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# The lymphatic absorption of fatty acids and output of phospholipids are lowered by estrogen replacement in ovariectomized rats

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*The lymphatic absorption of fatty acids and output of lipids during intraduodenal lipid infusion were compared in Sprague-Dawley ovariectomized rats with (OXE) and without (OXP) estradiol implants. A time-release estradiol pellet (25 μg/day/rat) was implanted subcutaneously in the OXE rats and a placebo pellet in OXP rats. At 4 weeks, the rats were fitted with lymph cannula and infused at 3.0 ml/hr via a duodenal catheter with a lipid emulsion containing 3.2 μCi $^{14}$C-linoleic acid ($^{14}$C-LA), 171 μmol LA, 488 μmol triolein, 520 μmol sodium taurocholate, and 1.5 μmol α-tocopherol in 24 ml phosphate buffered saline (pH 6.4). Lymph was collected at hourly intervals for 8 hours via a mesenteric lymph cannula. The average rate of lymph flow was reduced in OXE (1.4 ml/hr), compared with that of OXP rats (2.2 ml/hr). The total cumulative absorption of $^{14}$C-LA during the 8-hr period, as expressed in % dose, was significantly lower in OXE (26.9 ± 5.2%) than in OXP rats (33.6 ± 4.0%). The lymphatic outputs of fatty acids (16:0, 18:0, 18:1, 18:2, and 18:3) also were significantly reduced in OXE rats. The amount of $^{14}$C-LA absorbed into the lymph were highly correlated ($r = 0.85$, $P < 0.05$) with the amount of phospholipid (PL) released. The results provide the first evidence that the rate of lymphatic absorption of lipids is reduced by estrogen replacement in ovariectomized rats. This may be caused by a limited availability of PL to the enterocyte during chylomicron formation.* (J. Nutr. Biochem. 7:214–221, 1996.)

**Keywords:** estradiol; fatty acids; linoleic acid; lymphatic absorption; phospholipid; cholesterol

## Introduction

Increasing evidence indicates that estrogen (ES) alters body fat distribution in early puberty in females.[1] ES replacement therapy in postmenopausal women prevents the increase of body fat[2] and abdominal fat mass.[3] ES treatment in animals also affects adipose tissue metabolism[4] and causes a significant loss of body fat.[5,6] Studies have shown that ES decreases lipogenesis by altering lipogenic enzyme production and/or activity,[7] enhances lipolysis,[8] decreases lipoprotein lipase (LPL) activity,[9] and lowers the rate of fatty acid (FA) esterification in adipose tissue.[7] ES is known to increase the plasma concentrations of free (unesterified) fatty acids (FFAs)[4,6,10] and affects the FA composition of plasma lipids,[11,12] and to stimulate FA synthesis in the liver[13] and increase FA mobilization from the adipose tissue.[4,7,10,14,15] These observations together indicate that ES affects fat metabolism via multiple mechanisms.[16,17]

A drastic reduction in the rate of intestinal transport of triglyceride[18] and a 30 to 50% decrease in fat absorption[19] have been reported in male rats treated with pharmacological dosages of ethinyl estradiol. The mechanism(s) underlying such effects is poorly understood. In adult male rats, ES treatment decreases the rates of intestinal secretion of apolipoproteins including apo$B_{48}$. Evidence also indicates that ES reduces the secretion of bile acids,[20] although its effect on lipid absorption is not known. At present, little information exists concerning whether the intestinal absorption of fat is altered by ES replacement in ovariectomized females. Elucidation of the role of ES in intestinal fat absorption in females may provide new insight into the mechanism by which ES replacement affects the assimilation of dietary fat and weight gain coinciding with meno-

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pause.[3] In the present study, we used ovariectomized rats treated with a low dose of estradiol to investigate if treatment altered the rate of lymphatic lipid absorption, with particular attention to its relationship with the output of phospholipid.

## Methods and materials

### *Animals and diet*

Ten Sprague-Dawley female rats (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) weighing 208 ± 10 g were placed individually in stainless steel wire-bottom cages in a windowless room controlled at 22–24°C with the light period from 3:00 p.m. to 3:00 a.m. and the dark period from 3:00 a.m. to 3:00 p.m. The rats were cared for in an animal care facility in the Department of Foods and Nutrition, Kansas State University, accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The rats were fed a commercial standard rodent chow for 1 week and fed ad libitum for 3 weeks a diet formulated according to the recommendations of the American Institute of Nutrition.[21,22] The composition of the diet is shown in *Table 1*. All animals were allowed free access to deionized water.

### *Ovariectomy and estrogen implantation*

At the end of 3 weeks, rats were starved for 12 hours and ovariectomized under halothane anesthesia. On day 12 following ovariectomy, the rats were divided randomly into the following two groups: one group (OXE) of five rats with average weight of 242 ± 11 g, which were implanted subcutaneously at the back of the neck with a time-release 17β-estradiol pellet (25 μg/day, Innovative Research of America, Toledo, OH, USA), and the other group (OXP) of five rats (242 ± 8 g), which were implanted with a placebo pellet containing no estradiol.

