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Low zinc intake decreases the lymphatic output of retinol in rats infused intraduodenally with β-carotene

Sang K. Noh, Sung I. Koo[1,*]

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

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Abstract

Previously, we have shown that the lymphatic absorption of retinol is significantly decreased in rats fed a low zinc diet. This study was conducted to determine whether the absorption of β-carotene also is altered in zinc-deficient male rats. The absorption of β-carotene was estimated by determining the amount of retinol appearing in the mesenteric lymph during intraduodenal infusion of β-carotene. One group of rats was fed the AIN-93G diet but low in zinc (LZ; 3 mg/kg) and the other was fed the same diet adequate in zinc (AZ; 30 mg/kg). The LZ and AZ rats were trained to meal feed equal amounts of the diets twice daily. At 6 weeks, each rat with lymph cannula was infused via an intraduodenal catheter at 3 ml/h for 8 h with a lipid emulsion containing 65.0 nmol β-carotene, 565.1 μmol triolein, 27.8 kBq ^{14}C-triolein (^{14}C-OA), 72 mg albumin, and 396 μmol Na-taurocholate in 24 ml PBS (pH, 6.7). The lymphatic output of retinol over the 8-h period was significantly lower in LZ rats than in AZ rats. The absorption of ^{14}C-OA also was significantly lower in LZ rats. No significant differences were observed between groups in intestinal β-carotene 15,15′-dioxygenase, retinal reductase, and retinal oxidase activities. The findings demonstrate that low zinc intake or marginal zinc deficiency significantly lowers the absorption of β-carotene as estimated by lymphatic retinol output. The results also indicate that the decrease in retinol output in LZ rats is not linked to defects in β-carotene cleavage and subsequent conversion of retinal to retinol in the intestinal mucosa. This study suggests that zinc status is an important factor determining the intestinal absorption of β-carotene and hence the nutritional status of vitamin A.

Keywords: β-carotene; Intestinal absorption; Phospholipid; Retinol; Zinc; Rat

1. Introduction

The nutritional status of zinc influences vitamin A metabolism in animals and humans and the deficiencies of both zinc and vitamin A are frequently observed in undernourished populations [1,2]. Previously, we have shown that zinc deficiency in rats significantly lowers the intestinal absorption of fat and fat-soluble vitamins including retinol [3-8]. β-Carotene is the major source of vitamin A from plant-based diets for a large segment of the world's population and, especially, for those with limited resources in developing countries [9–12]. At present, however, no information is available concerning whether the nutritional status of zinc affects the intestinal absorption of β-carotene and/or its conversion to retinol in the intestine.

The present study was conducted to determine whether low zinc intake influences the intestinal absorption of β-carotene and the activities of 15,15′ dioxygenase and other enzymes involved in the intestinal conversion of β-carotene in rats. In this study, we used rats with lymph cannulae to indirectly assess the intestinal absorption of β-carotene by determining the lymphatic output of retinol during intraduodenal infusion of β-carotene.

2. Materials and methods

2.1. Animals

Eight-wk old male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN), weighing 231–245 g, were housed individually in polypropylene cages with stainless steel grid bottoms in a room controlled at 22–24°C and

* Corresponding author. Tel.: +1-860-486-3495; fax: +1-860-486-3674.

E-mail address: skoo@canr.uconn.edu (S.I. Koo).

[1] Current address: Department of Nutritional Sciences, University of Connecticut, Storrs, CT, USA

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Table 1
Composition of low-zinc diet[a]

Ingredient	Amount
	g/kg
Egg White	200.0
Cornstarch	396.5
Dextrinized cornstarch	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 from Dyets, Bethlehem, PA, according to the recommendations of the American Institute of Nutrition [13].

[b] Contained 0.02% tert-butylhydroquinone.

[c] With the omission of zinc, as purchased. The diet was supplemented with zinc carbonate to provide 3.0 mg zinc/kg diet. The mineral mix was modified to adjust for the mineral contents of egg white used in place of casein [14].

