17β-Estradiol Preserves Endothelial Vasodilator Function and Limits Low-Density Lipoprotein Oxidation in Hypercholesterolemic Swine

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**Background** Cardiovascular events are less prevalent in premenopausal women and women receiving estrogen replacement than in postmenopausal women or men. Endothelium-derived relaxing factor (EDRF) is an important local modulator of vascular tone, and abnormal endothelial function is related, in part, to the oxidative modification of low-density lipoprotein (LDL). Estrogens possess substantial antioxidant activity and inhibit LDL modification in vitro.

**Methods and Results** We investigated the effects of 17β-estradiol (E2) on endothelial vasomotor function in cholesterol-fed miniature swine. Animals underwent ovariectomy or a sham procedure and received E2 or placebo via implant yielding three groups: sham, ovariectomy (E2 placebo), and implant (E2 implant). After 16 weeks, coronary arteries were harvested and endothelial function was examined in vitro. Vessels from the sham and implant groups demonstrated preserved endothelium-dependent relaxation to bradykinin, substance P, and A23187. Vessels from the ovariectomy group exhibited impaired relaxation to bradykinin and substance P (P<.05 versus sham and implant groups) but not to A23187. Plasma E2 levels were strongly correlated with the response to bradykinin (R=.82, P<.001), substance P (R=.64, P<.01), and A23187 (R=.65, P<.01). Compared with the ovariectomy group, LDL derived from the sham and implant groups was markedly resistant to ex vivo oxidation (P<.05), and this effect correlated with preserved endothelium-dependent relaxation to bradykinin (R=.62, P<.03) and substance P (R=.61, P<.03).

**Conclusions** Thus, E2 preserves endothelial function in cholesterol-fed swine in association with protection of LDL against oxidative modification. These data suggest that E2 may, in part, favorably affect vascular function and coronary artery disease by virtue of its antioxidant properties. 


**Key Words** lipoproteins • 17β-estradiol • estrogens • relaxing factors • hypercholesterolemia

Coronary atherosclerosis and cardiovascular events are less prevalent in women than in age-matched men.1 After natural or surgical menopause, women are more likely to develop coronary artery disease than premenopausal women of the same age.2 Estrogen replacement therapy limits the development of coronary artery disease and clinical events in both ovariectomized and postmenopausal women.3 Putative mechanisms responsible for the "protective" role of estrogens include estrogen-mediated effects on lipid metabolism, coagulation parameters, and blood pressure.1 Oral estrogen therapy is associated with increased levels of high-density lipoprotein cholesterol (HDL-C) and decreased low-density lipoprotein cholesterol (LDL-C),4 although large-scale studies indicate that only 25% to 50% of the beneficial effect of estrogen is due to effects on lipoprotein levels.5,6

The coronary vascular endothelium plays a central role in regulating arterial tone7 and platelet function,8 in part through the actions of endothelium-derived relaxing factor (EDRF). Impairment of endothelium-dependent control of vascular tone develops early in atherosclerosis9 and may contribute to the development of myocardial ischemia.10 Abnormalities in endothelial control of vascular tone may be related, in part, to the oxidative modification of LDL. Exposure of normal arteries to oxidized LDL (ox-LDL) results in impaired endothelium-dependent arterial relaxation,11,12 and ox-LDL may inactivate EDRF directly.13

Recent evidence suggests that estrogens possess antioxidant activity that is related to the presence of a phenolic ring.14 Modification of LDL by copper ions, monocytes, and endothelial cells in vitro is inhibited by estrogens.15 In phospholipid microsomes, estrogens with a phenolic structure (ie, 17β-estradiol, estradiol) inhibit membrane phospholipid peroxidation in proportion to the extent of their membrane incorporation.16 Moreover, membrane estrogens are subject to conversion into their catechol analogues, which possess substantial antioxidant activity.14

There is evidence to suggest that estrogens influence endothelium-dependent regulation of vasomotor tone. In ovariectomized rabbits, 17β-estradiol treatment enhances endothelium-dependent relaxation to acetylcholine,17 and similar findings have been reported in canine coronary arteries with 17β-estradiol and progesterone in combination.18 The release of EDRF in response to vasoconstricting agents is enhanced in female rabbits compared with males or ovariectomized females,19 and
estrogen replacement modulates endothelium-dependent vasomotor function in pathological states. In spontaneously hypertensive rats, estrogen treatment lowers blood pressure and improves endothelium-dependent relaxation. Estrogen replacement (with 17β-estradiol) in atherosclerotic monkeys limits acetylcholine-mediated coronary vasoconstriction, and the acute administration of ethinyl estradiol has a similar effect.

