Intraduodenal Infusion of Lysophosphatidylcholine Restores the Intestinal Absorption of Vitamins A and E in Rats Fed a Low-Zinc Diet

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Our previous work has shown that the lymphatic absorptions of lipids and lipid-soluble vitamins, retinol and α-tocopherol (αTP), are lowered markedly in rats fed a low-zinc (LZ) diet in parallel with lower lymphatic phospholipid outputs. Phosphatidylcholine (PC), when infused enterally, restored the absorptions of fat and retinol, but further lowered the absorption of αTP in rats fed the LZ diet. This study was conducted to determine whether a luminal infusion of lysophosphatidylcholine, a product of PC hydrolysis by pancreatic phospholipase A_2 (PLA_2), would simultaneously restore the absorptions of retinol and αTP in LZ rats. Rats were trained to consume two meals per day and were divided into two groups. One group was fed an AIN-93G diet containing a LZ (3.0 mg Zn/kg), and the other was fed the same diet, but containing adequate zinc (AZ; 30.0 mg Zn/kg) for 6 weeks. Rats with lymph cannula were infused at 3.0 ml/hr for 8 hr with a lipid emulsion containing retinol, αTP, and ^{14}C-labeled triolein (^{14}C-oleic acid) with or without 1-oleoyl-2-hydroxy phosphatidylcholine (lysoPC) in 24 ml of PBS (pH 6.5). When the lipid emulsion without lysoPC was infused, the absorptions of retinol and αTP were significantly lower in LZ rats (retinol, 13.2 ± 1.5 nmol; αTP, 430.6 ± 66.8 nmol) than in AZ rats (retinol, 18.2 ± 1.0 nmol; αTP, 543.8 ± 58.9 nmol). The lower absorptions of the vitamins in LZ rats occurred in parallel with a significant decrease in ^{14}C-oleic acid absorption. When the emulsion containing lysoPC was infused, however, absorptions of the vitamins (retinol, 18.4 ± 3.0 nmol; αTP, 777.2 ± 92.1 nmol) in LZ rats were restored completely to the control levels (retinol, 20.4 ± 2.8 nmol; αTP, 756.3 ± 136.1 nmol). The results suggest that the luminal hydrolysis of PC to lysoPC by PLA_2 may be impaired in LZ rats, resulting in impaired absorption of fat and the fat-soluble vitamins. [Exp Biol Med Vol. 226(4):342–348, 2001]

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The nutritional status of zinc profoundly influences the plasma and tissue levels of vitamins A and E in animals and humans (1–4). In our recent studies we presented evidence that the intestinal absorptions of fat, retinol, and α-tocopherol (αTP) are impaired in rats fed a low-zinc (LZ) diet. Under the conditions of matched food intakes and body weights between rats fed a LZ diet and those fed an adequate zinc (AZ) diet, the intestinal absorption of vitamin A was lowered significantly in LZ rats (5–7). Similarly, Kim et al. (8) observed that the lymphatic absorption of vitamin E also was impaired in LZ rats. The precise mechanism(s) underlying such adverse effects of LZ intake (or marginal zinc deficiency) on the absorption of the fat-soluble vitamins is not known. Our earlier studies (9, 10) have shown that the enterocyte of zinc-deficient rats accumulates large lipid droplets in the enterocyte and fails to transport the lipids via chylomicrons into the lacteal. The tendency to form and accumulate large lipid droplets appears to be due to a lack of surface components such as phospholipid, which are required for chylomicron synthesis.

In keeping with these observations, we showed that the lymphatic output of phospholipid during active fat absorption was reduced significantly in LZ rats and that the decreased rates of lymphatic phospholipid output was correlated significantly with the decreases in the lymphatic absorptions of fat, retinol, and αTP (5, 6, 8). When LZ rats were infused intraduodenally with a lipid emulsion containing phosphatidylcholine (PC), the intestinal absorptions of fat and retinol were restored fully to the level of AZ rats (6). However, under the same experimental conditions we observed that the enteral infusion of PC further lowered the absorption of αTP in LZ rats, whereas the absorption of fat and phospholipid output were increased to the control levels (11). Previous studies also have shown that the presence of intact PC in bile-salt micelles does not inhibit the uptake of

retinol by Caco-2 cells (12), but interferes with the absorption of αTP as measured by using the intestinal segment perfused *in situ* (13). These observations clearly indicate that PC influences the absorption of the two fat-soluble vitamins via distinctly different mechanisms.

