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Effects of zinc and essential fatty acid deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids

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Effects of zinc (Zn) and essential fatty acid (EFA) deficiencies on the rates of lymphatic absorption of vitamin A and output of phospholipid (LP) were investigated. Twenty male rats were divided into four groups in a 2 × 2 factorial design: (1) Zn and EFA deficient (−Zn−EFA), (2) Zn deficient and EFA adequate (−Zn+EFA), (3) Zn adequate and EFA deficient (+Zn−EFA), and (4) Zn and EFA adequate (+Zn+EFA). At 9 weeks, rats with lymph cannula were infused at 3 mL/hr via an intraduodenal catheter with a triolein emulsion containing 5.4 μCi ^{3}H-retinol (^{3}H-ROH). The lymphatic absorption of ^{3}H-ROH was lowered markedly in −Zn rats. The rates of ^{3}H-ROH absorption in −Zn−EFA and −Zn+EFA groups were 0.44 and 0.58 nmol/hr, respectively, compared with 0.78 nmol/hr in +Zn−EFA and 0.98 nmol/hr in +Zn+EFA rats. Dietary EFA alone produced only a mild effect on ^{3}H-ROH absorption. Similarly, the effect of dietary Zn on the lymphatic output of PL was more pronounced than that of EFA. The output of PL was correlated closely with the absorption of ^{3}H-ROH (r = 0.90). No interaction between dietary Zn and EFA was noted in the lymphatic ^{3}H-ROH absorption or PL output. The lymphatic output of linoleic acid (LA) or arachidonic acid (AA) in −Zn rats was approximately 50% of that in their respective Zn adequate controls. However, the PL output in −Zn rats was not related to the amount of EFAs released in the lymph. Dietary Zn had no effect on the rate of intestinal PL synthesis, as determined by incubating a micellar solution of lysophosphatidylcholine (LPC) and ^{3}H-palmitic (^{3}H-PA) or ^{3}H-arachidonic acid (^{3}H-AA) in a jejunal loop in situ. Dietary EFA also did not affect the rate of ^{3}H-PA incorporation into PL. However, the rate of ^{3}H-AA incorporation into PL was significantly lower in −EFA rats than in +EFA rats. In −EFA rats, more ^{3}H-AA was distributed in phosphatidylethanolamine and less in phosphatidylcholine, compared with +EFA groups. The findings indicate that Zn deficiency has a pronounced adverse effect on the lymphatic absorption of retinol, with no interactive effect with dietary EFA. The lowering of ^{3}H-retinol absorption in −Zn rats is correlated highly with a decrease in lymphatic PL output. The decreased PL output is not due to a defect in the intestinal acylation of LPC but probably to impaired biliary secretion of PL into the intestine. (J. Nutr. Biochem. 6:595–603, 1995.)

Keywords: essential fatty acids; lymphatic absorption; phospholipid; vitamin A; zinc

Introduction

Earlier studies[1–5] have shown that the serum levels of vitamin A are lowered significantly in zinc-deficient (ZD) animals. A metabolic link between zinc (Zn) and vitamin A nutrition in humans is also suggested by a strong association between zinc and vitamin A status in premature infants,[6] malnourished children,[7–9] patients with liver cirrhosis,[10] viral hepatitis,[11] and cystic fibrosis.[12] A specific role of zinc in vitamin A metabolism is suggested further by the therapeutic effect of supplemental zinc in improving vision restoration in dim light[7,8] in children and normalizing dark adaptation thresholds in patients with liver cirrhosis[10,13–15] and cystic fibrosis.[12,15]

However, the mechanisms underlying the metabolic interaction between the two micronutrients have yet to be

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determined. Numerous studies, as reviewed by Smith,[5] have shown that the serum levels of vitamin A and retinol-binding protein (RBP) are reduced markedly in ZD animals. These findings suggest that the low levels of serum vitamin A observed in zinc deficiency may be due in part to a defect in RBP synthesis and hence mobilization of retinol from the liver via RBP. Previously,[16,17] we have shown that the intestinal absorption of lipids in general is impaired in ZD rats. Consistent with the finding, we recently have observed that the intestinal absorption of retinol also was lowered significantly in ZD rats.[18]

Although the primary cause for the impaired absorption of lipids or lipid-soluble nutrients in zinc deficiency has not been clearly defined, our studies[16,19,20] strongly suggest that the enterocyte of ZD rats fails to assemble "normal" chylomicrons because of the lack of the surface coat components, such as phospholipids (PL) and apoproteins. Numerous studies[21] involving ZD animals and humans have shown pronounced alterations in the fatty acid composition of PL especially phosphatidylcholine (PC). One of the prominent changes in PL fatty acids in ZD animals and humans is a decrease in essential fatty acids (EFAs), particularly, arachidonic acid (AA) (C20:4) with or without an increase in linoleic acid (LA) (C18:2), suggesting defects in the desaturation and elongation of the fatty acid.[22,23] In addition, another line of evidence indicates that in zinc deficiency polyunsaturated fatty acids (or EFAs) of membrane PL are increasingly susceptible to peroxidation both in vitro and in vivo.[21,24] Such alterations in EFA metabolism induced by zinc deficiency would limit the availability of EFAs for PL synthesis. Thus, a lack of EFAs in the liver would adversely affect the hepatic synthesis and biliary secretion of PL into the intestine, which is the primary source of PL utilized for the formation of chylomicrons in the enterocyte.[25] In fact, EFA deficiency in rats has been shown to interfere with intestinal fat absorption[26] and lower the concentrations of C18:2 and C20:4 in PC present in bile and intestinal microsomes. Mucosal failure to form appropriate PL for chylomicron coating is speculated to be a possible cause of lipid malabsorption.[26]

In the present study, the following questions were addressed: (1) Does zinc or EFA deficiency alter the lymphatic output of PL? (2) Is the absorption of vitamin A related to the amount of PL released into the lymph? and (3) Is the lymphatic PL output influenced by EFA availability, as altered by zinc or EFA deficiency? The present study utilized rats with lymph cannula in order to simultaneously determine the lymphatic absorption of retinol and lymphatic outputs of PL and fatty acids in lymph lipids following intraduodenal infusion of a triolein emulsion containing ^{3}H-retinol.