We sought to examine the effect of endogenous and exogenous estrogens on endothelium-dependent arterial relaxation in a hypercholesterolemic swine model. In particular, we hypothesized that estrogens may improve endothelial vasomotor function by virtue of their antioxidant properties. We report the effects of endogenous and exogenous estrogens on endothelium-dependent arterial relaxation as well as effects on plasma lipoproteins and LDL susceptibility to ex vivo oxidation.

Methods

Materials

Nitroglycerin was obtained from Solopak Laboratories, and 2,2'-azobis-(2-aminopropane) hydrochloride (AAPH) was purchased from Kodak, Inc. Glutaraldehyde, formaldehyde, oxtosamine, and cacoodylate were purchased from Polysciences, Inc. Bradycardin, substance P, calcium ionophore (A23187), and all other compounds were purchased from Sigma Chemical Co. Physiological saline solution (PSS) contained 118.3 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L MgSO4, 1.2 mmol/L KH2PO4, 25 mmol/L NaHCO3, 11.1 mmol/L glucose, 10 µmol/L indomethacin, and 0.026 mmol/L Na3EDTA. Phosphate-buffered saline (PBS) consisted of 10 mmol/L Na3PO4 and 0.15 mol/L NaCl (pH 7.4). A23187 was prepared and diluted in dimethylsulfoxide; all other reagents were prepared with distilled water.

Animals

Fifteen sexually mature female Yucatan miniature swine (Sus scrofa) 26 weeks old (30 to 40 kg) were used for these studies. Animals were subjected to either laparoscopic ovariec- tomy (n = 10) or laparoscopy and sham ovariotomy (n = 5) as described below. Ovariec tomized animals were randomized to receive 17β-estradiol or placebo via implant for 16 weeks yielding three groups of animals: (1) sham (sham ovariotomy), (2) ovariotomy (ovariectomy without hormonal replacement), and (3) implant (ovariectomy with 17β-estradiol replacement). After recovering from surgery (=1 week), animals consumed an atherogen diet containing 1% (w/w) cholesterol, 40% of calories as coconut oil, and 0.7% (w/w) sodium cholate for the study period. Dietary intake was limited to 1 kg/d, and plasma samples were obtained at the end of the study period for determination of fasting (18-hour) plasma lipoproteins and 17 β-estradiol levels. Animal protocols used for this study were approved by IACUC at both the West Roxbury VA Medical Center and the University of New Hampshire. Twelve of the 15 animals in this study were used concurrently for a study detailing the effect of estrogens on lipoprotein levels, lipoprotein composition, LDL oxidation, and antioxidant levels in hypercholesterolemic swine.

Laparoscopic Ovariotomy and Estrogen Replacement

Prophylactic benzathine penicillin (40 000 U/kg IM) was administered after surgery. The animals were sedated with 15 mg/kg xylazine IM (Mobay Co) and 2.5 mg/kg ketamine IM (Aveco), and anesthesia was maintained with 1.5% to 2.0% isoflurane (Anaquest) by nose cone. With aspetic technique, a laparoscope and trocar sleeves (US Surgical) were placed into the abdomen, the ovaries were identified, and ovariotomy was performed using a laparoscopic gastrointestinal anastomosis stapler (US Surgical). Estrogen replacement was administered via two subcutaneous implants constructed from 4-cm sections of sterile Silastic tubing (0.132-in. i.d., 0.183-in. o.d., Dow Corning Inc) containing 200 mg 17β-estradiol powder (Steraloids). Two implants produced plasma 17β-estradiol levels of approximately 200 to 250 pmol/L in a 45-kg animal corresponding to a high physiological 17β-estradiol level in a normal pig. Plasma 17β-estradiol concentrations were measured using standard radioimmunoassay on ether-extracted plasma. Antibody for these assays was kindly provided by Dr G. Niswender of Colorado State University.

