Review

Vascular consequences of menopause and hormone therapy: Importance of timing of treatment and type of estrogen

Raghvendra K. Dubey[a,b,c,*], Bruno Imthurn[a,b], Matthias Barton[d], Edwin K. Jackson[a,c,e]

[a]*Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA, United States*
[b]*Department of Obstetrics and Gynecology, Clinic for Endocrinology (D217, NORD-1), University Hospital Zurich,*
Frauenklinikstrasse-10, CH-8051, Zurich, Switzerland
[c]*Department of Medicine, University of Pittsburgh, Pittsburgh, PA, United States*
[d]*Medical-Policlinic, Department of Internal Medicine, University Hospital Zurich, Zurich, Switzerland*
[e]*Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA, United States*

Abstract

Premenopausal women have a lower risk for cardiovascular events, and mortality due to coronary vascular disease (CVD) in premenopausal women is rare. These facts suggest that endogenous estrogens, such as estradiol, protect the cardiovascular system, and several observational studies and a few small clinical studies conducted in healthy and younger postmenopausal women support this hypothesis. In contrast, two large randomized clinical trials (RCTs), using conjugated equine estrogens and conducted in older women with established CVD or without overt CVD, failed to demonstrate protection against CVD by exogenous estrogens. These divergent findings have resulted in confusion with regard to the association between estrogen deficiency and CVD in postmenopausal women. In order to reconcile these contradictory findings, it is necessary to examine the pathophysiology associated with age-dependent changes within the vessel wall and to compare the pharmacology of different types of estrogens. Understanding age-dependent changes in vascular pathology and the pharmacology of different estrogens may facilitate the development of therapeutic strategies for hormone replacement therapy (HRT) that would be effective in delaying vascular remodeling leading to CVD following menopause. In this review we provide an overview of the impact of menopause and estrogen deficiency on vascular remodeling and emphasize the importance of timing and type of estrogen to achieve maximum benefits with regard to reducing the risk of CVD.
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1. Evidence for a protective action of endogenous estradiol

Endogenous estrogens may attenuate CVD. Age-associated CVD lags by 10 years in premenopausal women compared with men (Fig. 1). Between 45 and 64 years of age, the prevalence of CVD in men is several times that of age-matched women [1,2]. Following menopause and loss of endogenous estradiol (major ovarian estrogen), these gender-based differences narrow [3,4]. Most women who enter menopause are asymptomatic for CVD, and 95% of the women who develop CVD do so after menopause.

Early loss of endogenous estradiol is associated with CVD. Most autopsy studies demonstrate increased CVD in young oophorectomized women [5]. The Framingham Study shows that women entering menopause early naturally or surgically have a greater likelihood of developing CVD compared to age-matched premenopausal women. The Nurses' Health Study reports an increased risk of CVD in women with bilateral oophorectomy not

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* Corresponding author. Department of Obstetrics and Gynecology, Clinic for Endocrinology (D217, NORD-1), University Hospital Zurich, Frauenklinikstrasse-10, CH-8051, Zurich, Switzerland. Tel.: +41 1 255 8608; fax: +41 1 255 4439.

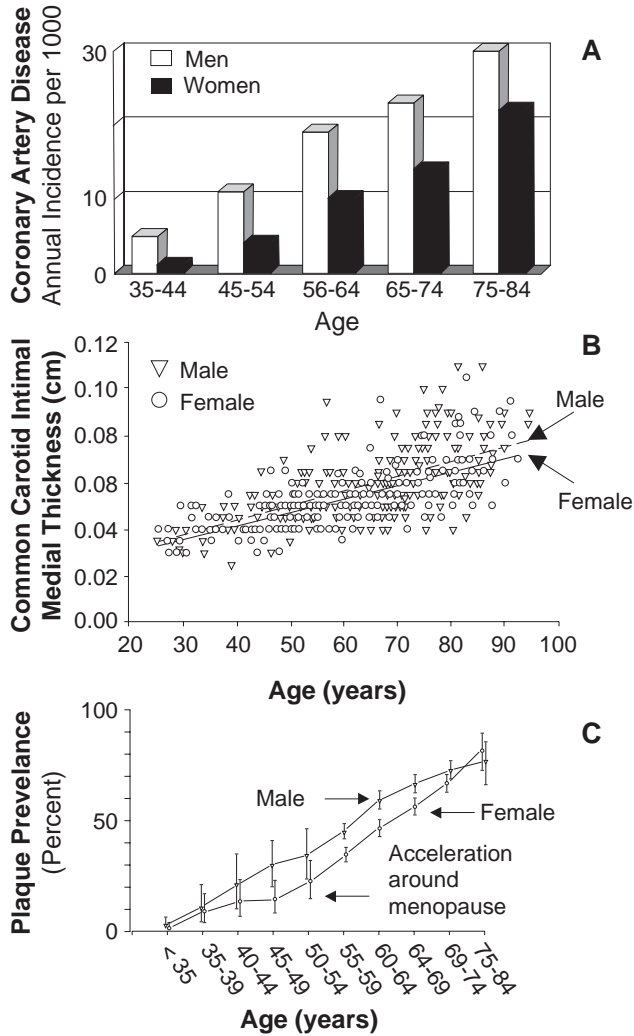*E-mail address:* Raghvendra.dubey@usz.ch (R.K. Dubey).

