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The lymphatic absorption of lipids is normalized by enteral phosphatidylcholine infusion in ovariectomized rats with estrogen replacement

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Estrogen (ES) replacement previously was shown to decrease the lymphatic absorption of fat with a simultaneous decrease in phospholipid (PL) output in ovariectomized (OX) rats. The present study was conducted to investigate whether intraduodenal infusion of phosphatidylcholine (PC) would normalize the absorption of ^{14}C-linoleic acid (^{14}C-LA) and unlabeled oleic acid (OA) in OX rats implanted s.c. with an estradiol pellet (OXE; 25 μg/day/rat), compared with OX rats implanted with a placebo pellet (OXP). Additionally, this study examined whether ES would alter the rate of fatty acid esterification into various lipids in the intestinal mucosa. Both groups were meal-trained and pair-fed for 7 weeks. Each rat with a lymph cannula was infused at 3 mL/hr via a duodenal catheter with a lipid emulsion containing ^{14}C-LA and triolein (380 μmol) with or without PC (41 μmol). Lymph was collected hourly for 8 hr via a lymph fistula. Without PC infusion, the total lymphatic absorption of ^{14}C-LA was significantly lower (26.0 ± 3.4% dose) in OXE rats than in OXP rats (31.8 ± 2.0% dose) with a close parallel decrease in OA output at 2 hr and thereafter. Also, the total lymphatic secretion of PL was significantly lower in OXE rats (26.5 ± 2.4 μmol) compared with OXP rats (37.1 ± 4.1 μmol). When PC was infused, the total lymphatic absorption of ^{14}C-LA and OA in OXE rats was restored completely to normal, whereas PC infusion had no effect on the lymphatic outputs of ^{14}C-LA or OA in OXP rats. The lymphatic outputs of PL were enhanced markedly in both groups with PC infusion. The hourly output of PL was correlated highly with the absorption of ^{14}C-LA (r = 0.81) and OA (r = 0.78). Regardless of whether lysophosphatidylcholine (lysoPC) was present in the intestinal lumen, the rates of mucosal ^{14}C-LA incorporation into triglycerides (TG), PL, and other lipids were not affected by ES treatment. The results indicate that the slower rate of lymphatic absorption of fatty acids in ES-treated rats is not due to alteration in mucosal fatty-acid esterification, but to a limited availability of PL to the enterocyte. This may be associated with an inhibitory effect of ES on the hepatic secretion of PL via the biliary route. (J. Nutr. Biochem. 8:152–161, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

Estrogen (ES) is known to influence fat metabolism via multiple mechanisms. ES treatment causes a significant loss of body fat in animals[1,2] and prevents the increase of body fat and abdominal fat mass in postmenopausal women.[3,4] Evidence indicates that ES inhibits lipogenesis,[5–7] suppresses lipoprotein lipase activity,[8,9] and increases fatty acid mobilization from the adipose tissue.[7,10,11]

ES treatment in male rats is shown to cause a drastic

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reduction in the rate of intestinal fat absorption with decreases in the lymphatic secretion of apolipoproteins.[12,13] In a recent study,[14] we showed that ES treatment in ovariectomized (OX) rats significantly lowers the lymphatic absorption of fatty acids. An important finding from that study[14] was that the lower fatty acid absorption was correlated strongly with a decrease in the lymphatic output of phospholipid (PL). This observation suggests that less PL may be available to the enterocyte of the ES-treated rats during fat absorption, which, in turn, may slow the rate of chylomicron formation. The finding also provides the first evidence that ES affects fat metabolism at the intestinal level. Such an effect of ES may not only alter the rate of energy influx from the intestine, but also have a significant impact on the subsequent metabolism and assimilation of dietary fat.

The limited supply of PL during fat absorption in ES-treated rats may be associated with a decrease in biliary PL secretion into the intestinal lumen. Available evidence indicates that ES decreases bile flow[15–18] and biliary lipid secretion,[19–21] which are accompanied by impaired synthesis and transport of bile acids.[15,16,22] ES treatment also is shown to decrease sinusoidal membrane lipid fluidity[17,18,23] and increase the membrane cholesterol-to-phospholipid molar ratio.[24,25] At present, however, little information is available concerning the mechanism underlying the effect of ES on bile flow. In addition, whether ES treatment alters the biliary secretion of PL during intestinal fat absorption is not known.

The lower intestinal absorption of fat also may be attributable partly to change in the rate of fatty acid esterification in the enterocyte. ES treatment in male rats is known to decrease the rate of fatty acid esterification in the intestinal mucosa during fat absorption.[13] Evidence also indicates that a significant reduction in mucosal PC, as observed in bile-diverted rats, is associated with a decrease in acyltransferase activity of the intestinal microsomes and that the mucosal activity of acyltransferase is restored when PC is infused luminally.[26,27]

Based on these observations, the present study was conducted to determine whether PL, when provided enterally, normalizes the rates of intestinal lipid absorption and of mucosal fatty acid esterification in ovariectomized rats with ES replacement.

Methods and materials

Experiment 1

Animals and diet. Twenty female Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN USA) initially weighing 201 ± 5 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room maintained at 22 to 24°C and a daily 12-hr light/dark cycle with the light period from 0330 to 1530 hr. The rats were housed in an animal care facility at Kansas State University, approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The rats were given free access to a nutritionally adequate diet (*Table 1*) formulated according to the AIN-93 recommendations[28] by Dyets Inc. (Bethlehem, PA USA) and deionized water until they were assigned to treatment groups. The deionized water was obtained from a water purification system (Millipore Corporation, Marlboro, MA USA) and delivered via a stainless-steel watering system.

Table 1 Composition of diet[1]

Ingredient	g/kg
Egg white	200
Corn starch	396.486
Dyetrose (dextrinized corn starch)	132
Dextrose	100
Cellulose	50
Soybean oil[2]	70.014
Mineral mix	35
Vitamin mix	10
Biotin (1 mg/g sucrose mix)	4
Choline bitartrate	2.5

[1]Formulated by Dyets, Bethlehem, PA, according to the recommendations of the American Institute of Nutrition.[28]
[2]Contained 0.02% tert-butylhydroquinone.

Estrogen implantation and meal feeding. At the end of 7 weeks, rats weighing 245 ± 9 g were ovariectomized under halothane anesthesia. After 2 weeks of postoperative recovery, the rats were assigned randomly to the following two groups with 10 rats each. One group, weighing 270 ± 10 g, was implanted s.c. with a 60-day-release 17β-estradiol pellet (1.5 mg/pellet; Innovative Research of America, Toledo, OH USA) (OXE) in the back of the neck, and the other, weighing 267 ± 11 g, was implanted with a placebo.

