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Estradiol replacement elevates the serum and tissue levels of α-tocopherol in ovariectomized rats

Hae Jung Chung and Sung I. Koo

Department of Foods and Nutrition, Kansas State University, Manhattan, KS USA

Time-course changes in α-tocopherol (αTP) in serum and selected tissues were examined in ovariectomized rats with estradiol (ES) replacement. Female rats (242 ± 7 g) were ovariectomized (day 0) and divided into two groups of five each; one group was implanted subcutaneously with a time-release ES pellet (OXE; 25 μg/day/rat) and the other with a placebo pellet (OXP). Rats were pair-fed twice daily a modified AIN-93G diet at 9:30 am and 4:30 pm. At day 0 and week 2, 4, and 6 of ES replacement, αTP, phospholipid (PL), and cholesterol in serum and HDL fraction were measured. HDL was isolated by affinity chromatography. ES produced a marked increase in total serum αTP and HDL cholesterol without affecting total serum cholesterol. The increase in serum αTP was caused by a selective increase in HDL αTP with no change in non-HDL αTP. HDL αTP in OXE rats was 10.3 ± 0.8 μmol/L at day 0 and rose to 15.7 ± 1.6 μmol/L at 2 wk with no further increase with time. αTP in the non-HDL fraction did not differ between groups. The αTP level in the non-HDL fraction was 11.1 ± 2.0 μmol/L at day 0 and remained unchanged with time. The marked rise in HDL αTP in OXE rats was associated with a parallel increase in HDL PL (r = 0.80). The concentrations of αTP in the liver (103.4 ± 12.1 nmol/g) and kidneys (52.1 ± 3.6 nmol/g) of OXE rats were also significantly higher than those in the liver (74.4 ± 14.4 nmol/g) and kidneys (21.1 ± 2.2 nmol/g) of OXP rats. The results provide the first evidence that ES replacement at a physiological dose produces a marked increase in the body status of αTP. The finding may explain partly the inhibitory effect of ES on the oxidative modification of serum lipoproteins and elevation of serum HDL in response to ES replacement. (J. Nutr. Biochem. 9:67–74, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Women are distinctly less susceptible to coronary heart disease (CHD) in their reproductive years than men.[1–4] However, ample epidemiologic evidence indicates that the risk of CHD rises substantially in postmenopausal and ovariectomized women.[1–6] Several studies[7,8] have shown that ES replacement therapy may reduce the incidence of CHD in postmenopausal women. The health benefits of ES replacement have been attributed largely to its effects on lipid and lipoprotein metabolism, which include an increase in serum (plasma) high-density lipoprotein (HDL) cholesterol and a decrease in low-density lipoprotein (LDL) cholesterol.[9–12] Such changes in lipoprotein profiles produced by ES have been equated to a reduction of 25 to 50% in CHD risk.[13] However, the mechanism whereby ES reduces the relative risk of CHD is multifactorial and may be associated with its favorable actions on blood pressure, prostaglandin metabolism, and blood coagulation factors.[6,7,14]

In addition, ES has been shown to exhibit antioxidant properties.[15–18] Recent evidence indicates that ES inhibits LDL oxidation,[19–23] suggesting that the antiatherogenic effect of ES may be explained partly by its action as an antioxidant. However, the antioxidant effect of ES on lipoproteins, as demonstrated in earlier studies,[19–22] has been determined mostly in model systems under in vitro conditions using Cu^{+2}. Furthermore, the concentrations of

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Address correspondence and reprint requests to Dr. Sung I. Koo at Department of Foods and Nutrition, Kansas State University, Manhattan, KS 66506, USA.
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655 Avenue of the Americas, New York, NY 10010

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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 biotin sucrose mix)	4
Choline bitartrate	2.5

[1]Formulated by Dyets, Bethlehem, PA USA, according to the recommendations of the American Institute of Nutrition.[31]
[2]Containing 0.02% tert-butylhydroquinone.

ES used in these in vitro studies were much higher than are found under physiological conditions. Thus, it remains unknown whether and how ES may function in vivo as an antioxidant in lipoproteins and cells.

Alpha-tocopherol (αTP) is a potent biologic antioxidant that also has been shown to inhibit the oxidation of lipoproteins including LDL.[24–26] Studies have suggested that supplemental vitamin E attenuates the development of atherosclerosis in animals[27] and may reduce the risk of CHD in humans.[28] The vitamin is carried by all circulating lipoproteins and can be taken up by cells via the apoB,E (LDL) receptor.[29] Recently, we observed that ES replacement in ovariectomized rats produced a significant increase in intestinal αTP absorption.[30] This finding suggests the possibility that ES may affect the transport of αTP via serum lipoproteins and also may influence the nutritional or body status of the vitamin. The postulated antioxidant action of ES on lipoproteins may be mediated in vivo partly via its interaction with αTP in lipoproteins and tissues. The present study was conducted to investigate whether ES replacement in ovariectomized rats influences the concentrations and distribution of αTP in serum and selected tissues.