Fig. 1. (A) Age-dependent incidence of coronary artery disease (CAD) in men and women (Framingham Heart Study). (B) Age-dependent intimal-medial thickening of common carotid in healthy male and female volunteers (Baltimore Longitudinal Study on Aging; with permission, [55]). (C) Prevelance of carotid atherosclerosis by age and sex (with permission [59]).

receiving hormone replacement therapy (HRT) [6]. Younger age at natural menopause is associated with CVD [7] and CVD mortality [8,9]. A recent population-based study demonstrates an inverse relationship between age at menopause and prevalence and extent of carotid atherosclerosis [10].

In premenopausal women, endogenous estradiol may abrogate age-related vascular remodeling. Age-associated vascular remodeling involves endothelial dysfunction, enhanced growth of intimal smooth muscle cells (SMCs), and increased prevalence of vascular plaques (Fig. 1). The same cellular processes participate in atherosclerosis [11]. Although it is difficult to separate vascular changes due to aging from those due to menopause, comparisons between men versus age-matched postmenopausal women and between age-matched premenopausal versus postmenopausal women suggest that endogenous estradiol delays

CVD [1–4,12,13]. In support of this conclusion, estradiol inhibits many processes involved in age-associated vascular remodeling, including SMC proliferation and endothelial dysfunction, and lowers cholesterol and improves vascular tone (reviewed in Refs. [14,15]).

Time since menopause, rather than type of menopause (natural or surgical), is the major factor associated with elevated subclinical atherosclerosis [16]. In women 35 years of age, only fatty streaks and minimal atherosclerotic plaques occur in coronary arteries [17]. Active progression of atherosclerotic lesions transpires in the following decade (45–55 years of age) when menopause usually occurs, and these lesions start to develop complications when women are ≥65 years of age [18,19]. In contrast, pathologic findings in younger premenopausal women indicate mild vascular disease with low-density fibrous tissue compared with high-density fibrous tissue found at later stages of atherosclerosis development [20]. Marked endothelial dysfunction and intimal thickening also occur following menopause [21]. A significant increase in the prevalence of plaques and intima-media thickening (IMT) arises 5–8 years after menopause (Fig. 2; [21]). A significant increase in total and LDL-cholesterol occurs within 3 years of natural menopause [22], and in women undergoing oophorectomy an increase in total and LDL-cholesterol occurs in the first 6 weeks after oophorectomy [23]. Menopause is associated with endothelial dysfunction, decreased endothelial-dependent relaxation, and a decline in flow-mediated dilation [24]. Ovariectomy blunts forearm vasodilation in response to intrabrachial acetylcholine [25], and sympathetic nerve activity increases during the early phases of menopause [26].
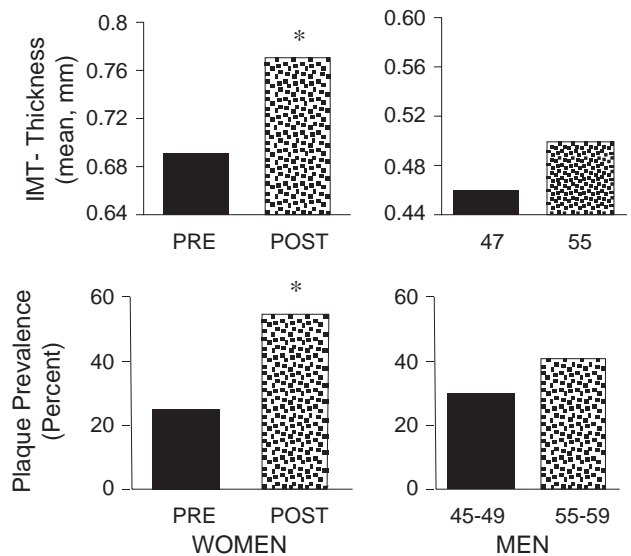

Fig. 2. Prevalence and degree of carotid atherosclerosis in premenopausal women and women 5–8 years after menopause, and in age-matched men after 8 years. The upper panel shows intima-media thickness and the lower panel shows prevalence of plaques. * significant increase versus premenopausal (PRE). (with permission [21,55,59]).

Hypertension may contribute to acceleration of atherosclerosis following menopause. Postmenopausal women >60 years old make up the majority of hypertensives and are more likely to develop CVD [27]. Surgical menopause following oophorectomy increases peripheral vascular resistance and blood pressure, and this effect is abrogated by HRT [28,29]. Sex hormones influence mechanisms involved in regulating blood pressure [30–32]. Compared to premenopausal women, the activity and synthesis of pro-hypertensive factors are increased, whereas the synthesis of blood pressure-lowering factors is decreased [30,32]. Studies demonstrate the blood pressure-elevating effects of androgens and suggest that increases in the androgen-to-estradiol ratio activate pro-hypertensive mechanisms [30]. Estrogens, but not androgens, induce favorable effects on the kidney, the organ that determines long-term levels of arterial blood pressure. There is increased salt sensitivity in postmenopausal women which may contribute to hypertension [33], suggesting that changes in renal function following menopause contribute to the increased risk of atherosclerosis. The effects of menopause on hypertension, and subsequently atherosclerosis, may vary between subjects. For example, the effects may be enhanced in women with diabetes mellitus (associated with increased testosterone levels) [34] or obesity [30].

2. Evidence that exogenous estrogens reduce menopause-associated vascular consequences

Cross-sectional and prospective studies demonstrate significant reductions in CVD in women taking conjugated equine estrogens (CEEs) [5], suggesting that exogenous estrogens prevent CVD. A meta-analysis of observational studies shows that HRT is associated with a one-third reduction in fatal CVD [35]. Use of unopposed CEE and CEE plus a progestin shows a relative CVD risk of 0.70 and 0.66, respectively [36].