Methods and materials

Experiment 1

Animals and diets. Sprague Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN USA) weighing 147 to 153 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room and were subjected to a light cycle with the light period from 3:00 p.m. to 3:00 a.m. and the dark period from 3:00 a.m. to 3:00 p.m. The 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 (AAALAC). The rats were acclimatized for 1 week and fed a commercial rat chow during this period. The animals were assigned randomly to the following four groups in a 2 × 2 factorial design, with Zn and EFA as the two dietary factors: (1) a Zn and EFA deficient group (−Zn−EFA), fed a diet containing 3.0 mg of Zn/kg and 5.0% of beef tallow as the sole source of fat; (2) a ZD and EFA adequate group (−Zn+EFA) fed a diet containing 3.0 mg of Zn/kg and 2.5% beef tallow and 2.5% corn oil; (3) a Zn adequate and EFA deficient group (+Zn−EFA), fed a diet with 31 mg of Zn/kg and 5.0% beef tallow; and (4) a Zn and EFA adequate group (+Zn+EFA), fed a diet with 31 mg of Zn/kg and 2.5% beef tallow and 2.5% corn oil. Except for Zn and fat, all diets were formulated as recommended by the American Institute of Nutrition (*Table 1*).[27,28] The −EFA and +EFA diets formulated as above provided 1.3 mg of EFA (C18:2 and C18:3) and 15.8 mg of EFA/g of diet, respectively. All animals were fed ad libitum for a period of 9 to 12 weeks and given free access to distilled-deionized water via a stainless-steel delivery system.

Cannulation of mesenteric lymph duct. After food was withheld for 24 hr, rats were anesthetized with halothane, and the mesenteric lymph duct was cannulated as described previously.[29] Briefly, an abdominal incision was made along the midline by using a cauterizer. The major intestinal lymph duct was cannulated with polyethylene tubing (SV.31 tubing; i.d. 0.50 mm, o.d. 0.80 mm; Dural Plastics, Auburn, Australia). An indwelling infusion catheter (Silastic medical grade tubing; i.d. 1.0 mm; o.d. 2.1 mm; Dow Corning, Midland, MI USA) was introduced via the gastric fundus into the upper duodenum and secured by a purse-string suture (4-O Silk; Ethicon Inc., Somerville, NJ USA). After the abdominal incision was closed, the rats were placed in stainless-steel restraining cages[30] in a heated chamber (30°C) for postoperative recovery for 44 to 48 hr. During this period, the rats were infused via the duodenal catheter with a maintenance solution (277 mM glucose, 144 mM NaCl, and 4 mM KCl) at a rate of 3 mL/hr by using an infusion pump (Harvard Apparatus, Model 935, South Natick, MA USA).

Table 1 Composition of zinc deficient diets*†

Ingredient	−Zn−EFA (g/kg)	−Zn+EFA (g/kg)
Egg white, spray dried	200	200
Sucrose	502.996	502.996
Corn starch	150	150
Beef tallow‡	50	25
Corn oil§	—	25
Cellulose	50	50
Mineral mix‖	35	35
Vitamin mix	10	10
Biotin	0.004	0.004
Choline bitartrate	2	2

*Purchased from Dyets (Bethelehem, PA USA).
†According to the recommendations of the American Institute of Nutrition.[27,28]
‡Fatty acid composition (%): C14:0, 3.1%; C14:1, 0.6%; C16:0, 26.8%; C16:1, 4.5%; C18:0, 24.9%; C18:1, 37.5%; C18:2, 2.6%; and C18:3, trace.
§Fatty acid composition (%): C14:0, trace; C16:0, 10.8%; C16:1 trace; C18:0, 2.1%; C18:1, 26.5%; C18:2, 60.0%; and C18:3, 0.6%.
‖With the omission of zinc, as purchased. The diet was supplemented with Zn to provide 3.0 mg of Zn/kg.

Measurement of lymphatic absorption of ^{3}H-retinol (^{3}H-ROH). After postoperative recovery, each rat was infused via the duodenal catheter with a lipid emulsion containing ^{3}H-retinol (^{3}H-ROH) at 3 mL/hr in subdued light. The lipid emulsion consisted of 5.4 μCi ^{3}H-ROH (specific activity; 44.9 Ci/mmol, NEN Research products, Du Pont, Wilmington, DE USA), 70 nmol retinol, 678 μmol triolein, 148 nmol α-tocopherol, and 396 μmol sodium taurocholate in 24 mL of phosphate buffered saline (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl, and 5 mM KCl/L, pH 6.4). The lipid emulsion was prepared by sonication (W-375, Heat Systems-Ultrasonics, Long Island, NY USA) under a constant stream of N_2 in subdued light. Lymph samples were collected in preweighed, ice-cold, centrifuge tubes each containing 4 mg of Na_2-EDTA. The lymph samples (100 μL) were taken at hourly intervals, mixed with scintillation liquid (ScintiVerse, Fisher Scientific Co., Fair Lawn, NJ USA), and counted to determine the ^{3}H radioactivity (Beckman LS-8100, Beckman Instruments, Fullerton, CA USA). Percentages of the injected dose appearing in hourly lymph volumes were computed.

Determination of the lymphatic output of phospholipid and the fatty acid composition of lymph lipids. PL was measured by the method of Raheja et al.[31] Lipids were extracted from the lymph samples by the method of Folch et al.[32] The lipid extracts were saponified and methylated as described by Slaver et al.[33] Fatty acids were analyzed by gas chromatography using a Hewlett-Packard Model 5880A GC (Hewlett-Packard, Palo Alto, CA USA) equipped with a flame-ionization detector. Methylesters of fatty acids were separated on a Stabilwax-DA capillary column (15 m × 0.53 mm i.d.; Resteck Corp., Bellefonte, PA USA) with helium flow at the rate of 13 mL/min. The standard was prepared with methyl-fatty acids (C16:0–C20:4) (Nu Chek, Elysian, MN USA), and methyl-nonadecanoic acid (C19:0) was used as the internal standard.

Vitamin A and zinc analysis. Blood samples (2 mL) were withdrawn from the orbital sinus[34] under halothane anesthesia. The liver was removed after exsanguination. To prevent decay and loss of vitamin A, all samples were handled in subdued light and in the cold. All-trans-retinyl acetate (20 ng) as the internal standard was mixed with each sample. Vitamin A standards (all-trans-retinol and all-trans-retinyl acetate) were purchased from Sigma Chemical Co. (St. Louis, MO USA). For serum vitamin A measurement, the extract from 100 μL of serum was filtered through the microfilter membrane (pore size: 0.45 μm TF; diameter: 13 mm; Millipore Corp., Bedford, MA USA), dried under N_2 gas, and dissolved in 100 μL of ethanol.