At present, however, the mechanism underlying such differential effects of PC on retinol and αTP absorption is far from clear. Evidence from *in vitro* studies suggests that the uptake of extremely hydrophobic lipids such as cholesterol from PC-containing mixed micelles is markedly inhibited (12). Furthermore, this effect of micellar PC is completely abolished by the presence of lysophosphatidylcholine (lysoPC) or addition of pancreatic phospholipase A_2 (PLA_2) to PC-containing bile-salt mixed micelles (12, 14). At present, it is not known whether substitution of lysoPC for intact PC in a lipid emulsion would overcome the inhibitory effect of PC on the absorption of αTP, another extremely hydrophobic lipid. It also remains to be answered whether the luminal hydrolysis of PC by pancreatic PLA_2 is impaired in zinc deficiency, thereby limiting the absorption of αTP and other lipids. The present study, therefore, was conducted to test if lysoPC as incorporated into a lipid emulsion would simultaneously restore the intestinal absorptions of αTP and retinol in LZ rats.

Materials and Methods

Animals. Eight-week-old male Sprague-Dawley rats weighing 192 to 214 g were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). They were housed individually in polypropylene cages with stainless steel grid bottoms in a room controlled at 22 to 24°C and 60 to 70% humidity and were subjected to a daily12-hr light:dark cycle (light from 2100 to 0900 hr). Rats were cared for in an animal facility of the Department of Human Nutrition at Kansas State University that was fully accredited by the American Association for the Accreditation of Laboratory Animal Care. During 2 weeks of acclimation, rats had free access to a nutritionally adequate AIN-93G diet (15) and deionized water as provided via a stainless-steel watering system. Animals were maintained in accordance with the animal care and use guidelines approved by the Kansas State University Institutional Animal Care and Use Committee.

Meal Feeding and Dietary Treatment. In an attempt to avoid any confounding effects of low food intake, a decrease in weight gain, and a change in feeding behavior that commonly result from feeding an LZ diet, rats were trained to completely consume two meals daily at 0930 and 1530 hr. After 2 weeks on the AIN-93G standard diet, rats were starved for 24 hr and given 5 g/meal of the same diet for the first 2 days and then a 1-g increment per meal for the next 2 consecutive days. On Day 5 of meal training, rats were given 7.0 g at 0930 hr and 7.5 g at 1530 hr. With this feeding schedule, rats consumed each meal completely within 90 min. After the meal training period, rats were assigned randomly to the following two groups: an LZ group fed a diet containing 3.0 mg of Zn/kg and an AZ group fed the same diet, but containing 30.0 mg of Zn/kg. Both LZ and AZ groups continued to be fed two meals per day of their respective diets for 6 weeks. The total amount of diet given (14.5 g/day) represented 85% of their normal food intake and was adequate to support growth and weight gain in both groups. A basal diet (Table I) was formulated by Dyets Inc. (Bethlehem, PA) according to the AIN-93G recommendations (15), with the following modifications: (i) egg white as the protein source and dextrose in place of sucrose; (ii) soybean oil was vitamin E-stripped; and (iii) the mineral mix was modified to adjust the mineral contents for the use of egg white as the protein source, according to the recommendations of Reeves (16). This basal diet contained 1.0 mg of Zn/kg and was supplemented with zinc carbonate to prepare the LZ and AZ diets.

Cannulation of the Mesenteric Lymph Duct. At the end of 6 weeks, rats were starved for 15 hr prior to surgery (17). While rats were under anesthesia (2.0% halothane in 2.0 l of oxygen/min delivered via a halothane vaporizer), a midline abdominal incision was made. The superior mesenteric lymph duct was cannulated with a vinyl tube (medical grade, 0.50 mm i.d., 0.80 mm o.d., Dural Plastics & Engineering, Dural, Australia). The cannula was fixed in place with ethyl cyanoacrylate glue (Elmer's Products, Columbus, OH) and externalized through the right flank. An infusion catheter (Silastic Laboratory tubing, 1.02 mm i.d., 2.16 mm o.d., Dow Corning, Midland, MI) was inserted into the upper duodenum via the gastric fundus and was secured in place with a purse-string suture (4-0 Silk, Ethicon, Somerville, NJ) around the fundic incision. The infusion catheter was exteriorized alongside the lymph cannula. The rats were placed in restraining cages and allowed to recover for 20 hr in a recovery chamber maintained at 30°C. During the post-operative recovery, phosphate buffered saline (PBS) containing glucose (in millimoles per liter: 277 glucose, 6.75 Na_2HPO_4, 16.5 NaH_2PO_4, 115 NaCl, and 5 KCl; pH 6.5) was infused through the duodenal cath-

Table I. Composition of Zinc-Deficient Basal Diet[a]

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

[a] Formulated and supplied by Dyets (Bethlehem, PA) according to the recommendations of the American Institute of Nutrition (15).
[b] Contained 0.02% tert-butylhydroquinone.
[c] With the omission of zinc, as purchased. The diet contained 1.0 mg zinc/kg diet. The mineral mix was modified to adjust for the mineral contents of egg white used in place of casein (16).

eter at 3.0 ml/hr via a syringe pump (Model 935, Harvard Apparatus, South Natick, MA).