The Nurses' Health Study was conducted in 121,700 female nurses aged 30–55 years [12,13]. The latest report involving 70,533 postmenopausal women followed for 20 years indicates that the overall relative CVD risk in current users of estrogens is 0.61 after adjustment for age and common cardiovascular risk factors [13]. The Nurses' Health Study suggests increased risk for ischemic stroke in HRT users.

3. Results of RCTs are inconsistent with observational studies

Randomized clinical trials (RCTs) were launched to verify that HRT reduces CVD. Two large placebo-controlled RCTs, the Heart and Estrogen/Progestin Replacement Study (HERS [37]) and the Women's Health Initiative Study (WHI [38]) tested the effects of HRT in secondary and primary prevention, respectively.

3.1. Secondary prevention trials

HERS enrolled 2763 women (mean age, 67 years) with documented CVD. Subjects were administered daily CEE (0.625 mg) and medroxyprogesterone (MPA; 2.5 mg) or placebo. HERS, after approximately 4.1 years of follow-up, found no difference in primary CVD outcome (nonfatal MI plus CVD death), even though HRT significantly reduced LDL and increased HDL [37]. A significant increase in adverse CVD events was found in the HRT group during the first year of treatment (52% excess cardiovascular events), and no protective effects were evident after an additional 2.7 years of follow-up [39].

The HERS findings were unexpected, but consistent with several smaller RCTs conducted for secondary prevention [40,41]. These smaller studies also showed either no protection or a slight increase in CVD events during the first year of HRT. Early adverse effects (MI and thromboembolic events) were also observed in the Coronary Drug Project conducted with CEEs (2.5 mg or 5.0 mg) in men [42]. The Women's Estrogen for Stroke Trial (WEST) examined the effects of estradiol on stroke rates in postmenopausal women and found no effect [43].

3.2. Primary prevention trials

The WHI study [38], conducted in healthy postmenopausal women between 50 and 79 years of age, was initiated to evaluate whether HRT was effective in primary prevention. It was a double-blind randomized study with two arms, one studying the impact of CEE (0.626 mg/day) plus MPA (2.5 mg/day) or placebo in 16,608 women with a uterus and the second studying the impact of CEE (0.625 mg/day) alone or placebo in 10,739 women without a uterus. The outcome of WHI was that neither estrogen nor estrogen plus progestin decreased CVD.

The WHI findings are supported by some, but not all, smaller primary prevention trials. A pooled analysis of smaller RCTs conducted in mostly young women potentially devoid of unrecognized CVD at baseline supported the WHI findings with no significant cardiovascular benefits of HRT [44]. In the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial there was a non-significantly higher incidence of cardiovascular and thrombotic events among women assigned HRT [45].

The Estrogen in the Prevention of Atherosclerosis Trial (EPAT) [46] demonstrated that oral administration of unopposed estradiol (1 mg/day) significantly reduced the progression in carotid artery atherosclerosis in healthy women (average age, 61 years). Similar to EPAT, unopposed estradiol therapy slowed the progression of atherosclerosis in the Asymptomatic Carotid Artery Progression Study [47], and 2 years of treatment with estradiol plus

desogestrel or CEE plus cyclic norgestrel [48] tended to decrease carotid IMT in healthy perimenopausal women (age 40–60 years).

In the Women's Estrogen–Progestin Lipid Lowering Hormone Atherosclerosis Regression Trial (WELL–HART) [49], 226 postmenopausal women (mean age, 63.5 years) who had at least one coronary artery lesion were randomly assigned to placebo, estradiol, or estradiol plus MPA. After a median of 3.3 years of follow-up, the change in stenosis was measured using quantitative coronary angiography. In contrast to EPAT [46], no significant effects of estradiol or estradiol plus progestin on the progression of atherosclerosis were found in older postmenopausal women with established coronary artery atherosclerosis. The different outcomes of the EPAT versus WELL–HART may largely be due to the subject population studied, i.e., healthy subjects versus those with established CVD.

Other primary prevention trials include the Puget Sound Group Health Cooperative (PSGHC; [50]), the Postmenopausal Hormone Replacement against Atherosclerosis (PHOREA; [51]), and the Women's International Study of Long Duration Oestrogen after Menopause (WISDOM). In the PSGHC trial, similar to the Nurses' Health Study, the relative risk for myocardial infarction in healthy women was increased more than twofold in new users of HRT as compared to women using HRT for 1 to 2 years [50]. In the PHOREA trial, which investigated HRT (1 mg/day estradiol plus 0.025 mg gestodene) in healthy postmenopausal women (age 40–70 years), no significant difference in carotid IMT was observed after 2 years [51]. The WISDOM trial is a primary prevention trial investigating the use of CEE (plus MPA in women with uterus) over a 10-year period, and similar to WHI it will assess the risk of coronary events. This study is ongoing and the results will help in assessing the cardioprotective role of estrogens in women. Finally, results should be available soon from the Estrogen and Graft Atherosclerosis Research study (EAGAR; secondary prevention trial), which is examining the effects of estradiol plus MPA to prevent graft occlusion in postmenopausal women who have recently undergone coronary artery bypass surgery.