60–70% humidity, and subjected to a daily 12-h light:dark cycle with the light period from 2100 to 0900 h. During 2 weeks of acclimation, rats had free access to a nutritionally adequate rodent diet [13] and deionized water as provided via a stainless-steel watering system. Rats were cared for in an animal facility of the Department of Human Nutrition at Kansas State University, accredited by the American Association for the Accreditation of Laboratory Animal Care, in accordance with the animal care and use guidelines approved by the Kansas State University Institutional Animal Care and Use Committee.

2.2. Meal feeding

In an attempt to equalize the food intakes, growth rates, and feeding patterns between LZ and AZ rats, all rats were trained to meal feed twice a day at 0930 and 1530 h, as previously described in our studies [5–8]. Briefly, after 2 weeks on the AIN-93G standard diet, rats were starved for 24 h and given 5 g/meal of the same diet for the first 2 days and 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 h and 7.5 g at 1530 h. With this feeding schedule, rats consumed each meal completely within 2 h. After the meal training period, rats were assigned randomly to the following two groups: 1) LZ group, fed the AIN-93 diet but low in zinc (3.0 mg of Zn/kg) and 2) AZ group, fed the same diet but adequate in zinc (30.0 mg of Zn/kg). Both 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. A basal diet (Table 1) was formulated by Dyets Inc. (Bethlehem, PA) according to the AIN-93G recommendations [13], with the following modifications: 1) egg white was used as the protein source and dextrose in place of sucrose and 2) 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 [14]. This basal diet contained 1.0 mg of Zn/kg and was supplemented with zinc carbonate to prepare the LZ and AZ diets.

2.3. Cannulation of the mesenteric lymph duct

At 6 weeks, rats were starved overnight for 15 h prior to surgery. The mesenteric lymph duct was cannulated as previously described in detail [5,15]. Postoperatively, the rats were placed in restraining cages and allowed to recover for 20 h in a recovery chamber maintained at 30°C. During the postoperative recovery, phosphate buffered saline (PBS) containing glucose (in mmol/L: 277 glucose, 6.75 Na_2HPO_4, 16.5 NaH_2PO_4, 115 NaCl, and 5 KCl; pH 6.7) was infused through the duodenal catheter at 3.0 ml/h via a syringe pump (Model 935, Harvard Apparatus, South Natick, MA).

2.4. Determination of lymphatic ^{14}C-labeled triolein absorption

Lymph was collected for 1 h prior to lipid infusion as the fasting lymph. Rats were then infused with a lipid emulsion containing β-carotene. The lipid emulsion consisted of 65.0 nmol all-trans-β-carotene (>99%, Acros Organics, Pittsburgh, PA), 27.8 kBq of [carboxyl-^{14}C]-triolein (specific activity, 4.14 GBq/mmol, DuPont NEN, Boston, MA), 565.1 μmol triolein (95%, Sigma Chemical, St. Louis, MO), 396.0 μmol sodium taurocholate (97%, Sigma Chemical, St. Louis, MO), and 72.0 mg bovine albumin (98%, Sigma Chemical, St. Louis, MO) in 24 ml of PBS buffer. The emulsion was freshly prepared under a gentle N_2 stream and subdued light prior to infusion. Lymph was collected at hourly intervals for 8 h in preweighed ice-chilled plastic tubes containing 30 μg of n-propyl gallate and 4 mg of Na_2-EDTA.

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 (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. Determination of lymphatic retinol output

Total retinol (free and esterified retinol) was extracted from lymph by the method of Ross [16]. Aliquots (100-μl) of lymph samples were saponified in 10 vol of 95% ethanol and 5% potassium hydroxide solution (Fisher Scientific, Pittsburgh, PA) containing 1% pyrogallol as antioxidant (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 was analyzed by a Beckman HPLC (Beckman Instruments, Fullerton, CA) equipped with a C-18 reversed-phase column (Alltima C18, 5 μm, 4.6 $\times$ 150 mm, Alltech Associates, Deerfield, IL). Methanol:water (99:1, v/v) was used as the mobile phase and propelled at 1 ml/min [17]. Detection was monitored at 325 nm (UV detector, Module 166, Beckman Instruments, Fullerton, CA). Under these conditions, retinol and retinyl acetate were eluted at 3.4 and 4.6 min, respectively. The concentrations of retinol from 2.79 to 13.26 pmol yielded a linear curve ($r = 0.99$).

