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Intraduodenal phosphatidylcholine infusion restores the lymphatic absorption of vitamin A and oleic acid in zinc-deficient rats

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Previously, the lymphatic absorption of retinol was shown to be lowered markedly in zinc-deficient (ZD) rats, in parallel with a decrease in phospholipid (PL) output. The present study investigated whether an intraduodenal infusion of PL would restore the intestinal absorption of vitamin A in ZD rats fed 3 mg of Zn/kg of diet, as compared with a zinc adequate pair fed (PF) rats fed 31 mg of Zn/kg. Both ZD and PF rats were trained for meal feeding and fed equal amounts of the respective diets twice daily for 5 weeks. Each rat with lymph cannula was infused at 3 mL/hr via a duodenal catheter with a triolein emulsion containing 5.4 μCi ^{3}H-retinol (^{3}H-ROH) and 70 nmol retinol with or without 40 μmol phosphatidylcholine (PC). The absorption of ^{3}H-ROH was measured by collecting lymph hourly for 8 hr during lipid infusion. When infused with no PC, the absorption of ^{3}H-ROH was significantly lower in ZD rats beginning at 2 hr. Until it plateaued at 5 hr, the retinol absorption in ZD rats occurred at 0.42 nmol/hr, which was about 50% of the rate observed in PF rats. The cumulative absorption of ^{3}H-ROH for 8 hr was 19.0 ± 1.4% in ZD and 29.7 ± 1.6% dose in PF rats. The cumulative PL secretion was 7.5 ± 1.1 μmol in ZD and 14.2 ± 1.1 μmol in PF rats. Also, the output of PL remained significantly lower at each hourly interval in ZD than in PF rats. The hourly PL output was correlated closely with the hourly ^{3}H-ROH absorption ($r = 0.80$, $P < 0.05$). When PC was infused, the rate of ^{3}H-ROH absorption and total absorption for 8 hr in ZD rats were restored completely to the PF level, with a simultaneous increase in lymphatic PL output. In both ZD and PF rats, the ^{3}H-ROH absorption increased at 0.97 nmol/hr prior to 5 hr and reached a maximum at 3.9 nmol/hr at 5 hr. The PL outputs for 8 hr in ZD and PF rats were 14.6 and 21.6 μmol, respectively. The lymphatic output of oleic acid was correlated highly with ^{3}H-ROH absorption ($r = 0.80$, $P < 0.05$). These findings provide the first evidence that a limited supply of PL to the enterocyte is a primary cause of the impaired absorption of retinol and oleic acid in ZD rats. This may be due to a defect in the biliary secretion of PL into the intestinal lumen. It is postulated that, because of the lack of PL, the enterocyte of ZD rats fails to form chylomicrons, principal carriers of dietary lipids and lipid-soluble nutrients. (J. Nutr. Biochem. 6:604–612, 1995.)

Keywords: intestinal absorption; phospholipid; vitamin A; zinc deficiency

Introduction

In previous studies,[1,2] we have provided evidence that the intestinal absorption of lipids is impaired in zinc deficiency. The impaired absorption appeared not to be due to defects in the luminal digestion of lipids or in the mucosal uptake of hydrolytic products, but primarily to the defective intestinal formation of chylomicrons, judging from the massive accumulation of large lipid droplets in the enterocyte.[1] These lipid droplets exhibited a strong tendency to coalesce within the cytoplasm of the intestinal epithelium, suggestive of altered composition of chylomicron coats caused by a lack of the surface materials such as phospholipid (PL). In subsequent studies[3,4] using marginally zinc-deficient (ZD) rats, we also observed the massive accumulation of large-sized lipid droplets in the enterocyte. The compositional analysis

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of chylomicrons showed a general lack of surface components such as PL and apoB. The findings further suggested that the "exit block" of large lipid droplets might be caused by the enterocyte's failure to form mature chylomicrons because of inadequate surface components.

Phosphatidylcholine (PC) of biliary origin is a major contributor to chylomicron surface PC.[5–7] The latter is derived largely from reacylation of lysophosphatidylcholine (LPC) absorbed after the hydrolysis of luminal PC.[5,6] Thus, an adequate supply of PC to the enterocyte is important for the formation and release of chylomicrons.[8] In a recent study,[9] we investigated whether zinc deficiency indeed causes a decrease in lymphatic PL output and whether the amount of PL secreted is related to the lymphatic absorption of retinol. The absorption of ^{3}H-retinol was tested in view of the evidence that in zinc deficiency serum levels of vitamin A are markedly lowered and clinical signs of vitamin A deficiency symptoms are manifested.[10,11] Data showed that the lymphatic secretion of PL was decreased drastically in ZD rats. In a close parallel with the decrease in PL output, the intestinal absorption of both retinol and oleic acid was significantly lowered in ZD rats.

The above observations suggest that an insufficient biliary secretion of PL into the intestine may be the probable cause of the low lymphatic output of PL and impaired absorption of lipids in zinc deficiency. To test whether the shortage of luminal PL is indeed rate-limiting, we investigated whether an intraduodenal infusion of PC would restore the intestinal absorption of lipids in ZD rats. In the present experiment, we trained zinc-deficient rats and pair-fed controls for meal feeding and compared the effects of intraduodenally infused PC on the rates of ^{3}H-retinol absorption and its relationship with the lymphatic output of PL.

