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Enteral infusion of phosphatidylcholine increases the lymphatic absorption of fat, but lowers α-tocopherol absorption in rats fed a low zinc diet☆

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Abstract

Our previous study has shown that the lymphatic absorption of both fat and α-tocopherol (αTP) is lowered markedly in rats fed a low zinc diet, with a parallel decrease in lymphatic phospholipid (PL) output. This study was conducted to determine if enteral infusion of phosphatidylcholine (PC) could restore lymphatic absorption of fat and αTP in zinc-deficient rats. One group of rats was fed an AIN-93G diet containing 3 mg Zn/kg (low zinc; LZ) and the other was fed the same diet but containing 30 mg Zn/kg (adequate zinc; AZ). Rats were trained to consume two meals daily of equal amounts of food. At 6 wk, each rat with lymph fistula was infused at 3 mL/h with a lipid emulsion containing 3.6 μmol αTP and 565 μmol [carboxyl-^{14}C]-triolein (^{14}C-OA), with or without 40 μmol 1,2-dilinoleoyl-PC in 24 mL PBS at pH 6.4. The lymphatic absorptions of fat and αTP were determined by measuring ^{14}C-radioactivity and αTP appearing in the mesenteric lymph collected hourly for 8 h. When the emulsion devoid of PC was infused, the absorptions of both ^{14}C-OA (41 $\pm$ 4% dose) and αTP (431 $\pm$ 55 nmol) in LZ rats were significantly lower than in AZ rats (48 $\pm$ 2% ^{14}C-OA dose and 581 $\pm$ 70 nmol αTP). When the emulsion containing PC was infused, the absorption of ^{14}C-OA was restored rapidly to normal in LZ rats, along with a parallel increase in lymphatic PL output. However, PC infusion further lowered the absorption of αTP to 311 $\pm$ 20 nmol/8 h in LZ rats and also lowered the absorption of αTP in AZ rats (347 $\pm$ 48 nmol/8 h). The results demonstrate that low zinc intake results in impaired intestinal absorption of both αTP and fat. The findings also indicate that PC significantly improves the intestinal absorption of fat, but inhibits αTP absorption, suggesting that PC affects the intestinal absorption of αTP and fat via distinctly different mechanisms.

Keywords: α-Tocopherol; Fat; Intestinal absorption; Phosphatidylcholine; Zinc

1. Introduction

The clinical manifestations of zinc deficiency in both animals and humans include hypogonadism, alopecia, dermatitis, ocular lesions, and retarded growth [1]. The clinical symptoms closely resemble in many respects those observed in the deficiencies of vitamins A and E. The importance of adequate zinc status in maintaining normal intestinal function has long been suggested by studies on hereditary human zinc deficiency disorder (acrodermatitis enteropathica), which is characterized by malabsorption and chronic diarrhea [2,3]. Previously, several studies have suggested that the animal's zinc status profoundly affects the body status of vitamin E and that dietary supplementation of the vitamin prevents the development of certain external symptoms of zinc deficiency [4,5]. In one study, [4] the plasma concentration of vitamin E was shown to be lower in zinc-deficient rats than in pair-fed controls. In the zinc deficient animals, the plasma levels of vitamin E failed to rise to the pair-fed control levels in response to increasing dietary vitamin E, suggesting a defect in intestinal absorption of the vitamin.

However, an unresolved question has been whether the impaired absorption of lipids in zinc deficient animals is due specifically to a compromised zinc status or to a generalized malnutrition produced by drastic decreases in food intake and weight gain. Using adult rats trained to consume two

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meals a day to match the food intakes and body weights between zinc-deficient and control rats, Noh and Koo [6] recently have provided direct evidence that the animal's zinc status per se is a critical determinant of the tissue concentrations of α-tocopherol (αTP). Feeding of a low zinc (LZ) diet (3.0 mg zinc/kg) for 6 wk significantly lowered the concentrations of αTP in the liver, kidney, heart, testis, and brain. Furthermore, the concentrations of αTP were correlated strongly with the zinc levels of the tissues.