Methods and materials

Animals and diet

Ten female Sprague-Dawley rats (Harlan Spargue Dawley, Inc., Indianapolis, IN, USA) initially weighing 209 ± 9 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. The rats were cared for in an animal care facility at Kansas State University accredited by the American Association for the Accreditation of Laboratory Animal Care. All procedures for animal use and care were approved by the Institutional Animal Care and Use Committee.

The rats were fed a nutritionally adequate diet (*Table 1*) formulated according to the AIN-93G recommendations.[31] Because OXE rats consumes significantly less food than OXP, as observed previously,[32] both groups of rats were pair-fed and trained to develop a meal feeding behavior. All rats were fed twice daily 4 g at 0930 and 8 g 1630 hr for 18 days, and, from day 19 on, 4 g at 0930 and 9 g 1630 hr. This amount of diet fed (13 g/day) represented 90% of their average daily food consumption before ovariectomy. Food was given in a glass jar with a stainless steel lid designed to prevent food spillage and waste. Each meal was consumed by both groups within 1 hr. All animals were given free access to deionized water from a water purification system (Millipore Corporation, Marlboro, MA USA) delivered via a stainless-steel watering system.

At 3 weeks, rats were ovariectomized under halothane anesthesia (2% halothane in 2 mL oxygen/min). After 18 days' postoperative recovery, the rats were assigned randomly to two groups of five each. One group (OXE), average weight 267 ± 2 g, was implanted subcutaneously in the posterior neck with a 60-day release 17β-estradiol pellet (ES) (25 μg/day; Innovative Research of America, Toledo, OH, USA) and the other (OXP), average weight 268 ± 3 g, was implanted with a placebo pellet containing no ES.

Collection of serum samples and removal of tissues

At day 0 and weeks 2, 4, and 6 after ES implantation, rats from each group were starved for 16 hr. Under halothane anesthesia, blood samples were withdrawn via the orbital sinus. After blood sampling at 6 weeks, the rats were killed under halothane anesthesia, and the liver, kidneys, heart, and lungs were excised and stored frozen at −70°C until used for analysis.

Isolation of HDL

Serum was separated by centrifugation at 1,000 × g for 60 min, and HDL was isolated from fresh serum by affinity chromatography.[33] Columns used for HDL separation (Isolab, Akron, OH USA) were packed with heparin-agarose as the affinity medium. Serum (300 μL) was placed on the filter disc of the affinity column and allowed to migrate into the medium. The HDL was eluted by adding 700 μL of 120 mM NaCl, followed by a wash with 800 μL of 120 mM NaCl. The non-HDL fraction (VLDL + LDL) was eluted with 1.8 mL of 500 mM NaCl. This procedure yielded greater than 98% recovery based on the amounts of cholesterol eluted in both fractions.

Determination of αTP, PL, and cholesterol

For all analyses, samples were prepared under subdued light. For αTP analysis, lipids were extracted from whole serum (50 μL), HDL fraction (200 μL), and tissues (300 mg) using the method of Folch et al.[34] A mixture of chloroform and methanol (2:1, v/v) containing butylated hydroxytoluene (0.45 mol/L methanol) was used for extraction. The lipid extracts were filtered through a syringe filter (nitrocellulose filter membrane; 0.45 μm TF, Sigma Chemical Co., St. Louis, MO USA). The solvents were evaporated under a gentle stream of nitrogen at 40°C. The dry residues were solubilized in methanol[35] and mixed thoroughly. αTP analysis was performed by using a Beckman HPLC system equipped with a reverse-phase column (Alltech Alltima C18, 5 μm, 4.6 × 150 mm) and Beckman System Gold Software (Beckman Instruments, Inc., Fullerton, CA USA). Alpha-tocopheryl acetate (αTP acetate) was used as the internal standard. Methanol was used as the mobile phase[35] and propelled at 2 mL/min. Detection was monitored at 292 nm. Under the conditions, αTP and αTP acetate were eluted at 4.1 and 5.3 min, respectively. The αTP concentrations in the non-HDL fraction (VLDL + LDL) were calculated by subtracting the HDL αTP from the αTP in whole serum.

For phospholipid (PL) analysis, lipids were extracted[34] from serum and tissue samples. PL was measured colorimetrically (UV-1201, Shimadzu Scientific Instruments Inc., Columbia, MD USA) by the method of Raheja et al.[36] Cholesterol was determined directly from fresh serum (10 μL) and HDL fraction (80 μL) by using an enzymatic method (Sigma Diagnostic kit, Sigma Chemical Co., St. Louis, MO USA).