Methods and materials

Animals and diet

Twenty mature Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN USA) weighing 250 to 260 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room and subjected to a light-dark cycle with the light period from 9:00 p.m. to 9:00 a.m. and the dark period from 9:00 a.m. to 9:00 p.m. The rats were cared for in an animal care facility at Kansas State University which was 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. To establish a meal feeding pattern, all rats were fed twice daily at 8:30 a.m. and 3:30 p.m. according to the following protocol. After food was withheld from rats for 24 hr, they were fed 5 g of a zinc-adequate diet per meal for the first 2 days, 6 g/meal for the next 4 days, and 7 g/meal for the next 7 days. With this feeding protocol, the rats completely consumed each meal within 3 hr. After this training period, the rats were divided into the following two groups and fed 7 g of their respective diets at 8:30 a.m. and 8 g at 3:30 p.m.: (1) a ZD group, fed a diet containing 3.0 mg of zinc/kg and (2) a pair fed (PF) group, fed a diet with 31 mg of zinc/kg. The diets were formulated by Dyets Inc. (Bethlehem, PA USA) according to the recommendations of the American Institute of Nutrition (*Table 1*).[12] The diet, as purchased, contained 1.0 mg of zinc/kg and was supplemented with zinc carbonate to provide 3.0 mg/kg. All animals were given free access to deionized water delivered via a stainless-steel watering system. The ZD rats were paired initially with PF rats of similar body weight. Both ZD and PF rats were fed equal amounts of the respective diets. Blood samples (2 mL) were withdrawn from the orbital sinus[13] at 3 and 4 weeks to monitor the zinc status of ZD rats.

Table 1 Composition of zinc-deficient diet*

Ingredient	g/kg
Egg white	200
Corn starch	396.486
Dyetrose (dextrinized corn starch)	132
Dextrose	100
Cellulose	50
Soybean oil†	70.014
Mineral mix‡	35
Vitamin mix	10
Biotin (1 mg/g of biotin sucrose mix)	4
Choline bitartrate	2.5

*Formulated and supplied from Dyets (Bethlehem, PA USA) according to the recommendations of the American Institute of Nutrition.[12]
†Contained 0.02% Tert-butylhydroquinone.
‡With the omission of zinc, as purchased. The diet was supplemented with zinc carbonate to provide 3.0 mg of zinc/kg of diet.

Cannulation of the mesenteric lymph duct

After food was withheld for 18 hr, the mesenteric lymph duct was cannulated as described previously.[14] With halothane anesthesia, an abdominal incision was made along the midline 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 placed via the gastric fundus into the upper duodenum and secured by a purse-string suture (4-0 Silk; Ethicon Inc., Somervelle, NJ USA). After the abdominal incision was closed, the rats were placed in restraining cages[15] 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 consisting of 277 mM glucose, 144 mM NaCl, and 4 mM KCl/L at the rate of 3 mL/hr by using an infusion pump (Harvard Apparatus, Model 935, South Natick, MA USA).

Measurement of the lymphatic absorption of ^{3}H-ROH

After postoperative recovery, each rat was infused with a lipid emulsion containing ^{3}H-ROH (specific activity: 42.1 Ci/mmol; NEN Research products, Du Pont, Wilmington, DE USA) at 3 mL/hr via the duodenal catheter in subdued light. The lipid emulsion consisted of 568 μmol triolein, 5.4 μCi of ^{3}H-ROH, 70 nmol ROH, 148 nmol α-tocopherol, and 396 μmol sodium taurocholate with or without 40 μmol egg yolk phosphatidylcholine (PC) (Purity: 99.0%; Sigma Chemical, St. Louis, MO USA) in 24 mL of phosphate buffered saline (PBS; 6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl, and 5 mM KCl; pH 6.4). Analysis by atomic absorption spectrometry showed that the lipid emulsion contained 0.008 ppm of zinc mainly contributed by the source of PC. This represented a total of 0.19 μg of zinc in 24 mL infused for 8 hr. As determined by gas chromatography (GC), the fatty acid composition of the egg yolk PC was (in % mass): 27% C16:0,

15% C18:0, 15% C18:1, 37% C18:2, and 6% C20:4. The lymph samples were collected hourly in preweighed ice-cold centrifuge tubes containing 4 mg of Na_2-EDTA. The hourly lymph samples (100 μL) were mixed with scintillation liquid (ScintiVerse; Fisher Scientific Co., Fair Lawn, NJ USA) and counted to determine ^3H radioactivity appearing in the lymph (Beckman LS-8100; Beckman Instruments, Fullerton, CA USA).

Determination of the lymphatic output of lipids and fatty acids

Triglyceride was determined enzymatically[16] after dilution (1:20, vol/vol) of lymph samples (100 μL) with 150 mM NaCl. Lymph phospholipid was measured by the method of Raheja et al.[17] For fatty acid analysis, lipids were extracted from the lymph samples by the method of Folch et al.[18] The lipid extracts were saponified and methylated.[19] Methylesters of fatty acids were separated on a Stabilwax-DA capillary column (15 m × 0.53 mm i.d.; Resteck Corp., Bellefonte, PA USA) in a Hewlett-Packard Model 5880A GC (Hewlett-Packard, Palo Alto, CA USA) equipped with a flame-ionization detector. Methyl-nonadecanoic acid (C19:0) (Nu Chek, Elysian, MN USA) was used as an internal standard for quantitation of fatty acids.

Vitamin A analysis

Blood samples (2 mL) were withdrawn from the orbital sinus[13] and serum separated by centrifugation at 1,000*g* for 60 min. After exsanguination, the liver was removed and blotted. To prevent decay and loss of vitamin A, all samples were handled in subdued light and in the cold. Aliquots (100 μL) of serum and lymph were extracted for lipids.[18] The extracts were filtered through the microfilter membrane (pore size: 0.45 μm TF; diameter: 13 mm; Analtech Associates, Inc., Deerfield, IL USA), dried under N_2, and dissolved in 100 μL of ethanol. Retinol and retinyl esters were separated by a normal-phase high performance liquid chromatography (HPLC) column (Absorbosphere HS, 4.6 mm × 25 cm; Analtech Associates, Inc.) and Beckman System Gold Software (Beckman Instruments). Hexane:2-propanol (95:5) was used as the mobile phase and propelled at 1 mL/min. Detection was monitored at 325 nm (UV-detector, Module 166, Beckman Instruments). Under these conditions, retinyl esters were eluted as a single peak at 3.1 min and retinol at 7.2 min. The standard curve (peak area versus nanograms of retinol or retinyl palmitate) was constructed by injecting retinoid standards. The concentrations of retinol from 2 to 24 ng and or retinyl palmitate from 20 to 240 ng yielded a linear curve (r = 0.99).