At present, how zinc deficiency results in lowering of the tissue levels of αTP is not clearly understood. We recently showed that the intestinal absorption of αTP is influenced profoundly by zinc status [7]. Under the same dietary conditions used in the study cited above, [6] we measured the lymphatic absorption of αTP by infusing rats intraduodenally with a lipid emulsion containing a physiological dose of αTP (3.6 μmol/8 h). A significant decrease in αTP absorption in the rats fed a LZ diet was clearly detectable. The total cumulative absorption of αTP in LZ rats was 63.7% of that in the controls fed an adequate zinc (AZ) diet. Consistent with this observation was a marked difference in the maximal rate of αTP absorption between LZ rats (67.0 nmol/h) and AZ controls (96.0 nmol/h). This finding indicates that the intestinal absorptive process for this fat-soluble vitamin is regulated by a mechanism sensitive to the animal's zinc status. One important observation from this study was that the lower rate of lymphatic αTP absorption was associated with a parallel decline in lymphatic phospholipid (PL) output. Under similar experimental conditions, [8,9] we also observed that the lymphatic absorptions of fat and retinol in LZ rats were impaired, with a decrease in lymphatic PL output. When phosphatidylcholine (PC) was provided intraduodenally, however, the absorptions of both fat and retinol were restored completely to normal [9].

In the light of the above observations, the present experiment was conducted to investigate whether the lymphatic absorption of αTP also could be normalized by infusing PC in rats fed a LZ diet. In this paper, we provide the first evidence that PC, when infused luminally, lowers drastically the lymphatic absorption of αTP but restores the absorption of fat (fatty acid) to normal, in LZ rats, indicating that luminal PC affects the intestinal absorption of fat and αTP via distinctly different mechanisms.

2. Materials and methods

2.1. Animals

Twenty male Sprague-Dawley rats, initially weighing 220 to 234 g, were obtained at 7 wk of age from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN), and housed singly in plastic cages with stainless steel wire bottoms in a room of controlled temperature (22–24°C) and lighting (lights off from 0900 to 2100 h). Rats were cared for in an animal facility of the Department of Human Nutrition at Kansas State University, which was accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Animals were maintained in accordance with the policies and guidelines for routine animal care and experimental procedures of the Kansas State University Institutional Animal Care and Use Committee. During the first week, rats were acclimated to the housing conditions and had ad libitum access to a zinc-adequate AIN-93G diet [10] and to deionized water as delivered via a stainless-steel automatic watering system.

2.2. Meal training and dietary treatment

In an attempt to minimize any confounding effects of a low food intake and a consequent reduction in body weight gain, which are commonly observed in zinc deficient animals, the rats were trained to completely consume two meals daily. This protocol was designed to match the amounts of food consumed and the feeding behaviors of both LZ and AZ rats. After 1 wk of acclimation, rats were given two meals per day at 0930 h and 1630 h. Initially, rats were starved for 24 h and were given 5 g/meal of the AZ diet for the first 2 d, with a 1-g increment per meal for the next two consecutive days. On day 5 of training, they were given 7 g at 0930 h and 7.5 g at 1630 h. With this feeding schedule, rats consumed each meal completely within 90 min. After the training period, rats were assigned randomly to the following two experimental groups: 1) a LZ group fed a diet low in zinc (3.0 mg of zinc/kg diet) and 2) an AZ group fed a diet adequate in zinc (30.0 mg of zinc/kg diet). Both LZ and AZ groups continued to be fed two meals per day of their respective diets for 5 wk. The total amount of diet given (14.5 g/d) represented 85% of their normal food intake and was adequate to support growth and weight gain in both groups. A zinc-deficient basal diet (Table 1) was formulated by Dyets Inc. (Bethlehem, PA) according to the AIN-93G recommendations, [10] with the following modifications: 1) egg white as the protein source and dextrose in place of sucrose; 2) beef tallow and soybean oil (1:1 by weight) as the fat sources. The soybean oil used was not vitamin E stripped; and 3) The mineral mix was modified to adjust the mineral contents with the use of egg white as the protein source, according to the recommendations of Reeves [11]. This basal diet contained 1.0 mg of zinc/kg. To prepare the LZ and AZ diets, the basal diet was supplemented with zinc carbonate. The LZ and AZ diets provided the following amounts of tocopherols (mg)/kg diet: 68.2 dl-α-tocopherol (all-rac), 2.7 RRR d-α-tocopherol, 0.3 β-tocopherol, 23.2 γ-tocopherol, and 6.7 δ-tocopherol.