For the analysis of unesterified retinol in the liver, 0.5 g of minced liver was extracted.[32] The extract (1.0 mL) was filtered and dried under N_2. For total liver vitamin A, 1 mL of the extract was lyophilized and saponified in 1 mL of an ethanolic solution containing 0.9 M potassium hydroxide.[35] The sample was heated for 25 min at 70°C in a standing water bath. After incubation, the sample was cooled on ice and vortexed for 1 min after addition of 3 mL of petroleum ether. The extract was filtered through the filter membrane, dried under N_2, and redissolved in 100 μL of ethanol. Vitamin A analysis was performed by using a reverse-phase high performance liquid chromatography (HPLC) column (Ultrasphere ODS, 4.6 mm × 25 cm, Beckman Instruments) and Beckman System Gold software (Beckman Instruments). Methanol:water (99:1) was used as the mobile phase and propelled at 1 mL/min.[36] Detection was monitored at 325 nm (UV Detector, Module 166, Beckman Instruments). Under these conditions, retinol and retinyl acetate were eluted at 5.1 and 7.4 min, respectively. A standard curve (peak area versus nanograms of retinoid) was constructed by injecting retinoid standards. The concentrations of retinol from 2 to 24 ng and of retinyl acetate from 20 to 240 ng yielded a linear curve (r = 0.99).

For zinc analysis, blood samples were centrifuged at 1,000g for 60 min. Serum samples (200 μL) were diluted with 400 μL of distilled-deionized water to determine zinc by atomic absorption spectrophotometry (Perkin-Elmer Co., Norwalk, CT USA) with air-acetylene flame. The zinc standards were prepared from a Fisher-certified reference standard solution (Fisher Scientific Co.).

Experiment 2

This experiment was conducted to determine whether the intestinal acylation of L-α-lysoPC is altered by dietary Zn or EFA. Unless otherwise stated, the experimental design, composition of diet, and experimental conditions were the same as described for Experiment 1. Forty mature Sprague Dawley rats (Harlan Sprague Dawley, Inc.) were fed ad libitum for 16 weeks.

Preparation of a ligated intestinal loop in situ. Two optically clear micellar solutions were prepared by a modification of the method of Johnston and Borgstrom[37]: one consisted of 0.8 mCi ^{3}H-palmitic acid (^{3}H-PA) (specific activity; 60.0 Ci/mmol; NEN Research Products, Du Pont), 0.1 mM unlabeled palmitic acid (PA), 0.1 mM L-α-lysoPC (palmitoyl), 2.2 mM glucose, 0.05 mM albumin, and 16.5 mM sodium taurocholate/L of phosphate buffered saline (pH 6.4). The other consisted of the same ingredients, but contained 0.8 mCi of ^{3}H-arachidonic acid (specific activity; 100.0 Ci/mmol, NEN Research Products, Du Pont) and 0.1 mM unlabeled arachidonic acid in place of ^{3}H-PA and unlabeled PA.

After a midline incision of the abdomen was made, a 15-cm long jejunal segment distal to the ligament of Treitz was ligated in situ. The lower end of segment was ligated with a suture (4-O Silk, Ethicon Inc.). Incubation medium (0.8 mL of a micellar solution as prepared above) was injected slowly into the loop through the upper end of the segment and closed with a suture and the abdomen was closed. The jejunal segment was incubated for 10 min in situ. During this period, less than 0.5% of the radioactivity escaped from the intestine into the blood circulation, and virtually no radioactivity was found in the liver. At 10 min, the segment was removed carefully and chilled immediately in ice-cold saline. The luminal content was collected by washing three times with 5 mL of ice-cold Krebs-Ringer phosphate buffer (pH 6.4) containing 18.3 mM sodium taurocholate. The intestinal segment was slit open, blotted with absorbent paper, and weighed.

Determination of ^{3}H-fatty acid incorporation into PL. An aliquot (200 μL) of the luminal washing was counted to measure the amount of radioactivity remaining in the intestinal lumen and the percent recovery of the injected dose was computed. The intestinal segment was homogenized (Eberbach Corp., Ann Arbor, MI USA) with 20 mL of Krebs-Ringer buffer. An aliquot (500 μL) of the homogenate was kept for protein analysis and the remainder was extracted for lipids.[32] The lipid extract (100 μL) was mixed with scintillation liquid (ScintiVerse, Fisher Scientific Co.) and counted to determine total ^{3}H radioactivity (Beckman LS-8100 Series Liquid Scintillation Systems, Beckman Instruments).

The distribution of ^{3}H radioactivity in PL was determined by separating the lipids by thin layer chromatography (TLC) on a Silica Gel G plate (20 × 20 cm; Analtech, Newark, DE USA) with hexane-diethylether-glacial acetic acid (70:30:2, vol/vol/vol).[16] For separation of phosphatidylcholine and phosphatidylethanolamine, lipid extract was applied to Silica Gel G plates and developed with chloroform-methanol-acetic acid-water (25:15:4:2, vol/vol/vol/vol).[38] The separated lipid was visualized with iodine vapor. The lipid spots on TLC plates were scraped into counting

vials and eluted with 1.0 mL of 100% ethanol for 10 min prior to mixing with the scintillation liquid (ScintiVerse, Fisher Scientific). From the lipid extract, fatty acids were analyzed by gas chromatography, as described above in Experiment 1.

Statistical analysis. The experimental design was a 2 × 2 factorial, where the factors were Zn and EFA, and the interaction also was tested. All data were expressed as mean ± SD, and the level of significance was determined at $P < 0.05$, unless otherwise stated. All statistical analyses were performed by using the SAS statistical software.[39] The correlation coefficients were determined by linear regression analyses.