Organ Chamber Methodology

Animals were killed with 120 mg/kg sodium pentobarbital IV (Anthony Products) after premedication with 100 IU/kg sodium heparin IV (Elkins-Sinn) and 20 mg/kg ketamine IM in accordance with the methods approved by the Panel on Euthanasia of the American Veterinary Medical Association.

After the animals were killed, the hearts were excised and immediately placed into ice-cold PSS. The epicardial coronary arteries were carefully dissected away from the myocardium, and the proximal half of each artery was cut into 3-mm rings (5 mm long, 3 mm diameter per ring), placed in an organ chamber (37°C, pH 7.4) containing 20 mL PSS, and suspended between two tungsten stirrups for the measurement of isometric tension with a force transducer (model FT-03, Grass Instrument Co) and a chart recorder (model RS 3800, Gould Instrument Co). The organ chambers were aerated with 95% O2/5% CO2, and each vessel was gradually stretched to 12 g of resting tension (optimal tension based on preliminary studies) and allowed to equilibrate for 1 hour before the introduction of vasoactive drugs. Vessel contraction was assayed in quiescent rings in response to PSS containing 10, 20, 40, and 80 mmol/L KCl (substituted for NaCl). In selected vessels, the endothelium was removed by gently rolling the vessel with a wooden applicator stick positioned in the vessel lumen. Vessels were contracted with the thromboxane mimetic U46619 (1 µmol/L), and vascular function was examined by the cumulative addition of bradykinin, calcium ionophore (A23187), substance P, or nitroglycerin. Representative rings were fixed in 3% glutaraldehyde for scanning electron microscopy to confirm the presence or absence of endothelium. With the exception of A23187, the order of agonists was randomized to control for cumulative effects. Vessel ring studies were performed by personnel blinded to hormonal therapy.

Lipoprotein Determination

Blood was drawn into tubes containing EDTA (1.5 mg/mL) and aprotinin (0.05%), and plasma was obtained after low-speed centrifugation at 4°C. Plasma total cholesterol (TC) and triglycerides (TG) were quantified using enzymatic methods. HDL-C was measured after phosphotungstic-MgCl2 precipitation of apoprotein B containing very-low-density lipoproteins (VLDL) and LDL. The LDL-C plus VLDL cholesterol (VLDL-C) fraction was determined from the difference between TC and HDL-C as described by Terpstra and colleagues.

Lipoprotein Isolation and LDL Susceptibility to Ex Vivo Oxidation

LDL (density = 1.009 to 1.040 g/mL) was isolated using single-spin gradient ultracentrifugation as described previously. The purity of the LDL fraction was assessed by agarose electrophoresis. Standard incubation of LDL to assess susceptibility to oxidation was performed at a LDL concentration of 0.05 mg/mL LDL protein in the presence of 10 µmol/L AAPH at 37°C. LDL oxidative susceptibility was assessed by continuous spectrophotometric monitoring at 234 nm and quantified by the lag-phase duration preceding the formation
Lipoprotein Levels in Miniature Swine

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<th>Group</th>
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<th>Implant</th>
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<tr>
<td>TC, mg/dL</td>
<td>101±7</td>
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<td>HDL-C, mg/dL</td>
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<td>LDL-C+VLDL-C, mg/dL</td>
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<td>264±65</td>
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<tr>
<td>TG, mg/dL</td>
<td>67±11</td>
<td>40±3</td>
<td>41±3</td>
<td>58±9</td>
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Plasma was collected from unanesthetized animals before and after 16 weeks of an atherogenic diet, and total cholesterol (TC), HDL cholesterol (HDL-C), LDL and VLDL cholesterol (LDL-C+VLDL-C), and triglycerides (TG) were determined as described in "Methods." Pretreatment values represent the mean±SEM of data from all 15 animals, whereas the sham, ovariectomy, and implant values are mean±SEM of 5 animals per group. *P<.05 vs pretreatment.