Serum and liver zinc analysis

Prior to analysis, the whole liver was minced finely with a blade razor, and 0.2 g of the minced tissue was solubilized in 25% tetramethylammonium hydroxide. Serum was diluted 1:3 with deionized water. Zinc was determined by atomic absorption spectrophotometry with an air-acetylene flame (Perkin-Elmer Co., Norwalk, CT USA). The zinc standards were prepared from a Fisher-certified reference standard solution (Fisher Scientific Co.).

Statistical analysis

Student t tests were used for single point data and the analysis of variance (ANOVA) along with the least significant difference (LSD) test of the SAS statistical package[20] for multiple-point data. All data were expressed as mean ± SD and the level of significance was determined at $P < 0.05$, unless otherwise stated.

Results

General observations

Figure 1 compares the average body weights of ZD and PF rats during 5 weeks of dietary treatment. During the first 3 weeks, no difference in the body weight was noted between the groups. The body weight of the ZD group was slightly lower at 4 weeks and reached 97% of that of PF controls at 5 weeks. Serum zinc was significantly lower in ZD rats (9.7 ± 0.8 μmol/L), compared with PF controls (17.7 ± 1.6 μmol/L). Liver zinc concentrations in ZD and PF rats were 15.4 ± 3.4 and 32.4 ± 2.6 μmol/g, respectively. No external signs of zinc deficiency were visible. The serum and liver concentrations of vitamin A are shown in *Table 2*. Under the conditions used, no difference was noted in the serum vitamin A level between groups. However, the liver concentration of vitamin A, as expressed in micromoles per gram of liver, was significantly lower in ZD (2.1 ± 0.1 μmol) than in PF (2.4 ± 0.2 μmol). The average liver weights in ZD and PF rats were 9.8 ± 0.7 and 10.5 ± 0.4 g, respectively, with no significant difference ($P > 0.05$). The total liver stores of vitamin A in ZD rats (18.5 ± 1.3 μmol) was significantly lower than those in PF rats (22.7 ± 2.2 μmol). The liver vitamin A was mostly in retinyl ester (98.6% in ZD and 98.9% in PF rats) with no significant difference between groups.

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

After postoperative recovery, a steady lymph flow was established at the rate of 1.5 to 2.0 mL/hr. The lymph flow was accelerated to the rate of 2.0 to 3.1 mL/hr after 3 hr of infusing the lipid emulsion. The peak of hourly lymph volume occurred between 5 and 6 hr. Without PC infusion, the average hourly lymph volumes in ZD and PF rats were 2.5 ± 0.3 and 2.0 ± 0.4 mL, respectively. With PC infusion, the hourly lymph volumes were 2.0 ± 0.2 in ZD and 2.3 ± 0.2 mL in PF rats.

Figure 2A compares the hourly lymphatic absorption of

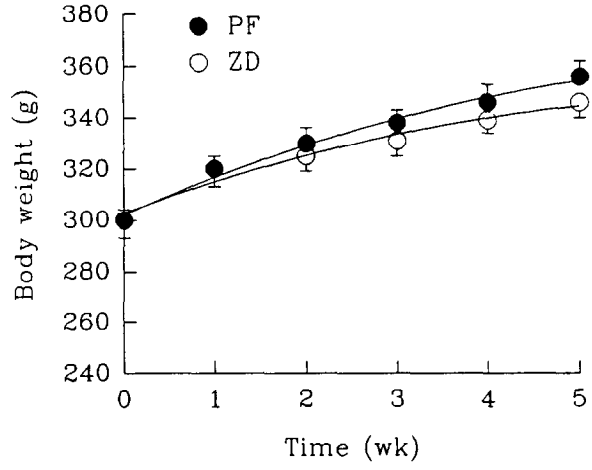

Figure 1 The average body weights of ZD and PF rats during a 5 week period. The body weights of ZD and PF rats did not differ until 3 weeks. The final body weight in ZD rats at 5 weeks was 97% of that of PF controls.

Table 2 Effect of marginal zinc deficiency on serum and liver vitamin A*

Dietary treatment	Serum (μmol/L)	Liver Total (μmol)	Liver g weight (μmol)	Liver RE† (%)
ZD	1.5 ± 0.1[a]	18.5 ± 1.3[a]	2.1 ± 0.1[a]	98.6 ± 0.2[a]
PF	1.7 ± 0.1[a]	22.7 ± 2.2[b]	2.4 ± 0.2[b]	98.9 ± 0.1[a]

*Mean ± SD, n = 5. Values in the same column not sharing a common superscript [a,b] are significantly different ($P < 0.05$).
†RE, retinyl ester.

^{3}H-ROH (% dose) and the rates of retinol absorption between ZD and PF rats not infused with PC. Starting at 2 hr, the absorption of ^{3}H-ROH was significantly lower in ZD rats than in PF rats ($P < 0.05$). Although the ^{3}H-ROH absorption of the ZD group increased gradually with time, it failed to catch up with that of PF rats. Prior to reaching its peak at 5 hr, the retinol absorption in ZD rats increased at the rate of 0.42 nmol/hr, which was less than half of the rate (0.88 nmol/hr) observed in PF controls. The cumulative absorption of ^{3}H-ROH in ZD rats was significantly lower at 2 hr and thereafter. At 8 hr, the total lymphatic absorption of ^{3}H-ROH was 19.0 ± 1.4% dose in ZD and 29.7 ± 1.6% in PF controls. In both groups, 92 to 93% of the lymph vitamin A was found in retinyl ester with no difference between the groups. *Figure 2B* compares the hourly lymphatic absorption of ^{3}H-ROH (% dose) and the rates of retinol absorption between ZD and PF rats infused with PC. The lymphatic absorption of ^{3}H-ROH in ZD rats was restored completely to the PF level, and the ^{3}H-ROH absorption in ZD rats occurred in a close parallel to that in PF rats at each hourly interval. At the peak of ^{3}H-ROH absorption, 5.5 ± 0.5% and 5.6 ± 0.4% of the dose were absorbed in ZD and PF rats, respectively. Up to 5 hr, the lymphatic retinol absorption increased rapidly at the rate of 0.97 nmol/hr in both groups. After peaking at 5 hr, the rate of absorption declined slowly at 0.27 nmol/hr in both groups. The cumulative amounts of ^{3}H-ROH absorbed in ZD and PF rats were 33.2 ± 0.7% and 33.2 ± 3.3% dose, respectively. In both ZD and PF rats, 93.0 to 93.5% of the lymph vitamin A was found in the form of retinyl ester.