2.3. Surgical procedure

At the end of 5 wk, an intraduodenal infusion catheter and a lymph cannula were inserted surgically, as detailed previously [12]. Briefly, animals starved overnight (15 h) were anesthetized with a halothane vaporizer (2.0% halo-

Table 1
Composition of 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[d]	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 [10].

[b] Contained 0.02% tert-butylhydroquinone and tocopherols (2.7 mg RRR d-α-tocopherol, 0.3 mg β-tocopherol, 23.2 mg γ-tocopherol, and 6.7 mg δ-tocopherol per 70 g).

[c] Without added zinc. The diet contained 1.0 mg zinc/kg. The mineral mix was modified to adjust for the mineral contents of egg white used in place of casein [11].

[d] Contained all-rac dl-α-tocopherol as the source of vitamin E.

thane in 2.0 L oxygen/min). After a longitudinal 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 proximal duodenum via the gastric fundus. It was secured in place with a purse-string suture (4-0 Silk, Ethicon, Somerville, NJ) around the fundic incision and was exteriorized. The animal was placed in a restraining cage and allowed to recover for at least 20 h in a recovery chamber maintained at 30°C. During the recovery period, phosphate buffered saline (PBS) (in mmol/L: 277 glucose, 6.75 Na_2HPO_4, 16.5 NaH_2PO_4, 115 NaCl, and 5 KCl; pH 6.4) was infused continuously through the catheter at 3 mL/h via an infusion pump (Harvard Apparatus, Model 935, South Natick, MA). For each experiment, as described below, five rats per group (LZ and AZ) were used with a total of 10 rats.

2.4. Determination of ^{14}C-oleic acid absorption

After recovery, rats were infused with a lipid emulsion, which consisted of 3.6 μmol αTP (all-rac-dl-α-tocopherol, 97%, Aldrich Chemical, Milwaukee, WI), 27.8 kBq of [carboxyl-^{14}C]-triolein (specific activity, 112.0 mCi/mmol, DuPont NEN, Boston, MA), 565 μmol triolein (95%, Sigma Chemical, St. Louis, MO), and 396 μmol sodium taurocholate with or without 40 μmol 1,2-dilinoleoyl PC (>99%, Avanti Polar Lipids, Alabaster, AL) in 24 mL of PBS. The lipid emulsions with PC and without PC were used to determine whether luminal PC infusion restores the intestinal absorption of αTP. Lymph samples were collected during lipid infusion at hourly intervals for 8 h in preweighed ice-chilled plastic tubes containing 30 μg of n-propyl gallate (Sigma Chemical, St. Louis, MO) 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 (^{14}C-OA) absorbed. The hourly rate of ^{14}C-OA absorption was expressed as percent (%) of the total dose of ^{14}C-radioactivity infused.

2.5. Measurement of lymphatic αTP absorption

An aliquot (100-μL) of the hourly lymph sample was pipetted into a glass test tube. Anhydrous sodium sulfate (150 mg; 99%, Acros Organics, Pittsburgh, PA) and 1.0 mL of acetone were added [13]. The contents were mixed vigorously on a vortex mixer. Following centrifugation at 1,000 $\times$ g at 4°C for 10 min, the organic phase was filtered through a PTFE syringe filter (0.45 μm, Alltech Associates, Deerfield, IL), dried under N_2, and redissolved in a defined volume of chloroform-methanol (1:3, v/v). An internal standard (α-tocopheryl acetate) was added into each sample to monitor recovery, which generally exceeded 94%. αTP was determined by a Beckman HPLC with System Gold software (Beckman Instruments) equipped with a C-18 reversed-phase column (Alltima C18, 5 μm, 4.6 $\times$ 150 mm, Alltech Associates, Deerfield, IL). Methanol was used as the mobile phase at 2.0 mL/min [14]. Detection was monitored at 292 nm (Module 166, Beckman Instruments, Fullerton, CA). Under these conditions, αTP was eluted at 4.1 min, and α-tocopheryl acetate at 5.3 min. A standard linear curve (peak area vs. ng αTP, $r = 0.999$) was generated by using αTP standards ranging from 110.5 to 442.3 pmol. The concentration of αTP per 100-μL lymph was used to calculate the amount of αTP absorbed into the lymph collected at hourly intervals.