### *Cannulation of mesenteric lymph duct*

Four weeks after estradiol implantation, rats were starved for 18 hours, the mesenteric lymph duct was cannulated, and a catheter was placed into the duodenum as described in our previous study.[23] Briefly, under halothane anesthesia, an abdominal incision was made along the midline with a cauterizer. The major intestinal lymph duct was cannulated with vinyl tubing (SV. 31 tubing; ID 0.5 mm; OD 0.80 mm, Dural Plastics & Engineering, Dural, N.S.W., Australia). An indwelling infusion catheter (Silastic medical grade tubing; ID 0.04 in.; OD 0.085 in., 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., Somerville, NJ, USA). After the abdominal incision was closed, the rats were placed in restraining cages[24] in a heated chamber (30°C) for postoperative recovery for 44 to 48 hours. During this period, the rats were fully awake and infused via the duodenal catheter with a maintenance solution consisting of 277 mM glucose, 144 mM NaCl, and 4 mM KCl at a rate of 3.0 ml/hr by using an infusion pump (Harvard Apparatus, Model 935, South Natick, MA, USA).

**Table 1** Diet composition[1,2]

| Ingredient | g/kg |
|---|---|
| Egg white, spray-dried | 200 |
| Corn starch | 150 |
| Dextrose | 502.996 |
| Cellulose fiber | 50 |
| Corn oil | 50 |
| Mineral mix | 35 |
| Vitamin mix | 10 |
| Biotin | 0.004 |
| Choline bitartrate | 2 |

[1]Purchased from Dyets Inc., Bethlehem, PA, USA.
[2]Formulated according to the American Institute of Nutrition recommendations,[21,22] except for the following modifications: Egg white and dextrose were used in place of casein and sucrose, respectively. Supplemental biotin was used in addition to the AIN vitamin mix, because egg white was used as the protein source.

### *Measurement of $^{14}$C-linoleic acid absorption*

After postoperative recovery, each rat was infused via the duodenal catheter with a lipid emulsion at 3.0 ml/hr. The lipid emulsion contained 3.2 μCi $^{14}$C-linoleic acid ($^{14}$C-LA) (specific activity: 53 mCi/mmol, radiochemical purity: 99.0%, MEN Research Products, Boston, MA, USA), 171 μmol linoleic acid (LA), 488 μmol triolein, 520 μmol sodium taurocholate, and 1.5 μmol α-tocopherol in 24 ml phosphate buffered saline (6.75 mM $Na_2HPO_4$, 16.5 mM $NaH_2PO_4$, 115 mM NaCl, and 5 mM KCl per L, pH 6.4). The lipid emulsion was prepared by sonication (W-375, Heat Systems-Ultrasonics, Long Island, NY, USA) under a constant $N_2$ stream. During infusion of the lipid emulsion, lymph was collected at hourly intervals for 8 hours via the lymph cannula into a preweighed ice-cooled plastic centrifuge tube containing 4.0 mg of $Na_2$-EDTA. At each hourly interval, the lymph output was measured by reweighing the tube.

Aliquots (100 μl) of hourly lymph were mixed with scintillation liquid (18 ml) (ScintiVerse, Fisher Scientific Co., Fair Lawn, NJ, USA) and counted to determine the $^{14}$C-radioactivity appearing in the lymph (Beckman LS-8000 series liquid scintillation systems, Beckman Instruments, Fullerton, CA, USA). Percentages of the injected dose in hourly lymph volumes were calculated and expressed as % absorption.

### *Lymph lipid analysis*

Total lipids from 100 μl hourly lymph samples were extracted[25] using a mixture of chloroform:methanol 2:1 (v/v) containing 10 mg of butylated hydroxytoluene/100 ml. The internal standard (C19:0) was added during lipid extraction. The lipid extracts were further saponified and methylated using methanolic $BF_3$, as described by Slaver and Lanza.[26] The methylesters of fatty acids were dissolved in petroleum ether and separated by gas chromatography (Hewlett-Packard model 5580A gas chromatography, Hewlett-Packard, Palo Alto, CA, USA) equipped with a Stabilwax-DA capillary column (15 m × 0.53 mm ID, Resteck Corp., Bellefonte, PA, USA). Qualitative and quantitative analyses of each fatty acid were achieved by comparing the retention times and the peak areas to the known standards. The standards were prepared from methyl-esters of fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3, C20:4, and C19:0 as the internal standard) (Nu-Chek Prep, Elysian, MN, USA).