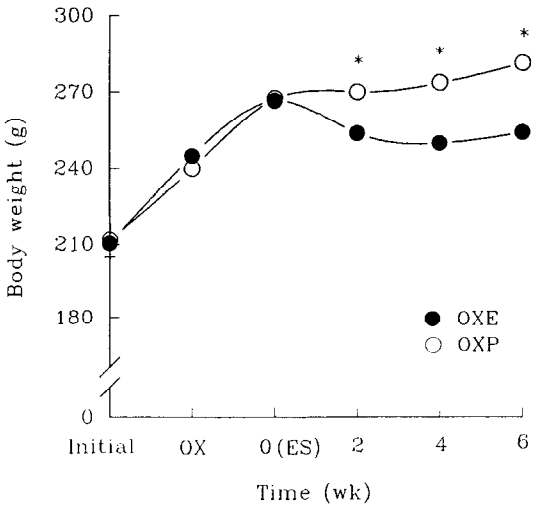

Figure 1 Time-course effect of ES implantation on average body weights. No significant differences were noted between groups in body weight at the start of experiment, at the time of ovariectomy, and at the time of ES implantation. After ES implantation, significant differences were observed at 2 weeks and thereafter. Whereas the average body weight of the OXE group decreased for the first 2 weeks and thereafter remained unchanged, the average body weight of the OXP group increased gradually throughout the experiment. Abbreviations: OXE, rats implanted with 17β-estradiol pellet; OXP, rats implanted with a placebo pellet; OX, time of ovariectomy; ES, time of estrogen implantation. All values are expressed as means ± SEM, $n = 5$. Asterisks (*) denote significant differences between groups at $P < 0.05$.

Statistical analysis

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

Results

General observations

Figure 1 shows changes in body weights of OXE and OXP rats over the course of the experiment. No significant differences were noted between groups in body weight at the start of the experiment (210 ± 13 g in OXE vs. 212 ± 5 g in OXP), at the time of ovariectomy (245 ± 7 g in OXE vs. 240 ± 8 g in OXP), and at the time of ES implantation (267 ± 2 g in OXE vs. 268 ± 3 g in OXP). After ES implantation, significant differences were observed at 2 weeks and thereafter. The average body weight of OXE rats decreased from 267 ± 2 g to 254 ± 4 g at 2 weeks and remained unchanged at 4 weeks (250 ± 4 g) and at 6 weeks (254 ± 5 g). The average body weight of the OXP group was significantly greater at 2 weeks and thereafter. As expected, the average weight of the uterus of OXE rats (0.73 ± 0.13 g) was significantly greater than that of OXP rats (0.14 ± 0.03 g).

Concentrations of αTP and PL in total serum and HDL

Table 2 shows the serum concentrations of αTP in OXE and OXP rats after ES replacement. At 2 weeks after ES replacement, total serum αTP concentration in the OXE group rose from 20.8 ± 2.6 μmol/L to 26.7 ± 2.8 μmol/L and remained at similar levels at 4 weeks (28.3 ± 1.4 μmol/L) and 6 weeks (26.8 ± 2.5 μmol/L). In contrast, the serum αTP concentrations in OXP rats remained unchanged from that observed at day 0. The total serum αTP concentrations in OXP rats were 20.1 ± 1.4, 22.1 ± 1.0, and 19.8 ± 1.2 μmol/L at 2, 4, and 6 weeks, respectively. The molar ratios of αTP (μmol) to cholesterol (mmol) in whole serum and the HDL fraction also were significantly higher in OXE rats than in OXP rats.

Figure 2A shows the αTP concentrations in HDL fractions from both groups. In the OXE group, average αTP concentration in HDL increased sharply from 10.3 ± 0.8 to 15.7 ± 1.6 μmol/L at 2 weeks after ES implantation. This

Table 2 Effect of estrogen on serum α-tocopherol and cholesterol standardized α-tocopherol[1]

	0 week[2]	2 week	4 week	6 week
Serum α-tocopherol (μmol)				
OXE	20.8 ± 2.6	26.7 ± 2.8—a	28.3 ± 1.4—a	26.8 ± 2.5—a
OXP	20.8 ± 2.6	20.1 ± 1.4—b	22.1 ± 1.0—b	19.8 ± 1.2—b
Serum α-tocopherol (μmol)/cholesterol (mmol)				
OXE	8.7 ± 0.5	9.8 ± 0.7—a	10.2 ± 0.8—a	10.1 ± 0.6—a
OXP	8.7 ± 0.5	7.7 ± 0.5—b	8.2 ± 0.8—b	7.7 ± 0.9—b
HDL α-tocopherol (μmol)/cholesterol (mmol)				
OXE	8.1 ± 0.6	10.1 ± 0.7—a	8.8 ± 0.8—a	10.0 ± 2.0—a
OXP	8.1 ± 0.6	7.3 ± 1.2—b	6.9 ± 0.7—b	7.1 ± 0.4—b
Non-HDL α-tocopherol (μmol)/cholesterol (mmol)				
OXE	9.7 ± 2.5	9.5 ± 1.1	13.4 ± 4.9	10.3 ± 2.8
OXP	9.7 ± 2.5	8.2 ± 1.8	10.1 ± 2.9	8.7 ± 2.0

[1]Mean ± S.D.; $n = 5$ per group. Values in the same column with different letters are significantly different ($P < 0.05$). OXE, rats implanted with estradiol pellet; OXP, rats implanted with a placebo pellet.
[2]Values at 0 week are identical for both groups and represent the pretreatment values before assigning ovariectomized rats to the OXE and OXP groups.