Histological Examination

In each animal, the midportion of the left anterior descending coronary artery was fixed with a solution of 10% formalin in PBS (pH 7.4) for 20 minutes. The coronary artery was gently cleaned of loose connective tissue, washed in cacodylate-sucrose buffer (10.26 g sucrose in 150 mL 0.1 mol/L cacodylate) for 5 minutes, and further fixed in glutaraldehyde-cacodylate solution (3% glutaraldehyde, 0.1 mol/L cacodylate) for 24 hours. Samples prepared in this manner were sectioned (0.2 mm), dehydrated, and embedded in paraffin by standard histological procedures. Sections were stained with resorcin fuchsin (for elastin) and subjected to morphometric analysis of intimal and medial area using an automated videomicroscopy system (Zeiss Instruments).

Data Analysis

Unless otherwise specified, all data are presented as mean±SEM. Vessel relaxation is expressed as the percent reduction in tension compared with that produced by 1 μmol/L U46619. 17β-Estradiol levels, lipoprotein levels, intimal proliferation, and LDL susceptibility to ex vivo oxidation were compared among treatment groups using ANOVA with a post hoc Newman-Keuls comparison. Dose-response relationships for bradykinin, substance P, A23187, and nitroglycerin were compared among treatment groups using ANOVA. The relation between vascular function and LDL resistance to oxidation or plasma 17β-estradiol levels was examined by relating lag phase or the logarithm of plasma 17β-estradiol levels to vessel relaxation in response to 0.1 μmol/L bradykinin or 3 mmol/L substance P using linear regression (Spearman). Statistical significance was accepted if the null hypothesis was rejected at the .05 level.

Results

Plasma 17β-Estradiol and Lipoprotein Levels

Plasma 17β-estradiol levels were consistent with hormonal treatment. Animals in the ovariectomy group demonstrated plasma 17β-estradiol levels that were near the limits of detection (20±6 pmol/L). Animals in the sham group had an intermediate plasma 17β-estradiol level of 86±29 pmol/L (P<.05 versus ovariectomy), whereas animals in the implant group demonstrated a significantly elevated plasma 17β-estradiol level of 239±37 pmol/L (P<.05 versus ovariectomy and sham groups).

Plasma lipoprotein profiles from the study animals are given (Table). Before animals consumed an atherogenic diet, plasma TC, LDL-C plus VLDL-C, HDL-C, and TG levels were 101±7, 43±3, 58±4, and 67±11 mg/dL, respectively. After 16 weeks of dietary treatment, plasma TC, LDL-C plus VLDL-C, and HDL-C increased significantly (P<.05), whereas plasma TG levels were unchanged (Table). There were no significant differences in plasma lipoprotein profiles among the different treatment groups.

Estrogens and Vascular Function

Animals in the sham, ovariectomy, and implant groups all demonstrated similar coronary artery contractile responses to 1 μmol/L U46619 of (mean±SD) 8.7±2.6, 10.6±2.7, and 9.8±3.3 g, respectively (P=NS). In a similar fashion, the contractile responses to 10, 20, 40, and 80 mmol/L KCl were not significantly different on the basis of hormonal treatment. The EC50 for KCl-mediated contraction in the sham, ovariectomy, and implant groups was 22.3±0.4, 21.8±0.5, and 21±0.4 mmol/L, respectively (P=NS).

The coronary artery responses to bradykinin are contained in Fig 1. Intact coronary arteries from the sham and implant groups demonstrated significant dose-dependent arterial relaxation in response to bradykinin with maximal relaxations of 86±11% and 91±5%, respectively (both P<.001 versus baseline). In contrast, vessels derived from the ovariectomy group demonstrated significant impairment of endothelium-dependent arterial relaxation relative to vessels from
Fig 2. Plot demonstrating the effect of endogenous and exogenous estrogens on substance P-mediated endothelium-dependent arterial relaxation in hypercholesterolemic swine coronary arteries. Arteries were harvested from animals that were subjected to ovariectomy (a), ovariectomy plus 17β-estradiol implant (A), or sham ovariectomy (e) and maintained on an atherogenic diet for 16 weeks. Vessels were harvested, prepared as described in "Methods," and contracted with the thromboxane mimetic U46619 (1 μmol/L). Vessels devoid of endothelium demonstrated no significant relaxation to substance P; the response at 3 nM is displayed (open symbols). Data are presented as mean±SEM and are derived from vessels harvested from five animals in each group. *P<.001 vs sham and implant groups.

