Cardiovascular Effects of Estrogen

Todd Tolbert and Suzanne Oparil

There is a strong link between menopause and increased cardiovascular disease incidence in women, and observational studies suggest that postmenopausal hormone replacement therapy (HRT) reduces cardiovascular disease risk. However, the only published prospective controlled trial of the effects of HRT on cardiovascular outcomes (Heart and Estrogen-Progestin Replacement Study, HERS) showed no net benefit of conventional HRT in women with established coronary disease. An angiographic study of HRT in a similar patient population showed no regression of established coronary lesions in the active treatment group. Fundamental mechanistic studies of the cellular and molecular events by which hormones protect (or fail to protect) blood vessels are needed to define the role of postmenopausal HRT in cardiovascular disease. Herein, we review studies from our laboratory using the rat carotid injury model showing that estrogen inhibits neointima formation through an estrogen receptor (ER)-dependent mechanism operative in the early period after vascular injury. We have demonstrated that activation of vascular smooth muscle cells and subsequent release of soluble factors including osteopontin stimulate the migration of adventitial fibroblasts in a luminal direction to eventually take up residence in the neointima, and furthermore, that production, release, or posttranslational processing of these factors are inhibited by estrogen through an ER-dependent mechanism. The relevant observational and prospective data on HRT in cardiovascular disease prevention are reviewed, and the cardiovascular effects of estrogen are discussed. Am J Hypertens 2001;14:186S–193S © 2001 American Journal of Hypertension, Ltd.

Key Words: Cardiovascular disease, estrogen, vascular injury.

Clinical Trials of Hormone Replacement Therapy and Cardiovascular Disease

Cardiovascular disease, including coronary artery disease (CAD) and stroke, is the leading cause of death in women.1 There is a strong link between menopause and an increased incidence of cardiovascular disease,2 and observational studies suggest that postmenopausal hormone replacement therapy (HRT), including various estrogen preparations with or without progestin (most commonly synthetic progestin), reduces cardiovascular disease risk by about half.1,3–9 These studies, although provocative, suffer from the limitations of their observational design, small patient numbers, and noncomparability of the two treatment groups: women who take estrogen are on average better educated, have higher incomes and better access to health care, and are healthier even before starting therapy.10,11 This deficiency in available data concerning the cardiovascular benefits (and possible risks) of HRT, combined with the known increased risk of breast and endometrial cancer and venous thromboembolism in long-term users of estrogen, has created controversy in the healthcare arena about the general advisability of hormone replacement.12

To overcome the limitations of observational studies, a number of prospective, randomized clinical trials, which examine the safety and efficacy of postmenopausal HRT, have been designed. The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial examined differences between placebo, unopposed estrogen, and estrogen/progestin regimens on selected heart disease risk factors in healthy postmenopausal women.13 Estrogen alone or in combination with a progestin improved lipoprotein levels, including decreased low-density lipoprotein cholesterol (LDL-C) and increased high-density lipoprotein cholesterol (HDL-C), and lowered fibrinogen levels compared to placebo. Similar improvements in cardiovascular risk profiles of women receiving estrogen were noted in the Ageing Women’s Project, a prospective randomized trial of unopposed estrogen, the selective estrogen receptor modulator raloxifene, and placebo in hysterectomized but otherwise healthy postmenopausal women.14 These results strengthened the conclusion, drawn from numerous observational studies, that postmenopausal HRT reduces cardiovascular disease risk (Table 1).

Received February 26, 2001. Accepted March 6, 2001.

From the The University of Alabama at Birmingham, Department of Medicine, Vascular Biology and Hypertension Program, Birmingham, Alabama.

Address correspondence and reprint request to Dr. Suzanne Oparil, Department of Medicine, Vascular Biology and Hypertension Program, 1034 Zeigler Research Bldg., 703 S 19th St, Birmingham, AL 35294-0007; e-mail: soparil@uab.edu

© 2001 by the American Journal of Hypertension, Ltd. Published by Elsevier Science Inc.
The results from the Heart Estrogen-Progestin Replacement Study (HERS), however, have added to the controversy over the role of HRT in cardiovascular diseases.\(^\text{15,16}\) HERS was the first large-scale, randomized clinical trial to test the efficacy and safety of hormone replacement on clinical cardiovascular disease outcomes in postmenopausal women. The study population included 2763 women with established CAD randomized to combined HRT or placebo that were followed for an average of 4 years. Overall, there was no significant difference between groups for the primary outcome, nonfatal myocardial infarction or coronary heart disease, death, or for several secondary cardiovascular end points. There was a statistically significant time trend, with more coronary heart disease events in the treatment group than in the placebo group in year 1 and fewer in years 3 and beyond (Fig. 1). Furthermore, post-hoc analysis of the HERS data showed no significant difference in the risk of stroke or transient ischemic attack between women receiving HRT and those receiving placebo.\(^\text{17}\) These results do not support instituting HRT in women with established coronary heart disease for the sole purpose of avoiding secondary events. HERS did not address the question of benefit (and risk) from estrogen alone or from combined hormone replacement in primary prevention, nor did it elucidate the mechanism of the apparently biphasic effect (early detriment, delayed benefit).