2.6. Phospholipid and zinc analyses

From 100-μl aliquots of lymph samples, phospholipid (PL) was measured colorimetrically (UV-1201 Spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD) by the method of Reheja et al [18], as detailed previously [5]. For zinc analysis, blood was collected via the orbital sinus [19] and serum was separated by centrifugation at 1,000 $\times$ g at 4°C for 60 min. A 250-μl serum sample was diluted 1:3 (v/v) with deionized water. Intestinal mucosa (300 mg) was digested in 2 ml of 25% tetramethylammonium hydroxide at 65°C for 30 min and diluted with 7 ml of deionized water prior to analysis. 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, Pittsburgh, PA).

2.7. Assay of β-carotene 15,15′-dioxygenase activity

The assay procedure was a modification of the method described by van Vliet et al [20]. Briefly, rats were killed with halothane overdose after a 15-h food deprivation. The first 30-cm intestinal segment (duodenum and jejunum) was immediately removed, slit open, and spread on an ice-cooled glass plate. Mucus was removed by flushing with ice-cold PBS buffer containing 16.5 mmol/L sodium taurocholate and blotting gently with absorbent paper three times. The intestinal mucosa was scraped off with a microscope slide. The mucosal scrapings were homogenized for 2 min with a Teflon pestle in 5 ml of ice-cold potassium phosphate buffer [in mmol/L: 100.0 potassium phosphate, 4.0 $MgCl_2$, and 1.0 dithiotreitol (Sigma Chemical, St. Louis, MO), pH 7.7]. The homogenate was centrifuged at 9,000 $\times$ g at 4°C for 20 min. The protein content of the supernatant was 11.1-16.3 mg/ml, as determined by using the BCA Assay Reagents (Pierce Chemical, Rockford, IL). The intestinal β-carotene 15,15′-dioxygenase (EC 1.13.11.21) assay was performed with 50 nmol all-trans-β-carotene and 3.5 mg of intestinal supernatant protein in 2 ml of potassium phosphate buffer [in mmol/L: 100.0 potassium phosphate, 30.0 NAD (Sigma Chemical, St. Louis, MO), 2.0 $MgCl_2$, 5.0 glutathione (Sigma Chemical, St. Louis, MO), 1.7 sodium dodecyl sulfate, 6.0 sodium taurocholate, 0.263 egg phosphatidylcholine (PC), and 0.25 α-tocopherol, pH 7.7]. The mixture was incubated in a shaking water bath at 37°C in the dark for 1 h. The reaction was stopped by adding 2 ml of ethanol.

2.8. Assay of retinal reductase and retinal oxidase activities

The assay procedure was essentially the same as described by Sundaresan et al [21]. The intestinal mucosal scrapings were homogenized for 2 min with a Teflon-glass homogenizer in 5 ml of 250 mmol/L sucrose solution containing 25 mmol/L KCl. The homogenate was centrifuged at 145,000 $\times$ g at 4°C for 1 h. The protein concentration of the supernatant preparation was 6.2-10.5 mg/ml. The supernatant was chosen as the source of retinal reductase (EC 1.1.1.1) and retinal oxidase (EC 1.2.1.3). The assay was performed with 500 nmol retinal (all-trans-retinal, Sigma Chemical, St. Louis, MO) in 2 ml of potassium phosphate buffer [100.0 mmol/L potassium phosphate, 30.0 mg Tween 40 (Acros Organics, Pittsburgh, PA), 0.5 mmol/L NADH (Sigma Chemical, St. Louis, MO), 1.0 mmol/L glutathione, pH 7.2] in a shaking water bath at 37°C in the dark for 1 h. The reaction was stopped by adding 5 ml of ethanol. Controls, with identical reaction mixtures, were run at zero time by adding 5 ml of ethanol. Retinal reductase activity was expressed as nmol of retinol formed $\times$ h^{-1} $\times$ mg $protein^{-1}$ while retinal oxidase activity was calculated from the difference between the control value and the sum of retinal and retinol values in each test tube.

