.29[a]	43.34 ± 1.49[a]	33.62 ± 4.18[b]

[1] Values are means ± SD, $n = 5$. [a,b,c] Values in a row without a common superscript differ ($P < 0.05$).

groups. The lymphatic output of 20:4 was decreased by SM infusion in a dose-dependent manner.

DISCUSSION

This study, using rats with lymph cannulae, provides direct evidence that SM, when infused luminally, profoundly influences the intestinal absorption of lipids. Egg SM lowered the intestinal absorption of CH in rats in a dose-dependent manner, along with parallel decreases in absorption of other lipids such as αTP and fatty acid (fat). It is noteworthy that SM did not affect retinol absorption. Our data also show that luminally infused SM failed to increase the lymphatic output of SM and that SM, at a higher dose, reduced the outputs of both SM and PC into the mesenteric lymph, suggesting a potential interactive effect on their luminal hydrolysis and/or uptake by the enterocyte.

Although the exact mechanism by which luminal SM

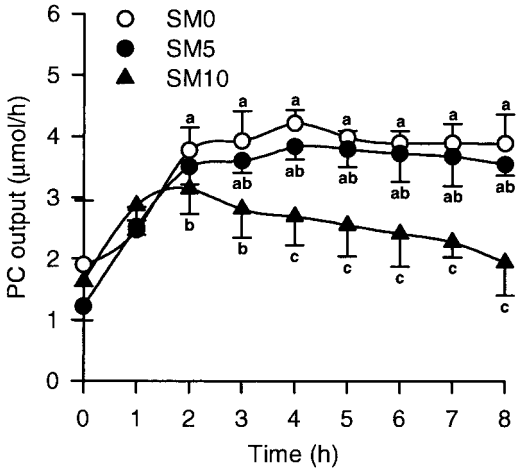

FIGURE 4 Hourly rates of lymphatic output of phosphatidycholine (PC) in rats during intraduodenal infusion of a lipid emulsion containing egg sphingomyelin (SM) at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0). All values are means ± SD, $n = 5$. Means with different superscript letters at each time differ, $P < 0.05$.

TABLE 4

Cumulative lymphatic outputs of fatty acids for 8 h during duodenal infusion of a lipid emulsion with sphingomyelin (SM) at 5 μmol/h (SM5) or 10 μmol/h (SM10), or with no SM (SM0) in rats[1]

Fatty acids	SM0	SM5	SM10
		μmol/8 h	
16:0	67.13 ± 7.01[bc]	93.31 ± 11.35[a]	71.39 ± 10.70[b]
18:0	18.49 ± 0.85[a]	18.94 ± 1.54[a]	14.17 ± 2.59[b]
18:1	548.1 ± 26.2[a]	469.2 ± 34.8[b]	403.6 ± 36.1[c]
18:2	48.51 ± 4.08[a]	45.46 ± 6.23[a]	32.84 ± 7.26[b]
20:4	28.38 ± 1.82[a]	25.12 ± 1.45[b]	16.28 ± 3.31[c]
22:6	3.04 ± 0.02[a]	3.12 ± 1.08[a]	1.42 ± 0.45[b]
Total	713.7 ± 32.0[a]	655.2 ± 53.3[a]	539.7 ± 59.2[b]

[1] Values are means ± SD, $n = 5$. [a,b,c] Values in a row without a common superscript differ ($P < 0.05$).

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 (28–30). Alkaline SMase, having a pH optimum at 9.0, is virtually inactive under the conditions of the gastric and duodenal lumen (30,31). Its activity, localized in the brush border membrane, has been shown to be maximal in the distal jejunum and lower in the ileum and colon (32). Alkaline SMase is nearly absent in the pancreatic juice, bile and liver of the rat (32). 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 (33) shows that the presence of intact PC in mixed micelles slows the transfer (desorption) of more hydrophobic lipids such as CH and αTP from the micellar matrix, whereas it does not affect 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 CH than PC does with CH (34,35). The tighter molecular packing, as produced via interaction of SM with CH, results in slower transfer (or desorption) of CH from SM vesicles or membranes than from those PC with matching acyl groups (34–38). Similarly, Eckhardt et al. (18), using Caco-2 cells in vitro, showed that milk SM is a more potent inhibitor of CH uptake than egg PC. They also observed that SM increases fecal CH excretion in mice fed a standard diet enriched with milk SM compared with those fed the same diet with egg PC. A synthetic dipalmitoyl-PC also decreases CH absorption compared with egg PC. It was postulated that SM decreases micellar solubilization and hence decreases concentrations of CH monomers available for uptake. In a recent study (39), we also found that milk SM, composed mainly of saturated longer chain (22–24 carbon) fatty acids, is more effective in inhibiting CH absorption than egg SM containing 18:1, 18:2 and 16:0 as major fatty acids, which is in agreement with the findings of Eckhardt et al. (18) and our previous observation that the degree of saturation and chain length of PC fatty acids is an important determinant of CH absorption (9).