Phospholipid was measured colorimetrically by the method of Raheja et al.[27] (UV-1201 Spectrophotometer, Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Total cholesterol was determined using o-phthalaldehyde as described by Rudel and Morris.[28] Free cholesterol was separated from total cholesterol by digitonin precipitation, and esterified cholesterol was calculated by subtracting free cholesterol from total cholesterol.[29] Phosphatidylcholine (purity: <99.0%) and cholesterol (purity: 99.0%) were purchased from Sigma Chemical Co., St. Louis, MO, USA and used

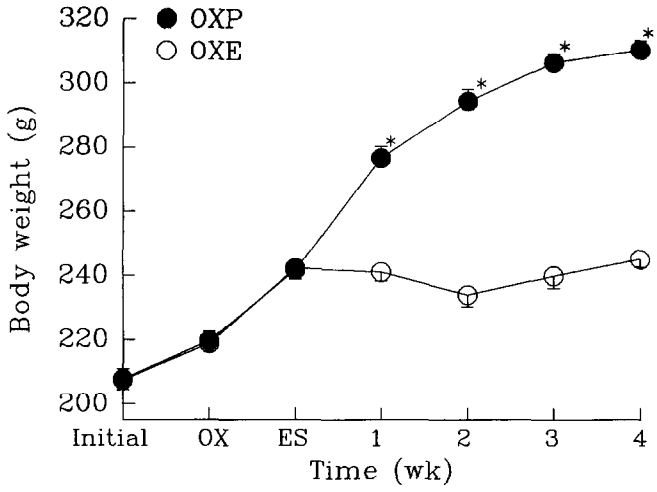


**Figure 1** Time-course effects of estradiol on averge body weights. The body weight of OXE rats were maintained at the pretreatment level throughout 4 weeks, whereas OXP rats gained weight steadily. *OXE, rats implanted with a time-release 17β-estradiol pellet; OXE,* rats implanted with a placebo pellet; OX, time of ovariectomy; ES, time of estrogen implantation. Asterisks (*) denote significant differences.

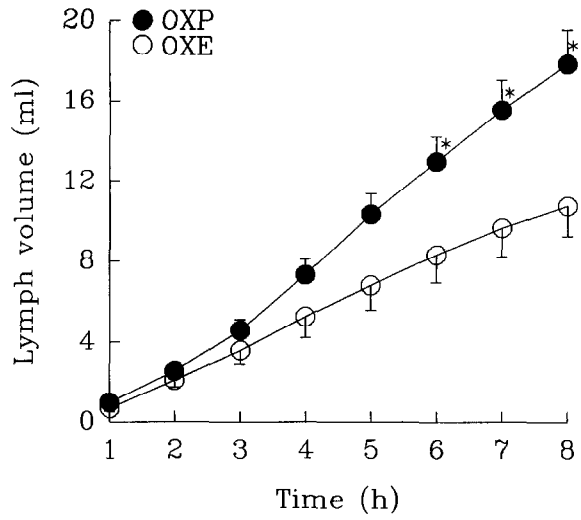


**Figure 2** Rate of lymph flow at hourly intervals for 8 hours. The average rates of lymph flow in OXE and OXP rats were 1.4 and 2.2 ml/hr, respectively. The total lymph flow for 8 hours (10.8 ml) in OXE rats was significantly lower than in OXP rats (17.8 ml). For acronyms, refer to *Figure 1.* Asterisks (*) denote significant differences.

as standards for the analyses of phospholipid and cholesterol, respectively.

### *Statistical analysis*

Student *t*-tests were used for comparison of single-point data between groups, and the analysis of variance (ANOVA) along with the least significant difference (LSD) test of the SAS statistical package[30] was used for multiple-point data. All data were expressed as mean ± SD, and the level of significance was determined at $P < 0.05$. The correlation coefficients were determined by linear regression analyses.[30]

## Results

### *General observations*

The time-course effect of ES on average body weights is presented by *Figure 1.* The body weights of OXE and OXP rats did not differ before ES treatment. The ovariectomized rats with ES implants (OXE) maintained their body weight with no significant loss or gain relative to that before ES treatment. In contrast, the ovariectomized rats without ES treatment (OXP) significantly and continuously gained weight with time. Significant differences were observed in body weight between the two groups at 1 week and thereafter. During ES treatment, OXE rats consumed significantly smaller amounts of food than OXP rats (*Table 2*). The changes in body weight were associated in general with variations in food intake. Food efficiency (g weight gain/g food) was significantly lower in OXE rats than OXP rats during the first 2 weeks after ES implantation, with no significant difference at 3 and 4 weeks.

### *Lymphatic absorption of $^{14}$C-linoleic acid ($^{14}$C-LA)*

The total volumes of lymph collected for 8 hours were 10.8 ml in OXE rats and 17.8 ml in OXP rats (*Figure 2*). The average rate of lymph flow was significantly lower in OXE rats (1.4 ml/hr) than in OXP rats (2.2 ml/hr). Significant differences in lymph flow were noted at 6 hr and thereafter. *Figure 3* presents the cumulative lymphatic absorption of

**Table 2** Food intake and food efficiency

| Group | OX[1] | ES[2] | Weeks after estradiol implantation 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| *Food intake (g/day)* | | | | | | |
| OXE | 14.3 ± 1.9[3] | 15.6 ± 1.5 | 14.3 ± 2.0[a] | 14.8 ± 2.1[a] | 16.6 ± 0.9[a] | 13.9 ± 1.6[a] |
| OXP | 15.4 ± 2.1 | 16.7 ± 1.3 | 19.0 ± 0.9[b] | 19.7 ± 1.2[b] | 19.9 ± 1.0[a] | 17.7 ± 1.5[b] |
| *Food efficiency (g body weight gain/g food)* | | | | | | |
| OXE | 0.11 ± 0.08 | 0.21 ± 0.03 | −0.01 ± 0.18[a] | −0.07 ± 0.07[a] | 0.04 ± 0.05 | 0.07 ± 0.04 |
| OXP | 0.11 ± 0.12 | 0.19 ± 0.05 | 0.28 ± 0.05[b] | 0.13 ± 0.03[b] | 0.09 ± 0.06 | 0.04 ± 0.05 |

[1]Day of ovariectomy.
[2]Day of estrogen pellet implanted.
[3]Mean ± SD, $n = 5$. Values in the same column not sharing a common superscript are significantly different ($P < 0.05$).