Lymphatic output of phospholipid

Figure 3A presents the hourly lymphatic output of PL in the rats infused with no PC. Even with infusion of glucose saline only, a marked difference in PL output was observed between groups. At 0 hr, the basal rates of PL secretion were 0.05 μmol/hr in ZD rats and 0.73 μmol/hr in PF controls. In ZD rats, the output of PL did not rise appreciably from the basal level during the first 2 hr of lipid infusion and remained significantly lower, compared with PF rats, at each hourly interval throughout 8 hr except at 5 hr. The maximal rates of PL output was 1.4 ± 0.2 μmol/hr in ZD and 2.0 ± 0.3 μmol/hr in PF rats. The cumulative amounts of PL secreted into the lymph in ZD and PF rats were 7.5 ± 1.1 μmol and 14.2 ± 1.1 μmol, respectively

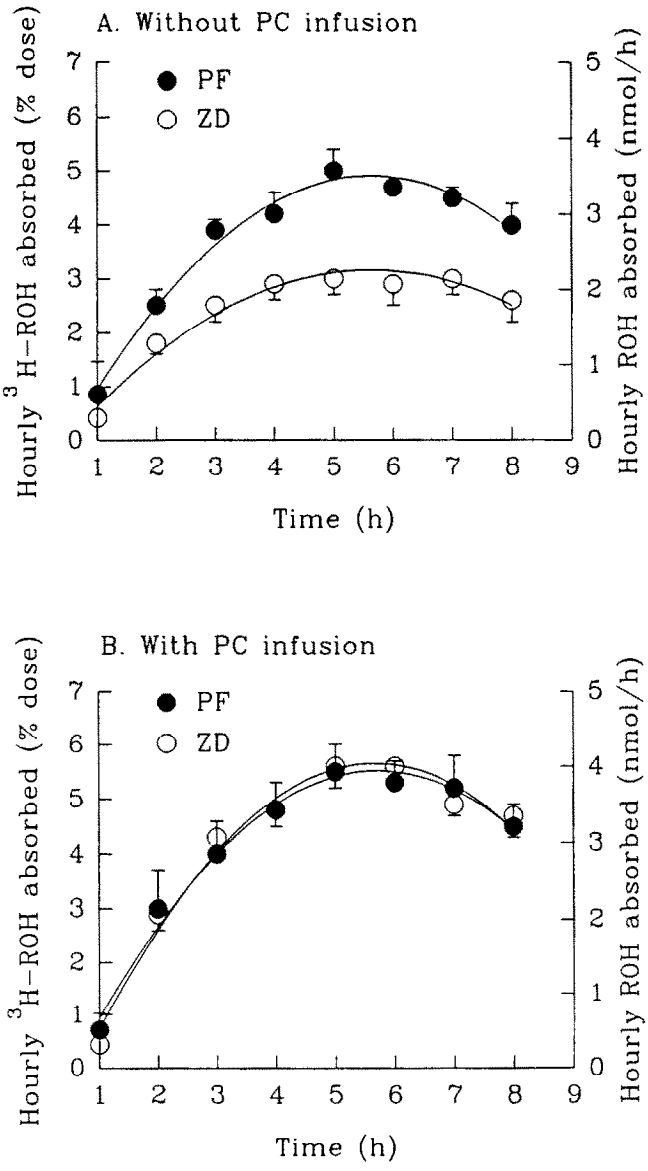

Figure 2 The lymphatic absorption of ^{3}H-ROH at hourly intervals for 8 hr. (A) When PC was not infused, the absorption of ^{3}H-ROH in the lymph was lowered markedly in ZD rats starting at 2 hr. Throughout 8 hr, the absorption of ^{3}H-ROH in ZD rats did not reach the control level in PF rats. From 1 to 5 hr, the rate of ^{3}H-ROH absorption in ZD was less than half of that observed in PF rats. (B) When PC was infused, the lymphatic absorption of ^{3}H-ROH in ZD rats was restored completely to that in PF rats at each hourly interval. Prior to reaching its peak at 5 hr, the retinol absorption increased rapidly at the rate of 0.97 nmol/hr in both groups.

(*Table 3*). When rats were infused with PC (*Figure 3B*), the hourly lymphatic secretion of PL rapidly increased to its maximal output at 4 hr in both groups. Except for 5 and 6 hr, the lymphatic secretion of PL was significantly lower in ZD rats than in PF rats. The maximal rates of PL output were 2.4 ± 0.3 μmol/hr in ZD and 3.1 ± 0.2 μmol/hr in PF rats. The cumulative output of PL was significantly lower in ZD rats. The total PL outputs for 8 hr were 14.7 ± 0.9 μmol in ZD and 21.6 ± 1.0 μmol in PF rats (*Table 3*). The PL output in ZD rats infused with PC was equal to that in the PF controls receiving no PC infusion.