Results

General observations

Body weights and serum Zn levels are shown in *Table 2*. The average final body weights in −Zn groups were significantly lower than those in +Zn groups. No significant difference in body weight was observed between −EFA and +EFA groups. Food intake was significantly lower in −Zn rats starting at 2 weeks and continuing through 9 weeks. No difference in food intake was noted between −EFA and +EFA rats over 9 weeks. The average daily food intakes of −Zn−EFA, −Zn+EFA, +Zn−EFA, and +Zn+EFA groups for the last 2 weeks were 11.3 ± 1, 12.0 ± 1, 17.3 ± 1, and 19.0 ± 1 g, respectively. Serum levels of Zn were significantly lower in −Zn rats (10.3 to 10.8 μmol/L), compared with Zn adequate controls (19.0 to 19.8 μmol/L). Dietary EFA had no effect on serum zinc. No external signs, such as alopecia and skin lesions, were visible at this stage of Zn deficiency.

Total serum and liver vitamin A concentrations are shown in *Table 3*. The serum levels of retinol were significantly lower in −Zn rats than in Zn adequate controls. Serum vitamin A levels were not affected by dietary EFA. The average levels of serum vitamin A in −Zn and +Zn rats were 1.4 ± 0.5 and 1.9 ± 0.3 μmol/L, respectively. The total content of vitamin A per whole liver was also significantly lower in −Zn rats, which was 63 to 64% of that in the Zn adequate controls. However, the concentrations of vitamin A per gram of liver were unaffected by Zn or EFA.

Table 2 Effects of dietary Zn and EFA on body weights and serum zinc levels*

Dietary treatment	Initial body weight (g/rat)	Final body weight (g/rat)	Serum zinc (μmol/L)
−Zn−EFA	153 ± 10	229 ± 10	10.3 ± 1.0
−Zn+EFA	147 ± 6	227 ± 8	10.8 ± 1.0
+Zn−EFA	152 ± 9	361 ± 10	19.0 ± 1.0
+Zn+EFA	148 ± 19	380 ± 16	19.8 ± 1.0
Zn	NS	0.0001	0.0001
EFA	NS	NS	NS
Zn × EFA	NS	NS	NS

*Mean ± SD, $n = 5$. NS, not significant ($P > 0.05$).

Lymphatic absorption of ^{3}H-retinol (^{3}H-ROH)

Figure 1A compares the hourly lymphatic absorption of ^{3}H-ROH for 8 hr. The effect of dietary Zn was more drastic than that of EFA. Starting at 2 hr postdosing, the absorption of ^{3}H-ROH was noticeably lower in −Zn rats than in +Zn rats ($P < 0.05$). From 1 through 5 hr, the absorption of labeled retinol rose at 0.44 and 0.58 nmol/hr in −Zn−EFA and −Zn+EFA rats and at 0.78 and 0.98 nmol/hr in +Zn−EFA and +Zn+EFA rats, respectively. In all groups, retinol absorption peaked at 5 and 6 hr. At the peaks, 3.1 and 4.2% of the dose were absorbed in −Zn−EFA and −Zn+EFA and 5.6 and 7.0% in +Zn−EFA and +Zn+EFA rats, respectively. The maximal rates of retinol absorption also were significantly lower in −Zn groups than in their respective Zn adequate controls. The maximal rates were 2.2 nmol/hr for −Zn−EFA and 2.9 nmol/hr for −Zn+EFA versus 3.9 nmol/hr for +Zn−EFA and 4.9 nmol/hr for +Zn−EFA rats. Dietary EFA also had a significant but mild effect on the lymphatic absorption of ^{3}H-ROH. A difference between −EFA and +EFA groups was observed only at 5 hr and thereafter. No interactive effect of dietary Zn and EFA was indicated. *Figure 1B* shows the cumulative absorption of ^{3}H-ROH for the 8-hr period. The cumulative lymphatic absorption of ^{3}H-ROH was significantly lower in −Zn rats, compared with their respective Zn adequate controls. The effect of dietary EFA was also significant, but much less pronounced than that of Zn. The total absorptions for 8 hr were 18 to 21% of the dose in −Zn groups and 33 to 39% in +Zn rats.

Lymphatic output of phospholipid

The lymphatic output of PL at hourly intervals for 8 hr is shown by *Figure 2A*. A more pronounced effect of dietary Zn on PL output was clearly evident, compared with that of EFA. The output of PL was significantly lower in −Zn groups at 2 hr postdosing than in Zn adequate controls. From 1 to 6 hr, the rates of PL secretion were 0.08 and 0.18 μmol/hr in −Zn−EFA and −Zn+EFA rats and 0.48 and 0.63 μmol/hr in +Zn−EFA and +Zn+EFA rats, respectively. Coinciding with the peak of ^{3}H-ROH absorption, the peak of PL output occurred between 5 and 6 hr. The hourly output of PL in −Zn rats remained nearly flat at 1 to 1.3 μmol/hr at its peak, whereas in +Zn rats it increased steadily at 2.5 to 3.0 μmol/hr. In −EFA rats, the lymphatic PL output was near the control (+EFA) level up to 5 hr and failed to reach the control (+EFA) level only at 6 hr as the absorption of ^{3}H-ROH peaked. After reaching its peak between 5 and 6 hr, the PL output declined with time in all groups as ^{3}H-ROH absorption began to level off. The hourly absorption of ^{3}H-ROH occurred in a close parallel with the amount of PL secreted into the lymph ($r = 0.90$, $P < 0.05$).

The cumulative lymphatic output of PL was markedly lower at 2 hr postdosing in −Zn rats than in +Zn rats (*Figure 2B*). The total amounts of PL released for 8 hr were 6 to 8 μmol in −Zn groups and 15 to 16 μmol in +Zn rats. Dietary EFA had no significant effect on cumulative PL output. Although the PL output tended to be lower in

Table 3 Effects of dietary Zn and EFA on serum and liver vitamin A*

Dietary treatment	Serum (μmol/L)	Liver		
		Weight (g)	Per liver (μmol)	Per gram of liver (μmol/g)
−Zn−EFA	1.3 ± 0.6	4.9 ± 0.3	9.8 ± 0.6	2.0 ± 0.1
−Zn+EFA	1.5 ± 0.4	6.5 ± 0.2	11.7 ± 0.6	1.8 ± 0.1
+Zn−EFA	2.1 ± 0.5	9.5 ± 0.5	15.2 ± 0.6	1.6 ± 0.1
+Zn+EFA	1.7 ± 0.1	9.3 ± 0.6	18.6 ± 1.1	2.0 ± 0.1
Zn	0.0001	0.0001	0.0001	NS
EFA	NS	0.0424	0.0057	NS
Zn × EFA	0.0030	0.0053	NS	NS

*Mean ± SD, n = 6 from Experiment 2. NS, not significant ($P > 0.05$).