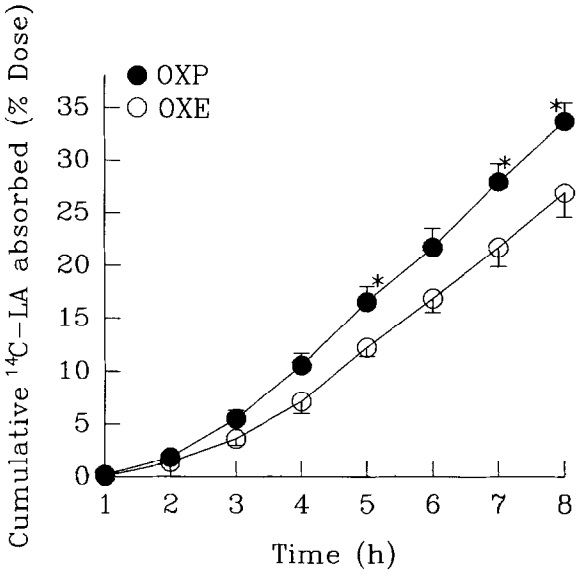


**Figure 3** Lymphatic absorption of $^{14}$C-LA. The total lymphatic absorption of $^{14}$C-LA was markedly lower in OXE rats (26.9 ± 5.2% dose) than that in OXP rats (33.6 ± 4.0%) for 8 hours. The significant differences were observed at 5 hours and thereafter. For acronyms, refer to *Figure 1*. Asterisks (*) denote significant differences.

$^{14}$C-LA for 8 hr. The total lymphatic absorption of $^{14}$C-LA was significantly lower in OXE rats (26.9 ± 5.2% dose) than in OXP rats (33.6 ± 4.0%). Significant differences were observed at 5 hr and thereafter. The specific activity of $^{14}$C-LA (dpm/mmol × $10^6$) increased with time in a similar pattern in both groups. The specific activities of $^{14}$C-LA in the hourly lymph varied from 0.5 at 1 hr to 4.3 at 8 hr with no significant difference between the groups at any time intervals. This indicated that the difference in $^{14}$C-LA absorption between the groups was not due to dilution of the tracer in the enterocyte. At 8 hr, the specific activity of $^{14}$C-LA was almost equal to that (4.2 dpm/mmol × $10^6$) in the lipid emulsion infused. The absorption of $^{14}$C-LA was correlated highly with the lymph volume ($r$ = 0.88, $P$ < 0.001), but was not correlated with the body weight if animals ($r$ = 0.53, $P$ > 0.113).

### *Lymphatic outputs of fatty acids*

*Figure 4* shows the lymphatic outputs of oleic (OA) and LA, which were included in the lipid emulsion infused. The output of OA, which was infused in the form of triolein, was significantly lower in OXE rats (780.0 ± 85.2 μmol) than OXP rats (894.5 ± 90.6 μmol). The average rates of lymphatic OA output in OXE and OXP groups were 97.5 μmol/h and 111.8 μmol/h, respectively. Similarly, the output of LA was significantly lower in OXE rats (105.9 ± 8.8 μmol) than OXP rats (119.7 ± 18.4 μmol). The average rates of lymphatic LA output were 13.2 μmol/hr in OXE and 15.0 μmol/hr in OXP rats. The lymphatic outputs of FAs, which were not included in the lipid emulsion, are shown by *Figure 5*. The outputs of palmitic (C16:0), stearic (C18:0), and linolenic (C18:3) acids were reduced significantly by ES treatment. The cumulative outputs of C16:0, C18:0 and C18:3 were 130.8 ± 8.8, 91.5 ± 6.2, and 32.6 ± 9.1 μmol in OXE rats and 195.0 ± 23.5, 161.8 ± 16.6, and 81.2 ± 16.2 μmol in OXP rats, respectively. The outputs of arachidonic acid (C20:4) were not significantly different between the groups.

