Brief Review

Hormone Replacement Therapy and Cardiovascular Disease
What Went Wrong and Where Do We Go From Here?

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Abstract—Observational studies in humans and experimental studies in animals and isolated cells supported the widely held belief that hormone replacement therapy protects the cardiovascular system from disease. To nearly everyone's astonishment, the Women's Health Initiative Study and the Heart and Estrogen/Progestin Replacement Study overturned the conclusion that hormone replacement therapy protects the cardiovascular system and, in fact, supported the opposite view that such therapy may actually increase the risk of cardiovascular disease. This review addresses 2 questions: what went wrong and where do we go from here? (***Hypertension*****. 2004;44:789-795.)**

Key Words: estrogen ■ hormones ■ cardiovascular diseases

The purpose of this review is to identify reasons for divergent conclusions between randomized clinical trials, such as the Heart and Estrogen/progestin Replacement Study (HERS)[1] and the Women's Health Initiative Study (WHI),[2] and observational, genetic, animal, and cellular studies with regard to the cardiovascular benefits of hormone replacement therapy (HRT).

Observational Studies Suggest HRT Has Beneficial Cardiovascular Effects

In modern societies, cardiovascular disease is the main cause of morbidity and mortality in men and women. Women, however, are comparatively spared. For example, in people younger than age 65 years, the prevalence of coronary heart disease (CHD) is several-fold higher in men.[3,4] Although CHD prevalence increases with age in both genders, before the age of 65 years the relationship between age and CHD prevalence is shifted rightward by 5 years in women;[5,6] compared with postmenopausal women, CHD deaths are rare in premenopausal women.[7]

Autopsy studies demonstrated increased CHD in oophorectomized young women,[8] and several studies demonstrated an increased risk of cardiovascular disease in women with bilateral oophorectomy without HRT compared with those receiving HRT.[9,10] Women with natural early-onset menopause who did not use HRT had a greater likelihood for CHD compared with age-matched premenopausal women.[9,11] Also, studies reported an inverse relationship between age at natural menopause and mortality from CHD[12,13] and carotid atherosclerosis.[14]

Bush et al demonstrated that HRT was associated with reduced all-cause mortality in postmenopausal women,[15] primarily because of favorable effects on high-density lipoprotein.[16] Barrett-Connor and Bush[8] reported that many, but not all, cross-sectional and prospective studies demonstrated a statistically significant reduction in CHD in women taking HRT, and Grady et al[17] presented a meta-analysis of published observational studies and reported that HRT was associated with one-third less fatal CHD. An up-to-date meta-analysis of 25 observational studies conducted between 1976 and 1996 showed that the relative risk for CHD in women who ever used HRT compared with never users was 0.70.[18]

The Nurses' Health Study was a comprehensive investigation conducted in 121 700 female nurses aged 30 to 55 years. In the latest report, compiled with data from 70 533 postmenopausal women followed-up for 20 years, the overall risk of CHD in current users of HRT was reduced, with a relative risk of 0.61 after adjustment for age and cardiovascular risk factors.[19] Short-term HRT use was associated with greater coronary benefit than long-term use.[19]

Genetic Studies Suggest That Estrogen Receptors Influence Cardiovascular Disease Risk

Estrogenic effects of HRT are mediated via estrogen receptors (ER), of which there are 2 types, ERα and ERβ. Vascular smooth muscle cells and endothelial cells express functional ERα and ERβ. Recent studies found that polymorphisms in ERα were associated with: premature CHD in a man;[20] CHD in postmenopausal women[21] and men;[22] pro-atherosclerotic profile of serum lipids in women with CHD;[23] and CHD in patients with familial hypercholesterolemia.[24] In-stent reste-

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nosis was significantly increased in women with an ERα polymorphism.[25] A polymorphism in the ERβ gene in postmenopausal Japanese women was associated with high blood pressure.[26]

Experimental Studies in Animals and Isolated Cells Suggest That Estrogen Protects the Cardiovascular System

Estrogen attenuates vascular pathology in most models of vascular disease, including vascular injury-induced neointima formation, allograft-induced vascular dysplasia, atherosclerotic diet-induced atherosclerosis, and vascular narrowing-induced neointima formation.[27]

In animals and isolated cells, estrogen induces multiple effects that in theory should reduce CHD risk.[27] For example, estrogen upregulates the synthesis of vasodilatory and growth inhibitory molecules such as nitric oxide, prostacyclin, cAMP, and adenosine. Also, estrogen downregulates the synthesis of vasoconstrictor molecules, growth inducers, and atherosclerosis inducers, such as endothelin, homocysteine, angiotensin II, renin activity, angiotensin-converting enzyme activity, catecholamines, and low-density lipoprotein. Moreover, estrogen inhibits mitogen-induced growth of vascular smooth muscle cells, cardiac fibroblasts, and glomerular mesangial cells.