2.6. Lymph PL and serum zinc analyses

Using 100-μL lymph samples, PL was measured colorimetrically (UV-1201 Spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD) by a modification [7] of the method of Raheja et al. [15]. For serum zinc analysis, blood was collected via the orbital sinus [16] at 2 and 4 wk, and serum was separated by centrifugation at 1,000 $\times$ g at 4°C for 60 min. Serum was diluted 1:2 (v/v) with deionized water. Zinc was determined by an atomic absorption spectrophotometer using an air-acetylene flame (Perkin-Elmer, Norwalk, CT). The zinc standards were prepared from a Fisher-certified reference standard solution (Fisher Scientific, Pittsburgh, PA).

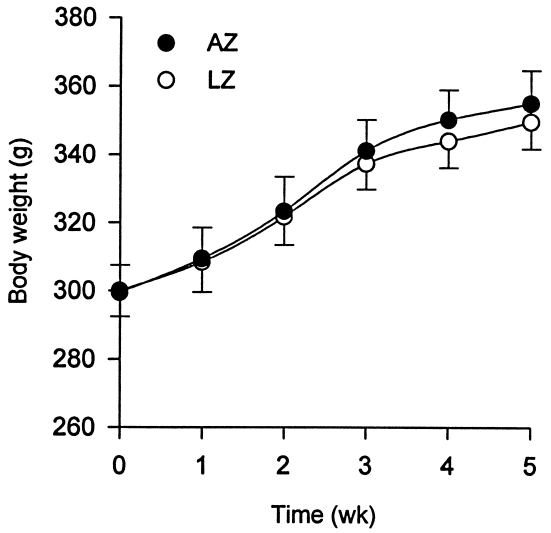

Fig. 1. Changes in the mean body weights of rats fed a diet low in zinc (LZ) and of those pair-fed a diet adequate in zinc (AZ) for 5 wk. Values are expressed as means ± SD, n = 10.

2.7. Statistical analysis

All statistical analyses were performed using PC SAS [17]. Student's t-test was used to compare group means at designated time intervals. ANOVA and the least significant difference test were performed to detect time-dependent changes within groups. The level of significance was determined at $P < 0.05$.

3. Results

3.1. Body weight and serum zinc

Under the meal-feeding conditions, both LZ and AZ rats continually gained weight throughout 5 wk (Fig. 1). No significant difference in body weight was noted between groups. The average body weight of LZ rats at 5 wk was at 98% that of AZ controls. Except for a slightly slower rate of weight gain, no external symptoms of zinc deficiency were detectable in LZ rats. The serum concentrations of zinc, as measured at 2 and 4 wk, were significantly lower in LZ rats (11.1 ± 0.7 μmol/L) than in AZ rats (20.2 ± 0.9 μmol/L) (Table 2).

3.2. Lymphatic absorption of ^{14}C-OA

The average hourly rates of lymph flow and the total lymph volumes for 8 h did not differ between LZ and AZ rats (Table 3), whether PC was infused or not. With no PC infusion, the rates of lymph flow in LZ and AZ rats were 2.4 ± 0.9 mL/h and 3.1 ± 0.4 mL/h, respectively, with no significant difference in total 8-h lymph volume between LZ (18.9 ± 7.1 mL) and AZ (24.4 ± 3.4 mL) groups. With PC infusion, the rates of lymph flow in LZ and AZ rats were 2.6 ± 0.2 mL/h and 2.7 ± 0.3 mL/h, respectively. The total volumes of lymph for 8 h were 20.4 ± 1.8 mL in LZ rats and 21.6 ± 2.3 mL in AZ rats, which did not differ significantly.

