Protection against atherosclerosis by estrogen is independent of plasma cholesterol levels in LDL receptor-deficient mice

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Abstract **Low density lipoprotein (LDL) receptor-deficient (LDLR−/−) mice consuming a high fat diet were used to assess the effect of endogenous and exogenous estradiol (E_2) on atherosclerosis. Sexually mature female mice were ovariectomized (OVX) and implanted with subcutaneous, slow-release pellets designed to release 6 μg/day of exogenous 17β-estradiol (17β-E_2), 17α-estradiol (17α-E_2), or placebo (E_2-deficient). Sham-operated control female (endogenous E_2) and male mice were studied as controls. Aortic atherosclerotic lesion area was reduced by physiologic amounts of both endogenous and exogenous E_2 compared to E_2-deficient female mice. Although plasma cholesterol levels were reduced by exogenous E_2 despite the absence of the LDL receptor, endogenous E_2 was not associated with any cholesterol changes. In contrast, only 17α-E_2 was associated with decreased fasting triglyceride. In subgroup analyses matched for time-averaged plasma total cholesterol, aortic lesion area was reduced by the presence of estradiol (E_2). E_2 protected LDLR−/− female mice from atherosclerosis and this protection was independent of changes in plasma cholesterol levels.**—Marsh, M. M., V. R. Walker, L. K. Curtiss, and C. L. Banka. **Protection against atherosclerosis by estrogen is independent of plasma cholesterol levels in LDL receptor-deficient mice.** ***J. Lipid Res.*** **1999.** 40: **893–900.**

Supplementary key words estradiol • estrogen • atherosclerosis • cholesterol • triglyceride • LDL receptor-deficient mice • mouse model • hormone replacement therapy • cervical carcinoma

Cardiovascular death is the most common cause of death in Western society accounting for more than 50% of all female deaths. Estrogen protects against cardiovascular disease and epidemiologic evidence has shown that postmenopausal hormone replacement therapy is associated with a reduction in the incidence of cardiovascular death by approximately 50% (1, 2). Although originally this protection in humans was thought to occur largely via estrogen-induced changes in plasma lipoproteins, these lipoprotein changes are likely to account for only a third of the protective effect of estrogen (3). Estrogen interacts with multiple factors that can potentially account for the effect of estrogen on atherosclerosis (reviewed in 4). Although many studies in multiple species have examined the effect of estrogen (largely in supraphysiological doses) on atherosclerosis, no studies in mice have distinguished the atheroprotective effects of estrogen from its cholesterol lowering effects. For example, in apoE-deficient mice, estrogen was atheroprotective although estrogen treatment reduced the total plasma cholesterol levels (5, 6).

The low density lipoprotein (LDL) receptor-deficient (LDLR−/−) mouse can, in part, mimic the human disease, familial hypercholesterolemia. In the absence of the LDL receptor, total plasma cholesterol levels are elevated by a high fat diet (7). Homozygous LDLR−/− mice on high fat diets develop extensive atherosclerosis throughout the aorta in a pattern representative of lesion development in humans (7, 8). Importantly, female LDLR−/− mice on a high fat diet develop less atherosclerosis than males (8). In this animal model the potential effects of estrogen on hepatic LDL receptors are avoided. Therefore, the LDLR−/− mouse represents a suitable mouse model for studying mechanisms of estrogen protection against atherosclerosis. This study evaluates the effects of estrogen on atherosclerosis that are independent of its effects on plasma cholesterol levels and establishes a new menopausal mouse model of atherosclerosis.

METHODS

Animal model

LDL receptor-deficient mice on a C57BL/6J background (LDLR−/−) were initially purchased from Jackson Laboratories (Bar Harbor, ME) and were bred in house. All studies were approved by The Scripps Research Institute Institutional Animal

Abbreviations: E_2, estradiol; LDLR−/−, low density lipoprotein receptor-deficient; OVX, ovariectomized; wt, weight.