4. The basis for discrepancies between observational studies and RCTs

4.1. Role of timing of HRT

In primates, CEE-induced vascular protection was attenuated when treatment was initiated after atherosclerosis was already established [52]. A 70% protection was observed when HRT was initiated simultaneously with an atherosclerotic diet in ovarectomized primates. A marginal delay in initiation of HRT, i.e., after moderate atherosclerosis, resulted in only a 50% protection. When HRT was begun 2 years into an atherosclerotic diet, no protection was observed [52]. Similar to the findings in primates, administration of estradiol prior and during, but not seven days after, balloon injury resulted in inhibition of neointima formation in rats [53]. Delayed delivery also failed to prevent neointima formation in rabbits [54].

The Nurses' Health Study supports the hypothesis that time when HRT is initiated influences cardiovascular benefit from HRT. Women who participated in this study were between 30 to 55 years of age, and ≈80% initiated HRT within 2 years of the onset of menopause [12]. In contrast to the Nurses' Health Study, women in HERS were 67 years of age and had been postmenopausal for many years at the time of enrollment. In HERS, the interval from menopause to randomization was 23 years versus 13 years in EPAT [46].

Although WHI was a primary prevention trial, similar to HERS, the participants in WHI were older (50–79 years) with only 10% of the participants between 50 and 54 years and 20% between 54 and 59 years. In women assigned HRT in WHI, 36% had hypertension, 49% were current or past smokers, and 34% were obese. The contention that the participants were healthy should be reconsidered.

The progression rate of vascular disease may depend on age at menopause and status of subclinical atherosclerosis. As shown in Fig. 3, intimal thickening increases with age and CVD extent [55]. In women who underwent bilateral oophorectomy, intimal thickening increases with years since menopause and reaches significance 15 years after menopause (Fig. 4) [16]. McGarth et al. [56] demonstrated that age-dependent progression of carotid intimal thickening in postmenopausal women is significantly reduced by HRT (Fig. 5). These findings suggest that estrogen plays a role in regulating intimal thickening. In addition to the impact of estrogen on intimal growth, estrogen reduces prevalence of
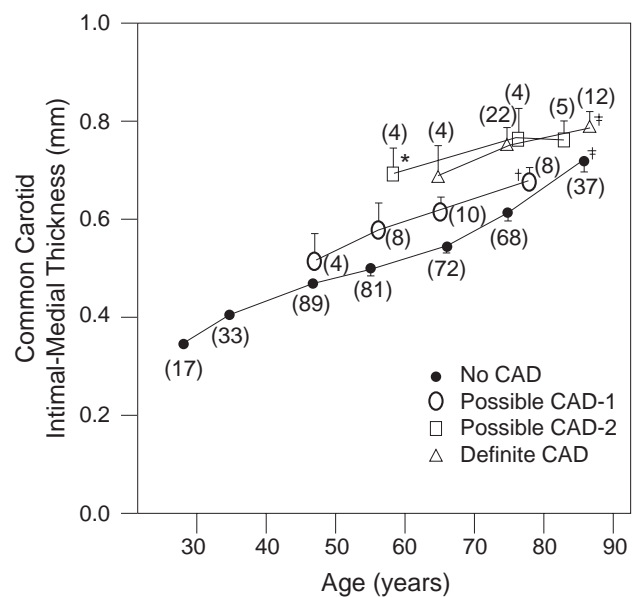

Fig. 3. Common carotid intimal-medial thickness as function of age, stratified by coronary artery disease (CAD) classification CAD2>CAD1>CAD (with permission [55]).
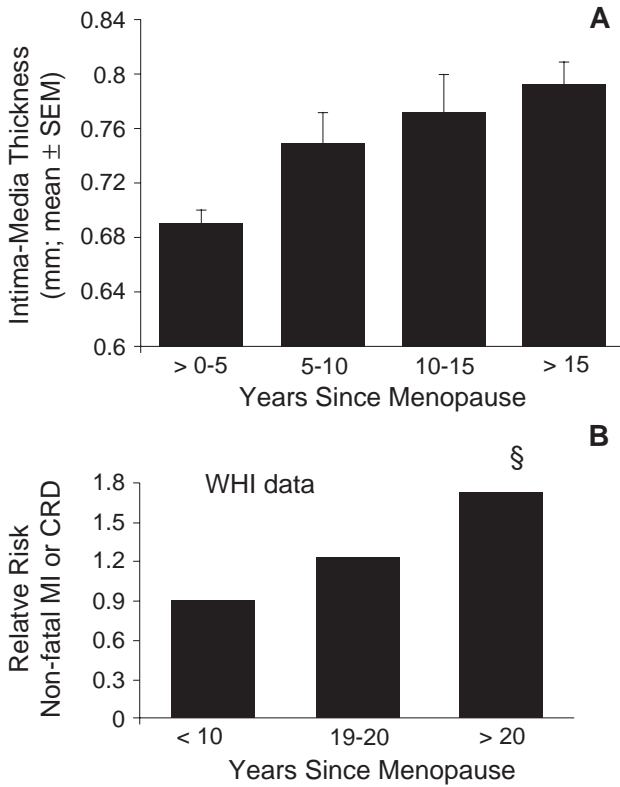
Fig. 4. (A) Time-dependent and early changes in intimal-medial thickening following menopause (with permission [17]). (B) Effect of time since menopause on incidence of myocardial infarction in women receiving HRT in WHI [38]. CRD, coronary related death.

the incidence of plaques is evident around perimenopause (40–50 years age) in women, and after 50 years of age (around menopause) a steep increase in the incidence of plaque is seen (Fig. 1). These observations suggest that estrogen slows vascular processes associated with CVD. Because in the absence of estrogen the progression of atherosclerosis may be rapid, the timing of initiation of treatment, with respect to onset of menopause, may have important ramifications on the therapeutic efficacy of HRT in preventing or delaying the progression of atherosclerosis and CVD. In WHI, the relative risk for non-fatal myocardial infarction increased as a function of years since menopause (Fig. 4).