2.9. HPLC analysis of β-carotene and retinoids

Lymph samples and enzyme assay mixtures were mixed with an equal vol of ethanol containing 1% pyrogallol (99%, Acros Organics, Pittsburgh, PA) and extracted twice with an equal vol of hexane. After a brief centrifugation, the upper phase was transferred into a vial, dried under N_2, and resuspended in chloroform:methanol (1:5, v/v). All-trans-retinyl acetate and β-apo-8′-carotenal (>98%, Fluka Chemical, Milwaukee, WI) were added as internal standards. HPLC analyses for β-carotene and retinoids were performed simultaneously using a Beckman HPLC automated system with System Gold Nouveau software equipped with a Beckman Model 168 photodiode-array detector and 507E autosampler (Beckman Instruments, Fullerton, CA). β-Carotene was monitored at 465 nm, β-apo-8′-carotenal at 380 nm, and other retinoids at 325 nm. β-Carotene and retinoids were separated on a C-18 reverse-phase column (Alltima C18, 5 μm, 150 $\times$ 4.6 mm, Alltech Associates, Deerfield,

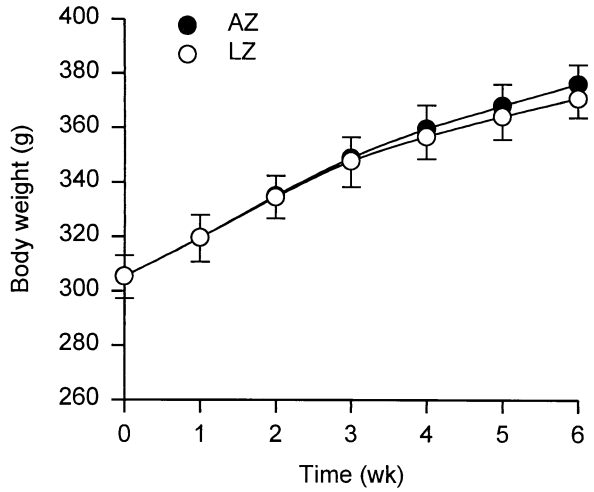

Fig. 1. Changes in the average body weights of rats fed diets low (LZ) and adequate in zinc (AZ) for 6 weeks. All values are expressed as means ± SD, n = 5.

IL) attached to a precolumn (Alltima C18, 5 μm, 7.5 × 4.6 mm). The mobile phase consisting of 100% methanol and 0.5% ammonium acetate was pumped at a flow rate of 0.5 ml/min for retinoids and after retinyl acetate peak, the flow rate was switched to 2.0 ml/min [22]. Under these conditions, retinol, retinal, retinyl acetate, β-apo-8′-carotenal, and β-carotene had retention times of 6.4, 7.3, 9.3, 11.4, and 27.9 min, respectively. The detection limit of β-carotene under the analytical conditions was 1.8 pmol per injection (50 μl).

2.10. Statistical analysis

Data were expressed as means ± SD. All statistical analyses were performed using PC SAS (SAS Institute, Cary, NC). Repeated measures ANOVA and the least significance difference test were used to compare group means at designated time intervals and time-dependent changes within groups. The level of significance was determined at $P < 0.05$.

3. Results

3.1. Body weight and serum zinc

Both LZ and AZ rats continually gained weight throughout 6 weeks despite 15% food restriction under the meal feeding conditions (Fig. 1). The average body weights did not differ between groups. At 6 weeks, the average body weight of LZ rats was at 98.6% that of AZ controls. The serum concentrations of zinc at 3 and 6 weeks were significantly lower in LZ rats than in AZ rats (Table 2), with no external deficiency symptoms observed in LZ rats.