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Care and Use Committee and comply with NIH guidelines. Mice were weaned at 28 days, initially fed ad libitum a standard mouse chow diet (Diet No. 5015; Harlan Teklad, Madison, WI), housed 2 per cage in autoclaved filter top cages with autoclaved water and kept in a 12-h light–dark cycle in a conventional clean room facility. All mice were anesthetized for surgery using inhalational methoxyflurane (Metofane; Malinckrodt Veterinary, Mudelein, IL) and intraperitoneal ketamine (Ketostat 100 mg/kg; Fort Dodge Laboratories Inc., Fort Dodge, IA). At sexual maturity (35–45 days old), bilateral ovariectomy (OVX) or sham ovariectomy (sham OVX) was performed. Sham-operated female (endogenous E_2; n = 14) and male (n = 21) mice served as controls. Female OVX mice were implanted with subcutaneous slow-release hormone pellets (Innovative Research of America, Toledo, OH) designed to release 6 μg/day for 90 days of exogenous E_2 as 17β-estradiol (exogenous 17β-E_2; n = 19) or 17α-estradiol (exogenous 17α-E_2; n = 12), or no E_2 as placebo vehicle alone (E_2-deficient; n = 15). One week after surgery mice were fed for 12 weeks an atherogenic high fat diet containing 1.25% cholesterol, 6% fat, minimum essential dietary requirements of vitamin E, and no cholate (Diet No. TD96335; Harlan Teklad). Venous blood was collected from the retro-orbital sinus under Metofane-induced anesthesia after an 8-h fast unless otherwise specified. Blood samples were collected 2 days before surgery on a chow diet, and on high fat diet at 18, 40, 60, and 84 days post surgery. The only nonfasting samples were collected at killing (84 days post surgery). Plasma was obtained by centrifugation of the whole blood at 5,000 rpm at 4°C for 10 min and stored at −80°C before analysis. Mice were weighed while anesthetized at each blood collection using a Ohaus portable standard LS200 scale (Ohaus Scale Corporation, Florham Park, NJ), precision 0.0025%.

Perfusion fixation and preparation of tissue for analysis of the extent of atherosclerosis

At killing, an incision was made in the inferior vena cava and a cannula was inserted into the left ventricle for infusion of phosphate-buffered saline (0.01 m, pH 7.4; PBS) followed by formal–sucrose (4% paraformaldehyde, 5% sucrose in PBS; Sigma Chemical Company, St. Louis, MO). Using a dissecting microscope (Zeiss, Oberkochen, Germany) the entire mouse aorta was dissected from the aortic valve to the bifurcation of the iliac artery. Adventitial fat was removed, the aorta opened longitudinally, and branches were severed to detach the aorta. Aortae were pinned flat onto smooth black dissecting wax with 0.2-mm diameter stainless steel pins (Fine Sciences, Foster City, CA) and neutral lipids were stained with Sudan IV (Sigma Chemical Co.) (8–10).

Measurement of uterine weight

The entire uterus was collected from all female mice to assess in vivo exposure to E_2. Serosal fat was removed with fine scissors using a dissecting microscope (Zeiss); the uterus was divided from the oviduct at the utero-tubal junction bilaterally and transected at the junction of the uterine body and cervix. After removal of luminal fluid, the entire uterus was wet-weighed on a micro-balance. Uterine weight was expressed as mg/gm body weight to correct for differences in total body weight.

Assessment of plasma cholesterol and triglyceride levels and FPLC profiles

Cholesterol and triglyceride were measured using enzymatic colorimetric method kits (Sigma Chemical Co.) according to the manufacturer's instructions. As an indicator of total cholesterol exposure during high fat feeding, time-averaged plasma total cholesterol levels were calculated for individual mice. Lipoproteins were fractionated by FPLC (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). For each treatment group a plasma pool was prepared of equal volumes of fasting plasma collected at day 40 from 4 mice with plasma cholesterol levels representative of the mean for that group. Plasma samples (100 μl) were applied to a Superose 6 column (1 × 30 cm; Pharmacia LKB Biotechnology Inc.), eluted with Tris-buffered saline, pH 7.4, containing 10 mm Tris-HCl, 0.15 m NaCl, and 0.01% EDTA, and collected in 0.5-ml fractions (n = 25). Total cholesterol content of each fraction was assayed as previously described (11) using Preciset cholesterol calibrator (Boehringer Mannheim, Indianapolous, IN) as standard.

Analysis of extent of atherosclerosis

The Sudan IV-stained aortae were photographed (Kodak elite 100 slide film) at a fixed magnification. Slides were digitized using a 35-mm slide scanner (Minolta QuickScan 35, Osaka, Japan) attached to a Power Macintosh (Apple Computer Inc., Cupertino, CA). Individual aortae were outlined electronically using Adobe Photoshop (Adobe Systems Inc., San Jose, CA) from the aortic valve to the bifurcation of the aorta to allow calculation of total aortic area. Lesion area was selected using a constant electronic filter designed to select the areas stained red (Chromatica; Chroma Graphics Inc., Burlingame, CA). Total aortic area and lesion area was calculated using NIH Image software (National Institutes of Health, Bethesda, MD) and lesion area was expressed as a percentage of total aortic area.