Fig 3. Plot demonstrating the effect of endogenous and exogenous estrogens on A23187-mediated endothelium-dependent arterial relaxation in hypercholesterolemic swine coronary arteries. Arteries were harvested from animals that were subjected to ovariectomy (a), ovariectomy plus 17β-estradiol implant (A), or sham ovariectomy (e) followed by an atherogenic diet for 16 weeks. Vessels were harvested, prepared as described in "Methods," and contracted with the thromboxane mimetic U46619 (1 μmol/L). Vessels without endothelium demonstrated no significant relaxation to A23187; the response at 1 μmol/L is displayed (open symbols). Data are presented as mean±SEM and are derived from vessels harvested from five animals in each group.

The coronary vessel response to substance P is shown in Fig 2. Intact coronary arteries from the implant and sham groups displayed significant endothelium-dependent arterial relaxation to substance P with maximal relaxations of 87±5% and 94±8%, respectively (P<.001 versus baseline; Fig 2). Vessels from animals in the ovariectomy group demonstrated significantly less endothelium-dependent arterial relaxation in response to substance P (maximal response, 43±12%) than vessels from either the sham or implant groups (P<.001; Fig 2). In the absence of endothelium, there was no significant relaxation in response to substance P (Fig 2).

The coronary vascular response to the receptor-independent calcium ionophore A23187 is presented in Fig 3. Vessels from all three treatment groups demonstrated significant dose-dependent relaxation in response to increasing doses of A23187 (P<.001 versus baseline). Maximal relaxations observed for the ovariectomy, sham, and implant groups were 82±6%, 95±6%, and 98±4%, respectively. In contrast to the responses seen with bradykinin and substance P, there was no significant difference in endothelium-dependent arterial relaxation on the basis of hormonal status (Fig 3). In vessels without endothelium, there was no significant relaxation in response to A23187 (Fig 3).

We investigated smooth muscle cell vasodilator function with the endothelium-independent vasodilator nitroglycerin (Fig 4). Deendothelialized vessels from all three treatment groups demonstrated significant dose-dependent arterial relaxation in response to nitroglycerin with maximal relaxations of 103±4%, 101±3%, and 100±3% in the ovariectomy, sham, and implant groups, respectively (P<.001 versus baseline). There was no significant difference in nitroglycerin-induced arterial relaxation among the three treatment groups (Fig 4). The response to nitroglycerin was similar in vessels with endothelium (data not shown). Thus, smooth muscle cell vasodilator function was similar in all three hormonal treatment groups.

Histological Examination

Previous studies in cholesterol-fed animals have demonstrated diminished atherogenesis in cholesterol-fed animals receiving estrogen compared with animals without estrogen.32,33 In the present study, such an effect might explain group differences in endothelium-dependent vessel relaxation. To investigate this possibility, we compared the extent of intimal proliferation (expressed as the ratio of intimal to medial area) in the mid–left anterior descending coronary artery from all three
treatment groups. Coronary sections from the ovariectomy group demonstrated the greatest intimal proliferation with an intimal-to-medial ratio of 0.198±0.05, whereas sections from the sham and implant groups produced ratios of 0.131±0.03 and 0.117±0.02, respectively. There were no statistical group differences in the degree of intimal proliferation among groups (ANOVA, P=NS).

LDL Susceptibility to Ex Vivo Oxidation

To test the hypothesis that estrogen may preserve endothelial vasodilator function by limiting oxidation of LDL, we examined LDL derived from all three treatment groups for its susceptibility to ex vivo oxidation by aqueous peroxyl radicals (Fig 5). The lag-phase duration preceding diene conjugation is inversely related to LDL susceptibility to oxidation.31 LDL isolated from the ovariectomy group was the most susceptible to ex vivo oxidation with a lag phase of 35±12 minutes. LDL derived from animals with exogenous estrogens (sham group) was significantly more resistant to oxidation with a lag phase of 85±15 minutes (P<.05 versus ovariectomy group). LDL from animals treated with 17β-estradiol implants were the most resistant to oxidation with a lag phase of 105±20 minutes (P<.05 versus ovariectomy), an effect that was not significantly different from the sham group. Thus, preserved endothelial-dependent arterial relaxation in these hypercholesterolemic swine was associated with enhanced LDL resistance to ex vivo oxidation.