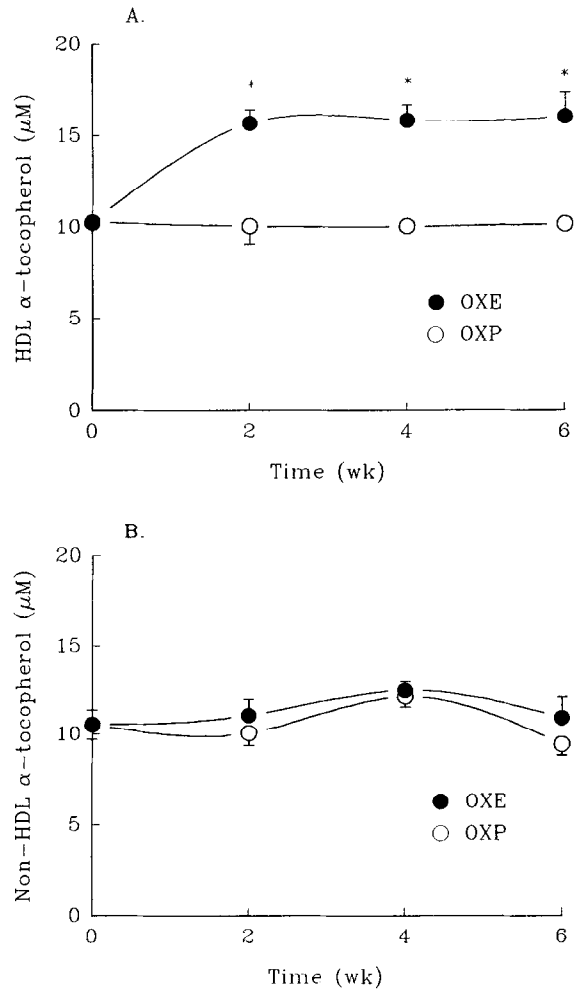

Figure 2 Changes in the concentrations of α-tocopherol (αTP) in the HDL and non-HDL fractions. In the OXE group, the αTP concentration in HDL increased sharply for first 2 weeks after ES implantation and this level was maintained throughout. In the OXP group, HDL αTP levels remained unchanged with time (*Figure 2A*). No significant differences were observed between groups in the αTP concentrations in the non-HDL fractions (VLDL + LDL) at the same intervals (*Figure 2B*). The increases in the total serum αTP concentrations in OXE rats were mainly due to increases in αTP concentrations in HDL fraction. All values are expressed as means ± SEM, $n = 5$. Asterisks (*) denote significant differences between groups at $P < 0.05$.

level was maintained at 4 weeks (15.8 ± 1.9 μmol/L) and 6 weeks (16.0 ± 2.9 μmol/L). In the OXP group, HDL αTP levels remained unchanged with time. The αTP concentrations in HDL in this group were 10.0 ± 2.2, 10.0 ± 0.8, and 10.4 ± 0.8 μmol/L at 2, 4, and 6 weeks, respectively. No significant differences were observed between groups in the αTP concentrations in the non-HDL fractions (VLDL + LDL) at the same intervals (*Figure 2B*). The increases in the total serum αTP concentrations in OXE rats were mainly attributable to increases in αTP concentrations in the HDL fraction. No differences were noted in the αTP concentrations in the non-HDL fractions. The αTP concentrations in the non-HDL fractions from OXE rats were 11.1 ± 2.0, 12.5 ± 1.1, and 10.1 ± 2.6 μmol/L at 2, 4, and 6 weeks after ES implantation, whereas those in OXP rats were 10.3 ± 0.8, 12.1 ± 1.4, and 9.4 ± 1.3 μmol/L at 2, 4, and 6 weeks, respectively. The cholesterol-standardized αTP values in both the HDL and non-HDL fractions also were significantly higher in OXE rats than in OXP rats (*Table 2*).

In OXE rats, the serum concentrations of PL rose significantly from 1.3 ± 0.1 at day 0 to 1.9 ± 0.1 mmol/L at 2 weeks and remained elevated at 4 weeks (2.2 ± 0.2 mmol/L) and 6 weeks (2.1 ± 0.2 mmol/L) after ES implantation. However, the PL concentrations in OXP group did not change with time. *Figure 3* shows the serum concentrations of PL in the HDL and non-HDL fractions. The PL concentrations in the HDL fraction were also markedly elevated by ES treatment (*Figure 3A*). Significant differences in the HDL PL concentrations were noted between groups. In OXE rats, the PL concentrations in serum HDL rose from 0.6 ± 0.1 at day 0 to 0.9 ± 0.3 mmol/L at 2 weeks, whereas in OXP rats the PL concentration did not