4.2. Role of type of estrogen used for HRT

Both HERS and WHI were conducted with CEEs. CEEs are a mixture of estrogens extracted from horse urine and also contain progestins, androgens, and other substances (Table 1). The main constituents of CEEs are specific equine estrogens and weak estrogenic molecules such as estrone, estrone sulfate, and estriol. In contrast to CEE, the major

plaque [21,57,58], and the time-curve for prevalence of plaques is shifted to the right in women (Fig. 1) [59]. In contrast to the linear increase observed in men, a plateau in
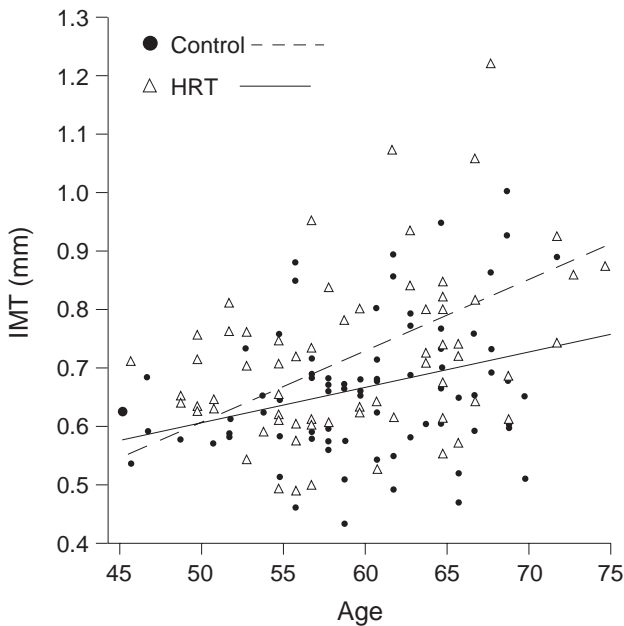

Fig. 5. Hormone replacement therapy (HRT) slows age-associated increase in intimal-medial thickness (IMT) in postmenopausal women (with permission [56]).

Table 1
Various constituents of conjugated equine estrogen

Estrogens
Sodium-estrone sulfate
Sodium-Equilinsulfate
Sodium-17α-Dihydroequilinsulfate
Sodium-17α-Estradiolsulfate
Sodium-17β-Dihydroequilinsulfate
Sodium-17α-Dihydroequileninsulfate
Sodium-17β-Hydroequileninsulfate
Sodium-Equileninsulfate
Sodium-17β-Estradiolsulfate
Sodium-delta 8,9-Dehydroestronsulfate

Progestins
5a-Pregnane-3β, 20β-diol
5α-Pregnane-3β, 16α, 20β-triol
5α-Preg-16-en-3β-ol-20-one
5α-Pregnane-3 β-ol-20-one
Sodium-4-Pregene-20-ol-3-one-Sulfate
3β-Hydroxy-5(10), 7-estradiene 17-one-3-Sulfate

Androgens
5α-Androstane-3β, 17α-diol
5α-Androstane-3β, 16β-diol
5α-Androstane-3β, 16α-diol
5α-Androstane-3β-ol, 16-one

Other substances
5,7,9 (10) Estratriene-3β, 17β-diol
17α-Dihydro-delta 8,9-Dehydroestrone
17β-Dihydro-delta 8,9-Dehydroestrone
5,7,9,(10) Estratriene-3β-ol-17-one
2-Hydroxyestrone
2-Methoxyestrone

endogenous estrogen lost during menopause is estradiol, and this is not present in CEE [45].

The effects of CEEs or other estrogens may be very different than those of estradiol. Binding affinity, selectivity for estrogen receptor (ER) subtypes, ER activation, and metabolism vary markedly among different estrogens [14]. Because ER dependent and independent mechanisms play a role in mediating the biological actions of estradiol on the cardiovascular system, CEEs and other estrogens may not mimic the cardiovascular effects of estradiol. For example, ethinyl estradiol, an estrogen compound, is known to induce deleterious effects on the cardiovascular system [14]. In a follow-up study in Uppsala, Sweden [60], the authors found a reduced risk of myocardial infarction for medium-potency estrogens (48%, estradiol [predominantly 2 mg]; 15.2% CEEs [predominantly 0.625 mg]; and 36.8% for both) compared with low-potency estrogens (oral estriol [predominantly 1 mg] or vaginal estriol/dienoestrol) (relative risk, 0.75), with a similar decrease in the subgroup that took estrogens with progestin (relative risk, 0.69).

In in vitro studies using human aortic SMCs, we demonstrated that in contrast to estradiol, estrone, estriol, estrone sulfate (major components of CEEs) were less potent in inhibiting mitogen-induced SMC growth and mitogen-activated protein kinase activity [61]. Because abnormal growth of SMCs plays a role in the remodeling processes associated with CVD, antiproliferative efficacy and potency of an estrogen may play an important role in defining the vascular protective actions of that estrogen. Indeed, in a non-human primate model, administration of CEE was shown to inhibit the progression of atherosclerosis, but had no effect on intimal hyperplasia after balloon injury [62]. Importantly, in EPAT, administration of estradiol to postmenopausal women with no evidence of CVD significantly reduced the progression of intimal thickening [46].