Previously, we observed that OXE rats consumed significantly less food than OXP rats. In order to delineate the specific effect of estradiol, both groups of rats should be pair fed and trained to develop a similar feeding behavior. To accomplish this, we used a method of meal feeding. All rats were fed twice daily at 0830 and 1530 hr. A meal feeding pattern was established as follows. To train them for meal feeding, the average daily food intake of OXE rats was monitored each day for 4 days, and the OXP rats were fed the same amounts of diet. After 4 days, food intake was restricted to 90% of the average. Both OXE and OXP rats were pair-fed 4.5 g/rat at 0830 and 6.0 g/rat at 1530. Each meal was consumed within 45 to 55 min by OXE rats and within 30 min by OXP rats.

Cannulation of lymph duct. After 7 weeks of estrogen implantation, food was withheld for 23 hr and anesthetized by using a halothane vaporizer with a constant supply of oxygen (2.0% halothane in 1.5 to 2.0 L oxygen/min). The mesenteric lymph duct was cannulated as described previously.[29,30] Briefly, after an abdominal incision was made along the midline by using a cauterizer, the major intestinal lymph duct was cannulated with a clear vinyl tube (medical grade, I.D. 0.5 mm, O.D. 0.8 mm; Dural Plastics and Engineering, Dural, Australia), and the cannula was secured with methyl cyanoacrylate. An intraduodenal infusion catheter was placed by passing a silicone tube (Silastic medical grade tubing; I.D. 1.0 mm, O.D. 2.1 mm; Dow Corning Medical Products, Midland, MI USA) through the gastric fundus into the proximal duodenum, and was secured by closing the fundic incision by a purse-string suture (4-0 silk; Ethicon Inc., Somerville NJ USA). Both the lymph cannula and duodenal catheter were exteriorized through the right flank. After the abdomen was closed, the rat was placed in a restraining cage, housed in a recovery chamber kept at 30°C to prevent hypothermia, and allowed to recover for 20 hr. During this period, a maintenance solution containing (in mM) 277 glucose, 144 NaCl, and 4 KCl was infused

via the catheter at a rate of 3 mL/hr by using an infusion pump (Harvard Apparatus, Model 935, South Natick, MA USA).

Lymph collection and measurement of ^{14}C-linoleic acid. After postoperative recovery, the rat was infused with a lipid emulsion containing 3.2 μCi ^{14}C-LA (specific activity: 53 mCi/mmol, radiochemical purity: 99.0%, NEN Research Products, Boston, MA, USA), 342 μmol LA, 380 μmol triolein, 402 μmol sodium taurocholate, and 3 μmol α-tocopherol with or without 41 μmol egg phosphatidylcholine (PC) (purity: 99.0%; Sigma Chemical Co., St. Louis, MO, USA) in 24-mL phosphate-buffered saline (PBS). The PBS buffer (pH 6.4) consisted of (in mM) 6.75 Na_2HPO_4, 16.5 NaH_2PO_4, 115 NaCl, and 5 KCl. The lipid emulsion was infused at 3 mL/hr via the intraduodenal catheter. Lymph was collected hourly for 8 hr into preweighed conical centrifuge tubes containing 25 mM disodium EDTA cooled in ice-filled beakers under subdued light.

From the hourly lymph samples, the ^{14}C radioactivity was determined in 100 μl aliquots after mixing with scintillation liquid (ScintiVerse; Fisher Scientific Co., Fair Lawn, NJ USA) by scintillation spectrometry (Beckman LS-8100; Beckman Instruments Inc., Fullerton, CA USA). ^{14}C-LA radioactivities appearing in hourly lymph samples were expressed as percent of the total ^{14}C radioactivity infused.

Determination of lymphatic phospholipid and fatty acids. Aliquots (100 μL) of lymph samples were used for the analysis of phospholipid (PL). PL was measured colorimetrically (UV-1201 Spectrophotometer; Shimadzu Scientific Instruments Inc., Columbia, MD USA) by the method of Raheja et al.[31] For the fatty acid analysis, lipids were extracted[32] from 100 μL of the hourly lymph samples using a mixture of chloroform:methanol 2:1 (v/v) containing 10 mg of butylated hydroxytoluene (10 mg per 100 mL of methanol). Methyl nonadecanoic acid (C19:0) was used as an internal standard. After being filtered through a syringe filter (nitrocellulose filter membranes; pore size: 0.45 μm TF, diameter: 13 mm; Sigma Chemical Co., St. Louis, MO USA), the lipid extracts were hydrolyzed with 1 mL of 0.5 N methanolic NaOH in the boiling water for 15 min. Fatty acids were saponified and methylated simultaneously with 2 mL of 14% methanolic BF_3 to prepare methyl esters of their constituent fatty acids, as described by Slover et al.[33] Fatty acid methyl esters (FAMEs) were redissolved in 100 μL of petroleum ether. Separation and quantitation of FAMEs were performed by capillary gas chromatography using a Hewlett-Packard model 5580A gas chromatograph (Hewlett-Packard, Palo Alto, CA USA), equipped with a Stabilwax-DA capillary column (15 m length × 0.53 mm ID, Resteck Corp., Bellefonte, PA USA).

Experiment 2

Intestinal esterification of ^{14}C-LA. To determine the effects of ES treatment and luminal PL on the mucosal esterification of ^{14}C-LA, a ligated jejunal loop was used in situ. The protocols concerning diet, ES implantation, and experimental conditions were the same as described for Experiment 1. In this study, 20 female rats were meal-fed twice daily 4 g/rat at 0830 and 9.0 g/rat at 1530 for 7 weeks after ES implantation.

Preparation of micellar solutions. Micellar solutions with or without PL were prepared by a modification of the method of Johnston and Borgström.[34] One micellar solution without PL contained 267 μCi ^{14}C-LA (specific activity: 58 mCi/mmol, radiochemical purity: 98.2%, NEN Research Products, Boston, MA USA), 133.0 μM unlabeled LA, 66.7 μM 2-monooleoylglycerol (C18:1, [cis]-9) (Sigma Chemical Co., St. Louis, MO USA), 2.2 mM glucose, 50.0 μM albumin, and 16.5 mM sodium-taurocholate per L of PBS (pH 6.4). The other with PL was prepared in the same manner except that 66.7 μM L-α-lysoPC (L-α-lysophosphatidylcholine, palmitoyl; purity: 99.0%; Sigma Chemical Co., St. Louis, MO USA) and 200.0 μM unlabeled LA were included.