### Table 1. Population studies of effects of HRT on cardiovascular risk factors, atherosclerotic disease, and cardiovascular outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Full Title</th>
<th>Intervention/End Point</th>
<th>Therapy</th>
<th>Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAVEAT</td>
<td>Coronary angioplasty versus excisional atherectomy trial</td>
<td>Atherectomy; angioplasty</td>
<td>E2 + P</td>
<td>Lower restenosis rate</td>
</tr>
<tr>
<td>HERS</td>
<td>Heart and estrogen/progestin replacement study</td>
<td>CV disease outcomes</td>
<td>E2 + P</td>
<td>↑ early events, ↓ later events</td>
</tr>
<tr>
<td>ERA</td>
<td>Estrogen replacement and atherosclerosis trial</td>
<td>Angiographic CAD progression</td>
<td>E2 + P</td>
<td>No difference in CAD progression</td>
</tr>
<tr>
<td>WHI</td>
<td>Women’s health initiative</td>
<td>Longitudinal outcomes</td>
<td>E2 + P</td>
<td>In progress</td>
</tr>
<tr>
<td>PEPI</td>
<td>Postmenopausal estrogen/progestin intervention trial</td>
<td>HDL</td>
<td>E2 + MPA</td>
<td>Smaller ↑ in HDL with MPA</td>
</tr>
<tr>
<td>FOS</td>
<td>Framingham offspring study</td>
<td>CV risk factors</td>
<td>E2 + P</td>
<td>↑ PAI-1 with E2 ± MPA</td>
</tr>
<tr>
<td>NHS</td>
<td>Nurses’ health study</td>
<td>E2 and E2 + P</td>
<td>E2 + P</td>
<td>Decrease major CAD</td>
</tr>
</tbody>
</table>

E2 = estrogen; P = progestin; CV = cardiovascular; PAI-1 = plasminogen activator inhibitor; CAD = coronary artery disease.

**FIG. 1.** Time trend for the primary end point (coronary heart disease [CHD] death plus nonfatal heart attacks) for the Heart Estrogen-Progestin Replacement Study (HERS). Relative hazard for developing a primary CHD event on hormone replacement therapy (HRT) compared with placebo (Pbo) in year 1 was 1.52 (95% confidence interval, 1.01 to 2.29); in year 2, 1.00 (0.67 to 1.49); in year 3, 0.87 (0.55 to 1.37); in years 4 and 5, 0.67 (0.43 to 1.04). For tests of continuous trend in log-relative hazard, \(P = .009\). (Data derived from Ref. 15.)
later benefit) of combined hormone replacement in women with atherosclerotic disease. Answers to the first questions will come from the Women’s Health Initiative (WHI), a randomized trial of estrogen and combined HRT for primary prevention, which includes 10 times as many treated women as HERS and a longer (9 years) period of treatment, completing in the year 2005. The last question can be answered only by further research in both human subjects and animal models.

Another prospective study of HRT and cardiovascular disease in postmenopausal women, the Estrogen Replacement and Atherosclerosis Trial (ERA), failed to demonstrate a protective effect of HRT on the progression of atherosclerosis after 3 years of treatment in postmenopausal women with angiographically documented CAD randomized to HRT (estrogen ± progestin) or placebo. These results were contrary to previous observational data that found a decreased incidence of angiographically defined coronary atherosclerosis in women on HRT compared to controls. Despite significant reductions in LDL-C and increases in HDL-C in women receiving estrogen or estrogen plus medroxyprogesterone acetate in ERA, there was no difference in the minimal coronary diameter at follow-up compared to those receiving placebo. The rates of clinical cardiovascular events were also similar between the two active treatment groups and the placebo group. Ongoing large, prospective studies, including The Million Women’s Study, the largest observational study to date on the effects of HRT on cardiovascular disease risk, and the Women’s International Study of long Duration Oestrogen after Menopause (WISDOM), will further define the role of HRT in cardiovascular disease.