Most animal studies conducted to elucidate the vascular protective actions of estrogen therapy used estradiol rather than CEEs. Importantly, studies conducted in animals provide strong support that estradiol protects against CVD. In almost all the models of atherosclerosis and neointimal thickening estradiol prevents pathological vascular remodeling processes and neointima formation [14].

Several studies indicate that the vascular protective effects of estradiol are mediated in part by ER-independent actions [63–66] and, therefore, the vascular protective effects of estradiol would not be mimicked by non-estradiol-based estrogens with a different pharmacological profile. Our studies indicate that metabolism of estradiol to methoxyestradiols is responsible for the anti-mitogenic effects of estradiol on vascular SMCs, cardiac fibroblasts, and glomerular mesangial cells [67]. Importantly, these effects of estradiol on cell growth are ER-independent [67]. Increased proliferation of these cell types leads to hypertension, vascular disease, left ventricular hypertrophy, and glomerulosclerosis. Thus, some of the cardiovascular and

renal protective effects of estradiol may be mediated via their conversion to methoxyestradiols. Direct evidence for this hypothesis comes from our finding that the anti-mitogenic effects of estradiol are lost in aortic SMCs cultured from COMT-knockout mice that cannot form methoxyestradiols [68]. The importance of estradiol metabolites in vasoprotection is further supported by our finding that in male obese ZSF1 rats that exhibit the metabolic syndrome, treatment with 2-hydroxyestradiol (precursor of 2-methoxyestradiol) decreases body weight, improves vascular endothelial function, decreases nephropathy, exerts antidiabetic actions, and lowers blood pressure and blood cholesterol [69].

Changes in the androgen-to-estradiol ratio following menopause may also play a role in defining the deleterious effects of menopause on the cardiovascular system. The decline in estradiol levels during menopause leads to a higher androgen-to-estradiol ratio in postmenopausal women; moreover, this ratio is lower in HRT users [70]. Androgens induce vasoconstriction and SMC growth and exacerbate diet-induced atherosclerosis, plaque formation, and pro-atherosclerotic arterial remodeling. These findings suggest that the increase in the androgen-to-estradiol ratio in postmenopausal women may be another mechanism which contributes to the acceleration of atherosclerosis observed in postmenopausal women [30–32]. Indeed, in women, high testosterone levels are associated with dyslipidemia [71], type-2 diabetes mellitus [34], and hypertension [72], suggesting that increases in androgen levels may accelerate CVD in postmenopausal women. In a case control study, compared to women with normal ovaries, more extensive atherosclerosis was observed in women with polycystic ovary syndrome, a condition associated with elevated androgen levels. Increased testosterone levels correlate with the degree of coronary atherosclerosis measured by angiography [73], and in a recent nested case-control prospective study a trend towards increased CVD risk was observed in women with higher androgen-to-estradiol ratios [74]. Together, the above findings suggest that acceleration of atherosclerosis in postmenopausal women may in part be due to unopposed actions of androgens due to decline in estradiol levels.

4.3. Role of polymorphisms (single nucleotide polymorphism [SNP])

Polymorphisms in the gene for the ER-α receptor are associated with: increased incidence of premature CVD in a man [75]; CVD in postmenopausal women [76] and older men [77]; pro-atherosclerotic profile of serum lipids in women with CVD [78]; and CVD in patients with familial hypercholesterolemia [79]. The incidence of in-stent restenosis is significantly increased in women with a polymorphism in the ER-α gene [80]. Polymorphisms in the ER-β gene in healthy postmenopausal women are associated with high blood pressure [81]. Although most studies have

found an association between polymorphisms in ER-α and ER-β genes and cardiovascular risk, there is also evidence for lack of an association. Matsubara showed that three polymorphisms in ERs were not associated with prevalence and severity of CVD and that these polymorphisms were unrelated to serum lipid levels [82]. An alternative mechanism that may be involved in regulating ER-dependent protection of the cardiovascular system is age-dependent methylation of ER [83].

The importance of SNPs in governing biological effects of estrogen and defining therapeutic efficacy of HRT has been reviewed by Herrington [84]. In a hallmark-study using subjects from the ERA trial, the authors demonstrated that ER-α polymorphisms can alter the response to HRT [78]. Subjects from both active arms of ERA (oral CEE and oral CEE plus MPA) and placebo were characterized with respect to selected ER-α polymorphisms. After adjusting for potential confounders, the 18.9% of women who had IVSI-401 C/C genotype in the active-treatment arms showed increases in HDL cholesterol more than twice the increase observed in other women. This effect was found in both groups, i.e., women receiving estrogen or estrogen plus progestin, and the effects were observed across racial groups [78]. A similar pattern of response was observed for the HDL-3 subfraction, and a trend for greater increase in apolipoprotein A-1 was evident [78]. In a subsequent report, women in the active treatment arms with ER-α IVS1-401 C/C genotype showed greater reductions in E-selectin but not CRP, suggesting that the effects were due to selective gene–drug interaction [85].