Esterification of ^{14}C-LA into mucosal lipids. After 7 weeks of ES implantation, food was withheld from both OXE and OXP rats for 13 hr before experiment. Under halothane anesthesia, an abdominal incision was made along the midline by using a cauterizer, and a 10-cm long jejunal segment of the small intestine distal to the ligament of Treitz was ligated in situ. The lower end of the segment was tied with a suture (4-0 Silk; Ethicon Inc., Somerville, NJ USA). Through the upper end, 0.8 mL of a micellar solution was injected slowly into the jejunal segment. After the loop was closed by ligation, the abdomen was closed with wound clips. The ligated jejunal segment was incubated for 10 min in situ. During this incubation period, less than 0.6% of the radioactivity was found to escape into the circulation. At 10 min, the rat was killed by cervical dislocation under anesthesia, and the segment was removed and chilled immediately in ice-cooled saline. The luminal content was emptied into a plastic tube and washed three times with 5 mL ice-cold Krebs-Ringer phosphate buffer (pH 7.4) containing 10 mM sodium taurocholate. The intestinal segment was slit open with fine scissors, blotted gently with absorbent paper, and weighed. A small portion of the adjacent segment was used for protein analysis. Protein was measured using the BCA Assay Reagents (Pierce Chemical Co., Rockford, IL USA) with bovine serum albumin as the standard.[35] Aliquots (200 μL) of the luminal washings were used to measure the radioactivity remaining in the intestinal lumen and percent recovery of the injected dose was calculated. The lipids were extracted from the jejunal segment and used for the measurement of ^{14}C radioactivity present in the intestinal mucosa.

Mucosal lipids were separated by solid phase extraction[36] into cholesteryl esters (CE), triglycerides (TG), mono- and diglycerides (MDG), free fatty acids (FFA), and phospholipids (PL) using an aminopropyl solid phase column (Bond Elut NH_2; Varian Sample Preparation Products, Harbor City, CA USA). Before lipid extracts were loaded, the columns were washed with 0.6 mL of acetone-water (7:1, v/v) and equilibrated by rinsing twice with 2.0 mL of hexane. Lipids dissolved in 300 μL of hexane-methyl tert-butylether-acetic acid (100:3:0.3, v/v/v) were loaded onto the column. CE fraction then was eluted using 5.0 mL of hexane and TG with 5 mL of hexane-chloroform-ethyl acetate mixture (100:5:5, v/v/v). Then, 5 mL of chloroform-isopropanol (2:1, v/v) and 5 mL of chloroform-methanol-acetic acid mixture (10:5:4, v/v/v) were applied successively to elute MDG and FFA. Finally, PL fraction was eluted with 5 mL of methanol-chloroform-water (10:5:4, v/v/v). Between each elution step, care was taken not to leave the column dry. Each fraction was dried under nitrogen and resolubilized in 0.5 mL of chloroform-methanol (1:2, v/v) and the ^{14}C radioactivity therein was determined after mixing with scintillation liquid (ScintiVerse, Fisher Scientific Co., Fair Lawn, NJ USA). The rates of ^{14}C-LA incorporation into different lipid classes were expressed in nmoles of LA/10 min/100 mg tissue protein.

Statistics. All statistical analyses were performed using PC SAS (SAS Institute, Cary, NC USA). Student's *t*-test was used to compare two group means.[37] Linear regression analysis was used to determine correlation between variables. Values in the text and tables are expressed as means ± SD, and values in figures (graphs) are expressed as means ± SEM. Significance was determined at $P < 0.05$, unless otherwise stated.

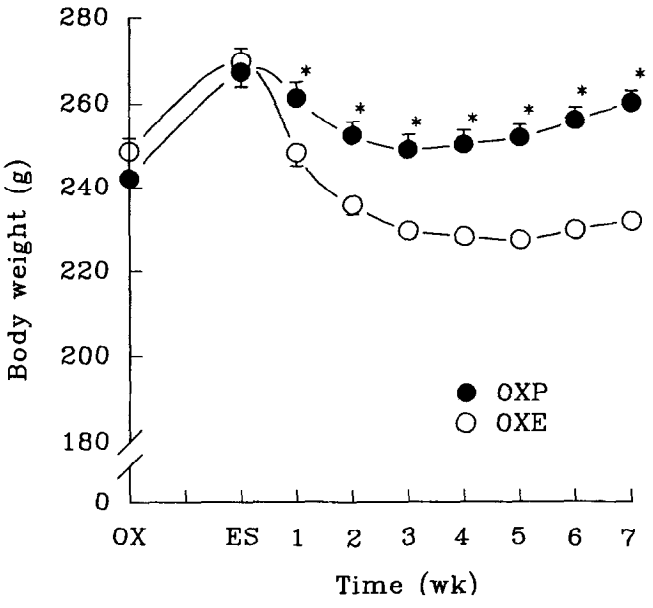

Figure 1 Time-course effect of ES treatment on average body weights. Rats were pair-fed twice a day (10.5 g/d) throughout the entire 7 weeks of ES treatment, and body weights were measured daily. No significant difference was noted in initial body weight (203 ± 4 g in OXE rats vs. 199 ± 6.0 g in OXP rats). After ES treatment, a significant difference was noted at 1 wk. At 7 weeks, the OXE rats weighed 232 ± 5 g, which was 89% of the body weight of OXP rats (260 ± 9 g). Abbreviations: OXE, rats implanted with estradiol pellet; OXP, rats implanted with a placebo pellet; OX, time of ovariectomy; ES, time of estrogen implantation. Data are expressed as means ± SEM, $n = 10$. Asterisks (*) denote significant differences between groups at $P < 0.05$.

Results

General observations

Figure 1 shows changes in body weights of OXE and OXP rats over the course of the experiment. Throughout the 7-week period of ES treatment, the body weights of both groups were monitored daily. No significant difference was noted between the groups in body weights at the start of experiment (203 ± 4 g in OXE rats versus 199 ± 6 g in OXP rats), at the time of ovariectomy (249 ± 10 g in OXE rats versus 242 ± 7 g in OXP rats), or at the initiation of ES treatment (270 ± 10 g in OXE rats and 267 ± 11 g in OXP rats). With ES implantation, the body weight of OXE rats began to decrease more rapidly than that of OXP rats, despite the fact that both groups were trained for meal feeding and pair-fed with equal amounts of the diet. A significant difference in body weight was observed at 1 week (248 ± 10 g in OXE rats and 261 ± 11 g in OXP rats) and thereafter. At 3 weeks after ES treatment, the average body weight of OXE rats was stabilized at 230 ± 7 g and did not change significantly throughout 7 weeks. At 7 weeks, OXE rats weighed 232 ± 5 g, which was 89% of the body weight of OXP rats (260 ± 9 g).

Lymphatic absorption of ^{14}C-LA

In response to lipid infusion, lymph flow was increased significantly and reached its peak in both groups during 3 to 5 hr. When PC was not infused, the average lymph flow rates were 2.4 ± 0.4 mL/hr in OXE rats and 3.1 ± 0.3 mL/hr in OXP rats. A significant difference in cumulative lymph volume was shown at 2 hr and thereafter (19.5 ± 3.4 mL in OXE rats and 24.8 ± 2.3 mL in OXP rats). With duodenal PC infusion, the average rates of lymph flow in OXE and OXP rats were 2.4 mL/hr and 2.7 mL/hr, respectively. No significant difference in total lymph flow was observed between groups (19.5 ± 4.0 mL in OXE rats and 21.3 ± 3.6 mL in OXP rats). PC infusion did not affect the rate of lymph flow or the total lymph volume.