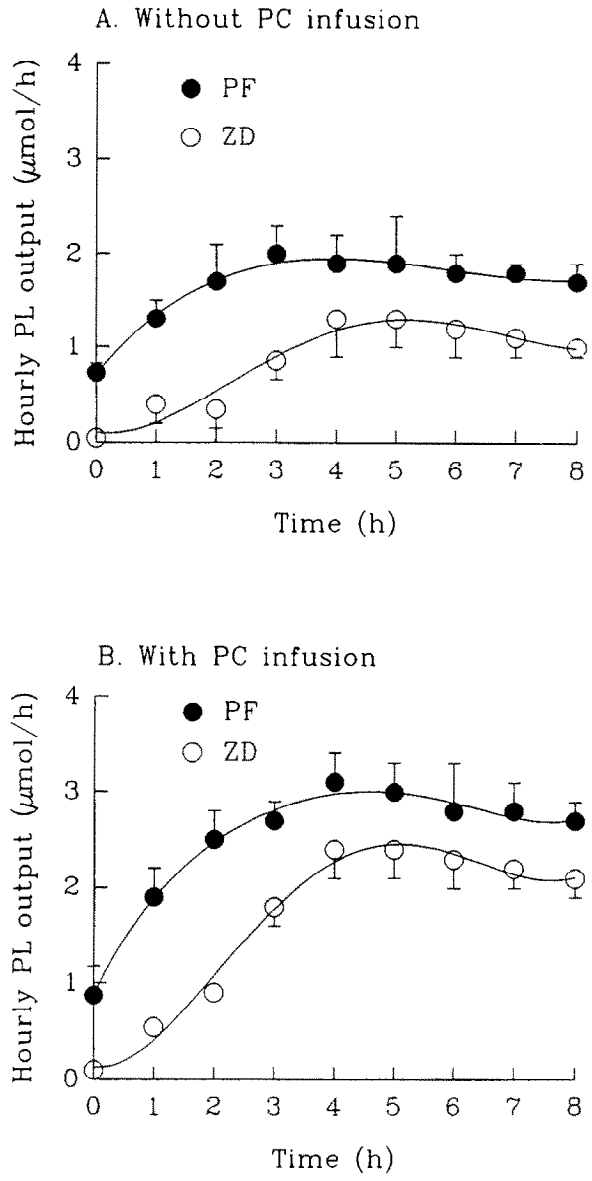

Figure 3 The lymphatic output of phospholipid (PL) at hourly intervals for 8 hr. (A) Without PC infusion, the rate of PL output did not rise appreciably from the basal level (0 hr) during the first 2 hr of lipid infusion and remained significantly lower than in PF rats at each hourly interval throughout 8 hr except 5 hr. The basal rates (at 0 hr) of PL secretion were 0.05 μmol/hr in ZD rats and 0.73 μmol/hr in PF controls. The maximal rates of PL output were 1.4 ± 0.2 μmol/hr in ZD and 2.0 ± 0.3 μmol/hr in PF rats. (B) With PC infusion, the maximal rates of PL output were 2.4 ± 0.3 μmol/hr in ZD and 3.1 ± 0.2 μmol/hr in PF rats. The total PL output for 8 hr was 14.7 ± 0.9 μmol in ZD and 21.6 ± 1.0 μmol in PF rats. The PL output in ZD rats infused with PC reached the level of the PF controls receiving no PC infusion (*Figure 3A*).

Lymphatic output of triglyceride and fatty acids

Table 3 shows cumulative lymphatic outputs of triglyceride (TG) and fatty acids for 8 hr. In the absence of PC infusion, the lymphatic output of TG in ZD rats was 165 ± 14 μmol, which was 68% of the PF level. Similarly, the lymphatic output of oleic acid (OA), as infused in triolein, was significantly lower in ZD rats (492 ± 20 μmol), compared with PF rats (828 ± 33 μmol). With PC infusion, the total TG output in ZD rats (303 ± 18 μmol) was restored completely to the control level (304 ± 11 μmol). The output of OA in ZD rats (817 ± 35 μmol) also was normalized to the control level (839 ± 31 μmol). The cumulative outputs of all other fatty acids were significantly lower in ZD, relative to PF rats, regardless of whether PC was infused. In both groups, PC infusion significantly increased the lymphatic secretion of linoleic and arachidonic acids, reflecting the high concentrations of the fatty acids in the egg PC infused.

Figure 4A illustrates the hourly lymphatic output of OA between ZD and PF rats infused with no PC. Starting at 1 hr, a significant difference was noted in OA output between groups. Between 1 and 5 hr, outputs of OA increased at the rate of 16 μmol/hr in ZD rats and at 26 μmol/hr in PF controls. When PC was infused (*Figure 4B*), the lymphatic output of OA was reduced slightly in ZD rats between 1 and 3 hr compared with PF controls. However, the secretion of OA in ZD rats was restored fully to the control level at 4 hr and thereafter. With PC infusion, the maximal rate of OA secretion in ZD rats was increased to 27 μmol/hr in ZD, which was nearly equal to that in PF rats (29 μmol/hr). The pattern of OA output closely followed that of TG.

The lymphatic output of arachidonic acid (AA) was reduced significantly in ZD rats, when they were not infused with PC (*Figure 5A*). Even at 0 hr with glucose saline infusion, the AA output was significantly lower in ZD (2.1 ± 0.3 μmol/hr) than in PF rats (3.3 ± 0.5 μmol/hr). In ZD rats, during the first 4 hr, the output of AA did not change above the basal level (0 hr), whereas a small immediate increase in AA output was evident in PF rats in response to lipid infusion. At its peak, the rates of AA output were 3.1 ± 0.5 μmol/hr in ZD and 5.0 ± 0.8 μmol/hr in PF rats. The total outputs for 8 hr were 21.4 ± 1.1 μmol in ZD and 33.7 ± 1.7 μmol in PF rats. With PC infusion, the outputs of AA began to rise slowly and were significantly above the basal level (0 hr) starting at 4 hr in both groups. The maximal rates of AA output were increased to 4.5 μmol/hr in ZD and 6.3 μmol/hr in PF rats. The cumulative secretion for 8 hr was significantly lower in ZD (27.3 ± 1.5 μmol) than in PF rats (39.2 ± 1.8 μmol). The pattern of AA release did not coincide with that of PL output. The hourly lymphatic outputs of linoleic acid (LA) followed virtually the same pattern as that of AA output (data not shown). In both groups, whether PC was infused or not, the average ratio of LA to AA was significantly greater in ZD rats (1.9 ± 0.1) than in PF rats (1.5 ± 0.1).