Table 2
Serum zinc and body weights in rats fed low zinc (LZ) and adequate zinc (AZ) diets for 5 wk[a]

	AZ	LZ
Serum zinc, μmol/L		
2 wk	19.1 ± 1.1	11.0 ± 1.1*
4 wk	20.2 ± 0.9	11.1 ± 0.7*
Body weight at 5 wk, g	355.0 ± 9.7	349.6 ± 7.9

[a] Means ± SD; n = 5 for serum zinc and n = 10 for body weight.
* Significantly different from AZ at the same time ($P < 0.05$).

During infusion of the lipid emulsion containing no PC, the intestinal absorption of ^{14}C-OA was significantly lower in LZ rats than AZ rats (Table 3). The average hourly rates of ^{14}C-OA absorption for 8 h were 5.1 ± 0.5% dose in LZ rats and 6.0 ± 0.3% dose in AZ rats. The total absorptions of ^{14}C-OA for 8 h were 40.7 ± 4.1% dose in LZ rats and 48.1 ± 2.2% dose in AZ control rats (Table 3, $P < 0.05$). The significant difference in cumulative ^{14}C-OA absorption was noticed beginning at 3 h of lipid infusion. When the lipid emulsion containing PC was infused, however, the hourly rates of ^{14}C-OA absorption in LZ rats were restored completely to those of AZ controls. The average hourly rates of ^{14}C-OA absorption in LZ and AZ rats during the 8-h period were 6.5 ± 0.3% dose and 6.7 ± 0.2% dose, respectively. The maximal rates of ^{14}C-OA absorption were 8.6 ± 0.5% dose/h in LZ rats and 9.4 ± 0.7% dose/h in AZ controls. The total 8-h absorptions of ^{14}C-OA in LZ and AZ rats were 51.7 ± 2.8% and 53.5 ± 2.0% dose, respectively (Table 3), which were not significantly different.

Table 3
Cumulative lymphatic absorptions of α-tocopherol (αTP) and triolein labeled with ^{14}C (^{14}C-OA) and output of phospholipid (PL) during duodenal infusion of a lipid emulsion with or without phosphatidylcholine (PC) in rats fed low zinc (LZ) and adequate zinc (AZ) diets[a]

	AZ	LZ
Without PC		
Lymph, mL/8 h	24.4 ± 3.4	18.9 ± 7.1
αTP, nmol/8 h	581.1 ± 69.9	430.8 ± 54.5*
% dose/8 h	16.3 ± 2.0	12.1 ± 1.5*
^{14}C-OA, % dose/8 h	48.1 ± 2.2	40.7 ± 4.1*
PL, μmol/8 h	34.7 ± 1.7	29.1 ± 3.5*
With PC		
Lymph, mL/8 h	21.6 ± 2.3	20.4 ± 1.8
αTP, nmol/8 h	347.3 ± 47.7	311.0 ± 20.2
% dose/8 h	9.8 ± 1.3	8.7 ± 0.6
^{14}C-OA, % dose/8 h	53.5 ± 2.0	51.7 ± 2.8
PL, μmol/8 h	38.1 ± 2.8	36.2 ± 2.7

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

3.3. Lymphatic absorption of αTP

The α-tocopherol present in the lymph should represent mostly all-rac dl-α-tocopherol, because the form of α-tocopherol infused was all-rac dl-α-tocopherol. It is possible that RRR-α-tocopherol might have been introduced into the intestinal lumen via the biliary route. However, the amount of α-tocopherol at the baseline (0 h) was negligible (3.0–6.0 nmol/h).