Without PC infusion, the average rates of ^{14}C-LA absorption were 3.2%/hr in OXE rats and 4.0%/hr in OXP rats. The cumulative absorption of ^{14}C-LA was significantly lower in OXE rats at 2 hr and thereafter (*Figure 2A*), compared with OXP rats. The total lymphatic absorption of ^{14}C-LA was 26.0 ± 3.4% dose in OXE rats and 31.8 ± 2.0% in OXP rats. With PC infusion, the lymphatic absorption of ^{14}C-LA in OXE rats rose to the level of OXP rats (*Figure 2B*). The average rates of ^{14}C-LA absorption were 4.2%/hr in OXE rats and 4.1%/hr in OXP rats. The lymphatic absorption in OXE rats during the first 3 hr of lipid emulsion tended to be lower, but was restored at 4 hr and thereafter (*Figure 2B*). With PC infusion, no significant difference was noted in the cumulative absorption of ^{14}C-LA between OXE and OXP rats except for the first 3 hr. The total 8-hr absorption of ^{14}C-LA was 33.3 ± 3.7% dose in OXE rats and 32.4 ± 3.4% in OXP rats. The intraduodenal infusion of PC significantly increased the absorption of ^{14}C-LA in OXE rats at 5 hr and thereafter, compared with OXE rats not infused with PC. PC infusion did not cause further increase in the ^{14}C-LA absorption in OXP rats (32.4 ± 3.4% dose) compared with OXP rats without PC infusion (31.8 ± 2.0%).

Lymphatic output of PL

The hourly lymphatic outputs of PL in OXE and OXP rats are presented by *Figure 3*. Without PC infusion, the lymphatic output of PL was significantly lower in OXE rats than in OXP rats. The average lymphatic outputs of PL in OXE and OXP rats were 3.3 μmol/hr and 4.6 μmol/hr, respectively. As shown in *Figure 3A*, the hourly outputs of PL gradually increased in both groups up to 5 hr. At 5 hr, the rates of PL output peaked at 4.3 ± 0.4 μmol/hr in OXE rats and at 6.0 ± 1.0 μmol/hr in OXP rats. A significant difference in PL output was observed between groups at 4 hr and thereafter. The average rate of lymphatic PL output in OXE rats was 3.0 μmol/hr during the first 5 hr of infusion and remained significantly lower than in OXP rats (4.2 μmol/hr). Without PC infusion, the cumulative outputs of PL were 26.5 ± 2.4 μmol in OXE rats and 37.1 ± 4.1 μmol in OXP rats, with significant differences between groups at 2 hr and thereafter. With PC infusion, the lymphatic outputs of PL rapidly increased in both groups. The rate of PL output remained significantly lower in OXE rats (4.9 μmol/hr) than in OXP rats (5.7 μmol/hr). The cumulative outputs of PL were 38.9 ± 3.3 μmol in OXE rats and 45.2 ± 2.5 μmol in OXP rats. PC infusion significantly increased the cumulative lymphatic outputs of PL in both groups, compared with the respective groups without PC infusion. The lymphatic output of PL (37.1 ± 4.1 μmol) in

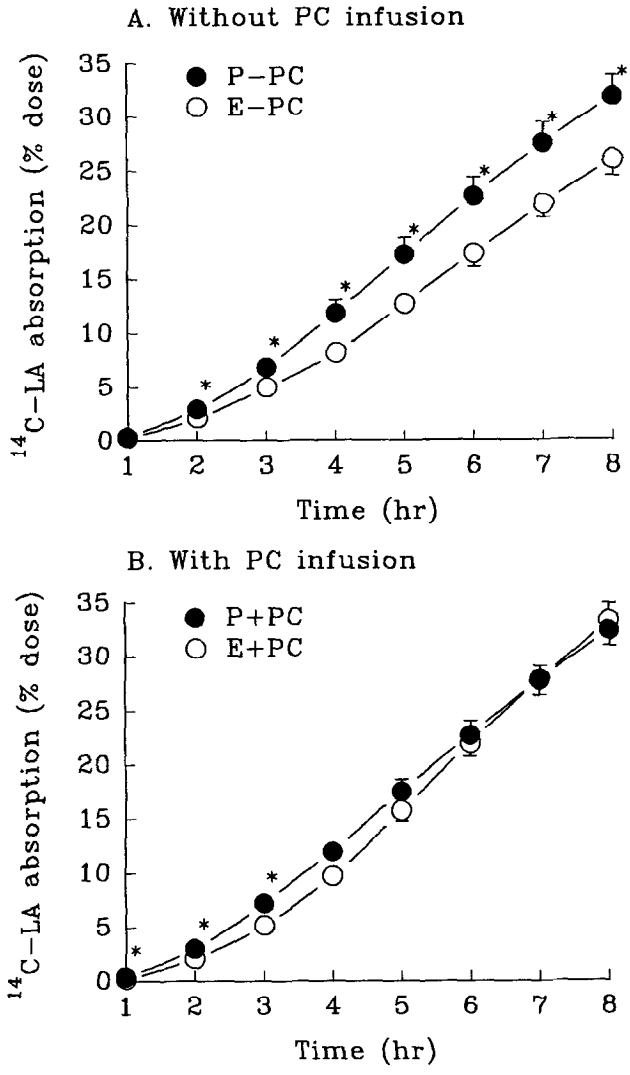

Figure 2 Cumulative absorption of ^{14}C-linoleic acid (^{14}C-LA) for 8 hr. (*A*) Without PC infusion, the cumulative lymphatic absorption of ^{14}C-LA in OXE rats was significantly lower at 2 hr and thereafter compared to OXP rats. The total absorptions of ^{14}C-LA were 26.0 ± 3.4% dose in OXE rats and 31.8 ± 2.0% in OXP rats. (*B*) With PC infusion, the total lymphatic absorption of ^{14}C-LA (33.3 ± 3.7%) in OXE rats reached the level of OXP rats (32.4 ± 4%). Abbreviations: P-PC, OXP rats without PC infusion; E-PC, OXE rats without PC infusion; P + PC, OXP rats with PC infusion; and E + PC, OXE rats with PC infusion. All values are expressed as mean ± SEM, n = 5. Asterisks (*) denote significant differences between groups at $P < 0.05$.