Effects of Hormones on Cardiac Remodeling and Performance

Human and animal studies have suggested a role for estrogen in cardiac performance and remodeling. In a study of healthy postmenopausal women treated de novo with 0.625 mg of conjugated estrogens and 2.5 mg of medroxyprogesterone acetate daily for 6 months, Taskin and colleagues noted significant increases in ejection fraction, improved diastolic function, and reduced left ventricular end-diastolic and end-systolic volumes by echocardiography. Other investigators have demonstrated significant reductions in left ventricular mass in women treated for more than 10 years with HRT compared to age-matched controls. Similar results were noted in a randomized trial of HRT versus placebo in hypertensive postmenopausal women. In this study, women randomized to transdermal 17β-estradiol had significantly greater reductions in left ventricular mass compared to controls on similar antihypertensive regimens. In another example of enhanced cardiac performance in the presence of estrogen therapy, Fraser et al demonstrated increased glucose oxidation and enhanced recovery of mechanical function in an ischemia–reperfusion model using isolated, perfused hearts from estrogen treated rats.

Direct Vascular Effects of Hormones

Definitive answers to questions about the effects of sex hormones on the cardiovascular system will come only from fundamental mechanistic studies of the cellular and molecular process by which estrogen and progestins protect (or fail to protect) blood vessels from damage. Although much has been learned about mechanisms of estrogen-induced vasoprotection from studies carried out in women, insights derived from animal experiments have been indispensable in advancing our understanding of the vascular effects of the sex hormones, particularly estrogen. It has been clearly shown that endothelial cells and vascular smooth muscle cells (VSMC) possess estrogen receptors (ER) and are thus targets for estrogen action. Furthermore, ER have been identified in cardiac myocytes and coronary arteries. A functional role for ER in coronary atherosprotection was suggested by one study in which ER were detected more frequently in the coronary arteries of premenopausal women free of atherosclerosis than in those with atherosclerotic disease. Cellular responses to estrogen may be elicited through both genomic and nongenomic mechanisms. For instance, ER stimulation has been shown to increase nitric oxide synthesis, and Clark et al recently demonstrated increased coronary blood flow and cardiac output in sheep in response to acute administration of both 17β-estradiol and conjugated equine estrogens.

Neointima formation occurs in response to vascular injury and can lead to stenosis. Most human and animal studies suggest that estrogen limits the proliferation of VSMC after vascular injury, thus inhibiting the neointimal response in acutely injured blood vessels. For example, 6-month results of the Coronary Angioplasty Versus Excisional Atherectomy Trial (CAVEAT I) showed that women undergoing atherectomy who received HRT (estrogen ± progestin) had significantly less late loss in minimal lumen diameter, larger lumen diameter, and lower restenosis rates (27% versus 57%, \( P = .038 \) for \( >50\% \) stenosis) than those not receiving estrogen. In contrast, estrogen had a minimal effect on restenosis after balloon angioplasty in this trial. Furthermore, two retrospective studies of women undergoing elective percutaneous transluminal coronary angioplasty (PTCA) showed improved survival and reduced cardiovascular event rates (death, nonfatal myocardial infarction, or nonfatal stroke) in women treated with hormone replacement; there was no difference between treatment groups in need for subsequent revascularization, suggesting no reduction in restenosis. These device-specific results are consistent with an inhibitory effect of estrogen on neointima formation, which is thought to play a greater role in restenosis after...
atherectomy than after balloon angioplasty, where later recoil is a major contributor. Estrogen treatment has been shown not to inhibit neointima formation in a nonhuman primate model with preexisting atherosclerosis. In this study, estrogen affected only a transient decrease in arterial cell proliferation rate immediately after balloon injury but did not alter either neointimal area or arterial remodeling. Interestingly, estrogen treatment administered concurrently with a high fat diet slowed the progression of experimentally induced atherosclerosis, but it did not modulate the acute injury response in the atherosclerotic artery. Although the possible explanations are numerous and complex, it is highly likely that this refractoriness of the injury response to estrogen in atherosclerotic vessels may reflect a greatly reduced density of ER, as reported by Losordo et al in diseased human coronary arteries. This mechanism could also contribute to the lack of benefit seen in the HERS trial early in the course of hormone replacement therapy.

Our laboratory has used balloon injury of the common carotid artery of the rat as an experimental model of localized and highly controllable vascular damage in which the response to injury can be studied in vivo. In this model, balloon inflation denudes endothelium and induces a highly reproducible intimal migration/proliferation of VSMC over the entire length of the affected vessel. Our recent observations suggest that balloon injury also induces activation/migration of adventitial cells into the neointima in this model and that these cells contribute to the neointimal response to injury.