Determination of ^{14}C-Oleic Acid Absorption. After post-operative recovery, rats were infused with a lipid emulsion consisting of 62.8 nmol retinol (all trans-retinol, 95%, Sigma Chemical, St. Louis, MO), 2.6 μmol αTP (all-rac-α-tocopherol, 97%, Aldrich Chemical, Milwaukee, WI), 0.75 μCi of [carboxyl-^{14}C]-triolein (specific activity, 112.0 mCi/mmol, DuPont NEN, Boston, MA), 565 μmol triolein (95%, Sigma Chemical), and 396 μmol sodium taurocholate with or without 40 μmol 1-oleoyl-2-hydroxy phosphatidylcholine (99%, Avanti Polar Lipids, Alabaster, AL) and 40 μmol linoleic acid (99%, Nu Chek, Elysian, MN) in 24 ml of PBS buffer. The amounts of retinol and αTP added to the lipid emulsion were set to approximate the rat's intakes of 61 nmol retinol and 2.5 μmol αTP that were provided by the 14.5-g diet (AIN-93G). Because the AIN-93G diet has no specific recommendation for phospholipid, the amount of lysoPC infused was to simulate the estimated intake of phospholipid through a typical diet in humans. The amount of lysoPC (40 μmol) added is equal to an intake of 60.0 mg/d/kg body wt., which is within the range (4–8 g) of daily phospholipid intake in human adults (18). The amount of bile salt (sodium taurocholate) in the emulsion was set at 0.89%, which is adequate to emulsify the lipids, but considered safe with no detrimental effect on the intestinal absorptive function (19).

Lymph was collected under subdued light at hourly intervals for 8 hr in pre-weighed ice-chilled plastic tubes containing 30 μg of n-propyl gallate and 4 mg of Na_2EDTA. From hourly fresh lymph samples, ^{14}C-radioactivity was determined in 100-μl aliquots after mixing with scintillation liquid (ScintiVerse, Fisher Scientific, Fair Lawn, NJ) 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-oleic acid absorbed. The hourly rate of ^{14}C-oleic acid absorption was expressed as the percentage (%) of the total dose of ^{14}C-radioactivity infused.

Measurement of Lymphatic Retinol Absorption. Total retinol (free and esterified retinol) was extracted from lymph by the method of Ross (20). A 100-μl lymph sample was saponified in 10 vol of 95% ethanol and 5% potassium hydroxide solution (Fisher Scientific, Pittsburgh, PA) containing 1% pyrogallol (99%, Acros Organics, Pittsburgh, PA) at 60°C for 20 min. After cooling, the contents were mixed vigorously with 20 vol of hexane and then 10 vol of water. After a brief centrifugation, the upper phase was transferred into a vial, dried under N_2, and resuspended in chloroform:methanol (1:3, v/v). All-trans-retinyl acetate as an internal standard was added into each sample to monitor extraction efficiency, which generally exceeded 95%. Retinol separation was performed by a Beckman HPLC with System Gold software (Beckman Instruments) equipped with a C-18 reversed-phase column (Alltima C18, 5 μm, 4.6 × 150 mm, Alltech Associates, Deerfield, IL). Methanol:water (99:1, v/v) was used as the mobile phase and propelled at 1 ml/min (21). Detection was monitored at 325 nm (UV detector, Module 166, Beckman Instruments). Under these conditions, retinol and retinyl acetate were eluted at 3.4 and 4.6 min, respectively. Standard curves were constructed by injecting pure retinol and retinyl acetate standards. The concentrations of retinol from 2.79 to 13.26 pmol yielded a linear curve (r = 0.99). The total amount of retinol absorbed into the lymph collected hourly was determined based on the concentration of retinol in 100 μl of lymph.