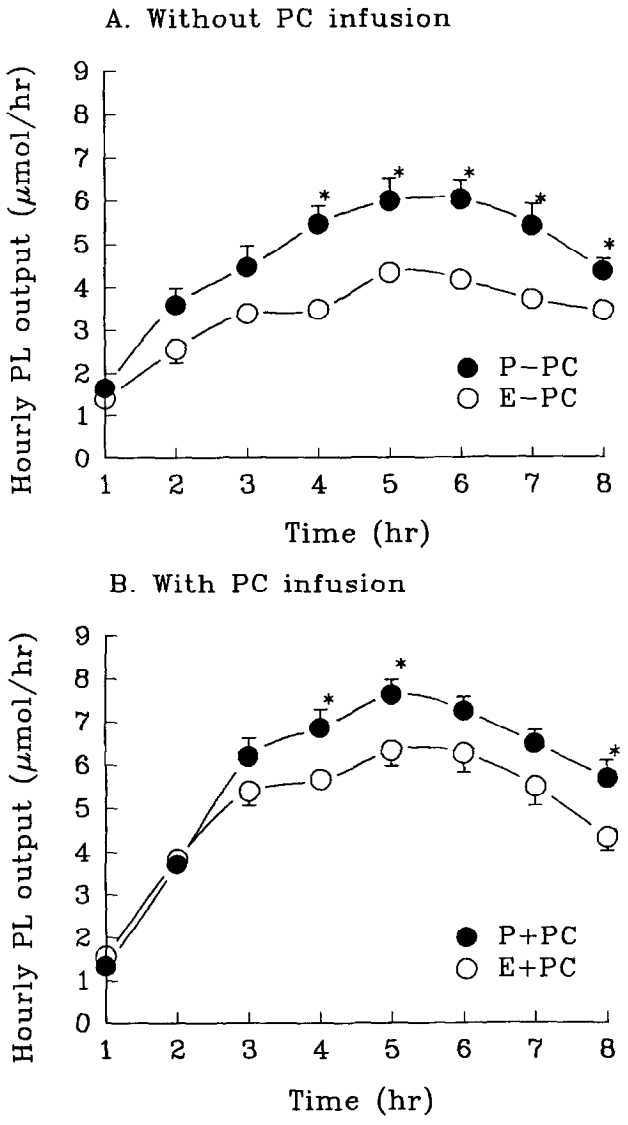

Figure 3 Hourly lymphatic output of phospholipid (PL) for 8 hr. (*A*) Without PC infusion, the output of PL in OXE rats was significantly lower compared to OXP rats at 4 hr and thereafter. The average outputs of PL were 3.3 μ/mol in OXE rats and 4.6 μmol/hr in OXP rats. (*B*) With PC infusion, the lymphatic outputs of PL were increased with an increment of 1.3 μmol/h in OXE rats and 0.8 μmol/hr in OXP rats compared to those not infused with PC. In both groups, the lymphatic output of PL peaked at 5 hr. For acronyms, refer to Figure 2. All values are expressed as mean ± SEM, n = 5 hr. For acronyms, refer to Figure 2. All values are expressed as mean ± SEM, n = 5. Asterisks (*) denote significant differences between groups at $P < 0.05$.

OXE rats with PC infusion was increased to the level of output in OXP rats not infused with PC (38.9 ± 3.3 μmol). The total increases in PL outputs with PC infusion were 46.7% in OXE rats and 22.1% in OXP rats.

Lymphatic output of fatty acids (FAs)

The total lymphatic outputs of individual FAs secreted into the lymph for 8 hr are summarized in *Table 2*. Generally, when PC was not infused, the lymphatic outputs of individual FAs were significantly lower in OXE rats than in OXP rats. However, PC infusion markedly enhanced the total outputs of individual FAs in OXE rats to the levels of OXP rats without PC infusion. PC infusion did not affect the total output of FAs in OXP rats.

Figure 4 shows the lymphatic outputs of oleic acid (OA) for 8 hr. OA was the major FA (62.2% in OXE rats and 60.7% in OXP rats) released into the lymph, because it was infused luminally in the form of triolein. In the absence of PC, the average OA outputs were 35.5 μmol/hr in OXE rats and 41.6 μmol/hr in OXP rats. At 2 hr and thereafter, the cumulative lymphatic output of OA was significantly lower in OXE rats (284.0 ± 22.3 μmol) than in OXP rats (332.4 ± 31.7 μmol) (*Figure 4A*). Similarly, the output of total

Table 2 Effect of estrogen on the total lymphatic outputs of phospholipid (PL) and fatty acids for 8 hr without and with luminal infusion of phosphatidylcholine (PC)[1]

Group	Fatty Acids in Lymphatic Lipids						
	PL	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4
				μmol			
Without PC							
OXE	26.5 ± 2.4[a]	46.3 ± 4.8[a]	19.0 ± 1.1[a]	284.0 ± 22.3[a]	87.3 ± 8.1[a]	4.4 ± 0.9[a]	16.0 ± 0.9[a]
OXP	37.1 ± 4.1[b]	58.9 ± 3.6[b]	23.2 ± 1.5[b]	332.4 ± 31.7[b]	105.9 ± 10.8[b]	6.2 ± 1.1[b]	21.1 ± 1.9[b]
With PC							
OXE	38.9 ± 3.3[a]	69.2 ± 7.4[a]	31.5 ± 3.8[a]	338.3 ± 34.4[a]	103.0 ± 8.8[a]	5.9 ± 1.2[a]	21.3 ± 3.7[a]
OXP	45.2 ± 2.5[b]	62.0 ± 7.5[a]	25.4 ± 4.2[b]	328.1 ± 19.2[a]	98.2 ± 8.0[a]	6.0 ± 1.4[a]	16.0 ± 2.9[b]

[1]Mean ± SD; $n = 5$ per group. Values in the same column not sharing a common superscript are significantly different ($P < 0.05$). OXE, rats implanted with estradiol pellet; OXP, rats implanted with a placebo pellet.

linoleic acid (LA) was significantly lower in OXE rats (87.3 ± 8.1 μmol) than in OXP rats (105.9 ± 10.8 μmol) at 2 hr and thereafter. Without PC infusion, the average rates of LA output were 10.9 μmol/hr in OXE rats and 13.2 μmol/hr in OXP rats. The lymphatic outputs of other FAs, which were not presented in the infused lipid emulsion, were significantly lower in OXE rats than in OXP rats (*Table 2*). The cumulative outputs of C16:0, C18:0, C18:3, and C20:4 for 8 hr were 46.3 ± 4.8, 19.0 ± 1.1, 4.4 ± 0.9, and 16.0 ± 0.9 μmol in OXE rats and 58.9 ± 3.6, 23.2 ± 1.5, 6.2 ± 1.1, and 21.1 ± 1.9 μmol in OXP rats, respectively.