−EFA rats than in +EFA controls, the difference was not significant ($P > 0.05$). The cumulative absorption of ^{3}H-ROH (% dose, *Figure 1A*) was highly correlated ($r = 0.98$, $P = 0.0001$) with the cumulative output of PL.

Lymphatic output of fatty acids

The cumulative outputs of individual fatty acids, as present in lymph lipids, are shown in *Table 4*. The amounts of both saturated and unsaturated fatty acids secreted into the lymph were significantly lower in −Zn groups than in their Zn adequate controls. In −Zn groups, the lymphatic output of OA, which was the sole fatty acid infused as triolein, was 68 to 73% of that in their respective Zn adequate controls. Dietary EFA did not affect the lymphatic output of OA, but reduced the outputs of all other fatty acids. The hourly output of OA was correlated closely with the absorption of ^{3}H-ROH ($r = 0.78$, $P < 0.05$). The total amounts of fatty acids secreted were 780 ± 51 and 927 ± 132 μmol in −Zn−EFA and −Zn+EFA rats and 1174 ± 143 and 1311 ± 99 μmol in +Zn−EFA and +Zn+EFA groups, respectively.

Both dietary Zn and EFA had pronounced effects on the lymphatic output of LA and AA. However, the effect of Zn was more pronounced than that of EFA (*Table 4*). The total output of LA in −Zn or −EFA groups was approximately 50% of that in respective +Zn or +EFA controls. The cumulative outputs of LA for 8 hr was 11.8 μmol in −Zn−EFA, 21.8 μmol in −Zn+EFA, 18.9 μmol in +Zn−EFA, and 40.3 μmol +Zn+EFA. The total outputs of AA also were affected similarly by dietary Zn and EFA. Significant interactive effects of dietary Zn and EFA were indicated for LA and AA output ($P < 0.01$).

The hourly outputs of LA and AA are graphically shown by *Figures 3A* and *3B*. Starting at 4 hr, the lymphatic outputs of both fatty acids were significantly lower in −Zn and in −EFA groups than in their respective controls. The hourly output of LA or AA in −Zn−EFA rats remained unchanged from 1 through 8 hr. The peak of LA output occurred between 5 and 6 hr in all other groups. At its peak, 1.6 and 3.6 μmol were released per hour in −Zn−EFA and −Zn+EFA rats and 3.3 and 6.0 μmol/hr in +Zn−EFA and +Zn+EFA rats, respectively. The AA outputs were 2.0 and 4.6 μmol/hr in −Zn−EFA and −Zn+EFA rats and 5.0 and 8.0 μmol/hr in +Zn−EFA and +Zn+EFA, respectively. The average ratio of LA to AA in lymph was significantly greater in −Zn groups (0.90 ± 0.1) than in +Zn groups (0.75 ± 0.1). No proportionate change in PL output was observed with the amount of LA or AA released into the lymph.

Incorporation of ^{3}H-fatty acids (^{3}H-FA) into phospholipid

The rate of incorporation of ^{3}H-palmitic (^{3}H-PA) or ^{3}H-arachidonic acid (^{3}H-AA) into PL was expressed in μmol ^{3}H-FA incorporated/min/100 mg of protein (*Table 5*). Dietary Zn had no effect on the rate of ^{3}H-FA incorporation into PL, regardless of whether ^{3}H-PA or ^{3}H-AA was used as substrate. Dietary EFA did not affect the rate of ^{3}H-PA incorporation, but altered the rate of ^{3}H-AA incorporation into PL. The rate of ^{3}H-AA incorporation was significantly lower in −EFA than +EFA rats. Dietary Zn and EFA altered the distribution of ^{3}H-PA between PC and PE. A greater proportion of ^{3}H-PA was utilized for PC and less for PE synthesis in −Zn or −EFA rats than in +Zn or +EFA controls. When ^{3}H-AA was used as the fatty acid substrate, less (75 to 78%) was incorporated into PC in −EFA than in +EFA rats (83 to 85%). Conversely, a significantly greater proportion of ^{3}H-AA (22 to 25%) was incorporated into PE in −EFA than in +EFA controls (15 to 17%). Dietary Zn had no effect on the distribution of ^{3}H-AA between PC and PE.

Discussion

In the present study, the effects of dietary Zn and EFA on the intestinal absorption of retinol were investigated by measuring directly the ^{3}H radioactivity appearing in the mesenteric lymph at hourly intervals during intraduodenal infusion of ^{3}H-ROH. The results showed that a marginal level of Zn deficiency in adult male rats markedly lowered the lymphatic absorption of ^{3}H-ROH. EFA deficiency exerted a mild effect on the absorption of ^{3}H-ROH. Also, compared with EFA, dietary Zn had a more pronounced effect on PL output. The absorption of ^{3}H-ROH was highly correlated ($r = 0.90$, $P < 0.05$) with the PL output, as measured at hourly intervals. The lymphatic secretion of LA and AA was influenced significantly by both dietary Zn