When PC was not infused intraduodenally, the lymphatic absorption of αTP occurred at a significantly slower rate in LZ rats (15.1 ± 2.2 nmol/h) than in AZ rats (22.8 ± 4.0 nmol/h) during the initial 4-h period (Fig. 2A). The average hourly rate of αTP absorption for the 8-h period also was significantly lower in LZ (53.8 ± 6.9 nmol/h) than in AZ (72.6 ± 8.7 nmol/h) rats. Consequently, the total 8-h absorption of αTP was lower significantly in LZ (430.8 ± 54.5 nmol; 12.1 ± 1.5% dose) than in AZ rats (581 ± 69.9 nmol; 16.3 ± 2.0% dose) (Table 3). When PC was infused, the rate of αTP absorption in LZ rats was reduced drastically and failed to rise with time after 1 h (Fig. 2B), which was in contrast to the immediate increase observed in the rate of ^{14}C-OA absorption. Likewise, PC infusion also reduced the rate of αTP absorption in AZ rats to the level of LZ rats, whereas it significantly increased the rate of ^{14}C-OA absorption. The peak rates of αTP absorption were observed at 2 h in both LZ (47.8 ± 10.3 nmol/h) and AZ (52.7 ± 8.8 nmol/h) rats. At 3 h and thereafter, the absorption rates in both groups began to decline gradually in a parallel fashion and did not differ between groups at any hourly interval. The average hourly rates of αTP absorption in LZ and AZ rats were 38.9 ± 2.5 nmol/h and 43.4 ± 6.0 nmol/h, respectively. The total absorptions of αTP for 8 h were 311.0 ± 20.2 nmol in LZ rats and 347.3 ± 47.7 nmol in AZ control rats, with no significant difference between groups (Table 3).

3.4. Lymphatic output of PL

When the lipid emulsion devoid of PC was infused, significant differences were observed between LZ and AZ groups in both the average rate of PL output and the total amount of PL released into the lymph. The average hourly rates of PL output were 3.6 ± 0.4 μmol in LZ rats and 4.3 ± 0.2 μmol in AZ rats. The rates of PL output peaked at 5 h at 4.3 μmol/h in LZ rats and 5.4 μmol/h in AZ rats, with a significant difference between groups. The total amounts of PL released into the lymph during 8 h were 29.1 ± 3.5 μmol in LZ rats and 34.7 ± 1.7 μmol in AZ controls (Table 3). When the lipid emulsion containing PC was infused, the lymphatic output of PL was increased rapidly in LZ rats and restored to the level of AZ controls. The average rates of PL output in LZ and AZ rats were 4.5 ± 0.3 μmol/h and 4.8 ± 0.4 μmol/h, respectively, with no significant difference between groups. The maximal hourly rates of PL output, as occurred at 5 h, were 5.6 ± 0.4 μmol in LZ and 6.1 ± 0.4 μmol in AZ rats. With PC infusion, no significant difference was observed in the 8-h cumulative outputs of PL between LZ (36.2 ± 2.7 μmol) and AZ (38.1 ± 0.4 μmol) rats (Table 3).