Discussion

By carefully controlling food intake, feeding pattern, and body weights of the rats in both groups in the present study, we have provided the first evidence that the intestinal malabsorption of vitamin A and lipids is attributable to the specific effect of zinc, rather than to a generalized effect of malnutrition. In addition, the present study clearly demonstrates that the major cause of the impaired absorption of retinol (or lipids) in ZD rats is an insufficient supply of PL to the enterocyte during chylomicron formation, which is a prerequisite for normal lipid absorption and transport from enterocyte to circulation.

Table 3 Effect of marginal zinc deficiency on the cumulative lymphatic outputs of phospholipid (PL), triglyceride (TG), and fatty acids for 8 hr*

Infusate	PL	TG	C16:0	C18:0	C18:1	C18:2	C20:4
				(μmol)			
Without PC							
ZD	7.5 ± 1.1[a]	165 ± 14[a]	47.7 ± 3.9[a]	32.2 ± 1.7[a]	492 ± 20[a]	36.5 ± 1.5[a]	21.4 ± 1.1[a]
PF	14.2 ± 1.1[b]	263 ± 13[b]	61.8 ± 5.3[b]	40.3 ± 1.3[b]	828 ± 33[b]	49.3 ± 2.5[b]	33.7 ± 1.7[b]
With PC							
ZD	14.7 ± 0.9[a]	303 ± 18[a]	51.7 ± 3.9[a]	35.0 ± 2.1[a]	817 ± 35[a]	45.0 ± 1.6[a]	27.3 ± 1.5[a]
PF	21.6 ± 1.0[b]	304 ± 11[a]	67.4 ± 2.1[b]	41.4 ± 1.9[b]	839 ± 31[a]	57.7 ± 2.1[b]	39.2 ± 1.8[b]

*Mean ± SD, $n = 5$. Values in the same column not sharing a common superscript [a,b] are significantly different ($P < 0.05$).

In the present experiment, we have shown that the lymphatic absorption of retinol is restored completed in ZD rats, when PC is infused intraduodenally. In the absence of infused PC, the cumulative ^{3}H-ROH absorption for 8 hr was 19.0 ± 1.4% in ZD rats and 29.7 ± 1.6% dose in PF controls. With PC infusion, however, the absorption of ^{3}H-ROH in ZD rats was restored completely to the control level. The total amount of ^{3}H-ROH absorbed for 8 hr was 33.2 ± 0.7% in ZD rats and 33.2 ± 3.3% dose in PF rats. Similarly, the lymphatic output of OA also was significantly lower in ZD rats without PC infusion, but restored fully to the PF level with PC infusion. The immediate and rapid increase in OA output with PC infusion suggests that luminal digestion of TG, membrane uptake of fatty acids, and resynthesis of TG in the enterocyte are not impaired in zinc deficiency. The finding also suggests packaging of the lipids (vitamin A and TG) into chylomicrons occurs normally in ZD rats, so far as PC is luminally available.

An important observation from this study was that the ^{3}H-ROH absorption and OA output occurred in a close parallel with the output of PL ($r = 0.80$, $P < 0.05$). A pronounced decrease in lymphatic PL secretion was clearly evident in ZD rats, even when the rats were infused only with glucose saline (baseline output). Furthermore, the initial PL output during the first 2 hr was delayed significantly in ZD rats, whereas there was an immediate rapid increase in PL output in PF rats, in response to the infusion of triolein without PC. When PC was infused, the rates of PL output rapidly increased from 0.1 (baseline rate) to 2.4 μmol/hr (maximal rate) in ZD rats and from 0.8 to 3.1 μmol/hr in PF rats. However, it should be noted that with PC infusion the total PL output in ZD rats reached the level (14.2 ± 1.1 μmol) of the PF rats not infused with PC. Also, the present data show that an increase in the PL output rate above 2.3 μmol/hr did not result in a further increase in ^{3}H-ROH absorption (or oleic acid output) in either ZD or PF rats. These findings show that the amount of luminal PC in ZD rats, as infused at 5 μmol/hr in the present study, was sufficient to meet the enterocyte's need for the synthesis and secretion of chylomicrons and to restore the rate of ^{3}H-ROH absorption to normal. This conclusion is in line with the previous observation[21] that luminal infusion of PC at the rate of 5 μmol/hr provided an adequate supply of PL for lipid absorption in bile fistula rats with no further increase in triglyceride output even at 20 μmol/hr.