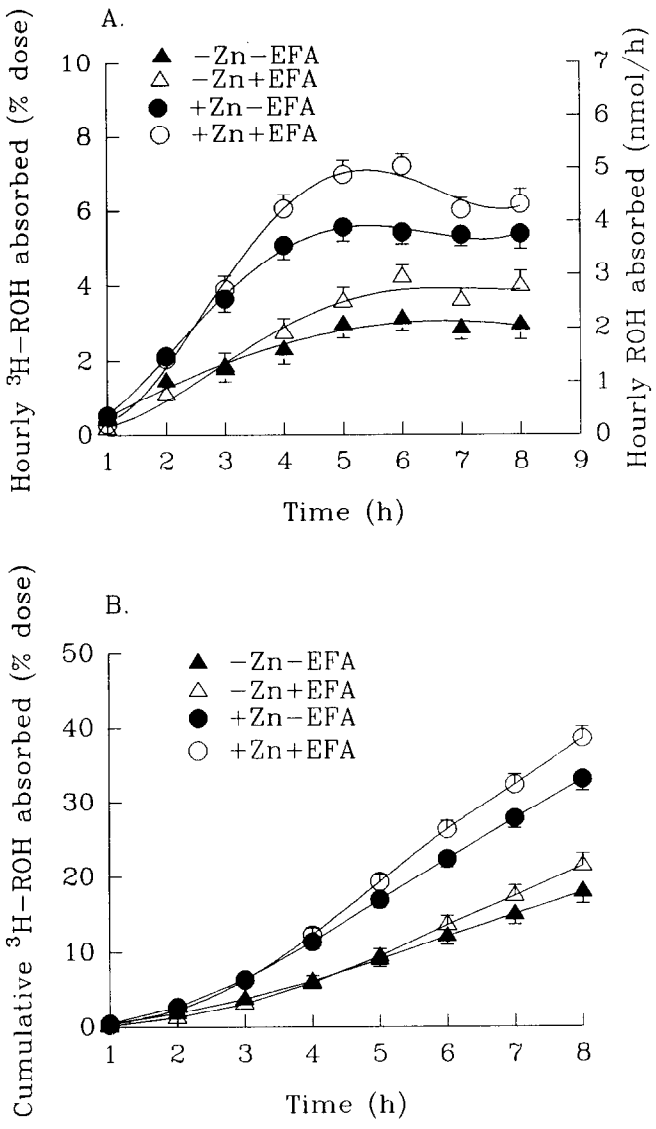

Figure 1 (A) The lymphatic absorption of ^{3}H-ROH at hourly intervals for 8 hr. The hourly absorption of ^{3}H-ROH was significantly lowered in −Zn rats at 2 hr and thereafter compared with their respective controls ($P < 0.05$). From 1 to 5 hr, retinol absorption occurred at 0.44 and 0.58 nmol/hr in −Zn−EFA and −Zn+EFA rats and 0.78 and 0.98 nmol/hr in +Zn−EFA and +Zn+EFA rats, respectively. Dietary EFA had a mild effect on ^{3}H-ROH absorption, which was evident starting at 5 hr. (B) the cumulative lymphatic absorption of ^{3}H-ROH for 8 hr. The amounts of ^{3}H-ROH absorbed for 8 hr were 18 and 21% of the dose in −Zn groups, and 33 and 39% in +Zn groups.

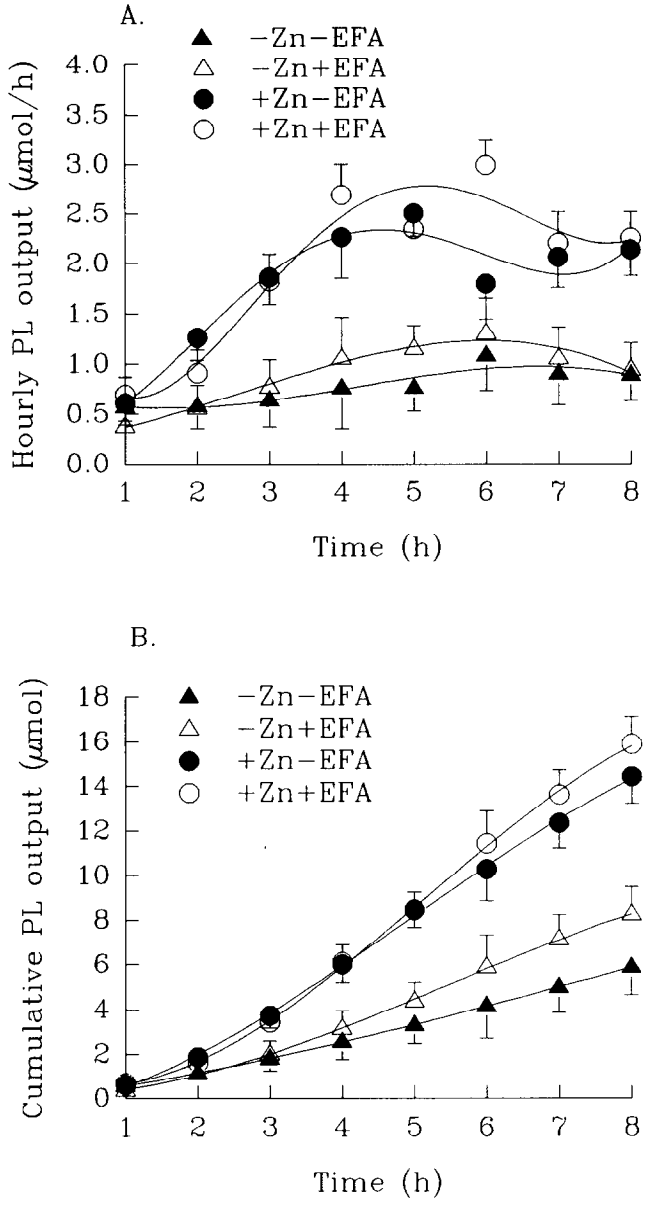

Figure 2 (A) The lymphatic secretion of phospholipid (PL) at hourly intervals for 8 hr. The PL output was lower in −Zn groups at 2 hr and thereafter compared with their respective controls ($P < 0.05$). At the peak, the rate of PL secretion remained nearly flat at 1 to 1.3 μmol/hr in −Zn groups compared with 2.5 to 3.0 μmol/hr in +Zn rats. Dietary EFA had a less pronounced effect on the PL output. A significant effect of EFA was observed only at 6 hr postdosing. (B) The cumulative lymphatic secretion of PL for 8 hr period. The total outputs of PL were 6 and 8 μmol in −Zn groups and 16 μmol in +Zn groups. However, dietary EFA had no significant effect on the cumulative output of PL, although it tended to be lower as a result of EFA depletion.

and EFA. However, the lymphatic output of PL was not associated with the amount of LA or AA secreted into the lymph.