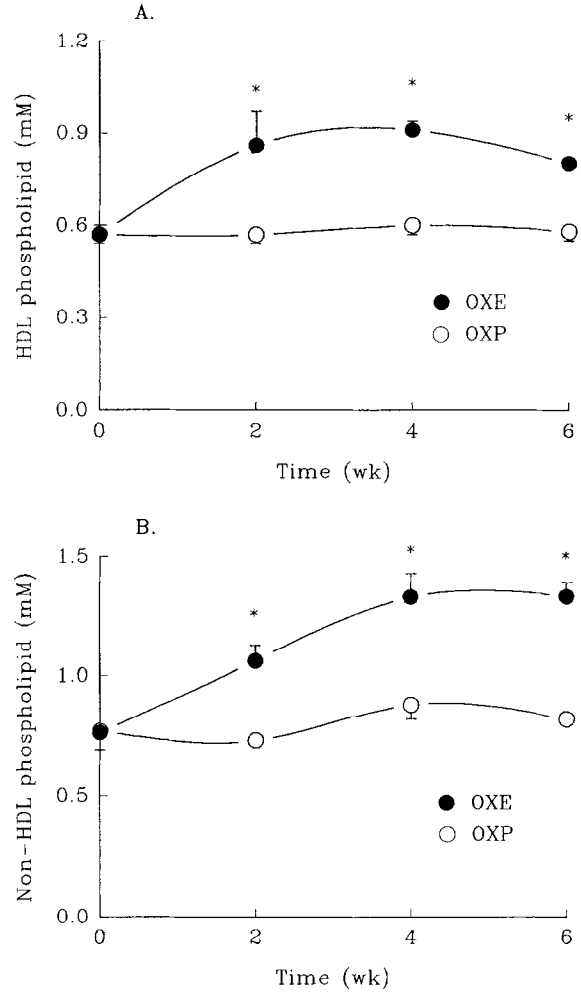

Figure 3 Changes in the concentrations of PL in the HDL and non-HDL fractions. In OXE rats, significant differences in PL concentrations in both fractions were noted between groups. In OXE rats, the levels of PL, as carried by serum HDL, rose from 0.6 ± 0.1 at day 0 to 0.9 ± 0.3 mmol/L at 2 weeks, whereas in OXP rats the PL concentration did not change with time (*Figure 3A*). Similar changes were observed in the levels of PL carried by the non-HDL fraction. All values are expressed as means ± SEM, $n = 5$. Asterisks (*) denote significant differences between groups at $P < 0.05$.

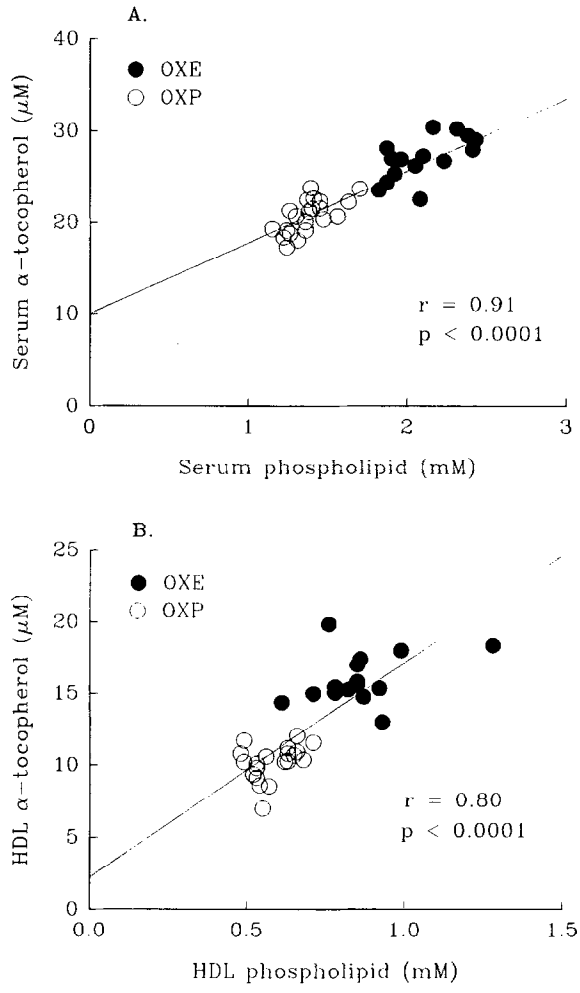

Figure 4 Correlation between serum αTP and PL concentrations. The levels of αTP were highly correlated with the levels of PL in whole serum (r = 0.91, $P < 0.0001$) (*Figure 4A*) and in the HDL fraction (r = 0.80, $P < 0.0001$) (*Figure 4B*).

change with time. The PL concentrations in the non-HDL fraction in OXE rats also were elevated significantly compared with those in OXP rats (*Figure 3B*).

The total serum concentrations of αTP were correlated highly with the serum concentrations of PL (r = 0.91, $P < 0.0001$) (*Figure 4A*). The concentrations of HDL αTP also were correlated significantly with serum HDL PL (r = 0.80, $P < 0.0001$) (*Figure 4B*).