### *Lymphatic output of phospholipid (PL)*

The output of PL in OXE rats was significantly and consistently lower throughout 8 hours (*Figure 6*). The total amounts of PL released for 8 hours in OXE and OXP rats

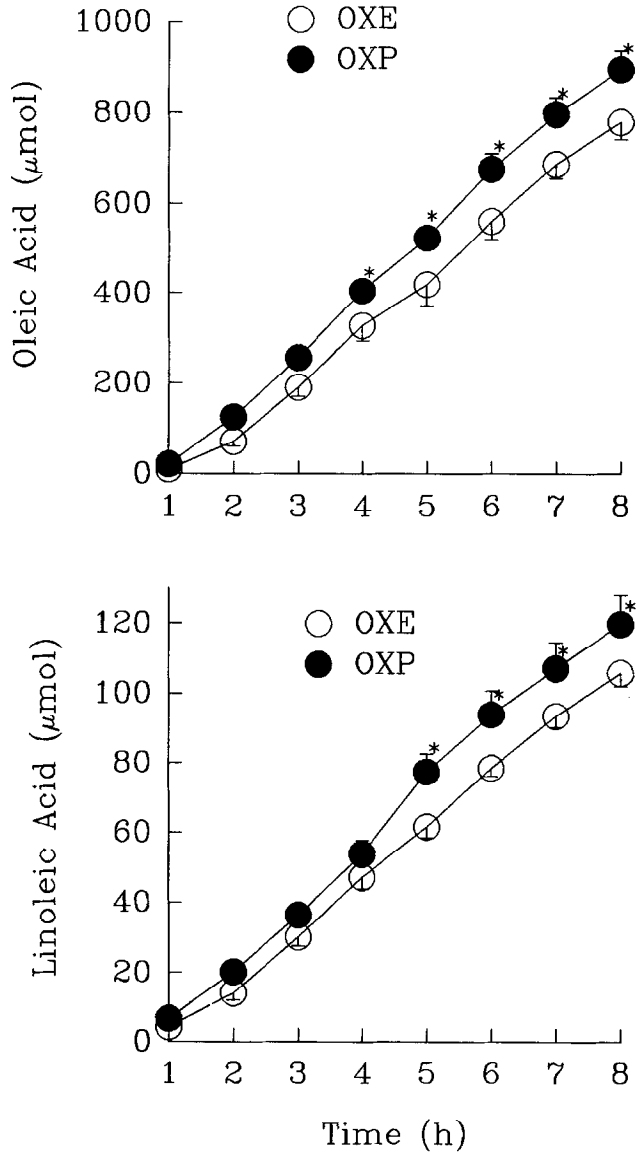


**Figure 4** Cumulative lymphatic outputs of oleic (upper panel) and linoleic acids (lower panel), which were included in the lipid emulsion infused. The total outputs of OA in OXE and OXP rats were 780.0 ± 85.2 μmol and 894.5 ± 90.6 μmol, respectively. The average rate of lymphatic OA output in OXE rats (97.5 μmol/hr) was significantly lower than in OXP rats (111.8 μmol/hr). The cumulative output of LA (lower panel) also was significantly lower in OXE rats (105.9 ± 8.8 μmol) than OXP rats (119.7 ± 18.4 μmol). The average rates of lymphatic LA output were 13.2 μmol/hr in OXE and 15.0 μmol/hr in OXP rats. For acronyms, refer to *Figure 1*. Asterisks (*) denote significant differences.