Measurement of Lymphatic αTP Absorption. From an aliquot of the hourly lymph sample, αTP was extracted by acetone with a slight modification (22). Briefly, lymph (100 μl) was pipetted into a glass test tube and 150 mg of anhydrous sodium sulfate (99%, Acros Organics) and 1 ml of acetone containing BHT (3.3 mg/100 ml) were added. The contents were mixed vigorously on a vortex mixer. Following centrifugation at 1000g at 4°C for 10 min, the organic phase was filtered through a PTFE syringe filter (0.45 μm, Alltech Associates), dried under N_2, and redissolved in chloroform:methanol (1:3, v/v). αTP acetate as an internal standard was added into each sample to monitor extraction efficiency, which generally exceeded 90%. αTP and α-tocopherol acetate were separated by a Beckman HPLC with System Gold software (Beckman Instruments) equipped with a C-18 reversed-phase column (Alltima C18, 5 μm, 4.6 × 150 mm, Alltech Associates). Methanol was used as the mobile phase (23) at 2 ml/min. Detection was monitored at 292 nm (Module 166, Beckman Instruments). Typical retention times were 4.1 min for α-tocopherol and 5.3 min for α-tocopherol acetate. αTP concentration was calculated using a standard curve with αTP concentrations ranging from 110.5 to 442.3 pmol. The total amount of αTP absorbed into the lymph collected hourly was determined based on the concentration of αTP in 100 μl of lymph.

Lymph Phospholipid and Serum Zinc Analysis. From 100-μl aliquots of lymph samples, phospholipid was measured colorimetrically (UV-1201 Spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD) by the method of Raheja et al. (24), as detailed previously (8). For serum zinc analysis, blood was collected via the orbital sinus (25) and centrifuged at 1000g at 4°C for 60 min. Zinc was determined by atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT). The zinc standards were prepared from a Fisher-certified reference standard solution (Fisher Scientific).

Statistical Analysis. All statistical analyses were performed using PC SAS (26). Data were expressed as means ± SD. Values obtained in the two groups were compared using a Student's t test at designated time intervals. The level of significance was determined at $P < 0.05$. ANOVA and the least significant difference test were performed to detect time-dependent changes within groups.

Results

Body Weight and Serum Zinc. Figure 1 shows changes in the body weights of LZ and AZ rats throughout the 6 weeks. The average body weight of LZ rats was not significantly different from that of AZ controls, as they were trained to consume the equal amounts of food at two meals per day. At 6 weeks the average final body weight of LZ rats was 97% that of AZ controls. Serum concentrations of zinc at 2, 4, and 6 weeks were significantly lower in LZ rats than in AZ rats (Fig. 2; $P < 0.05$). No external symptoms of zinc deficiency such as alopecia or skin lesions were detected throughout the experiment.

Lymph Flow. In response to lipid infusion, lymph flow was increased significantly and reached its peak in both groups at 3 to 5 hr. During infusion of the lipid emulsion without lysoPC, the average hourly rates of lymph flow for 8 hr were 2.8 ± 0.4 ml/hr in LZ rats and 2.6 ± 0.3 ml/hr in AZ rats, which were not significantly different (Table II). Similarly, during infusion of the emulsion containing lysoPC, the average rates in LZ and AZ rats were 2.9 ± 0.5 ml/hr and 3.3 ± 0.2 ml/hr, respectively. No difference was noted between groups.

Lymphatic Absorption of Retinol. When the lipid emulsion without lysoPC was infused, the total amount of retinol absorbed for 8 hr was significantly lower in LZ rats (13.2 ± 1.5 nmol; 21.0 ± 2.4% dose) than in AZ rats (18.2 ± 1.0 nmol; 29.0 ± 1.5% dose) at 2 hr and thereafter (Fig. 3A). The average hourly rate of retinol absorption also was significantly lower in LZ rats (1.65 ± 0.19 nmol/hr) than in AZ rats (2.28 ± 0.12 nmol/hr).

When the lipid emulsion containing lysoPC was infused, the lymphatic absorption of retinol rose sharply in LZ rats and reached the AZ control level. The cumulative absorptions of retinol in LZ and AZ rats were 18.4 ± 3.0 nmol (29.2 ± 4.8% dose) and 20.4 ± 2.8 nmol (32.5 ± 4.4% dose), respectively, which did not differ significantly (Fig. 3B). Also, the average hourly rates of retinol absorption did not differ between groups (2.3 ± 0.4 nmol/hr in LZ rats and 2.6 ± 0.3 nmol/hr in AZ rats). The maximal hourly rates of retinol absorption, observed at 3 hr, were 3.1 ± 0.8 nmol/hr in LZ rats and 3.1 ± 0.6 nmol/hr in AZ rats.