Statistics

All results were expressed as mean ± SEM unless otherwise stated. Normally distributed data were compared with ANOVA or unpaired *t*-test, whereas non-normally distributed data were compared with Kruskal-Wallis and Mann Whitney U tests, and correlations were calculated by Spearman rank correlation using Statview (SAS Insitute Inc. Cary, NC). A probability of 0.05 was regarded as significant.

RESULTS

Systemic effects of estrogen

At baseline (day 0), the body weight of female mice was significantly less than that of male mice ($P < 0.0001$). Although the body weights of endogenous E_2 female mice and female mice receiving exogenous E_2 were not different, these mice gained significantly less body weight than E_2-deficient mice. By 84 days, the body weight of the E_2-deficient female mice did not differ from that of male mice (**Table 1**). The rate of weight gain in mice exposed to exogenous E_2 increased between 60 and 84 days while that of endogenous E_2 mice remained constant, suggesting that the source of exogenous E_2, the pellets, was being exhausted prematurely (data not shown).

Uterine weight at day 84 differed among all the female groups (Table 1). Uteri from mice deficient in E_2 were consistently smaller than those exposed to either endogenous or exogenous E_2 ($P < 0.002$). Although endogenous E_2 mice had higher uterine weights than the OVX female mice exposed to exogenous 17β-E_2 ($P < 0.006$), these two treatment groups most closely approximated each other. The uteri of those OVX females receiving exogenous 17α-E_2 were largest ($P < 0.004$). Interestingly, exogenous 17β-E_2, but not 17α-E_2, was associated with the development of carcinoma of the cervix in 26% of the mice which ne-

TABLE 1. Effects of estrogen on body and uterine weight (wt) in LDLR−/− mice

E_2 Status	Surgery	n	Day 0 Body Wt	Day 84 Body Wt	Day 84 Uterine Wt
			g	*g*	*mg/g body wt*
Endogenous E_2	sham	14	14.7 ± 0.2	20.1 ± 0.4	2.61 ± 0.29[c]
Exogenous 17β-E_2	OVX	14	15.0 ± 0.4	22.1 ± 0.4	2.27 ± 0.91[d]
Exogenous 17α-E_2	OVX	12	14.2 ± 0.5	21.1 ± 0.3	7.98 ± 1.8[e]
E_2-deficient	OVX	15	14.7 ± 0.4	24.2 ± 0.5[a]	0.6 ± 0.19[f]
Male control	sham	21	16.9 ± 0.3	25.5 ± 0.4[b]	—

Results were expressed as mean ± SEM for all values.

[a] $P < 0.002$ E_2-deficient compared with endogenous and exogenous E_2 (17β- and 17α-E_2).

[b] $P < 0.0001$ male compared with endogenous and exogenous E_2 (17β- and 17α-E_2).

[c] $P < 0.006$ endogenous E_2 compared with exogenous E_2 and E_2-deficient.

[d] $P < 0.006$ exogenous 17β-E_2 compared with endogenous E_2, exogenous 17α-E_2, and E_2-deficient.

[e] $P < 0.004$ exogenous 17α-E_2 compared with endogenous E_2, exogenous 17β-E_2, and E_2-deficient.

[f] $P < 0.002$ E_2-deficient compared with endogenous E_2 and exogenous E_2 (17β- and 17α-E_2).

cessitated exclusion of these affected mice (n = 5) from this study. Therefore, the final number of exogenous 17β-E_2-treated female mice (n = 14) is referred to from here on. The carcinoma of the cervix was manifest as abdominal swelling with secondary renal tract obstruction due to carcinomatous invasion of the urethra and base of the bladder.

Estrogen effects on plasma cholesterol and triglyceride levels, and FPLC profiles

At baseline all mice were hypercholesterolemic on the chow diet with the fasting plasma cholesterol levels higher in the male (242.7 ± 7.2 mg/dl) than female (209.3 ± 3.3 mg/dl) mice ($P < 0.0001$). Fasting plasma total cholesterol increased approximately 4- to 5-fold on the high fat diet (**Fig. 1**). In the presence of physiologic endogenous E_2, fasting plasma cholesterol was not different from E_2-deficient female mice. However, fasting plasma cholesterol was reduced by exogenous E_2, both 17β- and 17α-E_2 ($P < 0.002$), compared to E_2-deficient female mice, despite the absence of the LDL receptor. In addition, fasting plasma cholesterol was higher in endogenous E_2 and E_2-deficient female mice ($P < 0.0002$) compared with male mice and did not differ between exogenous E_2 female and male mice.