The restoration of retinol absorption and lipid output in ZD rats by PC infusion provides clear evidence that a lack of luminal PC supply is the major defect responsible for the impaired absorption of lipids and lipid-soluble nutrients in zinc deficiency. However, the reason(s) for the decreases in the total PL output and the rate of PL output in ZD rats is not known. Even with PC infusion, the total lymphatic PL output and the rate of PL output in ZD rats did not reach that of the PF control infused with PC, as discussed above. Previous studies have suggested that, in the enterocyte, AA and LA are preferentially incorporated into PC at sn-2 position[22] and that these fatty acids may be the rate-limiting substrates for intestinal PL synthesis.[23] Our data showed that the lymphatic output of AA was significantly lower in ZD rats, with a higher ratio of LA to AA. This may be due to a defect in desaturation-elongation of LA in the intestine and/or in the liver, as previously observed in zinc deficient rats.[24,25] However, our data showed that the lymphatic PL output in either ZD or PF rats was not correlated ($P > 0.05$) with that of AA ($r = 0.05$) or LA ($r = 0.02$). This suggests that the availability of these fatty acids in the enterocyte is not a major determinant of PL output. Alternatively, the lower lymphatic output of PL may reflect a defect in delivery of luminal PL into the enterocyte, which involves the following major steps: (1) the hydrolysis of luminal PC to LPC; (2) mucosal reacylation of LPC to PC; (3) de novo synthesis of PC in the enterocyte; or (4) hepatic synthesis and biliary secretion of PC. Little information exists as to the effect of zinc on the luminal hydrolysis of PC, reacylation of LPC, and de novo synthesis of PC in the intestine. Further studies are needed to determine the exact mechanism(s) responsible. However, available information suggests that a major cause of the low lymphatic output of PL in ZD rats may be a defect in the hepatic production and/or biliary secretion of PC, because biliary PC is the major contributor to chylomicron PC[5–7] and a sufficient supply of PL via the biliary route is essential for production of normal chylomicrons during active fat absorption.[21,26–28] It is generally accepted that PC synthesized de novo in the enterocyte is not sufficient to sustain the high PC turnover during fat absorption.[26] Evidence suggests that the hepatic pool of PC may be reduced in zinc deficient animals.[29–31] In ZD rats[32] and mice,[33] the PL concentrations of liver microsomal membranes are lowered markedly. It has been shown that most of the PL secreted into the bile originates from the preformed pool of microsomal PL,[34] with only 7% derived from the de novo synthesis.[35] Thus, the low concentrations

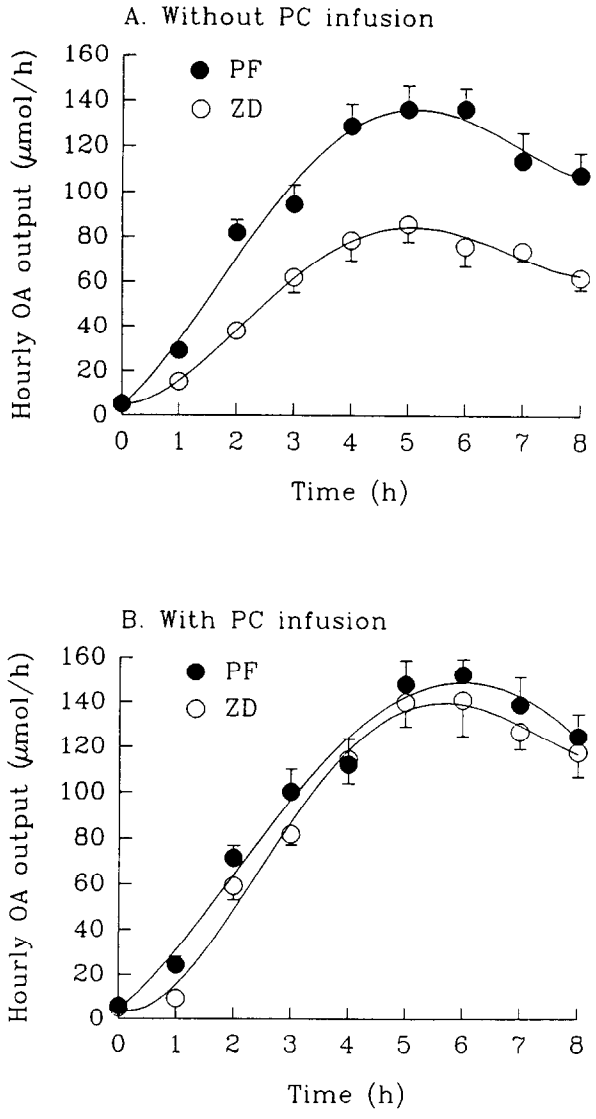

Figure 4 The lymphatic output of oleic acid (OA) at hourly intervals for 8 hr. (A) Without PC infusion, the OA output was significantly lower in ZD rats at each interval through 8 hr. The output of OA increased at a significantly slower rate in ZD rats (16 μmol/hr) than in PF rats (26 μmol/hr) up to 5 hr. (B) With PC infusion, the OA output was elevated to the level of PF controls. In Both ZD and PF rats, the maximal rate of OA secretion in ZD rats was increased to 27 μmol/hr in ZD, which was nearly equal to that in PF rats (29 μmol/hr). No difference in the cumulative output of OA was observed between ZD (817 ± 35 μmol) and PF rats (839 ± 31 μmol).