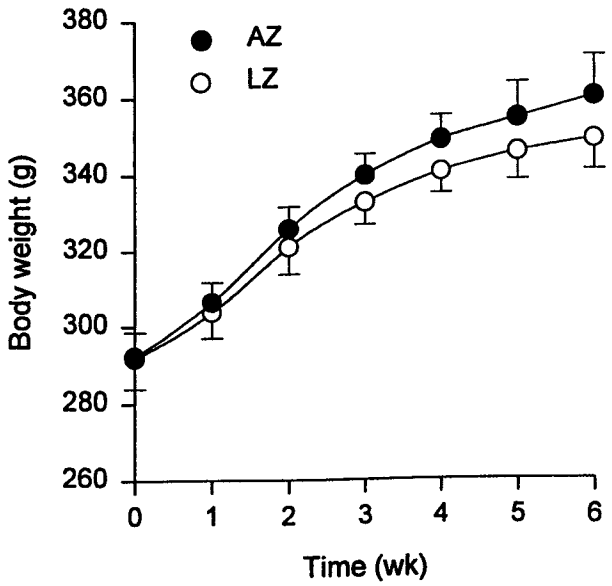

Figure 1. Changes in the mean body weights of rats fed an LZ diet and of those pair-fed an AZ diet for 6 weeks. All values are expressed as means ± SD, $n = 10$.

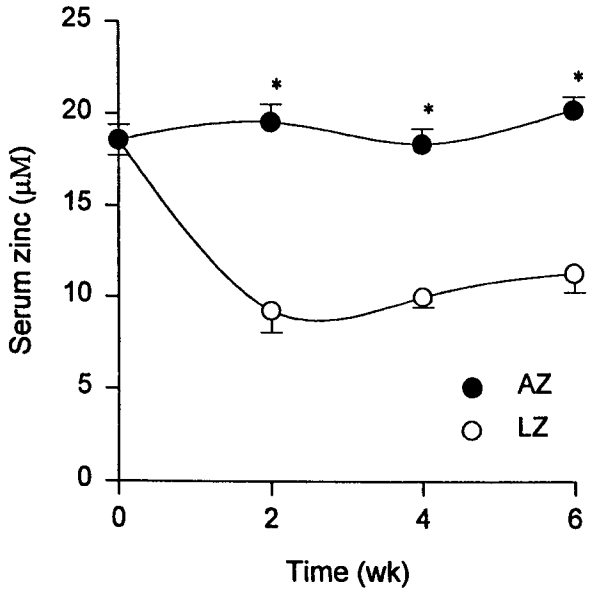

Figure 2. Serum concentrations of zinc in rats fed LZ and AZ diets. All values are expressed as means ± SD, $n = 5$. Asterisks (*) denote significant differences between LZ and AZ rats at the time interval at $P < 0.05$.

Table II. Cumulative Lymphatic Absorptions of Retinol, α-Tocopherol (αTP), and ^{14}C-Oleic Acid and Output of Phospholipid during Luminal Infusion of a Lipid Emulsion with or without Lysophosphatidylcholine (lysoPC) in Rats Fed Low-Zinc (LZ) and Adequate-Zinc (AZ) Diets[a]

	AZ	LZ
Without lysoPC		
Lymph, mL/8 hr	20.9 ± 2.1	22.0 ± 3.2
Retinol, nmol/8 hr	18.2 ± 1.0	13.2 ± 1.5*
% dose/8 hr	29.0 ± 1.5	21.0 ± 2.4*
αTP, nmol/8 hr	543.8 ± 58.9	430.6 ± 66.8*
% dose/8 hr	21.0 ± 2.3	16.6 ± 2.6*
^{14}C-oleic acid, % dose/8 hr	49.7 ± 3.6	38.3 ± 6.0*
Phospholipid, μmol/8 hr	32.0 ± 2.5	28.1 ± 1.0*
With lysoPC		
Lymph, mL/8 hr	26.4 ± 1.8	22.9 ± 4.3
Retinol, nmol/8 hr	20.4 ± 2.8	18.4 ± 3.0
% dose/8 hr	32.5 ± 4.4	29.2 ± 4.8
αTP, nmol/8 hr	756.3 ± 136.1	777.2 ± 92.1
% dose/8 hr	28.7 ± 5.2	29.5 ± 3.5
^{14}C-oleic acid, % dose/8 hr	57.5 ± 3.2	55.0 ± 3.5
Phospholipid, μmol/8 hr	35.0 ± 1.8	33.1 ± 1.3

[a] Means ± SD, $n = 5$. * Significantly different from AZ ($P < 0.05$).