Fasting plasma triglyceride levels also were increased on the high fat diet (**Fig. 2**). Only exogenous 17α-E_2 ($P < 0.008$) was associated with decreased fasting triglyceride levels compared to E_2-deficient female or male mice. Technical difficulties limited the reproducibility of plasma triglyceride estimation in plasma samples from nonfasting mice from day 84, therefore these data have not been presented. The plasma lipoprotein cholesterol distribution in female mice did not differ between E_2

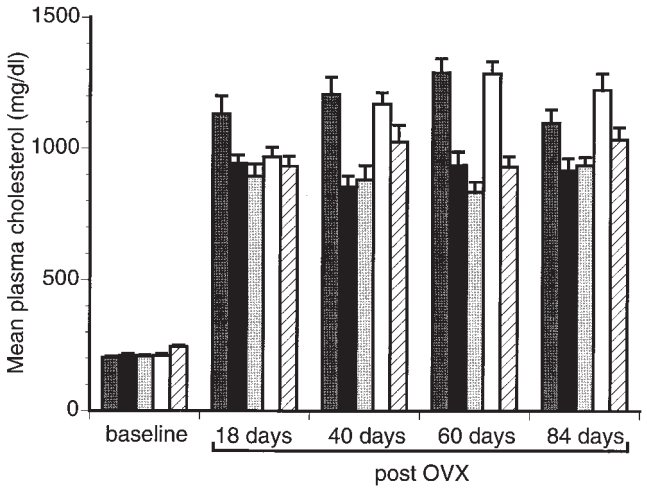

Fig. 1. Plasma total cholesterol (mean ± SEM) for each group during the 12-week study. Baseline measurements were performed while the animals were on the chow diet before any surgical intervention. All mice were fed ad libitum a high fat diet containing 6% fat, 1.25% cholesterol 1 week after surgery. The 84-day samples were collected in the non-fasting state. Sham-operated female (endogenous E_2; ■, n = 14) and male (male; ▨, n = 21) mice served as controls. Female OVX mice were implanted with subcutaneous slow-release hormone pellets designed to release 6 μg/day for 90 days of exogenous E_2 as 17β-estradiol (exogenous 17β-E_2; ■, n = 14) or 17α-estradiol (exogenous 17α-E_2; □; n = 12), or no E_2 as placebo vehicle alone (E_2-deficient; □, n = 15). Fasting plasma cholesterol was reduced by exogenous E_2 (17β-E_2 and17α-E_2) compared with E_2-deficient female mice ($P < 0.002$), and was higher in endogenous E_2 and E_2-deficient female mice than male mice ($P < 0.0002$).

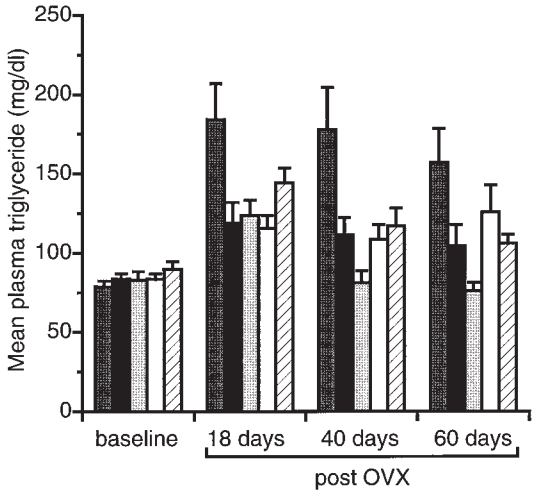

Fig. 2. Fasting plasma triglyceride (mean ± SEM) for each group during the 12-week study. Baseline measurements were performed while the animals were on the chow diet before any surgical intervention. All mice were fed ad libitum a high fat diet containing 6% fat, 1.25% cholesterol 1 week after surgery. Sham-operated female (endogenous E_2; ■, n = 14) and male (male; ▨, n = 21) mice served as controls. Female OVX mice were implanted with subcutaneous slow-release hormone pellets designed to release 6 μg/day for 90 days of exogenous E_2 as 17β-estradiol (exogenous 17β-E_2; ■, n = 14) or 17α-estradiol (exogenous 17α-E_2; □; n = 12), or no E_2 as placebo vehicle alone (E_2-deficient; □, n = 15). Only exogenous 17α-E_2 was associated with decreased fasting triglyceride ($P < 0.008$) compared with E_2-deficient female and male mice.