Unanticipated Results From Randomized Clinical Trials

HERS enrolled 2763 women with a mean age of 67 years and with documented CHD. The subjects took a single daily tablet of conjugated equine estrogens (CEE) (0.625 mg) and the progestin medroxyprogesterone (MPA) (2.5 mg) or placebo. After ≈4.1 years of follow-up, HERS did not detect an overall effect of HRT in primary CHD outcome (nonfatal myocardial infarction and CHD death combined).[1] During the first year of treatment, there was a statistically significant increase in adverse CHD events (52% excess cardiovascular events), and no protective effects were evident after an additional 2.7-year follow-up.[28] Although the HERS findings were unexpected, they were consistent with findings from several smaller trials conducted for secondary prevention.[29–35] Studies conducted with CEE or estradiol, with or without a progestin, showed either no protective effects or a slight increase in CHD events during the first year of use.

One criticism of HERS was that the participants had vascular disease at baseline. Clarkson et al demonstrated that the vascular-protective effects of HRT in primate models were quantitatively greater if HRT was started before the onset of atherosclerosis,[36–41] suggesting that HRT may only afford primary prevention.

The WHI is a set of clinical trials and an observational study, which together include >161 000 postmenopausal women. The clinical trials part of WHI was designed to allow randomized controlled evaluation of 3 distinct interventions, 1 of which was HRT. HRT was hypothesized to reduce the risk of CHD and other cardiovascular diseases. The HRT component of WHI[2] was conducted in "healthy" postmenopausal women who were between 50 and 79 years old and evaluated whether HRT was effective in primary prevention. It was a double-blind investigation with 2 arms, 1 studying the impact of CEE (0.625 mg/d) plus MPA (2.5 mg/d) or placebo in 16 608 women with an intact uterus and 1 studying the effects of CEE (0.625 mg/d) alone or placebo in 10 739 women without a uterus. The study design was to determine fatal and nonfatal heart disease, cancer, and osteoporotic fractures as the primary outcome. An increased incidence of invasive cancer in the estrogen plus progestin arm of the study led to the early termination of this arm after 5.2 years. In the estrogen–progestin arm, HRT increased risk of heart attacks and strokes. The CEE alone arm of the study was also terminated early because of futility or no beneficial effects on CHD and increased incidence of stroke. CEE alone increased risk of stroke and deep vein thrombosis.

A pooled analysis was conducted on 22 small randomized trials of HRT with a duration of 3 months to 3 years and a total number of 4124 women assigned to HRT, placebo, vitamin supplement, or no treatment.[42] The estrogens used in these studies were estradiol in 12 trials, ethinyl estradiol in 1 trial, CEE in 6 trials, estrone sulfate in 2 trials, estriol in 1 trial, and mestranol in 1 trial. The participants were mostly younger women with a low risk of unrecognized CHD. Analysis of the pooled data suggested that the calculated odds ratio for cardiovascular events in women assigned HRT was 1.39 (not significant). Similarly, in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, there was a nonsignificant higher incidence of cardiovascular and thrombotic events among women assigned to HRT.[43]

In contrast, a randomized placebo-controlled trial (Estrogen in the Prevention of Atherosclerosis Trial [EPAT]) by Hodis et al[44] demonstrated that oral micronized 17β-estradiol (estradiol, 1 mg/d) significantly reduced the progression of carotid artery atherosclerosis in healthy women (average age 61 years). However, estradiol did not affect progression in participants who took lipid-lowering medication, suggesting that the vascular protective effects of HRT are masked by other event-reducing therapies. Because other therapies known to reduce cardiovascular events were used by >50% of the subjects in HERS and WHI, this may in part explain the ineffectiveness of HRT in those studies. Similar to EPAT, estradiol therapy was shown to slow the progression of atherosclerosis in the Asymptomatic Carotid Atherosclerosis Prevention Study.[45]