Previously, a study (40) with Caco-2 cells demonstrated that SM content in the brush-border (apical) membrane of the enterocyte plays a regulatory role in the absorption of micellar cholesterol. When the apical membrane SM of Caco-2 cells is hydrolyzed by addition of exogenous SMase, cell uptake of CH from bile-salt micelles is markedly reduced (40). The authors concluded that, when the brush border membrane contains less SM, less micellar CH is taken up due to a decrease in the capacity of the membrane to accept and solubilize CH. A recent in vitro study with Caco-2 cells also showed that SMase treatment decreases the secretion of CH in large chylomicron-like lipoproteins (41). Based on these observations and our data here, it is possible that, because of its high affinity for CH, SM in mixed micelles in the intestinal lumen inhibits the transfer (desorption) of micellar CH to the enterocyte, whereas SM in the brush border membrane of the enterocyte increases the cell uptake and, hence, secretion of CH via lipoproteins. Thus, the SM content in mixed micelles and the brush border membrane of the enterocyte may regulate the entry of CH into the cell, whereas the movement of CH from the brush border membrane to the cell interior for absorption would require the hydrolysis of membrane SM, as demonstrated in intestinal cells and other cell types in vitro (42,43). Also, it is possible that SM may affect intracellular pathways that regulate cholesterol efflux from the enterocyte. Recent evidence suggests that ceramide, a product of SM hydrolysis by membrane SMase, may play a role in cholesterol efflux, as mediated by ATP-binding cassette transporter-1 (ABCA-1) (44). At present, it is unknown whether other sterol efflux proteins, ABCG5 and ABCG8 (45), are also influenced by this mechanism.

Our data show that SM at a higher dose (SM10) lowered the lymphatic outputs of both SM and PC, suggesting that luminal SM, at a critical concentration, may affect the rates of hydrolysis of both SM and PC. Recent studies in vitro (46–48) demonstrated that the addition of SM to dispersed PC liposomes inhibits the hydrolytic activity of type II secretory PLA_2, which is structurally similar to pancreatic PLA_2 (46), and that the substitution of ceramide for SM, a hydrolytic product of SM by SMase, enhances PLA_2 activity (47). Furthermore, incubation of SM-containing liposomes with SMase not only relieves the inhibition but also increases the enzyme activity (46–48). Thus, it is possible that excess SM, as observed in this study, may hinder the hydrolysis of luminal PC by inhibiting PLA_2 activity, and hence decreases its uptake by the enterocyte and output into the lymphatics. The inhibition of PC hydrolysis by SM also may result in impaired TG hydrolysis, as shown by previous studies (11–15), which may explain the decrease in lymphatic fat (or fatty acid) output in rats. At present, however, it is enigmatic that the infusion of SM at a higher dose fails to increase but rather markedly decreases the lymphatic output of SM. Further studies are warranted to determine the mechanism underlying this observation.

In summary, this study is the first to show that under in vivo conditions, enteral infusion of egg SM lowers the intestinal absorption of CH and αTP, without altering the absorption of retinol. At present, it remains to be determined whether SM regulates the uptake and absorption of CH and αTP via a similar mechanism. Egg SM also decreases the outputs of fat and phospholipid into the mesenteric lymph. This effect of SM may be associated with the slow hydrolysis of SM 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 SM, 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 SM intake affects the absorption of other lipids such as fat and fat-soluble nutrients.

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