Table 2
Serum zinc concentrations and body weights in rats fed diets low (LZ) and adequate in zinc (AZ) for 6 wk[a]

	AZ	LZ
Serum zinc, μmol/L		
3 wk	19.1 ± 1.1	9.2 ± 0.5*
6 wk	19.1 ± 1.0	9.6 ± 0.3*
Body weight at 6 wk, g	376 ± 12	371 ± 12

[a] Means ± SD; n = 5. * Significantly different from AZ at the same time ($P < 0.05$).

3.2. Lymph flow

When the lipid emulsion was infused, lymph flow increased rapidly and peaked at 4-5 h in both LZ and AZ rats. The rates of lymph flow, however, did not differ between the groups. The hourly rates were 3.0 ± 0.5 ml/h in LZ rats and 3.0 ± 0.4 ml/h in AZ rats. No differences in total volume of lymph were noted between groups (Table 3).

3.3. Lymphatic retinol output

Prior to infusion of the lipid emulsion, the basal (0 h) lymphatic outputs of retinol in LZ and AZ rats were 0.21 ± 0.04 nmol/h and 0.27 ± 0.05 nmol/h, respectively, with no significant difference between groups (Fig. 2). As the lipid emulsion containing β-carotene was infused intraduodenally, the lymphatic output of retinol began to increase rapidly in both groups (Fig. 2). However, the lymphatic output of retinol increased at a significantly slower rate (3.12 ± 0.29 nmol/h) in LZ rats than in AZ controls (3.91 ± 0.32 nmol/h) before reaching its peak at 5 h and 4 h, respectively. The average hourly rates over the 8-h period were 4.08 ± 0.33 nmol/h in LZ rats and 4.95 ± 0.49 nmol/h in AZ rats. The cumulative output of retinol also was significantly lower in LZ rats than in AZ rats at 2 h and thereafter (Table 3). Throughout the 8-h period of absorption, no intact β-carotene was detectable in the mesenteric lymph collected.

3.4. Lymphatic ^{14}C-OA absorption and PL output

The lymphatic absorption of ^{14}C-OA was significantly lower in LZ rats than in AZ rats. Up to 5 h, the absorption

Table 3
Cumulative lymphatic absorption of triolein labeled with ^{14}C (^{14}C-OA) and outputs of retinol and phospholipid during duodenal infusion of a lipid emulsion containing β-carotene in rats fed diets low (LZ) and adequate in zinc (AZ)[a]

Lipids	AZ	LZ
Retinol, nmol/8 h	39.6 ± 4.0	32.7 ± 2.6*
^{14}C-OA, % dose/8 h	53.0 ± 3.5	45.3 ± 3.8*
Phospholipid, μmol/8 h	32.1 ± 1.9	28.0 ± 2.4*
Lymph, mL/8 h	23.9 ± 2.9	24.2 ± 3.8

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

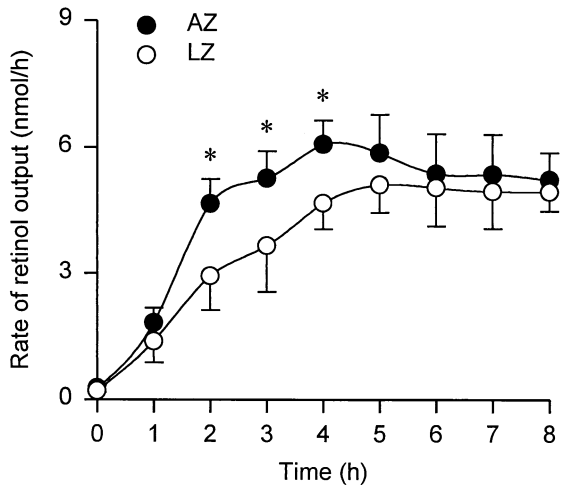

Fig. 2. The lymphatic output of retinol at hourly intervals for 8 h during intraduodenal infusion of lipid emulsion with β-carotene in rats fed diets low (LZ) and adequate in zinc (AZ). All values are expressed as means ± SD, n = 5. *Significantly different from LZ rats at the time point at P <0.05.

of ^{14}C-OA increased at 5.16 ± 0.39% dose/h in LZ rats, whereas it increased at 6.18 ± 0.35% dose/h in AZ rats with a significant difference between groups. The average hourly rates were 5.66 ± 0.48% dose/h in LZ rats and 6.66 ± 0.44% dose/h in AZ rats. The total cumulative ^{14}C-OA absorption for 8 h was significantly lower in LZ rats than in AZ rats (Table 3, $P < 0.05$).