More recently, Hodis et al published findings from a double-blind, placebo-controlled trial in 226 postmenopausal women (mean age 63.5 years) who had at least 1 coronary artery lesion (The Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial [WELL-HART]).[46] Participants were randomly assigned to placebo, micronized estradiol, or estradiol plus sequential MPA. After a median follow-up of 3.3 years, change in the percent stenosis was measured using quantitative coronary angiography. In contrast to the EPAT study,[44] no significant effects of estradiol or estradiol plus progestin on the progression of atherosclerosis was found in older postmenopausal women with established coronary artery atherosclerosis. The different outcomes of the EPAT versus the WELL-HART study may largely be caused by the subject population studied, ie, healthy subjects versus those with established

CHD, respectively. Data from the WHI study in postmenopausal women with hysterectomy and taking CEE suggest that younger women who use CEE may be at a reduced risk for CHD.[47] These observations are of primary importance in understanding the reasons for the divergent data on postmenopausal HRT.

Potential Factors Responsible for the Divergent Outcomes of HRT on CHD

Age and Pre-existing Disease

Because atherosclerosis and vascular remodeling is an age-dependent process,[48,49] a delay in HRT by even a few years may influence outcome. Studies conducted in nonhuman primates show that the effectiveness of HRT to protect against atherosclerosis is dependent on timing of the treatment and status of atherosclerosis. Early HRT administration caused a 70% protection in ovarectomized primates on an atherosclerotic diet, whereas delay in therapy until after development of moderate atherosclerosis resulted in only 50% protection. In primates that had received an atherosclerotic diet for 2 years before initiating HRT, HRT did not protect against atherosclerosis.[36] Administration of estradiol before and during, but not 7 days after, balloon injury resulted in inhibition of neointima formation in rats.[50] Delayed delivery failed to prevent neointima formation in rabbits.[51]

WHI was a primary prevention trial conducted in "healthy" women. However, similar to HERS the participants were older (50 to 79 years), with only 10% of the participants between 50 and 54 years old and 20% between 54 and 59 years old. There was no information on age at menopause of subjects, which may be important in defining cardiovascular status, which changes with age and more rapidly after menopause.[8] In women assigned HRT, 36% had hypertension, 49% were current or past smokers, and 34% were obese. Hence, it is possible that even though subjects were designated as healthy, the process of atherosclerosis likely was active in participants. Based on the primate data, a 6-year delay in HRT would be enough to reduce the protective actions of HRT.

If loss of hormones permits rapid progression of atherosclerosis, then early intervention with HRT, perhaps in the perimenopausal period, would be more effective than initiating therapy years after menopause. At age 35 years, women have minimal atherosclerotic plaques;[52] between 45 and 55 years of age, there is active progression of atherosclerotic lesions in the coronary arteries.[53] At 65 years of age, lesions begin to develop complications.[54] Therefore, in the later stages of atherosclerosis, the prothrombotic plaque-destabilizing effects of HRT may predominate, and it is feasible that HRT is beneficial only in younger women, before plaque complications set in.

In the Nurses' Health Study, which showed protective effects of HRT, ≈80% of women initiated HRT within 2 years of onset of menopause.[55] In contrast, the women in WHI and HERS were on average 63 and 67 years of age, respectively, and most likely had been postmenopausal for >10 years at the time of enrollment. Even the younger healthy participants (aged 50 to 59) in the WHI study probably had been menopausal ≈6 years before HRT.

Socioeconomic Status

In general, women who take HRT are more educated, wealthier, have healthier lifestyles, and have fewer cardiovascular risk factors.[56] A meta-analysis showed that the previously observed reduced risk for CHD among HRT users was lost when the statistical analysis included socioeconomic status.[57]

Type of Estrogen

HERS and WHI used CEE, which is a mixture of steroids extracted from pregnant equine urine and is of uncertain composition, but its primary active ingredients are sodium estrone sulfate, sodium equilin sulfate, and sodium 17α-dihydroequilinenin. After menopause, women lose estradiol (major ovarian hormone), whereas the levels of estrone (largely produced in peripheral tissue) remain unchanged. CEE does not replace estradiol.

Nomenclature obscures important distinctions between CEE and estradiol. By definition, any compound that can bind to and activate ERs is an "estrogen." Thus, both CEE and estradiol are estrogens; however, chemically, estradiol and CEE are different molecular entities. Thus, the pharmacological properties of various estrogens vary considerably and may influence the final outcome of studies evaluating the effects of HRT.