Lymphatic Absorption of αTP. When the lipid emulsion without lysoPC was infused intraduodenally, the total cumulative absorption of αTP for 8 hr was signifi-

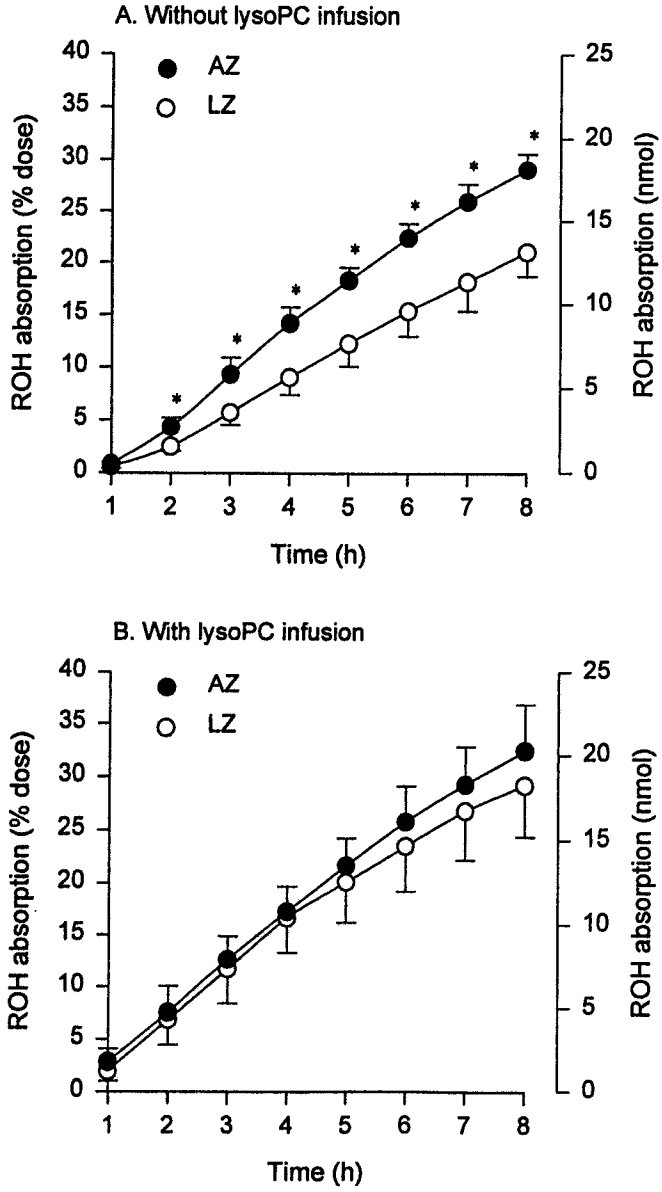

Figure 3. The lymphatic absorption of retinol (ROH) at hourly intervals for 8 hr during luminal infusion of lipid emulsion with or without lysoPC in rats fed LZ and AZ diets. All values are expressed as means ± SD, $n = 5$. Asterisks (*) denote significant differences between groups at the time interval at $P < 0.05$.