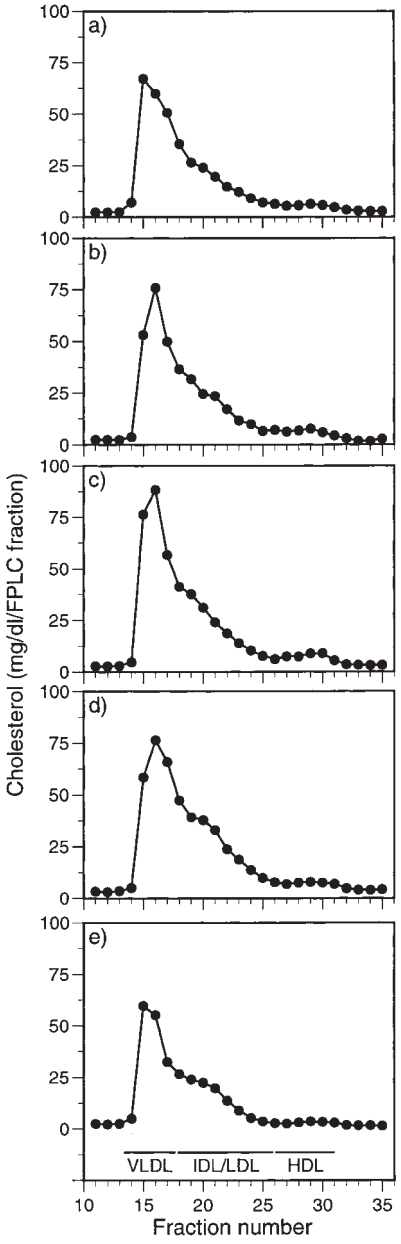

Fig. 3. Plasma FPLC profiles were similar in all mice irrespective of gender or E_2 status. Plasma collected 40 days post surgery was pooled from mice (n = 4) with plasma cholesterol levels representative of the mean for each treatment group. a) endogenous E_2 female, b) exogenous 17β-E_2, c) exogenous 17α-E_2, d) E_2-deficient, and e) male. Results expressed as cholesterol concentration (mg/dl) per fraction.

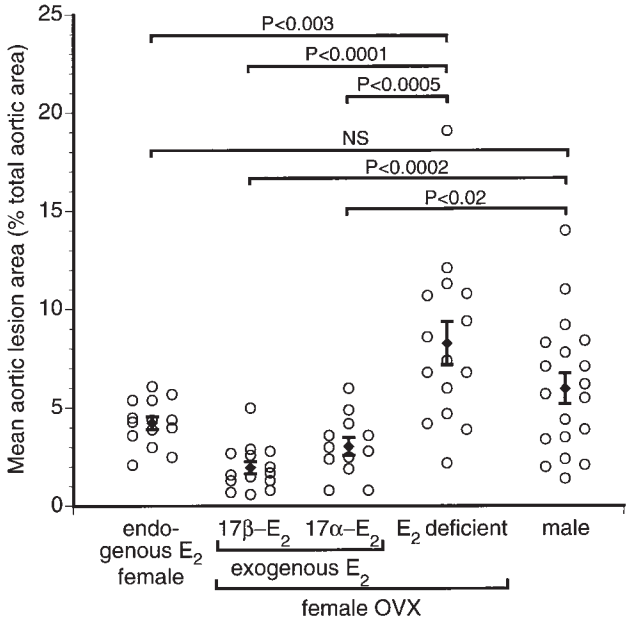

Fig. 4. Aortic lesion area and total aortic area were measured from the ascending aorta to the aortic bifurcation, and lesion area was expressed as % of total aortic area. Symbols represent individual data for each group (○) and mean ± SEM (◆). Treatment groups shown according to estrogen status: endogenous E_2 female (n = 14), female OVX exposed to exogenous 17β-E_2 (n = 14), 17α-E_2 (n = 12), or E_2-deficient (n = 15), and male mice (n = 21).

treatment groups (**Fig. 3 a**–**d**) and was similar to that of male mice (Fig. 3e).

Effect of estrogen on aortic atherosclerotic lesion area

Endogenous E_2 female mice with intact ovarian function and OVX female mice treated with exogenous 17β- and 17α-E_2 had reduced aortic atherosclerotic lesion area compared with E_2-deficient female mice ($P < 0.003$, $P < 0.0001$, and $P < 0.0005$, respectively) (**Fig. 4** and **Fig. 5**). While endogenous E_2 inhibited atherosclerosis to a lesser extent than exogenous E_2 (17β-E_2, $P < 0.0001$; 17α-E_2, $P < 0.02$), there was no difference in the atheroprotective effects of the exogenous 17β- and 17α-E_2. Exogenous E_2 (17β-E_2, $P < 0.0001$; 17α-E_2, $P < 0.02$) also reduced lesion area compared to male mice. Although endogenous E_2 female mice, tended to have less aortic lesion area than male mice, this did not reach statistical significance. More importantly, in subgroup analysis matched for time-averaged plasma total cholesterol, aortic lesion area was reduced ($P < 0.05$) in the presence of endogenous E_2 (n = 6) and exogenous 17β-E_2 (n = 5) compared with male controls (n = 9). No significant correlation was detected between atherosclerotic lesion area and time-averaged plasma total cholesterol (**Fig. 6**). While there was a weakly positive, but significant, correlation between body weight at killing and atherosclerotic lesion area ($r = 0.36$, $P < 0.05$), no significant correlations were found between atherosclerotic lesion area and age, or fasting plasma triglyceride.