Estrogens in CEE have different binding affinities for ERs, selectivities for ER subtypes, agonist activities for ERs, and metabolic products compared with estradiol.[27] Because both ER-dependent and ER-independent mechanisms play a role in mediating the pharmacological actions of estradiol on the cardiovascular system, CEE and other estrogens may not mimic the cardiovascular protective effects of estradiol.

Nonestradiol estrogens may be less able to counteract cardiovascular disease and may induce deleterious effects. For example, ethinyl estradiol, a nonestradiol estrogen, induces deleterious effects on the cardiovascular system.[27] A Swedish study[58] found a reduced risk of myocardial infarction for estradiol compared with oral estriol or vaginal estriol/dienoestrol.

In vitro studies using human aortic smooth muscle cells (SMCs) demonstrated that estrogens present in CEE were significantly less potent compared with estradiol in inhibiting mitogen-induced SMC growth and mitogen-activated protein kinase activity.[59] Because abnormal growth of SMCs plays a role in CHD, lack of antiproliferative actions by CEE may be responsible in part for the negative outcomes of HERS and WHI. In a nonhuman primate model, administration of CEE had no effect on intimal hyperplasia after balloon injury.[60] Importantly, in the EPAT study,[44] administration of estradiol to postmenopausal women without cardiovascular disease significantly reduced the progression of intimal thickening. These findings provide evidence that use of estrogens other than estradiol may be a critical factor contributing to the lack of protective actions of HRT.

Sequential metabolism of estradiol to catecholestradiols and ultimately to methoxyestradiols is responsible for the

antimitogenic effects of estradiol on vascular SMCs,[61] cardiac fibroblasts,[62] and glomerular mesangial cells.[63] Importantly, these effects of estradiol appear to be ER-independent.[61–63] The antimitogenic effects of estradiol are lost in aortic SMCs cultured from catecholamine-O-methyltransferase (COMT) knockout mice that cannot convert estradiol to 2-methoxyestradiol.[64] Increased proliferation of SMCs, cardiac fibroblasts, and mesangial cells lead 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, which have antimitogenic effects. The importance of estradiol metabolites in vasoprotection is further supported by findings that in obese ZSF1 rats that exhibit the metabolic syndrome, treatment with 2-hydroxyestradiol, the precursor of 2-methoxyestradiol, decreases body weight, improves vascular endothelial function, decreases nephropathy, exerts antidiabetic actions, and lowers blood pressure and blood cholesterol.[65]

Estradiol prevents neointima formation in mice lacking functional ERα and ERβ,[66,67] suggesting that the protective effects of estradiol on the cardiovascular system may be ER-independent. Estradiol prevents neointima formation in gonadectomized, but not intact, male rats,[68] even though male rats express ERs. In female rats, the inhibitory effects of estradiol are abrogated by MPA.[69] Because androgens and MPA are potent inhibitors of the enzyme responsible for the formation of 2-hydroxyestradiol,[70] these steroids may abrogate the cardiovascular protective effects of estradiol by blocking metabolism of estradiol to hydroxyestradiols and methoxyestradiols.

Estrogen may protect against cardiovascular disease by abrogating the effects of catecholamines. Sudhir et al[71] demonstrated that estradiol valerate decreased norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women, and Vogpatanasin et al[72] observed that transdermal estradiol, but not CEE, decreased sympathetic nerve discharge and blood pressure. In menopausal women, acute administration of estradiol and progesterone attenuated mental stress-induced cardiovascular responses and increases in plasma catecholamines.[73] Muscle sympathetic nerve activity measured by microneurography is reduced in women compared with age-matched men.[74] The inhibitory actions of estradiol on catecholamine spillover or sympathetic activation may be specific, because catecholestradiols inhibit tyrosine hydroxylase, a rate-limiting enzyme essential for catecholamine synthesis.[75] Based on these findings, it appears that estradiol, but not CEE, decreases basal sympathetic tone.