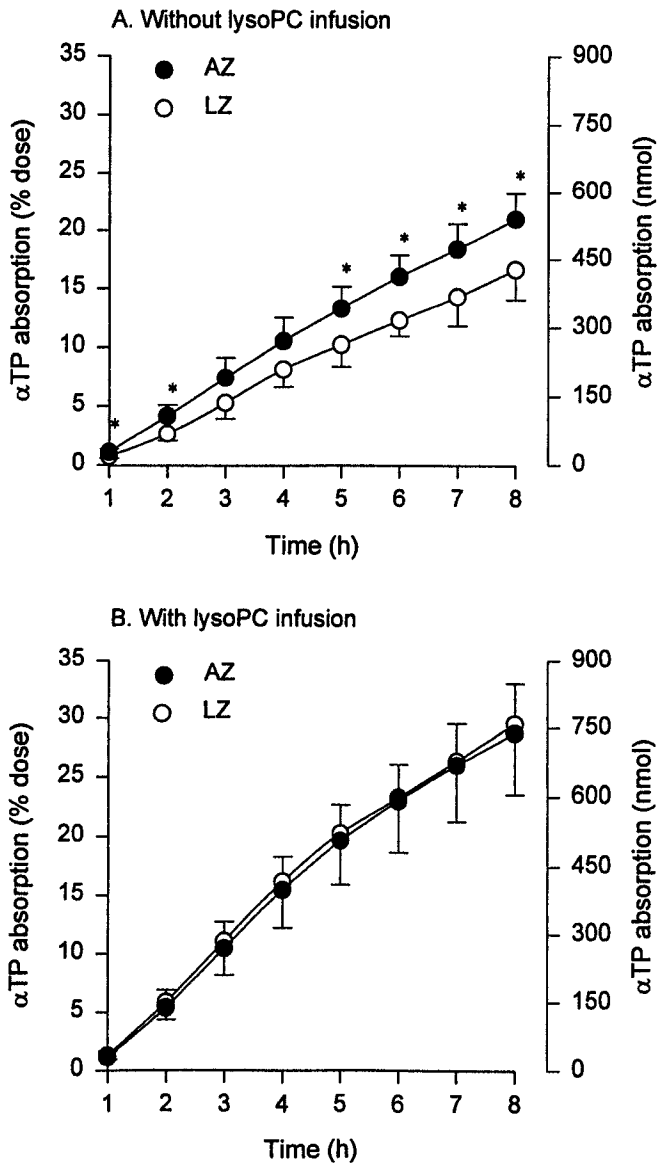

Figure 4. The lymphatic absorption of αTP at hourly intervals for 8 hr during luminal infusion of lipid emulsion with or without lysoPC in rats fed LZ and AZ diets. All values are expressed as means ± SD, $n = 5$. Asterisks (*) denote significant differences between groups at the time interval at $P < 0.05$.

cantly lower in LZ (430.6 ± 66.8 nmol; 16.6 ± 2.6% dose) than in AZ rats (543.8 ± 58.9 nmol; 21.0 ± 2.3% dose; Fig. 4A). The average hourly rates were 53.8 ± 8.3 nmol/hr in LZ rats and 68.0 ± 7.4 nmol/hr in AZ rats, which differed significantly.

When the lipid emulsion with lysoPC was infused, the rate of αTP absorption increased rapidly in LZ rats and was restored completely to the AZ level (Fig. 4B). The total amounts of αTP absorbed for 8 hr in LZ and AZ rats were 777.2 ± 92.1 nmol (29.5 ± 3.5% dose) and 756.3 ± 136.1 nmol (28.7 ± 5.2% dose; Table II). The average hourly rates of αTP absorption were 97.2 ± 11.5 nmol/hr and 94.5 ± 17.0 nmol/hr, respectively, with no significant difference between groups.

Lymphatic Absorption of ^{14}C-Oleic Acid. When the lipid emulsion without lysoPC was infused, the intestinal absorption of ^{14}C-oleic acid was significantly lower in LZ (38.3 ± 6.0% dose) than in AZ rats (49.7 ± 3.6% dose; Table II). The average hourly rates of ^{14}C-oleic acid absorption were 4.8 ± 0.8% dose/hr in LZ rats and 6.2 ± 0.5% dose/hr in AZ rats. When the lipid emulsion containing lysoPC was infused, however, the lymphatic absorption of ^{14}C-oleic acid in LZ rats was restored fully to the AZ control level. The total absorption of ^{14}C-oleic acid for 8 hr was 55.0 ± 3.5% dose in LZ and 57.5 ± 3.2% dose in AZ rats, with no difference between groups. The peak rates of ^{14}C-oleic acid absorption, observed at 5 hr, were 9.5 ± 0.8% dose/hr in LZ rats and 9.8 ± 0.7% dose/hr in AZ rats. The average hourly rates of ^{14}C-oleic

acid absorption in LZ and AZ rats for 8 hr were 6.9 ± 0.4% and 7.2 ± 0.4% dose, respectively, which were not significantly different.

Lymphatic Output of Phospholipid. Without lysoPC infusion, significant differences were noted between LZ and AZ groups at 5 hr and thereafter, in both the average rate (3.5 ± 0.1 μmol/hr in LZ rats versus 4.0 ± 0.3 μmol/hr in AZ rats) and the total amount of phospholipid output (28.1 ± 1.0 μmol in LZ rats versus 32.0 ± 2.5 μmol in AZ rats; Table II). With lysoPC infusion, the lymphatic output of phospholipid rapidly increased in LZ rats and was restored to the level of AZ controls. The total outputs of phospholipid for 8 hr was 33.1 ± 1.3 μmol in LZ rats and 35.0 ± 1.8 μmol in AZ rats (Table II). The average rates of phospholipid output were 4.1 ± 0.2 μmol/hr in LZ rats and 4.4 ± 0.2 μmol/hr in AZ rats, which did not differ significantly.