Plasma 17β-Estradiol, LDL Resistance to Oxidation, and Endothelium-Dependent Arterial Relaxation

We investigated the relation between endothelial vasodilator function and plasma 17β-estradiol levels in all 15 study animals. Fig 6 contains the relation of plasma 17β-estradiol levels and the extent of arterial relaxation to maximal doses of bradykinin, substance P, and A23187. The degree of arterial relaxation to 0.1 μmol/L bradykinin positively correlated with the logarithm of plasma 17β-estradiol levels with an R value of .82 (P=.00015), and vessel relaxation to substance P (3 nmol/L) also positively correlated to the logarithm of plasma 17β-estradiol levels with a correlation coefficient of .64 (P=.0097). Vessel relaxation to the receptor-independent calcium ionophore A23187 also correlated with the logarithm of plasma 17β-estradiol levels (R=.65, P=.0086), although over a much narrower range of vessel relaxation. Analysis of the slopes for regression lines relating plasma 17β-estradiol levels to vessel relaxation suggests that 17β-estradiol influences vessel relaxation in a rank order of bradykinin> substance P>A23187 in this model (slopes, 41±8, 28±9, and 15±5, respectively).

We further examined the relation between endothelium-dependent arterial relaxation and LDL resistance to oxidation in the 12 animals for which data on LDL oxidative resistance were available, and these results are presented in Fig 7. Endothelium-dependent arterial relaxation in response to maximal doses of bradykinin (0.1 μmol/L) and substance P (3 nmol/L) correlated with LDL resistance to oxidation (lag phase) with correlation coefficients of .62 and .61, respectively (P>.03 for both). LDL resistance to oxidation was not significantly correlated with plasma 17β-estradiol levels (R=.47, P=.123). There was no significant correlation between endothelium-dependent arterial relaxation and plasma lipoproteins (TC, LDL–C plus VLDL–C, HDL–C) or coronary artery intimal proliferation.

Discussion

The data presented here demonstrate that endogenous and exogenous estrogens preserve normal endothelium-dependent vasodilation in hypercholesterolemic swine. This preservation of vascular function was unrelated to any alteration in smooth muscle cell function as evidenced by the similar response to both nitroglycerin and KCl among the treatment groups. In this study, estrogens delivered as 17β-estradiol via a subcutaneous implant or through intact ovarian function had no significant effects on plasma cholesterol or
lipoproteins. Likewise, differences in endothelial vasomotor function were not associated with significant differences in the degree of intimal proliferation among treatment groups. Preservation of endothelial function was significantly related to both plasma 17β-estradiol levels and enhanced LDL protection from ex vivo oxidation.

Previous studies have examined the influence of estrogens on vascular function in normcholesterolemic animals. Gisclard and colleagues demonstrated that 17β-estradiol treatment enhanced endothelium-dependent relaxation to acetylcholine in ovariectomized rabbits, and in canine coronary arteries, 17β-estradiol and progesterone in combination improved vasorelaxation in response to acetylcholine. Basal release of EDRF appears enhanced in female rabbits compared to male or ovariectomized female rabbits. 17β-Estradiol also influences smooth muscle cell function. Jiang and colleagues observed impairment of endothelin-1-mediated contraction in rabbit coronary arteries treated with 17β-estradiol. In this model, the coronary artery constrictor response to BAY K 8644, a direct calcium channel agonist, was also inhibited by 17β-estradiol, suggesting that 17β-estradiol may act by preventing calcium channel function.

Estrogen replacement also modulates vasomotor dysfunction in pathological states. In the spontaneously hypertensive rat model, estrogen treatment lowers blood pressure and improves endothelium-dependent relaxation. In ovariectomized monkeys consuming an atherogenic diet, Williams and colleagues demonstrated that 17β-estradiol replacement abolished acetylcholine-mediated coronary vasoconstriction and that the acute administration of ethinyl estradiol was also associated with a less marked contractile response to acetylcholine.