DISCUSSION

These studies have demonstrated that E_2 reduced atherosclerosis independent of plasma cholesterol levels in LDLR−/− mice fed a high fat diet. Lesion development and inhibition by E_2 occurred despite the shortened length of high fat feeding (12 weeks), dictated by the limited duration of hormone release from the slow-release pellets.

To differentiate the estrogen receptor-mediated effects from nonreceptor-mediated events in this model, both ex-

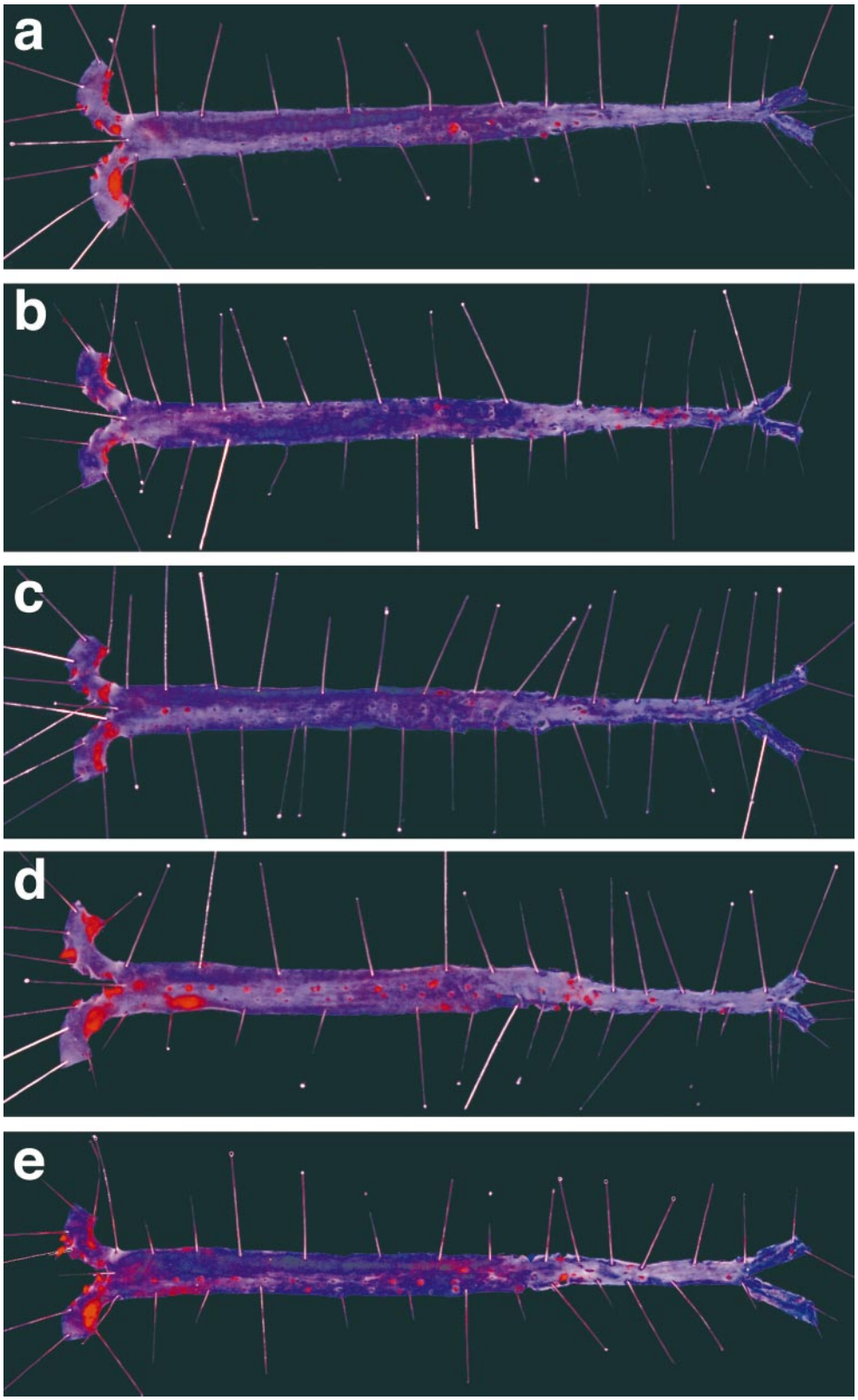

Fig. 5. Sudan IV stained en face aortae representative of the mean lesion area of each group: a) endogenous E_2, b) exogenous 17β-E_2, c) exogenous 17α-E_2, d) E_2-deficient, and e) male. The red areas indicate fatty streaks. Aortae are oriented with the aortic arch on the left and bifurcation on the right.