These observations demonstrate that the impaired absorption of retinol is associated primarily with a limited availability of PL during chylomicron formation in the enterocyte of Zn-deficient rats. The decreases in lymphatic retinol absorption and PL secretion were produced to a large extent by Zn deficiency per se, although the possibility cannot be ruled out that EFA deficiency secondary to Zn depletion may have an additive inhibitory effect on PL secretion. These findings are in line with the hypothesis that the impaired absorption of lipids in Zn deficiency is due to a lack of the surface coat materials such as PL during chylomicron assembly within the intestinal epithelium.[16,17,19,20] PL is not only a major surface component of chylomicrons, but also speculated to be an important stimulus to the intestinal synthesis of apoB-48.[40] The limited availability of PL and apoB-48 during chylomicron assem-

Table 4 Effects of dietary Zn and EFA on the cumulative lymphatic outputs of fatty acids*

Dietary treatment	Total	C 16:0	C 18:0	C 18:1	C 18:2	C 20:4
			(μmol)			
−Zn−EFA	780 ± 50	53.4 ± 4.1	18.3 ± 2.5	685 ± 66	11.8 ± 2.1	13.6 ± 1.2
−Zn+EFA	927 ± 132	72.5 ± 7.9	23.9 ± 2.1	784 ± 93	21.8 ± 2.8	25.0 ± 2.5
+Zn−EFA	1174 ± 143	85.8 ± 10.7	34.1 ± 5.3	1006 ± 104	18.9 ± 3.3	28.6 ± 2.6
+Zn+EFA	1311 ± 99	114.2 ± 11.2	38.0 ± 3.1	1068 ± 117	40.3 ± 4.3	50.9 ± 3.6
Zn	0.0001	0.0001	0.0001	0.0007	0.0001	0.0001
EFA	0.0360	0.0011	0.0280	NS	0.0001	0.0001
Zn × EFA	NS	NS	NS	NS	0.0017	0.0084

*Mean ± SD, $n = 5$. NS, not significant ($P > 0.05$).

bly may explain the accumulation of abnormally large lipid droplets in the enterocyte of Zn deficient rats,[16,19,20] with impaired lipid absorption.[16–18] The observations are remarkably similar to those described in human β-lipoprotein deficiency[41] and in rats treated with protein-synthesis inhibitors.[42]

Chylomicron PL is mostly (80%) PC and is derived largely from biliary PL secreted into the intestinal lumen.[25] Although the enterocyte is capable of synthesizing PL de novo, the PL produced via this pathway alone is not adequate to support a normal rate of lipid absorption.[43] The majority of mucosal PC is produced from reacylation of lysoPC absorbed after the hydrolysis of luminal PC of biliary origin.[44,45] Our findings indicate that dietary Zn has no effect on the intestinal synthesis of PC via lysoPC reacylation. Thus, the decrease in PL secretion observed in Zn-deficient rats is most likely due to an impaired synthesis and/or secretion of PL from the liver via the biliary route. Although no information exists regarding the specific role of Zn in PL synthesis in the liver, evidence suggests that Zn deficiency lowers the concentration of PL in microsomal membranes. In Zn-deficient rats,[46] the PL concentration (expressed in μg/mg of protein) of liver microsomal membranes was shown to be significantly lower, whereas the molar ratios of cholesterol/PL were significantly increased, relative to those of pair-fed controls. Similar changes in membrane PL were observed in Zn deficient mice.[47]

Numerous studies[21] involving Zn-deficient animals and humans with acrodermatitis enteropathica have demonstrated decreases in LA and AA incorporated into PL. The present data also showed marked decreases in the lymphatic output of these fatty acids and a significant increase in the average ratio of lymph LA to AA in −Zn groups. These changes may reflect primarily the reduced food intake and hence EFA intake in −Zn rats. The daily average intakes of EFAs (mostly C18:2) were 189.6 and 350.0 mg in −Zn+EFA and +Zn+EFA rats and 14.7 mg and 22.5 mg in −Zn−EFA and +Zn−EFA groups, respectively. The differences in dietary EFA intake tended to reflect the lymphatic outputs of LA and AA. In addition, the decreased output of LA and AA in −Zn rats may be partly due to a defect in desaturation and elongation of LA to AA[22,23] and increased susceptibility of the fatty acids to peroxidation due to zinc deficiency.[21,24] A previous study[26] reported that the intestinal absorption of lipids was impaired in EFA-deficient rats. The possibility was suggested that the defect might be linked to a lack of biliary PL secreted into the intestine.

Thus far, however, whether the lymphatic secretion of chylomicron PL is influenced by a change in EFA availability has not been clarified. Our data show that the lymphatic secretion of PL is not related to the amount of LA or AA released into the lymph (*Figures 2* and *3*). For example, the total PL secreted in +Zn−EFA rats was 91% of that in +Zn+EFA rats, while the amounts of LA and AA secreted in +Zn−EFA group were 47 and 57% of those in +Zn+EFA controls. Similarly, the PL output in −Zn−EFA rats was 71% of that of −Zn+EFA rats, whereas the outputs of LA and AA in −Zn−EFA rats were 54 and 48%, respectively, of those of −Zn+EFA rats. More importantly, the overall patterns of hourly PL output between groups did not coincide with that of EFA outputs. Therefore, our results clearly demonstrate that the total amount of PL secreted into the lymph, whether in −Zn or in +Zn rats, is independent of dietary EFA intake and the amounts of EFAs available to the intestinal mucosa. The decrease in PL output observed in −Zn rats was due primarily to Zn depletion, rather than to a lack of EFA available to the enterocyte. Consistent with this conclusion is our recent finding of a significant decrease in lymphatic PL output in Zn-deficient rats, when their food intake, feeding behavior, and body weight were matched closely with those of pair-fed controls by training them for meal feeding.[48] Under these conditions, an intraduodenal infusion of PC in Zn-deficient rats completely restored the lymphatic absorption of retinol with an increase in lymphatic PL output. These observations indicate that the impaired retinol absorption and lymphatic PL secretion are caused by Zn deficiency, rather than by reduced food intake or secondary effects of malnutrition.