We used the rat carotid injury model to test whether there is a sexual dimorphism in the neointimal response to balloon injury, and if so, whether this dimorphism is estrogen or androgen dependent. We hypothesized that the sex hormones modulate neointima formation in response to balloon injury of the carotid artery. Our initial study demonstrated that 1) neointima formation after balloon injury of the carotid artery was significantly greater in intact male Sprague-Dawley rats than in age-matched intact females; 2) gonadectomy of male rats did not alter the neointimal response; 3) gonadectomy of female rats was associated with a more robust neointimal proliferative response to injury, comparable to that seen in the male; and 4) estradiol replacement markedly attenuated neointima formation in gonadectomized rats of both sexes (Fig. 2). Serum estradiol levels in estrogen-treated male and female rats were in the physiologic (for females) range. Thus, the sexual dimorphism of neointima formation after balloon injury of the rat carotid artery is estrogen dependent. The vasoprotective effect of estrogen in this model was subsequently determined to be ER dependent by the demonstration that it was completely inhibited by the nonselective ER antagonist ICI 182,780.

The most dramatic of our findings was the observation that 17-estradiol inhibited the neointimal response to vascular injury by about 60% in gonadectomized rats of both sexes, an effect greater than that previously observed in our laboratory with mithramycin, an inhibitor of the neointimal response to injury through the downregulation of c-myc expression, and comparable to results observed with a variety of other agents in the rat carotid injury model. The protective effects of estrogen on the neointimal response to vascular injury were blunted in subsequent experiments by the coadministration of the synthetic progesterone medroxyprogesterone acetate (MPA) (Fig. 2).

To define the cellular mechanisms by which estrogen...
inhibits neo-intima formation after vascular injury, it is necessary to identify the critical estrogen-sensitive chemoattractants/growth factors/cytokines involved, along with their cellular sites of origin and of action. Importantly, recent in vitro and in vivo studies in our own laboratory have shown that activated VSMC release soluble factors that stimulate the migration of adventitial fibroblasts in a luminal direction to eventually take up residence in the neo-intima, and furthermore, that production, release, or posttranslational processing of these factors are inhibited by estrogen through an ER-dependent mechanism operative in the early period after vascular injury. Moreover, we have shown that short-term systemic administration of 17-β-estradiol in the period immediately preceding and after vascular injury is effective in preventing neo-intima formation in the balloon-injured rat carotid artery. Identification and characterization of these factors are ongoing in our laboratory.

To this end, we used activated rat aortic smooth muscle cells in culture to demonstrate that stimulation of adventitial fibroblast migration/adhesion occurs in vitro through an estrogen inhibitable, ER-dependent mechanism that requires gene transcription and new protein synthesis. Preliminary evidence suggests that synthesis of osteopontin (OPN), an extracellular matrix protein, by activated VSMC may play an important role in this process. Serum-activated VSMC in culture express high levels of OPN mRNA and protein, and estrogen inhibits OPN expression by VSMC. Both VSMC-conditioned media and OPN were found to direct adventitial fibroblast migration. Taken together with the observation that anti-OPN antibody inhibits fibroblast migration directed by VSMC-conditioned media, these findings suggest that estrogen-inhibitable OPN expression in damaged VSMC of balloon-injured vessels may direct migration of adventitial fibroblasts into the media.

Ongoing studies in our laboratory continue to delineate the cellular/molecular characteristics of the estrogen-modulated early response pathway to endoluminal vascular injury. In addition to the increased production of OPN, we have demonstrated robust expression of inducible nitric oxide synthase (NOS II) in activated VSMC, which like OPN, is inhibited by estrogen. Furthermore, we have made exciting preliminary observations that implicate fibroblast growth factor-1 (FGF-1) and its receptor FGFR-1β in the vascular injury response through a signaling pathway that involves increased expression of OPN and NOS II and is negatively modulated by estrogen in an ER-dependent mechanism. Other investigators have demonstrated similar inhibitory effects of estrogen through ER-dependent mechanisms on VSMC biology using cultured human aortic smooth muscle cells. These effects include inhibition of fetal calf serum-induced DNA synthesis, cell proliferation, collagen synthesis, platelet-derived growth factor-induced VSMC migration, and mitogen-activated protein kinase activity. Our future efforts will be directed to investigation of the functional interaction between FGF-1, FGFR-1β, OPN, and nitric oxide in the context of vascular injury and its modulation by estrogen.