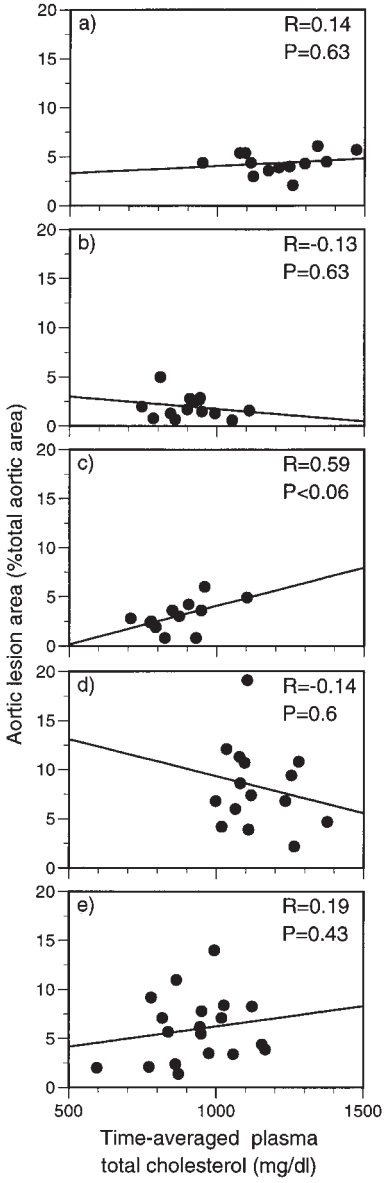

Fig. 6. There was no significant correlation between aortic lesion area and overall cholesterol exposure expressed as time-averaged plasma total cholesterol for individual treatment groups a) endogenous E_2 female (n = 14), b) exogenous 17β-E_2 (n = 14), c) exogenous 17α-E_2 (n = 12), d) E_2-deficient (n = 15), and e) male (n = 21). Shown for each group are Rho and *P* values.

ogenous 17β- and 17α-E_2 were tested. Classically, 17β-E_2 is the most potent agonist at the classical estrogen receptor, estrogen receptor α, whereas the α isomer is significantly less active (12). Atheroprotective effects mediated via the estrogen receptor were expected to have been significantly greater in exogenous 17β-E_2–treated mice, however, the interpretation has been complicated by the recent identification of the estrogen receptor β (13). In the mouse there are at least three isoforms of the estrogen receptor β (14), and there are no published data yet regarding the preferred agonist(s) at these receptor(s). Supraphysiologic doses of E_2 have been shown to prevent the vascular injury response to carotid artery injury in C57BL/6J mice (15), independent of the estrogen receptor α (16). Although the response to vascular injury differs from that of the atherosclerotic response to high fat feeding, the protective effect of E_2 may have occurred via the estrogen receptor β. In our studies, exogenous 17α-E_2 tended to be less potent than 17β-E_2 in reduction of aortic atherosclerotic lesion area, but this difference was not statistically significant. Therefore, it is not appropriate to conclude that the cholesterol-independent atheroprotective effects of estrogen occurred via non-receptor-mediated pathways, because vascular protective effects mediated via the β receptor(s) (16) cannot be excluded.

Higher body weights were associated with E_2-deficient female mice and male mice. The body weights of mice with intact ovarian function (endogenous E_2) were not different from mice treated with exogenous E_2 and this indicated that E_2 delivery was most likely adequate in the absence of toxicity. Estrogen in physiologic doses has effects on body fat composition and body weight in humans (17) and on body weight and food consumption in a variety of animal species including primates (18) and rodents (19). Although increased food consumption by the E_2-deficient female mice is a possible explanation for their higher body weight compared to female mice exposed to endogenous and exogenous E_2, a purely dietary factor is unlikely to explain the differences in atherosclerosis as the total plasma cholesterol of endogenous E_2 female mice and E_2-deficient female mice were identical despite the higher body weight of the E_2-deficient female mice. Our observations of the mouse body weights are in contrast to those shown in apoE-deficient mice on chow diet where only exogenous E_2, in higher doses than used in this study, but not endogenous E_2 (5, 6) was associated with reduced body weight compared to E_2-deficient female mice. Similarly, when apoE-deficient mice received a Western high fat diet, only exogenous 17β-E_2 in doses more than twice that used in this study reduced body weight compared to E_2-deficient female mice. Moreover, body weight was not altered by an approximately 3.5-fold higher dose of exogenous 17α-E_2 than that used in the present study. The mechanism(s) of these apparently different effects of E_2 between the genetic phenotypes is not clear. Nevertheless, previous studies have shown that apoE-deficient mice gained more weight than LDLR−/− female mice of similar age exposed to a high fat diet for the same time period (8).