Discussion

The present study confirmed that absorptions of the two fat-soluble vitamins are lowered significantly in rats fed a LZ diet. The data also provide clear evidence that an intraduodenal infusion of lysoPC, as incorporated into a lipid emulsion, normalizes the intestinal absorption of both retinol and αTP in LZ rats. The infusion of lysoPC increased the absorption of retinol by 39.4% in LZ rats and 12.1% in AZ rats. It also increased the lymphatic absorption of αTP by 80.5% in LZ and by 39.1% in AZ rats. These observations indicate that although luminal lysoPC enhances the intestinal absorptions of the vitamins in both LZ and AZ rats, its availability is more critical for the normal absorptions of the fat-soluble vitamins in LZ rats. Thus, our findings here and previous observations (6, 8), taken together, indicate that the luminal availability of lysoPC, rather than intact PC, is limited in LZ rats, resulting in impaired absorptions of fat and the fat-soluble vitamins. Although intact PC can be taken up directly by the brush-border membrane of the enterocyte (27), most of the PC of biliary or dietary origin is hydrolyzed by pancreatic PLA_2 to lysoPC, which in turn facilitates micellar formation in the intestinal lumen, and hence, uptake of micellar lipids. LysoPC, once taken up by the enterocyte, is reacylated to PC, which is utilized for formation of the PC coat of chylomicrons (28). Our previous study (5) clearly showed that the reacylation of lysoPC in the intestinal mucosa is not affected in LZ rats under similar experimental conditions. In addition, we recently observed that the rate of biliary PC secretion and the total amount of biliary PC output remain unaffected in LZ rats (33.7 ± 4.9 μmol/8 hr) compared to AZ controls (31.0 ± 1.9 μmol/8 hr), with no significant changes in other phospholipid classes and their fatty acid compositions (S.K. Noh and S.I. Koo). Thus, our observation here strongly suggests that a defect in luminal PC hydrolysis is the primary cause of the impaired absorption of fat and the fat-soluble vitamins observed in LZ rats.

Pancreatic PLA_2 hydrolytically removes the sn-2 fatty acyl moiety from phospholipids. Although the enzyme is known to require Ca^{++} for its activity (29), evidence also indicates that the enzyme binds zinc avidly *in vitro* and its activity is stimulated markedly by zinc in the presence of Ca^{++} and bile salts (30). The zinc-induced stimulation of pancreatic PLA_2 activity appears to be metal-specific, based on the observation that its activity is not affected by other divalent cations such as Mg^{++}, Fe^{++}, or Cr^{++}, and that the activity of structurally similar group II PLA_2 from snake venom is unaffected by zinc (30). Thus, it is probable that pancreatic PLA_2 activity is sensitive to the animal's zinc status.

However, it still remains to be understood how the luminal infusion of lysoPC restores the absorptions of both vitamins in LZ rats, whereas PC, infused in a lipid emulsion, enhances the intestinal absorption of retinol (6), but interferes with the absorption of αTP (11). Earlier studies suggest that the presence of PC in bile salt micelles expands the size of micelles and retards the diffusion of micelles across the unstirred water layer (31, 32), thereby limiting the uptake of micellar lipids (33). However, recent studies using Caco-2 cells showed that the addition of PC into mixed micelles does not inhibit the absorption of relatively less hydrophobic lipids such as retinol, fatty acid, and monoacylglycerol, whereas it markedly reduces the absorption of cholesterol, an extremely hydrophobic lipid (12). Although both retinol and αTP are fat-soluble, αTP, a 29-carbon lipid, is more hydrophobic than retinol, a 20-carbon alcohol with a shorter prenyl side chain. Thus, it is probable that αTP is transferred from the PC-containing micellar matrix at a much slower rate than retinol, and hence, is less readily available for uptake by the enterocyte. Previously, it also has been shown that the rate of transfer of a lipid from PC bilayer vesicles decreases with increasing hydrophobicity of the lipid (34). This may partially explain why luminally infused PC does not limit the absorption of retinol, but slows the absorption of αTP. Furthermore, evidence shows that substitution of lysoPC for intact PC in bile-salt mixed micelles or addition of pancreatic PLA_2 enhances the uptake of lipids regardless of their hydrophobicity (12, 14). A recent study using rat intestinal cells *in vitro* showed that the PLA_2-dependent hydrolysis of the surface PC in a lipid emulsion is required for the hydrolysis of the core triacylglycerol by pancreatic lipase and for stimulation of cholesterol uptake (35). These observations indicate that the initial hydrolysis of PC is a critical prerequisite step for lipolysis in the intestinal lumen and subsequent uptake of the lipolytic products and fat-soluble nutrients. These findings are in line with our observation that lysoPC simultaneously enhances the absorption of fat, retinol, and αTP.

In summary, the present study confirms our earlier findings that the intestinal absorptions of fat and the fat-soluble vitamins, retinol and αTP, are regulated by a mechanism sensitive to the animal's zinc status. Our observations here provide new evidence that an intraduodenal infusion of lysoPC simultaneously restores the intestinal absorption of

fat, retinol, and αTP in rats fed an LZ diet. The results suggest that a possible defect in PLA_2 activity, as produced by zinc deficiency, may limit the availability of lysoPC, which in turn slows the rate of intestinal chylomicron formation and absorptions of lipids and lipid-soluble vitamins.

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