Concentrations of αTP and PL in tissues

The concentration of αTP in the liver was increased significantly in OXE rats (103.4 ± 12.1 nmol/g) compared with that in OXP rats (74.4 ± 14.4 nmol/g) (*Table 3*). The αTP concentration in the kidney of OXE rats (52.1 ± 3.6 nmol/g) was more than double the concentration in the kidney of OXP rats (21.1 ± 2.2 nmol/g). The αTP concentration in the lung tended to be elevated in OXE rats (61.7 ± 10.4 nmol/g) compared with that in OXP rats (46.3 ± 9.8 nmol/g), although the difference was not statistically significant. Also, the αTP concentration in the heart did not differ between groups.

Table 3 Effect of estradiol replacement on the concentrations of α-tocopherol and phospholipid in selected tissues[1]

	OXE	OXP
α-Tocopherol (nmol/g tissue)		
Liver	103.4 ± 12.1—a	74.4 ± 14.4—b
Kidney	52.1 ± 3.6—a	21.1 ± 2.2—b
Lung	61.7 ± 10.4	46.3 ± 9.8
Heart	20.7 ± 5.2	23.2 ± 5.6
Phospholipid (μmol/g tissue)		
Liver	26.4 ± 1.6—a	29.3 ± 2.0—b
Kidney	31.3 ± 2.0—a	37.0 ± 1.7—b
Lung	20.3 ± 1.0	20.1 ± 1.5
Heart	29.7 ± 1.8—a	33.2 ± 2.5—b
α-Tocopherol/phospholipid (nmol/μmol)		
Liver	3.9 ± 0.6—a	2.5 ± 0.4—b
Kidney	1.7 ± 0.2—a	0.6 ± 0.1—b
Lung	3.1 ± 0.5	2.3 ± 0.5
Heart	0.7 ± 0.2	0.7 ± 0.2

[1]Mean ± S.D.; $n = 5$ per group. Values in the same row with different letters are significantly different ($P < 0.05$). OXE, rats implanted with estradiol pellet; OXP, rats implanted with a placebo pellet.

The liver concentration of PL was slightly but significantly lower in OXE rats (26.4 ± 1.6 μmol/g) than in OXP rats (29.3 ± 2.0 μmol/g). Likewise, the PL concentrations in the kidneys and heart also were lower in OXE rats than in OXP rats, whereas no difference was detected between groups in the PL concentration of the lungs (*Table 3*). The molar ratio of αTP (nmol) to PL (μmol) in the liver and kidney also were significantly higher in OXE rats than in OXP rats. The αTP/PL ratios for the heart and lungs did not differ between groups.

Total serum and HDL cholesterol concentrations

No significant differences in total serum cholesterol concentrations were observed between the two groups at the designated intervals. Also, in both groups, the serum concentrations of cholesterol did not change with time after ES implantation throughout the experiment. The serum cholesterol concentrations in OXE rats were 2.7 ± 0.0, 2.8 ± 0.2, and 2.6 ± 0.2 mmol/L at 2, 4, and 6 weeks, respectively. Those in OXP rats were 2.6 ± 0.2, 2.8 ± 0.2 and 2.6 ± 0.2 mmol/L at the same intervals, respectively. However, significant differences in HDL cholesterol were noted between groups at 2 weeks and thereafter (*Figure 5A*). ES replacement produced a marked increase in serum HDL cholesterol without affecting total serum levels. The HDL cholesterol level, as measured on the day of ES implantation, was 1.27 ± 0.01 mmol/L in both groups. With time, the levels of HDL cholesterol in the OXE group rose significantly to 1.60 to 1.80 mmol/L. In OXP rats, however, the serum levels of HDL cholesterol remained unchanged, as determined at 2, 4, and 6 weeks. No significant differences were noted in non-HDL cholesterol levels between groups or within groups with time (*Figure 5B*).

Discussion

The present study provides the first evidence that ES (17β-estradiol) replacement in ovariectomized rats mark-