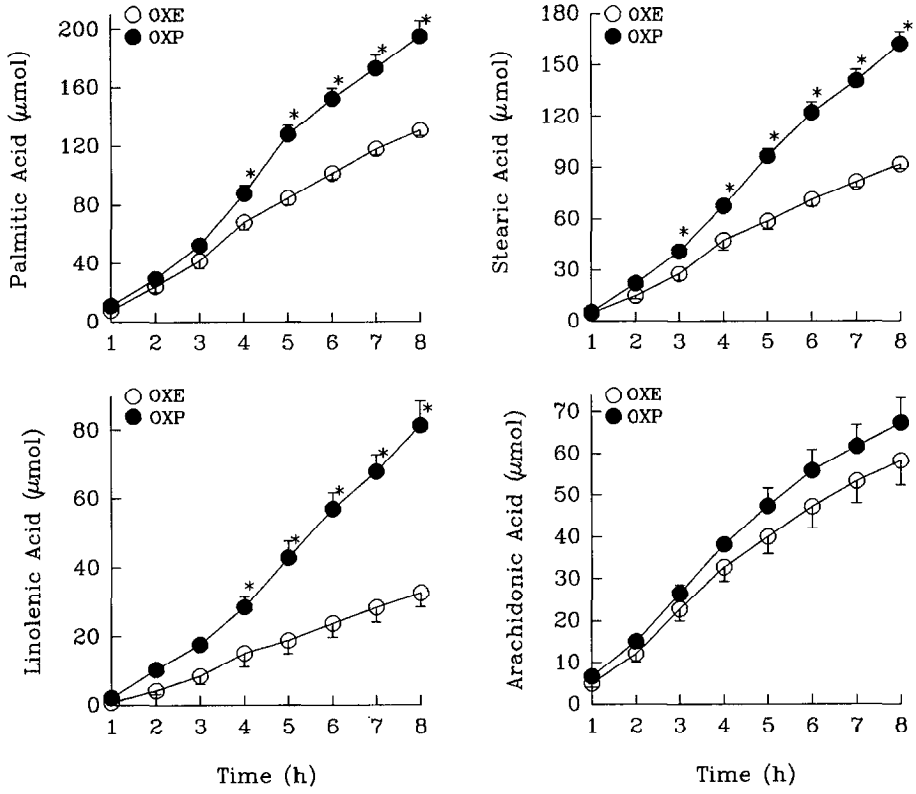


**Figure 5** Cumulative lymphatic outputs of endogenous FAs. The outputs of palmitic (C16:0), stearic (C18:0), and linolenic (C18:3) acids were significantly lower in OXE rats. The total outputs of C16:0, C18:0 and C18:3 for 8 hours were 130.8 ± 8.8, 91.5 ± 6.2, and 32.6 ± 9.1 μmol in OXE rats and 195.0 ± 23.5, 161.8 ± 16.6, and 81.2 ± 16.2 μmol in OXP rats, respectively. No difference in arachidonic acid (20:4) output was noted between the groups. For acronyms, refer to *Figure 1*. Asterisks (*) denote significant differences.

were 48.5 ± 7.2 μmol and 71.0 ± 11.7 μmol, respectively. The total amount of PL secreted in OXE rats was 68.4% of that in OXP rats. The average rates of PL output were 6.1 μmol/h in OXE rats and 8.9 μmol/h in OXP rats. The lymphatic output of PL was correlated highly with the amount of $^{14}$C-LA absorbed ($r = 0.85$, $P < 0.001$) and the amounts of individual FAs released into the lymph ($r = 0.95$, $P < 0.001$). However, no correlation was noted between PL output and body weight ($r = 0.15$; $P > 0.725$).

### *Lymphatic output of cholesterol*

*Figure 7* shows the cumulative lymphatic outputs of free cholesterol (FC) and esterified cholesterol (EC). No significant differences were observed in FC between OXE and OXP groups. However, the output of EC was significantly lower in OXE rats starting at 4 hours than in OXP rats. During 8 hours, the total outputs of EC in OXE and OXP rats were 5.0 ± 1.0 μmol and 7.6 ± 1.4 μmol, respectively. The lymphatic output of total cholesterol (TC) was significantly lower in OXE rats (8.7 ± 2.1 μmol) than in OXP rats (11.6 ± 1.7 μmol). The ratios of FC to TC tended to be higher in OXE rats, but no significant differences were noted between the groups at any hourly intervals.

## Discussion

The present results demonstrate that ES replacement in ovariectomized rats lowers the lymphatic absorption of $^{14}$C-LA and OA, which was infused in the form of triolein. Additionally, data also show that the lymphatic outputs of FAs (C16:0, C18:0, and C18:3) and cholesterol, which were not included in the lipid emulsion, were lowered significantly in rats treated with ES. The lymphatic absorption of $^{14}$C-LA was correlated highly with the lymphatic output of PL ($r = 0.85$; $P < 0.001$) and lymph flow ($r = 0.88$, $P < 0.001$). Likewise, the outputs of individual FAs, exogenous and endogenous, also were correlated significantly with the lymphatic PL output and lymph flow. In both groups, the absorption of $^{14}$C-LA was not related to the body weight ($P > 0.113$) of rats, and the lower $^{14}$C-LA absorption in OXE rats was not attributable to the difference in body weight between the groups. In the present experiment, the dose of total FA infused was set at 204 μmol/hr, which was well below the normal absorptive capacity (500 μmol/hr) of the rat small intestine, as estimated previously.[31]

The present study provides the first evidence that ES has an inhibitory effect on the intestinal absorption of lipid in ovariectomized rats. The marked decrease in PL output in ES-treated rats suggests that ES may reduce the intestinal absorption of lipids by altering the availability of PL for chylomicron formation during lipid absorption. This possibility is strengthened further by our recent observation that an intraduodenal infusion of phosphatidylcholine (PC) in OXE rats fully restored the lymphatic absorption of $^{14}$C-LA to the level observed in OXP rats (unpublished data). The absorption of dietary fat requires the intestinal formation of

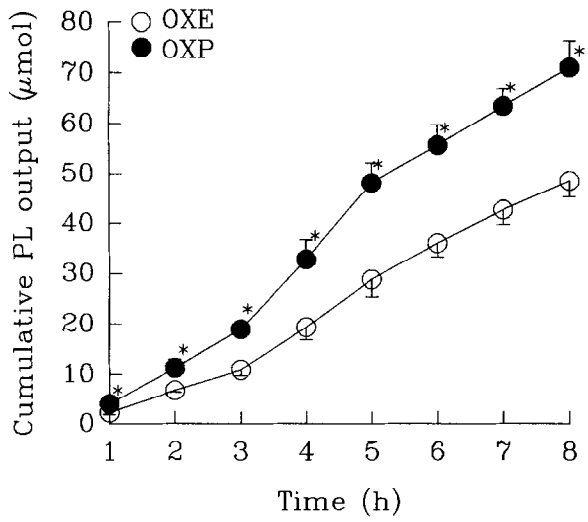


**Figure 6** Lymphatic output of PL during lipid infusion for 8 hr. The average rates of PL output were 6.1 μmol/hr in OXE rats and 8.9 μmol/hr in OXP rats. The total amounts of PL released for 8 hours in OXE and OXP rats were 48.5 ± 7.2 μmol and 71.0 ± 11.7 μmol, respectively. The total amount of PL secreted in OXE rats was 68.4% of that in OXP rats. The output of PL in OXE rats was significantly lower throughout 8 hours. For acronyms, refer to *Figure 1*. Asterisks (*) denote significant differences.