A question may be raised as to whether the impaired absorption of retinol in −Zn or −EFA is due to an alteration in the esterification of retinol in the enterocyte, which is critical step for its incorporation into chylomicrons. The intestinal esterification of retinol by lecithin-retinol acyltransferase (LRAT) requires PC and retinol bound to cellular retinol binding protein (CRBP-II) as the substrates. At present, whether Zn deficiency specifically alters the activity of intestinal LRAT and/or synthesis of CRBP-II is not known. The possibility exists that the lack of cellular PL, as suggested by the lower PL output in −Zn rats, might limit the availability of PC for LRAT. However, the EFA or

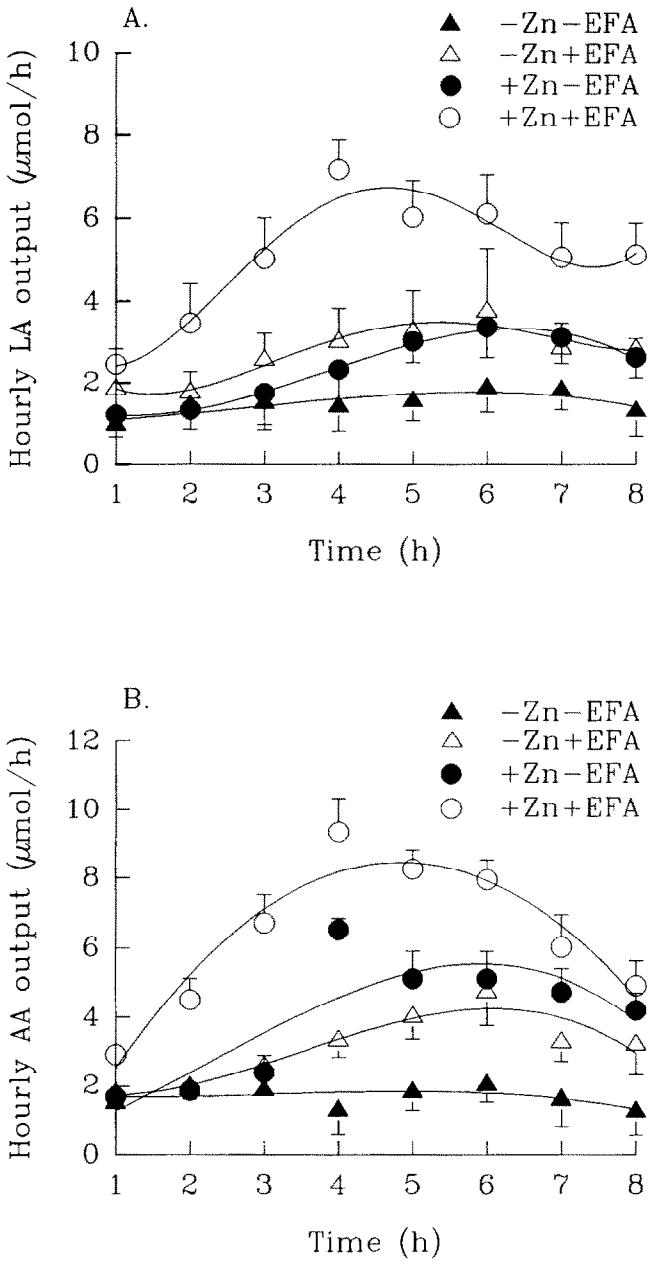

Figure 3 (A) The output of linoleic acid (LA) at hourly intervals for 8 hr. From 1 to 6 hr, the output of LA increased significantly in all rats except in −Zn−EFA rats. The effects of dietary Zn and EFA were evident starting at 4 hr. At their peaks, the rates of LA output were 1.6 and 3.3 μmol/hr in −Zn−EFA and −Zn+EFA rats, and 3.6 and 6 μmol/hr in +Zn−EFA and +Zn+EFA rats, respectively. (B) The output of arachidonic acid (AA) at hourly intervals for 8 hr. The output of AA remained flat in −Zn−EFA rats throughout 8 hr, in contrast to its rapid increase with time in +Zn+EFA rats. At their peaks, the rates of AA output were 2 to 4.6 μmol/hr in −Zn groups, and 5 to 8 μmol/hr on their Zn adequate controls. Dietary EFA had a pronounced effect on the rate and amount of AA released into the lymph, particularly during the first 5 hr.

polyunsaturated fatty acid moiety of PC may not be rate-limiting for retinol esterification in the intestinal mucosa, because LRAT transfers the 1-acyl group of PC,[49] which is predominantly saturated fatty acids. In the small intestine, the majority (>90%) of retinol is esterified to saturated and

Table 5 Effects of dietary Zn and EFA on the rate of ^{3}H-fatty acid incorporation into total phospholipid and distribution between phosphatidylcholine (PC) and phosphatidylethanolamine (PE)*

Dietary treatment	Incorporation of ^{3}H-FA into total PL (μmol/min²)	Distribution of ^{3}H-FA PC (%)	Distribution of ^{3}H-FA PE (%)
^{3}H-palmitic acid			
−Zn−EFA	2.6 ± 0.2	78.3 ± 3.0	21.7 ± 3.0
−Zn+EFA	2.2 ± 0.3	74.2 ± 4.3	25.8 ± 4.3
+Zn−EFA	2.4 ± 0.2	73.8 ± 0.5	26.2 ± 0.5
+Zn+EFA	2.3 ± 0.2	70.2 ± 4.4	29.8 ± 4.4
Zn	NS	0.0132	0.0132
EFA	NS	0.0225	0.0225
Zn × EFA	NS	NS	NS
^{3}H-arachidonic acid			
−Zn−EFA	0.39 ± 0.05	78.0 ± 3.1	22.0 ± 3.1
−Zn+EFA	0.48 ± 0.02	84.8 ± 2.3	15.2 ± 2.3
+Zn−EFA	0.42 ± 0.03	74.9 ± 3.0	25.1 ± 3.0
+Zn+EFA	0.52 ± 0.03	83.2 ± 1.9	16.8 ± 1.9
Zn	NS	NS	NS
EFA	0.0001	0.0001	0.0001
Zn × EFA	NS	NS	NS

*Mean ± SD, n = 5. NS, not significant ($P > 0.05$).
†Per 100 mg of tissue protein.

monounsaturated fatty acids and only a minor portion to polyunsaturated fatty acids and the proportions of the ester fatty acids remain relatively constant even when the fatty acid composition of diets is varied.[50,51]

In summary, the present study provides the first evidence that the lymphatic absorption of vitamin A is impaired in Zn-deficient rats. The decrease in retinol absorption was associated with a parallel decrease in lymphatic PL output. The decreased output of PL is not attributable to a lack of EFAs or a defect in intestinal PL synthesis via reacylation of lysoPC. The present findings suggest that the marked decrease in lymphatic PL output in Zn deficiency may be due primarily to a defect in the hepatic synthesis and/or secretion of PL via the biliary route during lipid absorption. Further studies are warranted to examine the effect of Zn status on the hepatic synthesis and biliary secretion of PL into the intestinal lumen and its impact on intestinal chylomicron formation.

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