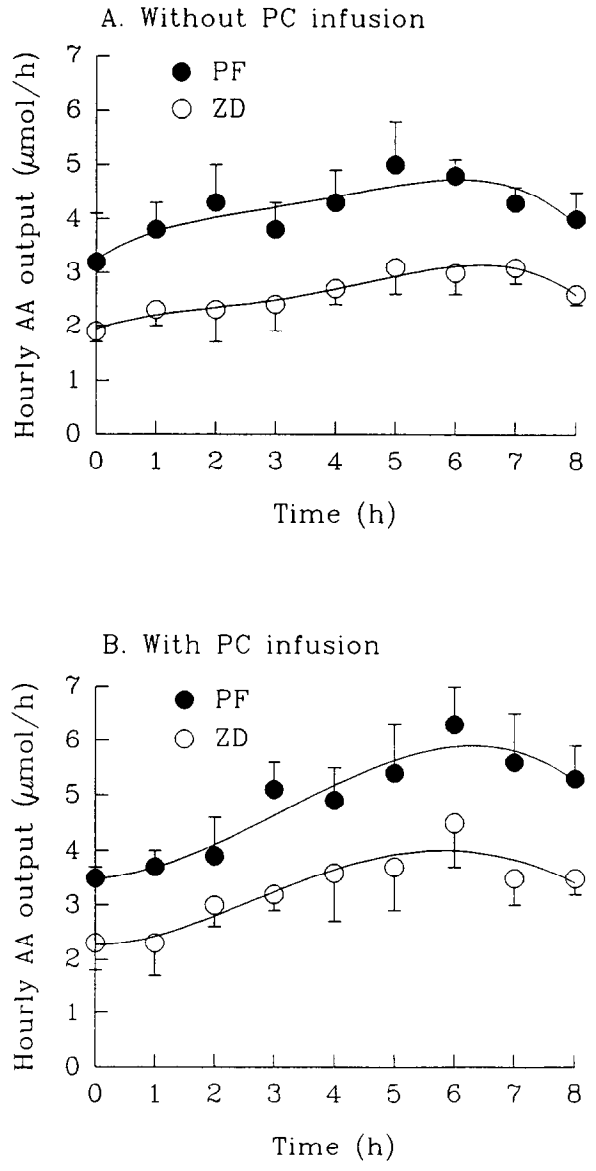

Figure 5 The lymphatic output of arachidonic acid (AA) at hourly intervals for 8 hr. (A) Without PC infusion, the lymphatic output of AA was markedly reduced in ZD rats at each hourly interval. Even at 0 hr, the AA output was significantly lower in ZD rats (2.1 μmol/hr) than in PF rats (3.3 μmol/hr). At its peak, the rate of output was 3.0 μmol/hr in ZD rats, and 5.0 μmol/hr in PF rats. The total output for 8 hr was 21.4 ± 1.1 μmol in ZD and 33.7 ± 1.7 μmol in PF rats. (B) With PC infusion, the maximal rate of AA output was increased to 4.5 μmol/hr in ZD and 6.3 μmol/hr in PF rats. The cumulative secretion for 8 hr was significantly lower in ZD (27.3 ± 1.5 μmol), compared with PF rats (39.2 ± 1.8 μmol).

of PC in the liver microsomes in ZD rats may limit the amount of PC released into the bile. In addition, the translocation of PC from microsomes to the canalicular membrane may require a proper assembly of the microtubules,[36] which may be a zinc-dependent process. Zinc has been shown to induce and stimulate the polymerization of monomeric tubulin in vitro.[37]

One may argue that the lower absorption of retinol in ZD rats might be caused by a defect in retinol esterification rather than in biliary secretion of PC and chylomicron assembly per se. Whether zinc depletion alters the intestinal activity of LRAT[38] and/or production of cellular retinol binding protein II (CRBP-II)[39] is currently unknown. However, our data suggest that a decrease in LRAT activity or lack of CRBP-II is not a likely cause for the impaired absorption of retinol in ZD rats, because no appreciable difference was observed between ZD and PF groups in lymphatic retinyl ester output. Approximately 93% of the retinol absorbed was found esterified in both groups. The possibility exists, however, that in the enterocyte of ZD rats

the amount of PC may be rate-limiting as a substrate for LRAT.

Thus the present observations indicate that a common defect responsible for the impaired absorption of retinol and OA resides in the assembly and secretion of chylomicrons due to a lack of surface PL, as postulated previously.[1–4] PC in the enterocyte not only contributes to the surface coat of chylomicrons, but may also play an important role in the intestinal synthesis of apoproteins. Deprivation of PC was shown to cause an overall decrease in protein synthesis in the intestinal mucosa.[40] Also, biliary PC may play a role in regulating the intestinal synthesis of apoB-48.[41] This suggests that PL available to the enterocyte during chylomicron formation may be an important stimulus to the intestinal synthesis of apoB-48. Recent studies have shown that zinc itself may play a crucial role in intestinal production of apoB-48. ApoB-48 is derived from the post-transcriptional editing of apoB mRNA, which involves the conversion of the CAA glutamine codon at 2153 of apoB mRNA to UAA, an in-frame stop codon.[42,43] Evidence indicates that the apoB mRNA editing enzyme in rats[44,45] and humans[46] is zinc-dependent. The marked decrease in the apoB-48 content of lymph chylomicrons from marginally ZD rats[4] suggests that the mRNA editing may be responsive to the zinc status of rats. Thus, the massive accumulation of large lipid droplets in the enterocyte and impaired lipid absorption, as observed in ZD rats,[1–4] may be attributable to the lack of cellular PL and apoB-48.

Finally, our data indicate that the liver concentrations of vitamin A are significantly lower in ZD rats. This may be attributable in part to the impaired absorption of dietary vitamin A. Our recent study[47] also has suggested that dietary vitamin A newly taken up by the liver may be susceptible to a more rapid degradation in ZD rats. Previously, other investigators have observed an increase in liver vitamin A in ZD rats,[10,48,49] which may be caused by a defect in mobilization of liver vitamin A via retinol-binding protein (RBP). A possible impairment in the hepatic synthesis of RBP in ZD rats has been suggested by Smith et al.[10] The discrepancy between these studies may stem from a difference in experimental conditions including the extent of zinc deficiency and feeding protocols used.

In summary, the present observations indicate that zinc deficiency lowers the lymphatic absorption of retinol and OA. The lowering of retinol absorption is accompanied by a decrease in lymphatic PL output. PC, when infused intraduodenally, restores the lymphatic absorption of both retinol and OA in ZD rats. The decrease in PL output is independent of the availability of AA or LA. The present finding strongly suggests that a limited supply of PL via the biliary route is a major cause of the impaired absorption of lipids. It is postulated that in zinc deficiency the enterocyte fails to assemble chylomicrons at a normal rate due to the lack of cellular PL and probably apoB-48. Further investigation is warranted to define the specific role of zinc in PL metabolism, intestinal apoB-48 production, and chylomicron assembly. A marginal stage of zinc deficiency in meal-trained rats, as produced here, may serve as a valuable model to probe the role of zinc and its possible interactive effect with PL in the intestinal assembly and secretion of chylomicrons.

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