When PC was infused luminally, the average rates of OA outputs in OXE and OXP rats were 42.3 μmol/hr and 41.0 μmol/hr, respectively (*Figure 4B*). The cumulative OA output in OXE rats (338.3 ± 34.4 μmol) was restored completely to the level of output in OXP rats (328.1 ± 19.2 μmol). OA was the primary FA present in lymph lipids (59.4% in OXE rats and 61.3% in OXP rats). PC infusion significantly increased the lymphatic output of OA in OXE rats at 2 hr and thereafter, but had no such effect in OXP rats. Similarly, with PC infusion, the total lymphatic output of LA (103.0 ± 8.8 μmol) in OXE rats reached the level of output in OXP rats (98.2 ± 8.0 μmol). PC infusion did not cause any significant change in LA output in the OXP group compared with the OXP rats without PC infusion (105.9 ± 10.8 μmol). The cumulative outputs of the endogenous FAs, C16:0 and C18:3, in OXE rats reached the levels of output in OXP rats, and the outputs of C18:0 and C20:4 rose significantly higher than those in OXP rats. The cumulative outputs of C16:0, C18:0, C18:3, and C20:4 were 69.2 ± 7.4, 31.5 ± 3.8, 5.9 ± 1.2, and 21.3 ± 3.7 μmol in OXE rats and 62.0 ± 7.5, 25.4 ± 4.2, 6.0 ± 1.4, and 16.0 ± 2.9 μmol in OXP rats, respectively.

The hourly lymphatic output of PL was correlated highly with the hourly absorption of ^{14}C-LA ($r = 0.81$, $P < 0.0001$) and the hourly OA output ($r = 0.78$, $P < 0.0001$) (*Figure 5*). The hourly PL output also was correlated significantly with outputs of other FAs ($r = 0.73$ with C16:0, $r = 0.71$ with C18:0, $r = 0.61$ with C18:3, and $r = 0.50$ with C20:4, $P < 0.001$).

Esterification of ^{14}C-LA into mucosal lipids

Figure 6 shows the effects of luminal lysoPC on the rate of ^{14}C-LA esterification into lipids in the intestinal mucosa of

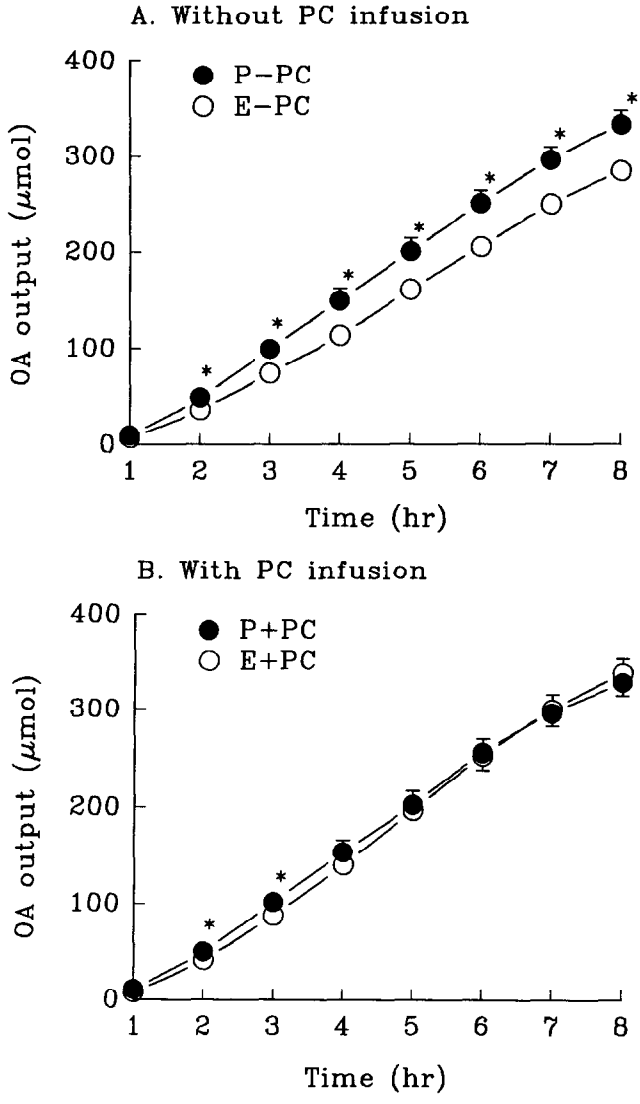

Figure 4 Cumulative lymphatic output of oleic acid (OA) for 8 hr. (*A*) Without PC infusion, the cumulative output of OA was significantly lower in OXE rats (284.0 ± 22.3 μmol) at 2 hr and thereafter compared to OXP rats (332.4 ± 31.7 μmol). (*B*) With PC infusion, a significant difference between groups was observed at 2 and 3 hr, but no significant difference in OA output was noted thereafter. The total outputs of OA were 338.3 ± 34.4 μmol in OXE rats and 328.1 ± 19.2 μmol in OXP rats. For acronyms, refer to *Figure 2*. All values are expressed as mean ± SEM, $n = 5$. Asterisks (*) denote significant differences at $P < 0.05$.

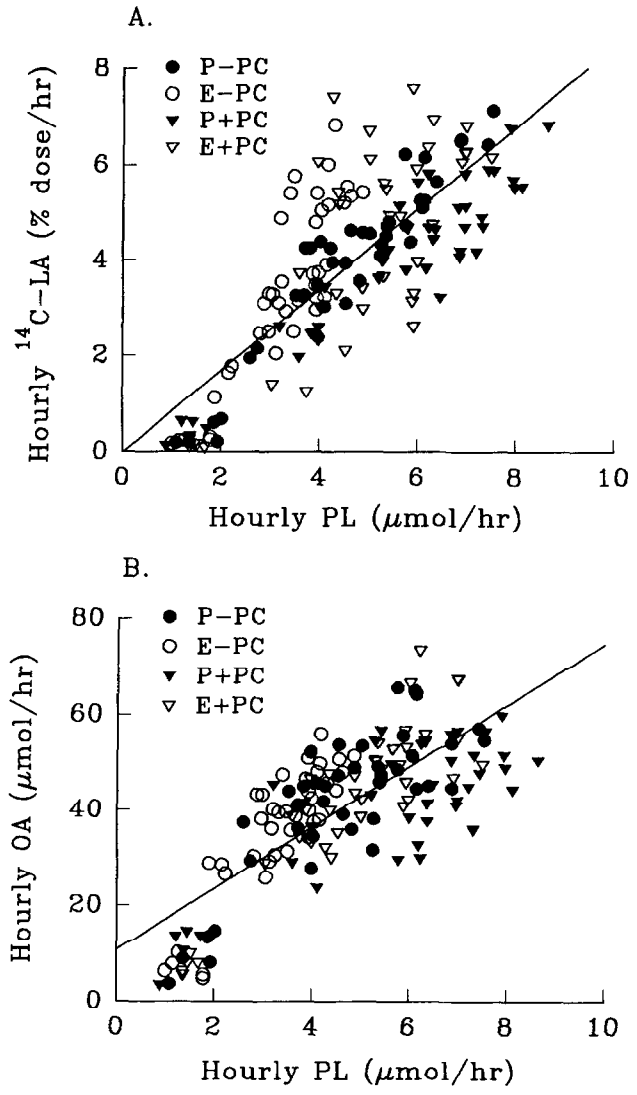

Figure 5 (*A*) Correlation between the hourly lymphatic absorption of ^{14}C-linoleic acid (^{14}C-LA) and the hourly lymphatic output of PL for 8 hr. The absorption of ^{14}C-LA in % dose is highly correlated (0.81, $P < 0.0001$) with the hourly lymphatic output of PL secreted into the lymph. (*B*) Correlation between the hourly lymphatic output of OA and the hourly lymphatic output of PL for 8 hr. A strong correlation ($r = 0.78$, $P < 0.0001$) was observed between the hourly output of OA and the hourly output of PL secreted into the lymph. For acronyms, refer to *Figure 2*.