The results presented here are in general agreement with the previous work of Williams and colleagues. Those investigators found that subcutaneous 17β-estradiol therapy limited the degree of pathological coronary artery constriction to intracoronary acetylcholine infusion in ovariectomized cynomolgus monkeys fed cholesterol for 30 months. The observations of Williams and colleagues are consistent with an enhanced release of EDRF that opposed the direct smooth muscle effect of acetylcholine; however, these findings may also reflect 17β-estradiol-mediated effects on vascular smooth muscle cell function. This point is particularly germane in light of observations demonstrating 17β-estradiol-mediated inhibition of coronary artery constriction by Jiang and coworkers.

In some respects, the data presented here differ from the previous work of Williams and coworkers. In that report, study animals did not demonstrate significant coronary artery vasodilation in response to either acetylcholine or nitrroglycerin. In contrast, coronary arteries from our animal subjects did demonstrate enhanced endothelium-dependent arterial relaxation with 17β-estradiol treatment. The present study examined vascular responses in precontracted coronary arteries ex vivo, whereas Williams and coworkers studied coronary arteries in vivo that were subject to autoregulation. It is possible that the differences in experimental conditions in these two studies may explain in part the differences in vascular reactivity.

In the study by Williams and colleagues, 17β-estradiol treatment was associated with a 33% reduction in plasma cholesterol that may have contributed to the observed effect on coronary vasomotor function. In contrast, the present study demonstrated a significant improvement in endothelium-dependent arterial relaxation without any effect on plasma lipoprotein levels. In women, oral estrogen therapy lowers plasma LDL and raises plasma HDL, whereas transdermal estrogen therapy has no effect. Our animals received 17β-estradiol via subcutaneous implant and thus may have been spared any 17β-estradiol-mediated effects on lipoproteins. Moreover, the similarity in lipoprotein profiles in our implant and sham animals is consistent with observations of other investigators in cholesterol-fed rabbits and monkeys treated with subcutaneous estrogen or estrogen-progesterone combinations.

In this study, we observed no significant association between 17β-estradiol levels and coronary artery intimal proliferation, whereas Williams and colleagues observed less intimal proliferation in estrogen-treated animals. It is possible that our study did not contain a sufficient number of animals to demonstrate such an association. Alternatively, it is also possible that similar lipoprotein levels among the treatment groups produced similar degrees of intimal proliferation with 16 weeks of cholesterol feeding.

In the present study, animals without endogenous or exogenous estrogens demonstrated abnormal endothelium-dependent arterial relaxation in response to the EDRF agonist bradykinin. This finding is not consistent with some previous studies using hypercholesterolemic swine. Possible explanations for this discrepancy may be related to differences in animal populations, duration of treatment, and the presence or absence of
atherosclerosis. In this study, we used only female swine, whereas other studies primarily have used males. Previous studies that failed to demonstrate abnormal bradykinin-mediated vasorelaxation used animals fed cholesterol for only 9 (Reference 37) or 10 (Reference 38) weeks, whereas animals in the present study received an atherogenic diet for 16 weeks. Moreover, we observed early coronary atherosclerotic lesions in our study animals, whereas others have not. In fact, in cholesterolfed pigs, Shimokawa and Vanhoutte observed impaired bradykinin-mediated arterial relaxation in coronary arteries with atherosclerotic lesions, whereas in the same animals, coronary segments without lesions demonstrated normal responses to bradykinin.

We observed a significant association between plasma 17β-estradiol levels and the extent of endothelium-dependent arterial relaxation in response to bradykinin, substance P, and A23187. Vessel relaxations to bradykinin and substance P were more highly dependent on plasma 17β-estradiol than relaxations to A23187. This observation would suggest that in hypercholesterolemia, membrane-receptor interactions and/or signal transduction may be influenced by the presence of 17β-estradiol. Similar findings have been reported in ovariectomized rabbits and dogs. Mechanistic etiologies for the “facilitation” of EDRF action in the presence of 17β-estradiol might include an increased availability of membrane receptors, increased cellular levels of constitutive nitric oxide synthase, limited degradation of EDRF, or limitation of LDL oxidation in vivo, which attenuates LDL-mediated endothelial dysfunction.