**Systemic Cardiovascular Effects of Hormones**

In addition to its direct actions on vascular cells and tissues, estrogen has many systemic effects, including alteration of serum lipid concentrations, coagulation and fibrinolytic systems, platelet aggregation, adhesion molecules, and growth factors. The effects of estrogen on serum lipid concentrations have been suggested by numerous observational studies and were confirmed in randomized trials such as PEPI and HERS. These include reductions in total cholesterol and LDL-C and increases in HDL-C and triglycerides. For years, it was postulated that estrogen-induced effects on serum lipid profiles were responsible for the improved cardiovascular outcomes noted in observational studies. However, this has not held true in randomized controlled trials, which thus far, have failed to show reductions in cardiovascular end points despite favorable changes in lipids. A small study that assessed the lipid-lowering effects of HRT initiated in the first versus the fifth year after surgically induced menopause may have shed light on this issue. This study showed that women in whom HRT was initiated later failed to have significant reductions in LDL-C or increases in HDL-C compared to women started on HRT within 1 year of menopause. It is possible that women with established atherosclerotic disease after menopause, such as the participants in HERS, fail to receive the full lipid-lowering benefit of HRT because of its initiation later in life.

In a separate post hoc analysis of the HERS data, Shlipak and colleagues noted a significant reduction in apolipoprotein a (Lp(a)) levels in women taking HRT compared to placebo. Furthermore, they found that relative hazards for cardiovascular events were lower for women using HRT who had baseline Lp(a) levels in the highest three quartiles compared to women taking placebo. The interaction of Lp(a) and HRT on the incidence of cardiovascular events in the HERS population and in other women at risk is an area that needs further investigation.

The antioxidant properties of estrogen have been investigated as a possible mechanism for its lipid-lowering effects, and the results have been inconsistent. Wen et al examined the effect of combined estrogen and progestin on LDL-C oxidation in healthy postmenopausal women and found no significant antioxidant effect. In vitro studies, however, have shown that physiologic concentrations of 17-β-estradiol inhibit oxidative modification of LDL-C. Other investigations into the lipid-lowering effects of estrogen have revealed that it enhances postprandial clearance of chylomicrons and chylomycin remnants and that HRT increases the ratio of arachidonic acid (20:4 n-6) to linoleic acid (18:2 n-6), thus increasing the precursor for eicosanoids with important cardiovascular functions.

Important biologic effects of estrogen relevant to car-
diovascular disease include its actions on the hemostatic system. Bar et al.\(^5\) found that ADP-mediated platelet aggregation was significantly inhibited by 17-β-estradiol in platelet rich plasma. Furthermore, this effect was reversed by the addition of ICI 182780 and 14-hydroxy tamoxifen, indicating that estrogen may regulate platelet function through binding to a nonnuclear receptor (platelets are devoid of nuclear components) with ligand-binding properties similar or identical to the wild-type ER. Numerous studies have shown decreased fibrinogen and plasminogen activator inhibitor-1 levels and increased fibrinolytic activity in estrogen-treated postmenopausal women.\(^58–62\) However, simultaneous increases in thrombin and fibrin generation have also been demonstrated, indicating the possibility of more generalized activation of the coagulation system, thus offering a potential explanation for the increased incidence of venous thrombosis with the use of HRT.\(^62\)

Effects of estrogen on adhesion molecules, cytokines and growth factors have been identified and implicated in the pathogenesis of cardiovascular disease. For example, the adhesion molecule ICAM-1, which is involved in the early stages of atherosclerosis, is expressed at lower levels in postmenopausal women taking oral estrogen therapy.\(^63\) Likewise, fasting plasma homocysteine levels were significantly reduced compared to placebo by a regimen of estrogen plus progestin but not by estrogen alone in a study of healthy postmenopausal women.\(^64\) Vascular endothelial growth factor (VEGF) levels were also reduced in normocholesterolemic postmenopausal women on estrogen but not in hypercholesterolemic women or controls.\(^65\) Other growth factors, including insulin-like growth factor I\(^66\) and transforming growth factor-β\(^67\) are increased by estrogen. The significance of these changes in growth factor expression and their impact on cardiovascular disease remain to be elucidated. Finally, a number of recent investigations have demonstrated that C-reactive protein level, an independent predictor of cardiovascular morbidity and mortality, is increased in a rapid and sustained manner after the initiation of HRT.\(^68–70\) The early elevation of C-reactive protein may play a role in the increased cardiovascular events noted in the first year of HRT in HERS.\(^15\)

Estrogen has a wide variety of effects on the cardiovascular system. Its actions are mediated by both rapid, non-genomic mechanisms and genomic mechanisms that involve gene regulation and protein expression. The role of estrogen in vascular biology is an evolving field with many questions remaining to be addressed. Continued fundamental mechanistic research using animal models is needed, as are more prospective clinical trials of estrogen therapy for the primary and secondary prevention of cardiovascular diseases.

References