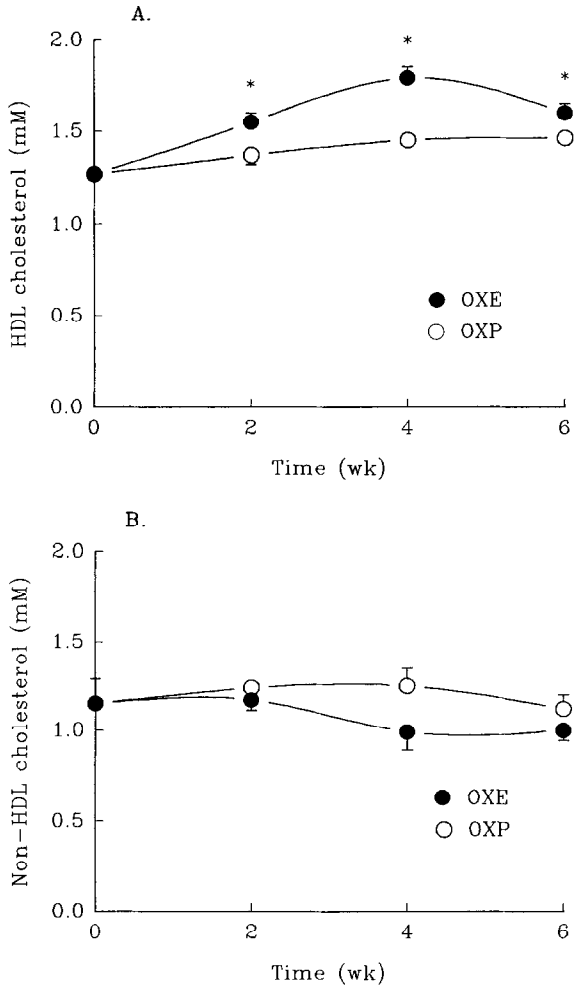

Figure 5 Changes in the concentrations of cholesterol in the HDL and non-HDL fractions. In OXE rats, a significant increase in serum HDL cholesterol was observed at 2 weeks after ES replacement with no change in total serum cholesterol. In OXP rats, the levels of HDL cholesterol did not change significantly throughout the experiment (*Figure 5A*). No significant differences were noted in non-HDL cholesterol between groups at any time intervals (*Figure 5B*). All values are expressed as means ± SEM, $n = 5$. Asterisks (*) denote significant differences between groups at $P < 0.05$.

edly elevates the serum levels of αTP and that this increase is attributable to a selective increase in αTP carried by HDL. The molar ratios of αTP/cholesterol in whole serum and in the HDL fraction also were significantly higher in OXE rats than in OXP rats. In addition, the present results demonstrate that ES replacement significantly increases the concentrations of αTP in the liver and kidney, major sites of vitamin E storage.[38] In this study, both OXE and OXP rats were trained to meal-feed and pair-fed a nutritionally adequate diet.[31] This method of feeding was used to match the feeding patterns and amounts of diet consumed by both groups. Thus, the increases in serum and tissue αTP concentrations observed in OXE rats are attributable specifically to the action of ES, rather than to a difference in food intake or feeding behavior of rats. Despite pair feeding, the OXE rats gained a significantly less weight than the OXP rats. This phenomenon has been observed in our earlier studies using ovariectomized rats.[32,39] Also, in postmenopausal women, estrogen replacement therapy has been shown to prevent the increase in body fat and abdominal fat mass.[40,41] Whether the lower body weight or reduced weight gain in the OXE rats is partly related to the increases in serum and tissue αTP concentrations is not known.

At present, the precise mechanism by which ES elevates the serum and tissue levels of αTP remains to be elucidated. One mechanism may be associated with an increase in the intestinal absorption of αTP. In a recent study,[30] we have found that ES produces a significant increase in the intestinal absorption of the vitamin. In this study[30] using the same dosage of ES and experimental conditions as described in the present experiment, we measured the intestinal absorption and biliary secretion of αTP simultaneously using ovariectomized rats equipped with lymph and bile cannulas. The lymphatic absorption of αTP was significantly greater (25 ± 3%) in OXE rats than in OXP rats (17 ± 4%), as determined during intraduodenal infusion of αTP in a lipid emulsion. Likewise, the amount of the vitamin secreted into the bile during the same period was increased twofold in OXE rats (40 ± 8 nmol) compared with the amount released (21 ± 5 nmol) in OXP rats. The marked increase in biliary αTP output in OXE rats is consistent with the increased concentration of αTP in the liver, as demonstrated in the present study. The increase in serum HDL αTP in OXE rats may be explained partly by the increased secretion of the vitamin from the liver via VLDL and its subsequent transfer to HDL during VLDL lipolysis by endothelial lipoprotein lipase.[29] Whether αTP is incorporated directly into nascent HDL in the liver and released as such into the circulation is not clear. However, the secretion of αTP via nascent HDL is thought to be insignificant.[29] The increase in intestinal αTP absorption in OXE rats suggests a specific regulatory role of ES in the intestinal formation of chylomicrons and subsequent metabolism of the vitamin. Previous studies[32,39] have suggested that ES regulates the amount of phosphatidylcholine or specific phospholipid species secreted into the bile, which in turn may alter the makeup of the surface phospholipids of chylomicrons formed in the enterocyte. The increase in intestinal αTP absorption is likely mediated by an increased incorporation of αTP into chylomicrons and/or an increased rate of chylomicron formation. Further studies on the mechanism of ES action in chylomicron formation in the enterocyte and lipid absorption are in progress.