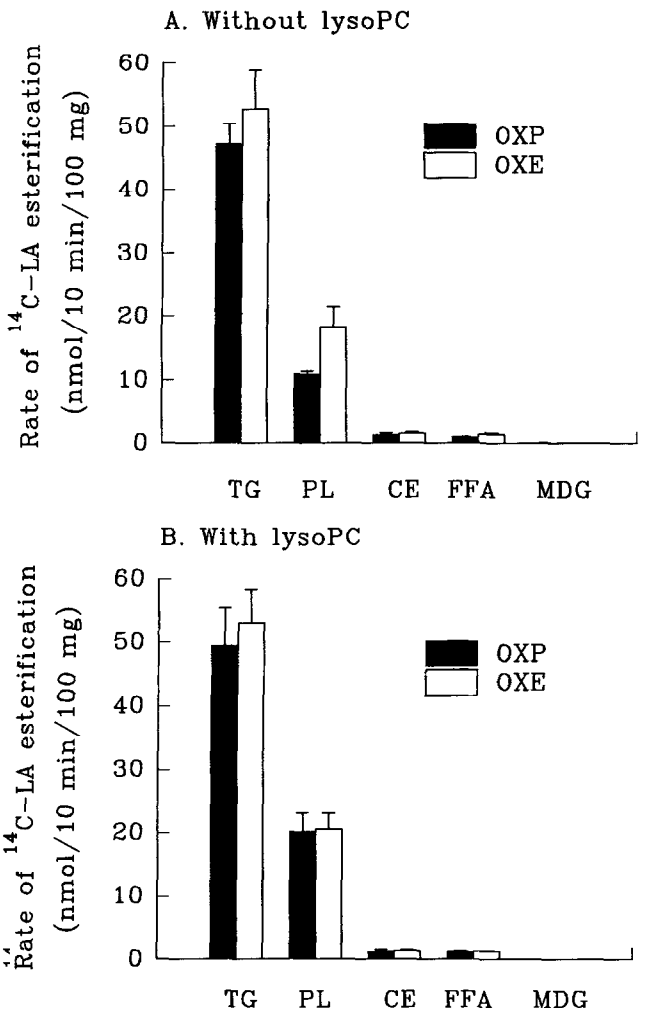

Figure 6 Rate of ^{14}C-LA esterification into lipids in the intestinal mucosa. All values were expressed in nmoles of ^{14}C-LA incorporated/10 min/100 mg tissue protein. (*A*) In the absence of lysoPC, most of ^{14}C-LA was incorporated into TG (71.7 ± 5.9% in OXE rats and 77.7 ± 2.9% in OXP rats) and PL (24.3 ± 5.4% and 18.2 ± 3.3%, respectively). No significant difference in the rate of ^{14}C-LA esterification into TG or PL was noted between groups. (*B*) In the presence of lysoPC, the rates of ^{14}C-LA esterification into TG and PL were 53.0 ± 12.2 and 20.5 ± 5.6 nmol, respectively, in OXE rats and 49.5 ± 12.9 and 20.3 ± 6.4 nmol, respectively, in OXP rats. No significant differences were shown between OXE and OXP rats. Abbreviations: TG, triglyceride; PL, phospholipid; CE, cholesteryl ester; FFA, free (unesterified) fatty acid; and MDG, mono- and diglycerides. All values are expressed as mean ± SEM, $n = 5$.

OXE and OXP rats. Each rat was injected with a micellar solution containing monoolein and free fatty acid in a 1:2 molar ratio with and without lysoPC. No significant differences in the rate of ^{14}C-LA esterification were observed between OXE and OXP rats, regardless of whether lysoPC was present in the incubation medium. In the absence of lysoPC, most of the ^{14}C-LA (96% in both groups) was incorporated into TG (71.7 to 77.7%) and PL (18.2 to 24.3%) (*Figure 6A*). In OXE rats, the rates (nmol esterified/10 min/100 mg tissue protein) of ^{14}C-LA esterification into TG, PL, CE, and MDG were 52.8 ± 13.5, 18.2 ± 7.3, 1.5 ± 0.5, and 0.1 ± 0.0, respectively. In OXP rats, the rates of ^{14}C-LA esterification into TG, PL, CE, and MDG were 47.3 ± 6.9, 10.9 ± 1.2, 1.4 ± 0.5, and 0.1 ± 0.0 nmol, respectively. In both groups, 1.0 to 1.4% of ^{14}C-LA remained unesterified, with no significant difference between groups. When lysoPC was present in the micellar solution (*Figure 6B*), the proportions of ^{14}C-LA esterified into PL were increased slightly from 24.3% to 26.9% dose in OXE rats and significantly from 18.2% to 27.9% in OXP rats. When expressed in nmol of ^{14}C-LA esterified/100 mg protein/10 min, the rates of ^{14}C-LA esterification into TG, PL, CE, and MDG were 53.0 ± 12.2, 20.5 ± 5.6, 1.3 ± 0.5, and 0.1 ± 0.0 nmol in OXE rats, respectively. In OXP rats, the rates of ^{14}C-LA incorporation into TG, PL, CE, and MDG were 49.5 ± 12.9, 20.3 ± 6.4, 1.3 ± 0.6, and 0.1 ±

0.0 nmol, respectively. No significant differences were noted between groups in each lipid class.

Discussion

The present data indicate that ES treatment in OX rats markedly reduces the lymphatic absorption of both exogenous and endogenous FAs, which is consistent with our previous finding.[14] When both OXE and OXP rats were infused luminally with PC, the lymphatic absorption of FAs in OXE rats was normalized to the levels in OXP rats, whereas PC infusion in OXP rats did not influence the lymphatic absorption of FAs. Also, luminal PC infusion significantly enhanced the lymphatic outputs of PL in both groups. No differences in the rate of ^{14}C-LA esterification into the mucosal lipids were shown between OXE and OXP rats regardless of whether lysoPC was present in the intestinal lumen.