chylomicron, which consists of a core of neutral lipids (largely triglyceride of dietary origin) and a surface coat of PL monolayer and apolipoproteins. Thus, during active fat absorption, a sufficient supply of PL via the biliary route is necessary to meet the increased demand for the formation and release of chylomicrons into the lymphatics.[32–34] Although the enterocyte is capable of synthesizing PC de novo, it is not sufficient to sustain the high PC turnover during active fat absorption.[35,36] Ample evidence shows that fat absorption is reduced in rats deprived of biliary or dietary PL.[35–39] Because the lipid emulsion infused in the present study contained no PL, the PL released into the lymph is thought to be largely of biliary origin. The decrease in the lymphatic PL secretion observed in OXE rats may reflect a reduction in the amount of biliary PL released into the intestinal lumen. However, the precise mechanism underlying the effect of ES on the biliary secretion of PL awaits further investigation. It is of interest to note that, in contrast to the reduced secretion of PL into the lymph, the fasting levels of plasma PL were shown to be elevated markedly in OXE rats, compared with those of OXP rats.[6] Similar observations also were reported in other studies.[40–42] It is well known that ES promotes the hepatic synthesis and secretion of PL into the plasma via lipoproteins such as high density lipoproteins (HDL).[42,43] It is possible that the ES-induced stimulation of the hepatic release of lipoproteins may result in an increased drainage of liver PL into the plasma and, consequently, may limit the amount of PL released via the bile into the intestinal lumen during fat absorption. This possibility is currently under investigation.

ES treatment also may delay or inhibit the esterification of FFAs in the intestinal mucosa. It has been shown[19] that ES treatment in male rats significantly increased the concentrations of FFAs and decreased the concentration of tri-

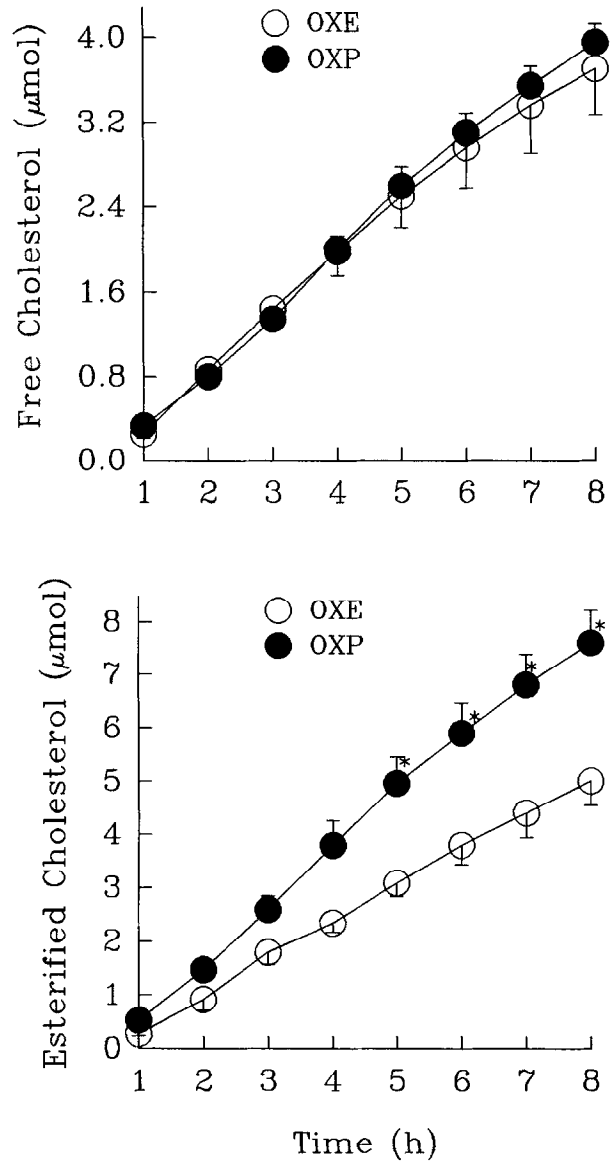


**Figure 7** Cumulative lymphatic outputs of free cholesterol (FC) (upper panel) and esterified cholesterol (EC) (lower panel). No significant differences in the output of FC were observed between OXE rats (3.7 ± 1.0 μmol) and OXP rats (3.9 ± 0.4 μmol) during the 8-hr period. The lymphatic output of EC was significantly lower in OXE rats than that in OXP rats at 4 hours and thereafter. The total lymphatic output of EC for 8 hours was 5.0 ± 1.0 μmol in OXE and 7.6 ± 1.4 μmol in OXP rats. For acronyms, refer to *Figure 1*. Asterisks (*) denote significant differences.