The hourly rate of PL output also was significantly lower in LZ rats than in AZ rats. Coinciding with the peaks of retinol and ^{14}C-OA absorption, the maximal hourly rates of PL, as occurred at 4 h, were 4.2 ± 0.2 μmol/h in LZ rats and 4.8 ± 0.3 μmol/h in AZ rats. The total amount of PL released for 8 h were 28.0 ± 2.4 μmol in LZ rats and 32.1 ± 1.9 μmol in AZ rats, with significant differences between groups at 5 h and thereafter (Table 3, $P < 0.05$).

3.5. Activities of intestinal β-carotene 15,15′-dioxygenase and retinal reductase and oxidase

No significant difference was observed in intestinal β-carotene 15,15′-dioxygenase between LZ and AZ rats (Table 4). Also, no differences were noted in activities of retinal reductase retinal oxidase between groups. However, mucosal zinc was significantly lower in LZ rats than in AZ rats (Table 4, $P < 0.05$).

4. Discussion

Previous studies have demonstrated that the intestinal absorption of intact β-carotene is minimal (0–2%) in rats because the rat intestine is highly efficient in converting β-carotene to retinol [23,24]. In the present study, virtually no β-carotene was detectable in mesenteric lymph, whereas the lymphatic output of retinol steadily increased with time both in the LZ and AZ rats. This study shows that the lymphatic output of retinol during intraduodenal infusion of β-carotene is significantly lowered in rats fed a diet low in zinc (3 mg zinc/kg diet). The lower retinol output was associated with simultaneous decreases in lymphatic fat (^{14}C-OA) absorption and PL output. At present, the mechanism underlying this effect of zinc is unknown. The intestinal absorption of β-carotene is governed by several major steps, which include the solubilization of β-carotene into mixed bile-salt micelles, micellar diffusion across the unstirred water layer, uptake by the enterocyte, incorporation into chylomicrons, and lymphatic secretion [23–25]. A defect in any of the luminal and intracelluar processes would potentially slow the rates and amounts of β-carotene uptake and absorption. Upon its uptake by the enterocyte, β-carotene is converted into retinal as the primary product by β-carotene 15,15′-dioxygenase and subsequently to retinol prior to incorporation into chylomicrons as retinyl esters [24,26]. The efficiency of conversion of β-carotene in the enterocyte, however, varies substantially depending on animal species [23]. Our observations here showed that the activity of mucosal β-carotene 15,15′-dioxygenase in LZ rats tended to decrease in LZ rats but did not significantly differ from that of controls, even though the mucosal concentration of zinc was markedly lowered in LZ rats. It remains to be seen if the activity of the enzyme is reduced in more advanced zinc deficiency. Also, the activities of retinal reductase and retinal oxidase were unchanged in these rats. These observations suggest that the conversion of β-carotene to retinal and subsequently to retinol in the intestinal mucosa is not attributable to the lower lymphatic output of retinol in LZ rats.

Based on findings from our previous studies [3–8,15], we postulated that the impaired absorption of fat and fat-

Table 4
Mucosal β-carotene 15,15′-dioxygenase, retinal reductase, and retinal oxidase activities and zinc levels of intestines of rats fed diets low (LZ) and adequate in zinc (AZ)[a]

Group	β-Carotene dioxygenase	Retinal reductase	Retinal oxidase	Zinc
	pmol retinal · mg protein^{-1} · h^{-1}	nmol retinol or retinoic acid · mg protein^{-1} · h^{-1}		nmol zinc · mg protein^{-1}
AZ	55.2 ± 11.4	7.8 ± 1.0	20.2 ± 2.1	2.0 ± 0.2
LZ	48.1 ± 5.7	7.8 ± 0.6	18.4 ± 3.3	1.3 ± 0.2*