In tandem with the overall improvement of αTP status by ES replacement, ES also may play a role as an antioxidant in protecting the vitamin against oxidation in lipoproteins and cells. In model systems under various in vitro conditions,[19–23] the antioxidant properties of ES and its metabolites have been well demonstrated. The antioxidant activity of ES has been attributed to its phenolic hydroxyl (OH) group of the aromatic ring, which is capable of reducing peroxy radicals. Under in vitro conditions,[18] ES has been shown to regenerate tocopherols from tocopheroxy radicals. Such an action of ES, if it occurs in vivo, also may prevent further oxidation of the tocopheroxy radical to tocopheryl quinone, thus sparing αTP. For ES to act as antioxidant, it must be able to interact with αTP present in lipoproteins. Generally, it has been believed that most of plasma ES

(approximately 98%) exists bound tightly to sex-hormone binding globulin (SHBG), corticosteroid-binding globulin (CSBG), and less tightly to albumin, with only about 2% of circulating ES being unbound.[42] According to this view, the unbound form of ES is thought to be the biologically active form that is taken up by cells via passive diffusion. However, more recent evidence emerging from steroid-lipoprotein binding studies strongly suggests that serum lipoproteins are significant carriers for ES.[43,44] Of the total serum ES in females, 8 to 9% is carried by serum lipoproteins, with HDL being the major carrier. It has been estimated that about 5.0% of the total serum ES is distributed in HDL, 2.5 % in LDL, and 1.1 % in VLDL; whereas 1.6% exists unbound; and the remainder is bound to SHBG, CSBG, and albumin.[44] Furthermore, the results from those studies[43,44] demonstrate that ES bound to HDL are subsequently esterified at C-3 with fatty acids (largely 18:2). This reaction is presumed to be catalyzed by lecithin:cholesterol acyltransferase (LCAT), allowing fatty acyl esters of ES to enter the hydrophobic core of the HDL particle. The ES esters carried by HDL were found to be transferable to other lipoproteins such as VLDL and LDL in a manner similar to cholesterol ester.[43,44] In conjunction with the suggested role of HDL as an ES carrier, it should be noted that HDL in human females also is a major transporter of αTP, carrying about 55.0% of the total plasma αTP, whereas in males about 60% of the plasma αTP is carried by LDL.[45] These observations suggest that serum lipoproteins, particularly HDL, may be significant physiologic carriers of circulating ES as well as αTP. While being carried by the lipoproteins, ES may interact directly with αTP on the surface of a specific lipoprotein particle. The increase in HDL αTP produced by ES replacement, as demonstrated by the present study, may be explained in part by a possible protective effect of ES on HDL αTP. Furthermore, the above findings suggest that the cellular uptake of ES and αTP may be mediated by the apoE and apoB,E (LDL) receptor dependent mechanisms.[43,44,46,47]

Evidence indicates that HDL serves as the principal carrier of lipid hydroperoxides in human plasma in vivo.[48] Thus, the HDL particles enriched with αTP, as produced by ES replacement, are likely better protected against free radicals and lipid peroxidation. Oxidized HDL loses its ability to remove cellular cholesterol, resulting in accumulation of unesterified cholesterol in macrophages, which may stimulate atherogenesis.[49,50] It has been shown that even a low dosage of supplemental αTP (150 mg/day) significantly reduces the propagation rate of HDL oxidation.[26] In addition, HDL also may serve as the primary donor of αTP to LDL during circulation. HDL αTP has been shown to be readily transferable to LDL.[51] In conjunction with the above observations, the ES-induced increase in HDL αTP, as shown by the present study, is of physiological and clinical significance. In fresh plasma from healthy human males and females, HDL was found to carry 85% of the plasma cholesterol ester hydroperoxides and all of the detectable phospholipid hydroperoxides, whereas plasma LDL lipids were virtually peroxide free.[48] HDL has been shown to inhibit the oxidative modification of LDL, possibly by a rapid exchange of oxidized lipids from LDL with intact lipids from HDL[52] and the transfer of αTP to LDL.[51]

The αTP content of LDL has been shown to be a critical determinant of the oxidative susceptibility of LDL.[24–26] The oxidation of LDL is believed to occur within the subendothelial layer of the arterial wall and oxidized LDL is internalized by macrophages via the scavenger receptor, which facilitates the accumulation of lipids in the developing foam cell.[23] In addition, HDL particles carrying lipid hydroperoxides have been shown to be removed more rapidly than the unoxidized lipids by cultured hepatocytes,[48] suggesting that HDL may play an important role in the hepatic clearance of circulating oxidized lipids. The HDL enriched with αTP, as produced by ES replacement, would be more effective in quenching free radicals and inhibiting the generation of oxidized lipids. Furthermore, the hepatic capacity to detoxify the oxidized lipids would be increased by ES treatment, as suggested by the marked increase in hepatic αTP concentration in OXE rats.

In summary, the present study provides new evidence that ES replacement in ovariectomized rats produces a marked increase in serum αTP, with a selective elevation in αTP carried by HDL. ES replacement also produces significant increases in the αTP concentrations in the liver and kidneys, suggesting that ES enhances the overall nutritional or body status of the vitamin. The increase in HDL αTP produced by ES replacement may inhibit the oxidation of lipoprotein lipids and have an additional protective effect against CHD. The present study and previous work by others suggest the possibility that ES may be carried by HDL and act as an antioxidant interacting with αTP. The mechanism underlying the action of ES in αTP metabolism awaits further investigation.

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