Many forms of estrogen demonstrate antioxidant activity, and inhibition of LDL oxidation in vivo may serve several important functions that would explain in part the preserved endothelial vasomotor function demonstrated here. Normal arteries exposed to ox-LDL develop impaired endothelial-dependent relaxation. Oxidation of LDL is associated with the intraparticle conversion of lecithin to lyssolecithin, presumably through phospholipase A2 activity intrinsic to apolipoprotein B, and the production of lyssolecithin during oxidative modification of LDL is particularly important in the development of an abnormal vascular response. Depletion of lyssolecithin from ox-LDL through incubation with defatted albumin or treatment with phospholipase B attenuates the development of abnormal vasomotion. Moreover, direct incubation of normal arteries with lyssolecithin results in abnormal endothelium-dependent arterial relaxation.

The impairment of EDRF action due to ox-LDL and lyssolecithin is partly related to defective receptor-mediated EDRF release. Normal arteries exposed to ox-LDL at concentrations of 50 μg/mL or less demonstrate a deficit in response to receptor-dependent EDRF agonists, whereas the response to the calcium ionophore A23187 remains largely intact. Exposure of endothelial cells to lyssolecithin results in diminished EDRF release, as determined by a vessel ring bioassay. Incubation of bovine aortic endothelial cells with lyssolecithin leads to impaired intracellular phosphoinositide hydrolysis and calcium release from intracellular stores in response to bradykinin. Thus, 17β-estradiol-mediated limitation of LDL oxidation in vivo could result in diminished lyssolecithin accumulation in the vascular wall and preserved receptor-dependent EDRF-mediated arterial relaxation. Our observation relating LDL susceptibility to oxidation by aqueous peroxyl radicals and vessel relaxation to substance P and bradykinin appears to support this contention.

Modified LDL may also affect EDRF action in atherosclerosis through its effects on leukocyte recruitment. Oxidized LDL is chemotactic for monocytes and prevents macrophage egress from atherosclerotic vessels. Vessel wall macrophages are a potential source of oxygen-derived free radicals that may serve as a source of continued LDL oxidation and EDRF inactivation. Moreover, the ox-LDL so formed may contribute to continued macrophage recruitment and may inactivate EDRF directly.

Abnormalities in endothelium-dependent relaxation may also be related to the inflammatory response associated with atherosclerosis and hypercholesterolemia. Several cell types within atherosclerotic vessels are inflammatory in nature and capable of releasing oxygen-derived free radicals. Investigation into the chemical nature of EDRF suggests that EDRF is either nitric oxide or a related redox form that combines readily with a number of chemical species, including oxygen and superoxide anion, leading to a loss of biological activity. One must consider that the estrogen-mediated preservation of EDRF action presented here may be a consequence of the free radical-scavenging characteristics of estrogens vis-à-vis superoxide anion. Alternatively, estrogens may in some way inhibit superoxide production by endothelial cells. In fact, hypercholesterolemic vessels appear to produce excess superoxide anion, and enhanced degradation of superoxide anion in atherosclerotic rabbits improves the response to endothelium-dependent vasodilators.

In summary, 17β-estradiol preserves receptor-mediated endothelium-dependent coronary arterial relaxation in hypercholesterolemia. In our experimental model, this effect is independent of 17β-estradiol-mediated changes in plasma lipoprotein levels, vascular morphology, and smooth muscle cell function. Because impairment of EDRF action may contribute to altered local control of vasomotor tone and platelet function, these results support the hypothesis that 17β-estradiol may limit the clinical expression of coronary artery disease by virtue of its effects on EDRF action and metabolism. With regard to the mechanisms responsible for our observations, the beneficial effects of 17β-estradiol treatment on vasomotor function were associated with marked group differences in the susceptibility of LDL to ex vivo oxidation. Furthermore, LDL susceptibility to oxidation correlated significantly with the extent of receptor-dependent EDRF-mediated arterial relaxation. These findings support the hypothesis that 17β-estradiol preserves endothelial function in hypercholesterolemia, in part by limiting LDL oxidation in vivo and thereby preventing the deleterious effects of ox-LDL on agonist-mediated EDRF release and EDRF degradation.

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