Uterine weight was assessed to measure in vivo E_2 action as conventional assay methods of plasma E_2 cross-react with multiple estrogenic compounds and the plasma requirements for the more specific extracted E_2 methods were too high (20). The very low weight, atrophic uteri of E_2-deficient female mice confirmed the absence of estrogenic stimulation and verified complete ovariectomy. Exogenous 17β-E_2 female mice had smaller uteri than the endogenous E_2 female mice suggesting that the exogenous dose of E_2 at completion of the study was within or below the normal physiologic range. Although the greater uterine weight of the exogenous 17α-E_2 female mice was inconsistent with its known decreased potency at the classic estrogen receptor (21), this inconsistency can be ex-

plained by the previously demonstrated differential effects of acute and chronic exposure and different capacity of 17α-E_2 to induce its own metabolism. Specifically, chronic 17α-E_2 exposure in rodents results in greater uterine size than acute 17α-E_2 treatment (21, 22), and 17α-E_2 is less potent than 17β-E_2 in inducing hepatic metabolism of estrogen compounds resulting in prolonged circulation of 17α-E_2 (21). The high incidence of carcinoma of the cervix in exogenous 17β-E_2 female mice was consistent with previously published data in mice of C57Bl background (reviewed in 23). Initially reported in the 1930s (24, 25), carcinoma of the cervix in mature mice in response to E_2 exposure is species and strain specific, and is related to the duration of estrogen exposure.

Although fasting plasma cholesterol in endogenous E_2 female mice was not different from that in E_2-deficient mice, exogenous E_2 (17β- and 17α-E_2) reduced fasting plasma cholesterol compared to E_2-deficient mice, despite the absence of the LDL receptor. In addition, fasting plasma cholesterol was higher in endogenous E_2 and E_2-deficient female mice than male mice and was not different between exogenous 17β- or 17α-E_2 female and male mice. Moreover, nonfasting plasma cholesterol (84 days) in endogenous E_2 female mice and male mice was not different. Endogenous E_2 female mice have intact ovarian function and therefore intact progesterone and androgen production which can elevate plasma cholesterol (reviewed in 26). These hormonal differences may contribute to the greater plasma cholesterol levels observed in the endogenous E_2 female than in the OVX exogenous E_2 female mice. Although the plasma cholesterol of high fat-fed LDLR−/− female mice has generally been reported to be higher than that of male mice (7, 8), differences in diet composition and the duration of feeding make direct comparisons difficult. Our data in LDLR−/− mice differ from that reported in apoE-deficient mice, where only high doses of exogenous E_2 reduced plasma cholesterol compared to E_2-deficient mice (5, 6), and plasma cholesterol levels were not altered by exogenous 17α-E_2 (5). Although the duration of high fat feeding was similar in one of these studies (5), the dietary composition was different. In our study only exogenous 17α-E_2 was associated with decreased fasting triglyceride. The variability observed within groups was a major contributing factor to the lack of detectable differences in fasting plasma triglyceride between E_2-replete and E_2-deficient female mice. In contrast to observations in apoE-deficient mice (5), where the VLDL peak was lower with E_2 exposure, the plasma FPLC profiles in our study of LDLR−/− mice were not altered by E_2 or gender. Together the data from the LDLR−/− and apoE-deficient mice suggest that the E_2 lowers cholesterol by additional mechanism(s) that are independent of effects of E_2 on induction of both LDL receptor (27, 28) and apoE (29) expression. Whereas apoE-deficient mice have intact LDL receptors that can be induced by E_2, apoE-dependent uptake is impeded while the converse is true in the LDLR−/− mouse. Estrogen effects on atherosclerosis that are independent of cholesterol lowering are numerous. These include: vasodilation, decreased peripheral resistance, reduced vascular reactivity to vasoconstrictive stimuli, reduced smooth muscle proliferation, protection of lipoproteins from oxidation, and modulation of inflammatory mediators (reviewed in 4, 30, 31). Results of this study indicate that the LDLR−/− mouse represents an appropriate model for further investigation of the mechanisms through which estrogen protects against atherosclerosis.

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