SNPs of other genes can also modulate the actions of estrogen or HRT by influencing drug–gene interactions. Estrogens increase the risk of venous and arterial thrombotic events, and this is associated with genetic polymorphisms [86]. Genetic mutations, such as Factor-V Leiden and prothrombin 20210A, may increase the risk of venous and arterial thrombotic events when estrogen levels are increased [87]. Recent examination of data from HERS revealed that women with factor-V Leiden polymorphism who were assigned HRT had substantially higher rates of venous thromboembolic events than women receiving HRT who lacked factor-V Leiden polymorphism, or women with factor-V Leiden polymorphism who were assigned placebo [88]. Presence of the prothrombin 20210G→A mutation in women with hypertension was found to be a significant factor for myocardial infarction in a case control study [87]. Women with the mutant prothrombin allele who were using HRT had almost 11-fold greater risk of nonfatal myocardial infarction, compared with current HRT users without the prothrombin variant [87].

Polymorphisms in other genes of the coagulation/fibrinolytic cascade may also play an important role in regulating estrogen induced thrombosis via altered drug–gene interaction. The sequence variants in Factor-VII gene, the prothrombin gene, factor-V, fibrinogen, and the gene for plasminogen activator inhibitor may be important [84,86].

Finally, since estradiol is metabolized to multiple biologically active endogenous metabolites, mutations in CYP450 isozymes or COMT may play a role in defining estradiols cardiovascular protective effects. Polymorphisms of CYP1B1 are associated with estrogen-induced breast cancer [89], suggesting that screening of patients for SNPs may identify patients with increased risk of estrogen-induced cancer.

4.4. Role of progestin

Progestins are administered sequentially or continuously with estrogen. The progestins/gestagens used clinically vary in chemical properties and possess varying degrees of androgenic and glucocorticoid activity [90]. Via glucocorticoid receptors, progestins can potentiate vascular procoagulant effects of thrombin by increasing thrombin receptors in SMCs [90]. Hence, the negative findings of HERS and one arm of WHI may have been due in part to concomitant use of MPA. In support of this idea, in the PEPI trial, CEE caused beneficial effects on LDL and HDL levels that were attenuated by MPA [45]. Because increased LDL and decreased HDL are associated with cardiovascular disease, the interpretation is that MPA may abrogate the protective effects of estrogens on the cardiovascular system. However, this interpretation is not supported by the observations that CEE and CEE plus MPA are equipotent in inhibiting atherosclerosis in non-human primates [91]. Similar to MPA, the anti-atherosclerotic effects of CEE were not abrogated by progesterone in cynomolgus monkeys [91]. However, in contrast to these studies, administration of CEE, but not CEE plus MPA, caused anti-atherosclerotic effects in monkeys [91].

The effects of progestins in attenuating the protective actions of estrogens on atherosclerosis are unclear. In cynomolgus monkeys given continuous estradiol or estradiol plus cyclically administered progesterone for 30 months, the anti-atherosclerotic effects were similar [91]. Loss of protective effects was observed in monkeys administered CEE plus MPA (no protection) as compared to those treated with CEE alone (72% reduction in coronary artery atherosclerosis [91]). In rabbits the protective actions of CEE or estradiol on atherosclerosis were not reversed by MPA nor by other progestins (norethindrone acetate and hydroxyprogesterone caproate; [91]). In most observational studies with positive outcomes, including the Nurse's Health Study [13], comparable reductions in CHD risk were found with HRT regardless of whether HRT included a progestin.

Intimal thickening plays a role in vascular remodeling and is associated with atherosclerosis. In a rat model, MPA abrogated the ability of estradiol to attenuate balloon-injury induced intimal thickening [32]. In contrast to these findings, progesterone and MPA inhibited mitogen-induced proliferation of SMCs in vitro. Also, in the Atherosclerosis Risk in Communities Study, the reductions in intimal-

medial thickness were similar in women receiving estrogen alone or estrogen plus MPA [92].

The above findings suggest that factors other than MPA are involved in the lack of protective actions observed in RCTs. The termination of the estrogen alone arm of WHI supports the notion that factors other than MPA are involved.

4.5. Role of interactions with lipoproteins

Ovarian dysfunction at the onset of menopause and increased CVD incidence are associated with increases in LDL, and decreases in HDL and estradiol [93]. Thus interactions between hormones and lipoproteins may participate in maintaining vascular homeostasis.

Estrogens influence the vascular effects of LDL cholesterol. Estradiol, which is a phenol with anti-oxidant properties, prevents the oxidation of LDL and VLDL to oxLDL and oxVLDL, and protects the vasculature against the deleterious effects of ox-lipids [14]. Estradiol prevents compromise of the endothelial barrier mediated by mmLDL and attenuates accumulation of mmLDL and oxLDL in the artery wall [94]. TNF-α-mediated oxidation and accumulation of LDL in the artery wall is prevented by estradiol [95]. Estradiol increases the catabolism of LDL, apo-B100, and beta VLDL via LDL-R-dependent and -independent mechanisms [14,96]. Also, estrogen: increases expression of LDL-R, increases clearance of VLDL, decreases LDL production, decreases LDL particle size, increases clearance of light and dense LDL, increases expression of VLDL and LDL-R in left ventricles of the heart, induces HMG-CoA reductase activity, and induces sterol-27-hydroxylase activity which decreases LDL production [14,96,97]. Estrogen-induced removal of VLDL is associated with increased activities of hepatic lipase, lipopotein lipase, and expression of LDL-R [14]. There is evidence for cross talk between ERs and LDL-R. Up-regulation of ERs is associated with an increase in LDL-R expression, and this effect can be blocked by the ER antagonist, tamoxifen [14].