A previous study[14] showed that ES replacement in OX rats significantly lowered the lymphatic absorption of lipids, and that the lowering of the intestinal fat absorption was correlated highly with a significant decrease in lymphatic PL output. The finding suggested that limited PL availability to the enterocyte during chylomicron formation might be a probable cause of the reduced fat absorption in ES-treated rats. An important observation from the present study is that a luminal PC infusion in OXE rats increased the lymphatic absorption of FAs to the levels in OXP rats. This finding provides evidence that ES treatment of OX rats limits the supply of PL during chylomicron formation, probably via the biliary route. PC infusion significantly increased the lymphatic outputs of PL in both OXE and OXP rats. Our data showed that the cumulative outputs of PL for 8 hr were 38.9 ± 3.3 μmol in OXE rats and 45.2 ± 2.5 μmol in OXP rats. With PC infusion, the rate of PL output in OXE rats (4.9 μmol/hr) reached the level of the OXP rats not infused with PC (4.6 μmol/hr). Despite the significant rise in PL output in OXP rats relative to OXE rats, no further increase in FA absorption was observed. These findings suggest that the average PL output of 4.6 μmol/hr is sufficient to meet the intestinal demand for chylomicron formation and to support the normal rate of fat absorption. This conclusion is in line with the previous observation[38] that an increase in PL infusion from 5 to 20 μmol/hr resulted in no further increase in TG output in bile-diverted rats. In the present study, PC was infused at 5.1 μmol/hr.

ES has been shown to decrease the biliary secretion of lipids including PL and alter the lipid composition of plasma membranes of the liver.[16,18,21,24] Also, ES increases the ratio of cholesterol to PL in the liver cell membranes,[22,26,39] with a decrease in membrane lipid fluidity. ES treatment in human subjects and in animals reduces bile flow and decreases the secretion of bile acids.[23] Bile salts represent the driving force for the hepatic secretion of PC into bile and stimulate biliary PC synthesis in the liver.[40] Thus, the biliary secretion of PL into the intestinal lumen may be reduced as a result of decreased bile flow, which occurs during ES treatment.[41] The reduced secretion of PL into the intestine may result in a decrease in the lymphatic absorption of lipids by reducing the availability of PL for chylomicron formation. Thus, the restoration of intestinal lipid absorption with an intraduodenal PC infusion in OXE rats further supports the conclusion that an insufficient supply of PL via the biliary route may be the major cause of the decreased absorption of lipids. However, at present, no direct evidence is available concerning whether ES inhibits the hepatic secretion of PL into the intestinal lumen during fat absorption. Several studies[42–44] show that ES treatment in OX rats significantly increases the levels of plasma PL. This increase is associated with ES-induced stimulation of the hepatic synthesis and secretion of PL into the plasma via lipoproteins such as high density lipoproteins.[44,45] The increased demand for PL for the hepatic synthesis of lipoproteins may reduce the amount of hepatic PL released into bile and, therefore, limit the availability of PL for the formation of chylomicron surface coat.

The possibility also exists that the limited supply of PL via the biliary route, as observed with ES treatment, may reduce the intestinal synthesis of chylomicron apoproteins, particularly, apoB_{48}.[46,47] O'Doherty et al.[27] showed that dietary or biliary deprivation of PL decreased the intestinal synthesis of proteins during fat absorption. This decrease was restored by the luminal infusion of PC. Subsequently, Davidson et al.[48,49] showed that the intestinal synthesis of apoB_{48} is reduced markedly in bile-diverted rats, implicating PL as a possible regulatory signal for apoB_{48} synthesis. Krause et al.[12] demonstrated that the lymphatic output of apoB was significantly lower in adult male rats, when treated with a pharmacologic dose of ES. The above observations taken together suggest that the reduced biliary secretion of PL into the intestinal lumen with ES treatment may cause a decrease in the intestinal synthesis of apoB_{48} and, hence, slow the rate of chylomicron formation. Thus, the reduced rate of fat absorption in OXE rats may be attributable to a slower rate of chylomicron formation because of limited availability of the PL and apoB_{48} needed to assemble the surface coat of chylomicrons.

Luminal PC is hydrolyzed to lysoPC and fatty acid, which are absorbed into the mucosal cells. In the intestinal mucosa, lysoPC is mainly reacylated to PC.[50] When biliary or dietary PC was readily available, the direct reacylation of absorbed lysoPC was shown to be the major source of chylomicron PL.[51] In the present study, the hourly lymphatic output of PL was correlated strongly with the hourly rate of ^{14}C-LA absorption ($r = 0.81$) and the hourly output of OA ($r = 0.78$), which was infused in the form of triolein. The normalization of the rates of both labeled LA and unlabeled OA with PC infusion in OXE rats indicates that the luminal hydrolysis of TG, mucosal uptake of fatty acids, and resynthesis of TG in the enterocyte are not affected by ES treatment. It is also evident that the formation of chylomicron and the packaging of TG into chylomicrons occur normally in OXE rats, so far as PC is provided luminally.

Previous work suggested that ES treatment may decrease the rate of FA esterification in the intestinal mucosa.[13] When male rats treated with a pharmacologic dose of ES were injected duodenally with ^{14}C-palmitate, 75% of ^{14}C was found in FFA and only 5% in TG in the intestinal mucosa, compared with 49% in FFA and 23% in TG in control rats. The finding suggested that the decreased rate of FA esterification into mucosal TG in ES-treated rats could

slow the rate of TG incorporation into chylomicrons. Rodger et al.[26] showed a reduction in the acyltransferase activity of the intestinal mucosa of bile-diverted rats. This was associated with a marked decrease in microsomal PC content. A luminal PC infusion restored acyltransferase activities,[27] implying that PL may be a rate-limiting factor for FA esterification.[13] However, the present study indicated that a physiological dosage of ES in the intestinal mucosa of OX rats did not alter the rate of ^{14}C-LA esterification. Regardless of whether lysoPC was present in the incubation medium, ES treatment did not affect the rate of ^{14}C-LA incorporation within the enterocyte of OXE rats. Also, the addition of lysoPC to the incubation medium in both OXE and OXP rats did not increase the rate of ^{14}C-LA incorporation into PL in the intestinal mucosa, indicating that a physiological dose of ES in OX rats, as used in the present study, does not alter the mucosal synthesis of PL from lysoPC. Therefore, the decreased FA absorption in OXE rats is not attributable to alteration in the rate of FA esterification or lysoPC reacylation.

In summary, the present observations demonstrate that, in OXE rats, an intraduodenal PC infusion fully restores the lymphatic absorption of FAs, and the intestinal incorporation of FAs into lipids is not altered by ES treatment in the mucosa. The results strongly suggest that a limited supply of PL via the biliary route may cause a delay in chylomicron formation and slow the rate of lymphatic fat absorption in OXE rats. Studies are currently in progress to determine the rate of biliary PL secretion during enteral fat infusion as affected by ES treatment. The slower rate of fat absorption from the intestine, as observed with ES treatment, may alter significantly the metabolism of dietary fat, fat distribution, and ultimately weight gain. The finding suggests that estrogen may play a regulatory role in intestinal fat absorption by modulating the secretion of PL via the bile, and may explain partly the inhibitory effect of estrogen replacement on lipogenesis and weight gain in postmenopausal women.

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