Progestin in the HRT Regimen

In women with an intact uterus, estrogens are given in combination with a progestin. The negative findings of HERS and one arm of WHI may have been caused in part by concomitant use of MPA. In support of this idea, in the PEPI trial, CEE caused beneficial effects on low-density lipoprotein and high-density lipoprotein levels that were attenuated by MPA.[43] Because increased low-density lipoprotein and decreased high-density lipoprotein 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 nonhuman primates.[39] Similar to MPA, the anti-atherosclerotic effects of CEE were not abrogated by micronized progesterone in cynomolgus monkeys.[37] However, in contrast to these studies, administration of CEE, but not CEE plus MPA, caused anti-atherosclerotic effects in monkeys.[37]

In one arm of WHI in women taking estrogen alone, no protective effects were observed even though lipids were favorably changed. Moreover, the Nurses Health Study demonstrated a similar risk reduction for CHD among women taking CEE alone and those taking CEE plus MPA. However, there was an increase in stroke risk in women taking CEE plus MPA versus women never using HRT.[19]

Studies by Williams et al suggested that MPA abrogates the vascular benefits of estrogen.[76] These investigators demonstrated that acetylcholine caused vasoconstrictor responses in estrogen-deprived monkeys not receiving HRT; however, a vasodilatory response was observed in monkeys treated with estrogen alone. The beneficial effect of estrogen was reduced by 50% by co-administration of MPA.[76]

In contrast, in brachial artery studies conducted in women, the vasodilatory effects of CEE were only marginally or not attenuated by MPA. In this regard, one study showed a small, but significant, attenuation of CEE-induced brachial artery dilatation by MPA.[77] However, 2 other well-conducted studies did not find any attenuation of CEE-induced dilatation by MPA or micronized progesterone.[78,79]

Whether progestins attenuate the anti-atherosclerotic effects of estrogen is also unclear. In cynomolgus monkeys, chronic estradiol or estradiol plus progesterone had similar anti-atherosclerotic effects.[37] In contrast, loss of protective effects were observed in monkeys administered CEE plus MPA as compared with those treated with CEE alone (72% reduction in coronary artery atherosclerosis).[39] However, studies conducted in rabbits[80] indicated that the protective actions of CEE or estradiol on atherosclerosis were not prevented by MPA. The protective actions of estradiol were not attenuated by other progestins such as norethindrone acetate and hydroxyprogesterone caproate.[81,82] In most observational studies with positive outcomes, including the Nurse's Health Study,[19] comparable reductions in CHD risk were found with HRT regardless of whether HRT included a progestin.

In a rat model, MPA abrogated the ability of estradiol to attenuate balloon injury-induced intimal thickening,[69] a process independent of lipids. This suggests that MPA may block the protective actions of estradiol that are mediated via its direct interaction with vascular cells. In contrast, progesterone and MPA inhibited mitogen-induced proliferation of SMCs in vitro. Also, in the Atherosclerosis Risk in Communities Study, reductions in intimal-medial thickness were similar in women receiving estrogen alone or estrogen plus MPA.[83]

The totality of the evidence indicates that MPA is not responsible for the lack of protective actions observed in

clinical trials. The termination of the estrogen alone arm of the WHI study supports the notion that factors other than MPA are involved.

Route of Administration of HRT

Oral, but not transdermal, estrogen increases C-reactive protein and IL-6 levels.[84,85] Because CEE is given orally, whereas estradiol is often administered transdermally to humans and subcutaneously to animals, it is possible that the differences in outcome between CEE and estradiol are caused in part by the route of administration.

Where Do We Go From Here?

The conflicting results between large randomized clinical trials (HERS and WHI) versus observational studies, genetic studies, smaller clinical trial, animal studies, and studies in isolated cells teach an important lesion, ie, we do not yet know enough regarding the effects of HRT on the cardiovascular system. We should not walk away from HRT research. Rather, we should increase research support of basic science and integrative and organ systems pharmacology and physiology aimed at better-understanding how HRT affects the cardiovascular system. We should address a number of important basic questions regarding HRT, including: (1) are the effects of estradiol a class effect shared by all ER agonists, or is estradiol pharmacology unique and different from the pharmacology of CEE and other estrogens; (2) what is the role of estradiol metabolites in the cardiovascular system and how is the production of estradiol metabolites regulated; (3) do progestins affect the metabolism of estradiol or otherwise affect the pharmacological profile of estrogens; (4) does oral versus nonoral estrogen administration differentially affect cardiovascular risk and if so how; and (5) is it important to initiate HRT during the perimenopausal period before the onset of vascular disease?

HERS and WHI have left the HRT research community confused. Rather than despair, we should roll up our sleeves and get back to work. The good news is that there are bound to be some interesting discoveries yet to be uncovered and new approaches yet to be developed to improve the lives of postmenopausal women.

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