Foam cell/fatty streak formation is involved in plaque pathogenesis and the interaction of LDL, macrophages, and SMCs. Estrogens can interfere with several biochemical events associated with these processes. Incubation of human THP-1 macrophages with estradiol reduces the uptake and metabolism of ^{125}I-labeled human acLDL, suggesting that estrogen reduces degradation of oxLDL via scavenger receptors [14]. In vivo studies in primates demonstrate that the rate of LDL degradation is decreased in arteries in response to estradiol. Estrogen reduces cholesterol accumulation and esterification, an important step in foam cell formation [14]. Pharmacological concentrations of estradiol inhibit migration of monocytic cells stimulated with oxidized-LDL by inhibiting secretion of MCP-1 by monocytic cells [14]. Estradiol inhibits oxLDL-induced adhesion of monocytes to endothelial cells [14]. Because estradiol downregulates the expression of adhesion molecules, it is

feasible that estradiol inhibits the adhesion process by preventing oxLDL induced expression of VCAM-1 and ICAM-1 [14].

HDL-C is more closely related to cardiovascular disease in women than LDL-C and HDL-C is the best predictor of CVD risk in women. Apo-AI accounts for most of the protein in HDL, and plasma concentrations of apo-AI are increased in premenopausal women and in postmenopausal women treated with estrogens [98]. Clinical studies provide evidence for estrogen-induced increases in the rate of production of HDL subfractions and decreases in the plasma clearance of HDL [98]. Studies in rodents showed that estradiol increases hepatic apo-AI mRNA levels and increases hepatic rates of apo-AI transcription [14]. In hepatoblastoma cells, pharmacological concentrations of estradiol increase apo-AI secretion and apo-AI mRNA levels by increasing the rate of apo-AI mRNA transcription without effecting apo-AI mRNA stability [14]. Similar to HDL, estrogen induces synthesis of apolipoprotein-E [99], which may confer vasoprotection. In apo-E-deficient mice, estradiol prevents both fatty streak formation and atherosclerotic lesions [100]. Therefore, mechanisms other than apo-E synthesis are involved in mediating the estradiol-induced vasoprotective effects.

Estradiol via ERs induces the expression of ATP-binding cassette-A1 (ABC-A1) transporter [101], and thus estradiol may facilitate reverse-cholesterol transport responsible for the movement of cholesterol from peripheral cells, including macrophage-derived foam cells in the arterial wall, back to the liver, where cholesterol is catabolized [102]. This action of estradiol may attenuate formation of foam cells, early players in the formation of arterial lesions. Acquisition of cholesterol and phospholipid protects the nascent lipid-poor apo-A1 particles from rapid catabolism, thus enabling their transition to mature HDL [102]. Patients with Tangier disease are heterozygous for ABC-A1 mutations and have dramatically low levels of HDL and low cholesterol efflux capacity. This suggests that the ABC-A1-mediated lipid secretory pathway corresponds to a rate limiting step in the production of HDL [102]. Hence, ABC-A1 acts as a gatekeeper for cholesterol flux from tissues. The recent reports that cholesterol can directly induce foam cell like phenotype in vascular SMCs [103] and that ABC-A1 is expressed and participates in reverse-cholesterol transport [102] suggest that estradiol-induced ABC-A1 expression in these cells protects against endothelial damage and atherosclerosis. Thus, estrogen-mediated up-regulation of the ABC-A1 expression may contribute to accelerated removal of cholesterol from peripheral tissues, and inhibit progression of atherosclerosis [104]. It should be noted that it is the functional HDL pool, not necessarily the total amount of HDL, that is important in removing arterial cholesterol. Decreased levels of HDL may result from fast turnover of smaller HDL particles. Tibolone, a hormone therapy drug widely used in Europe, reduces total HDL levels by almost 30%

without changing the cholesterol efflux potential [104] and without increasing the incidence of atherosclerosis.

Estrogen down regulates scavenger receptor-B1 (SR-B1) which is involved in the transfer of HDL2 particles to the liver, suggesting that estrogen-induced increases in HDL are associated with impaired rather than improved elimination of HDL-cholesterol [105]. Also, estrogens reduce hepatic lipase gene expression and activity [106], which would result in increases in HDL concentration and production of larger HDL particles. Oral, but not transdermal, estrogen therapy increases levels of serum amyloid-A (SAA) and alters HDL composition to contain higher SAA levels [107]. Because elevated levels of SAA predict adverse prognosis in healthy postmenopausal women, this mechanism could interfere with estrogen's protective effect. This suggests that the route of HRT plays an important role in minimizing adverse effects of estrogen. As pointed out by Herrington and Parks, increases in HDL levels may not always result in cardiovascular protection [108]. Because ERs play a major role in mediating the effects of estrogen on HDL synthesis [84], factors influencing ER function can significantly influence the effects of HRT on HDL. A study by Herrington and colleagues [78] demonstrates that a polymorphism of the ER-α can dramatically influence the effects of estrogen on HDL levels and may play a critical role in defining the protective effects of HRT in individual subjects receiving HRT.

5. Conclusion and future directions for treating vascular consequences of menopause

In conclusion, it is likely that the timing of the initiation of HRT and the status of cardiovascular health determines in part whether HRT will protect against CVD. Also, it is likely that transdermal estradiol, rather than oral CEEs, would be more effective in achieving cardiovascular protection. Finally, polymorphism/SNPs may importantly influence the outcome of HRT via altered drug–gene interactions, and, in the future diagnostic SNP-screening may help tailor HRT for the individual's needs, thereby increasing efficacy and safety.

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