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

soluble vitamins in LZ rats is caused by the limited availability of lysophosphatidylcholine (lysoPC) in the intestinal lumen, which is due to a defect in pancreatic PLA_2 activity. This hypothesis is based on our observations 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 h) compared to AZ controls (31.0 ± 1.9 μmol/8 h) with no significant changes in PL classes and their fatty acid compositions (unpublished data), and that an intraduodenal infusion of lysoPC immediately restores the absorption of fat and fat-soluble vitamins in LZ rats [7,15]. Evidence from in vitro studies with intestinal cells [27–29] strongly suggests that the initial hydrolysis of biliary or dietary PC to lysoPC by pancreatic PLA_2 is a step necessary for the efficient hydrolysis of triacylglycerol by pancreatic lipase/colipase [27–29], micellar formation and diffusion across the unstirred water layer [30,31], and stimulation of lipid uptake by the enterocyte [27–29]. The hydrolysis of PC to lysoPC is particularly critical for the intestinal uptake and absorption of extremely hydrophobic lipids such as β-carotene [32], α-tocopherol [15], and cholesterol [27,29], because the presence of intact PC in bile salt micelles slows the transfer (uptake) of those more hydrophobic lipids, without affecting the transfer of other less hydrophobic lipids such as fatty acids and monoacylglycerol [27,33]. Furthermore, lysoPC, whether provided in lipid emulsions or produced from luminal hydrolysis of PC by pancreatic PLA_2, stimulates the intracellular packaging and release of lipids into the lymphatics by facilitating reacylation of absorbed lipids and formation and secretion of chylomicrons [34,35]. In keeping with our hypothesis, Sugawara et al. [32] reported that lysoPC in mixed micelles or addition of pancreatic PLA_2 to the culture medium containing micellar PC markedly enhanced β-carotene uptake by Caco-2 cells, whereas intact PC suppressed its uptake in a dose-dependent manner.

At present, however, no definitive explanation is available for how the animal′s zinc status may influence the activity of pancreatic PLA_2 and, hence, the luminal availability of lysoPC. Evidence from an in vitro study [36] indicates that pancreatic PLA_2 binds zinc, and its activity is markedly enhanced by zinc in the presence of bile salts and Ca^{++}. It also is possible that zinc deficiency may adversely affect the production and/or secretion of PLA_2 from the pancreas, as suggested by a profound decrease in zymogen granules and quiescent morphological characteristics of the exocrine pancreas of zinc deficient rats [37]. Further studies are warranted to elucidate the exact mechanism involved.

Studies have shown that the serum levels of vitamin A are significantly lower in zinc deficient animals [38–40] and humans, particularly, premature infants and growing children [41–43]. Supplemental zinc and vitamin A have been shown to be effective in improving vision restoration in dim light in undernourished children [44] and women [45–47] in developing countries. Our data here demonstrate that the intestinal absorption of β-carotene (or retinol derived from it) is adversely affected by low zinc intake (or marginal zinc deficiency). This finding is consistent with the previous observations that β-carotene supplementation failed to raise the serum levels of retinol and eliminate night blindness in undernourished pregnant and lactating women [45], who are highly susceptible to zinc deficiency [45–47].

In summary, this study provides the first direct evidence that the animal's zinc status is an important determinant of β-carotene absorption, as assessed by the lymphatic output of retinol during intraduodenal β-carotene infusion. Our findings here suggest that the intestinal conversion of β-carotene to retinol remains unaffected. This study aimed at producing a mild zinc deficiency under the conditions of matched food intakes, body weights, and feeding patterns. Thus, it remains to be understood whether the intestinal and/or hepatic conversion of β-carotene to retinol is impaired in more advanced stages of zinc deficiency. The exact mechanism underlying the effect of zinc on intestinal β-carotene absorption awaits further investigation.

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