glyceride (TG) in the intestinal mucosa following an intraduodenal infusion of a lipid emulsion. This finding suggests that the rate of re-esterification of absorbed FAs into TG is reduced in the intestinal mucosa of ES-treated animals, which in turn may slow the rate of TG incorporation into chylomicrons. The slower rate of FA esterification may be caused by the limited availability of PL to the enterocyte. In previous studies,[34,44] a 35 to 40% decrease in the activities of mono- and di-acylglycerol acyltransferases was observed in the intestinal microsomes from bile-diverted rats. The decreases in enzyme activities were associated with a marked decline in microsomal PC.[34] The addition of PC to

the microsomes from these rats[34] or intraduodenal infusion of PC along with sodium taurocholate[44] resulted in a complete restoration of the enzyme activities. Thus, a reduced availability of PL to the enterocyte in ES-treated rats, as suggested by the lower lymphatic output of PL in OXE rats, may slow the mucosal esterification of OA and LA and may partly explain the decreases in their lymphatic outputs in OXE rats. This mechanism also may account for the reduced amounts of endogenous FAs released into the lymphatics in OXE rats, although the exact sources of endogenous FAs found in lymph remain unclear. The endogenous FAs appearing in lymph lipids potentially could be derived from plasma, bile, and enterocytes. Evidence suggests that the quantitative contribution of plasma FAs to chylomicron lipids may be minimal. In rats[45] as well as in humans,[46] it has been shown that FAs taken up from the plasma are not incorporated in significant amounts into intestinal lipoproteins. These findings are consistent with our observation here that the lymphatic outputs of endogenous FAs were lower, despite a marked elevation in plasma FFAs in ES-treated rats.[6] Whether ES specifically alters the secretion of lipids into the lymph via the biliary route and the mucosal esterification of endogenous FAs[47] should be investigated in future studies.

The decrease in FA absorption in OXE rats also may be linked partly to a reduced rate of lymph flow. Tso et al.[48] showed that the rate of lymph flow significantly influences the intestinal transport of chylomicrons, and a decrease in lymph flow reduces the rate of intestinal lipid absorption. They found that the intestinal chylomicron transport rate was related positively to lymph flow, when the intestinal lymph flow was below 40 μl/min (2.4 ml/hr). Our results also showed that the lymph flow (1.4 ml/h in OXE rats and 2.2 ml/hr in OXP rats) was highly correlated ($r = 0.88$, $P < 0.001$) with the lymphatic absorption of $^{14}$C-LA. Furthermore, the lymph flow was correlated significantly with the amounts of PL ($r = 0.75$, $P < 0.001$) and FAs released into the lymph ($r > 0.74$, $P < 0.001$). Our findings are consistent with the observation of Krause et al.[18] that the intestinal transport of TG was lowered in ES-treated male rats, with a concomitant reduction in lymph flow rate. The findings strongly suggest that the reduced rate of lymph flow in OXE rats may be another important factor contributing to the decrease in lymphatic lipid absorption. In normal animals,[49,50] a rise in lymph flow following fat feeding probably is related to both enhanced blood flow to the intestine and increased intestinal capillary permeability. Whether ES treatment produces alterations in these mechanisms remains to be determined.

Finally, the present data also demonstrate, for the first time, that the lymphatic output of cholesterol is lowered by ES treatment. The reduced cholesterol output was due to a decrease in EC with no change in FC. Because the lipid emulsion infused was devoid of cholesterol, lymph cholesterol may be of biliary, enterocyte, and/or plasma origin. Currently, no information is available as to whether ES treatment in ovariectomized rats alters the biliary or intestinal secretion of cholesterol or lymphatic filtration of plasma lipoproteins. Further study is needed to explain the lower lymphatic output of endogenous cholesterol in ES-treated rats. Regardless of the origin of lymph cholesterol, however, the significant decrease in lymphatic EC output observed in OXE rats suggests a possible inhibitory effect of ES on mucosal cholesterol esterification. Although no direct evidence for such an effect of ES has been demonstrated, a marked inhibition of cholesterol esterification and stimulation of CE hydrolysis were shown in liver microsomes preincubated in vitro with estradiol.[51] The esterification of absorbed cholesterol in the enterocyte is catalyzed primarily by acylCoA:cholesterol acyltransferase (ACAT) and is suggested to be an important regulator of its incorporation into chylomicrons and, hence, the absorption of cholesterol into the lymphatics.[52] Whether the activity of ACAT is influenced by ES remains to be determined.

In summary, the present study demonstrates that ES replacement in ovariectomized rats at a physiological dosage significantly reduces not only the lymphatic absorption of exogenous FAs, but also the lymphatic output of endogenous lipids. The reduced lymphatic output of lipids is correlated highly with the decrease in PL output and lymph flow. These findings suggest that ES replacement may reduce the hepatic synthesis and/or secretion of PL via the bile into the intestinal lumen. The limited availability of PL to the enterocyte may decrease the rate of FA esterification into TG and delay the formation of the chylomicron surface coat. The lower rate of intestinal absorption of FAs in the ES-treated rats may reduce the rate of energy influx from the intestine and may have a significant impact on the rates of fat assimilation and metabolism.

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