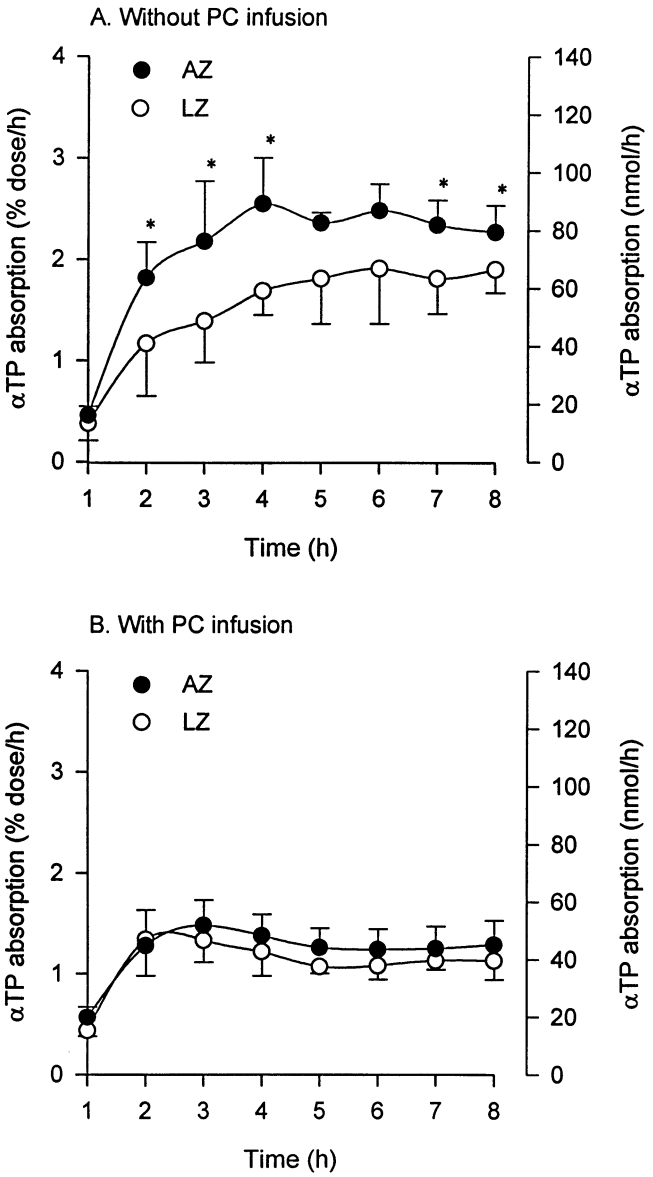

Fig. 2. The lymphatic absorption of α-tocopherol (αTP) at hourly intervals for 8 h during luminal infusion of lipid emulsion with or without phosphatidylcholine (PC) in rats fed diets low (LZ) and adequate in zinc (AZ). All values are expressed as means ± SD, n = 5. Asterisks (*) denote significant differences at the time interval at $P < 0.05$.

4. Discussion

The present study confirmed our previous finding [7] that feeding of a low zinc diet significantly lowers the intestinal absorptions of fat and αTP. A new finding from the present study is that luminally infused PC further lowers the lym-

phatic absorption of αTP in LZ rats, whereas it restores the absorption of fat and lymph PL output to the control levels. These observations clearly indicate that the intestinal absorptions of fat and αTP are mediated by a mechanism sensitive to the animal's zinc status, but show that luminal PC influences their absorptions via distinctly different mechanisms.

At present, the mechanism underlying the adverse effect of low zinc intake on αTP absorption is not understood clearly. Furthermore, the precise nature of the inhibitory effect of luminal PC on αTP absorption is unknown. Based on our earlier observations, [18–22] we previously proposed that, in zinc deficiency, the enterocyte fails to form chylomicrons at a normal rate during fat absorption because of a lack of the surface PC coat required for the formation of chylomicrons, the principal carriers of fat and fat-soluble vitamins. Under physiological conditions, PL (mostly PC) secreted via the biliary route is a major contributor to chylomicron PC. Therefore, a sufficient supply of biliary PC is essential for the production of chylomicrons in the intestinal absorptive cells and hence for lipid absorption [23]. Evidence suggests that the PL concentrations of liver microsomal membranes are lowered markedly in zinc deficient rodents [24–26]. Because most of the PL released into the bile originates from the preformed pool of microsomal PL, the low concentrations of PL in the liver microsomes may limit the amount of PC secreted into the bile and hence into the intestinal lumen during fat absorption. The limited availability of PC may result in impaired absorption of fat and fat-soluble vitamins [7–9,27]. This view also is supported by a significantly lower lymphatic output of PL and a rapid restoration of lymphatic absorption of fat by intraduodenal infusion of PC in LZ rats, as shown by the present and previous studies [7,9]. Luminally infused PC not only contributes to the surface coat of chylomicrons produced in the enterocyte, but also may serve as a direct signal that triggers the intestinal secretion of apoB-48 and triacylglycerol [28, 29]. Evidence also indicates that intestinal apoB mRNA editing is zinc-dependent [30–32]. A modest decrease in apoB mRNA editing activity has been observed in zinc deficient hamster [33]. However, Nassir et al. [34] demonstrated that apoB mRNA editing in the rat intestine and liver is preserved in zinc deficiency. Thus, a defect in the intestinal formation of apoB-48 may not be a direct cause of the impaired absorption of lipids observed in marginal zinc deficiency.

The present finding that luminally infused PC interferes with αTP absorption is somewhat unexpected, given that, under the same experimental conditions, the lymphatic absorption of fat and PL output in LZ rats are increased to normal by luminal infusion of PC. These PC-mediated increases were more pronounced in LZ rats, restoring fat absorption and PL output to control levels. However, the adverse effect of PC on αTP absorption appears to be mediated via a mechanism(s) independent of the animal's zinc status, because PC infusion inhibited αTP absorption in both LZ and AZ rats. The exact mechanism underlying the inhibitory effect is not readily apparent. The intestinal absorption of αTP is influenced by a number of luminal and intracellular factors, including the rate of luminal lipid hydrolysis, formation and diffusion of micelles through the unstirred water layer, interaction of αTP with other lipids incorporated into bile-salt micelles, its uptake and incorporation into chylomicrons, and their entry into the lymphatic system [35]. As discussed above, PC is important for the formation and secretion of chylomicrons and hence for stimulation of lipid transport from the enterocyte. In our own studies, we have shown repeatedly that PC infusion produces a rapid increase in fat absorption [9,12]. Thus, it is unlikely that the inhibition of αTP absorption by PC is associated with its interference with the intracellular processing and packaging of fat and the fat-soluble vitamin into chylomicrons. The site of interaction between PC and αTP appears to be in the intestinal lumen. In the intestinal lumen, lipids of endogenous or exogenous origin are present in various physical states, including an oil phase consisting of unhydrolyzed lipids and an aqueous phase consisting primarily of lipid hydrolytic products solubilized in mixed micelles. Micellar solubilization is thought to be necessary for diffusion of αTP across the unstirred water layer and subsequent uptake via the brush-border membrane [35]. Previous work showed that PC alone has no ability to solubilize αTP, but, in the presence of bile salts, it greatly enhances the solubility of αTP and increases the size of micelles [36]. The expansion of the micellar size induced by PC was found to be associated with the decrease in αTP absorption, as measured by using the rat small intestine perfused in situ [37]. Evidence from other studies also showed that inclusion of PC in bile-salt micelles increases their size and slows the rate of diffusion of mixed lipid micelles across the unstirred water layer [38].

At present, however, it still remains unclear how luminal PC, as infused in a lipid emulsion, enhances the lymphatic absorption of fat (fatty acid), but decreases the absorption of αTP. The differential effect of PC may be explained by the degree of its hydrophobic interactions with fatty acid and αTP within the micellar lipid matrix in which they are incorporated. Previously, the rate of desorption (transfer) of a lipid from PC-containing vesicles was shown to be a function of its hydrophobic interactions with PC and other lipids within the matrix [39]. For example, using Caco-2 cells in vitro, Homan and Hamelehle [40] showed that the presence of PC in mixed micelles reduced markedly the uptake of cholesterol, an extremely hydrophobic lipid, whereas micellar lysophosphatidylcholine (lysoPC) or hydrolysis of PC by phospholipase A_2 (PLA_2) abolished completely such an effect of PC. The PC in mixed micelles, however, did not affect adversely the uptake of oleic acid and monooleoylglycerol [40]. These in vitro observations are consistent with our findings here that luminally infused PC slows the lymphatic absorption of αTP, whereas it enhances the absorption of fat (fatty acid) [7–9]. This effect

of luminal PC may explain partly the reason why enteral infusion of PC restores the absorption of fat to normal in LZ rats, [9] whereas it fails to improve the absorption of αTP in both LZ and AZ rats, as observed here. We recently observed that lysoPC, when infused enterally, simultaneously restored the absorption of αTP and fat in LZ rats to the control levels [41]. This finding suggests that luminal PC hydrolysis by pancreatic PLA_2 may be defective in LZ rats. Lindahl and Tagesson [42] demonstrated that pancreatic PLA_2 avidly binds zinc in vitro and its activity is markedly stimulated by zinc in the presence of Ca^{+2} and bile salts. Thus, it is probable that zinc deficiency or low zinc intake may result in a decrease in pancreatic PLA_2 activity. Such a defect would not only hinder PC hydrolysis, but also limit the availability of PC in the enterocyte needed for the formation and secretion of chylomicrons, [23,43] resulting in impaired absorption of lipids and lower lymphatic output of PL in LZ rats.

In summary, the present study clearly demonstrates that low zinc intake or compromised zinc status results in impaired intestinal absorption of fat and vitamin E, along with a parallel decrease in lymphatic fat absorption and PL output. The data provide convincing evidence that intraduodenal infusion of PC further lowers the absorption of αTP in LZ rats, whereas it restores the absorption of fat. This effect of PC appears to be independent of the rat's zinc status. The results indicate that PC influences the intestinal absorptions of fat and αTP via distinctly different mechanisms.

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