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Review article

Can a healthy endothelium influence the cardiovascular effects of hormone replacement therapy?

Kwang Kon Koh*

Division of Cardiology, Gil Heart Center, Gachon Medical School, 1198 Kuwol-Dong Namdong-Gu, 405-760 Inchon, South Korea

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Abstract

Emerging clinical and observational evidences suggest that estrogen confers physiologic benefits that are receptor mediated and depend on the integrity and functional status of the endothelium within the coronary vasculature. In postmenopausal women, estrogen replacement therapy (ERT) and hormone replacement therapy (HRT) regimens can enhance the lipoprotein panel; blunt the expression of numerous cytokines, chemokines, and other proinflammatory mediators of endothelial injury and vascular smooth muscle cell proliferation; up-regulate endothelial nitric oxide synthase activity and nitric oxide production; and augment fibrinolysis potential and vasodilator capacity (diminish arterial resistance). Advancing age and atherosclerotic injury to the vessel wall tend to deplete estrogen receptors, compromise endothelial function, promote thrombus formation, and thus potentially diminish the efficacy of ERT and HRT. Therefore, optimizing the clinical benefits of these regimens in postmenopausal women depends largely on promoting a healthy endothelium through life-style modifications that diminish coronary risk.
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1. Introduction

Cardiovascular disease (CVD), which encompasses coronary artery disease (CAD), stroke, and peripheral vascular disease is the leading cause of death in US women and leads to more fatalities than the next seven leading causes of mortality combined [1]. With the projected upward spiral in the median age of the US population, CVD will likely assume ever larger clinical and public health dimensions. The cardioprotective effects of estrogen, which decrease the risk factors for the development of CVD, are amply documented [2]. For example, a recent metaanalysis of prospective observational studies showed that current users of estrogen replacement therapy (ERT) or hormone replacement therapy (HRT) experience a 35% reduction in the relative risk of CAD [3].

The sharp contrast between the above mentioned observational data and the neutral results reported by investigators in the Heart and Estrogen/progestin Replacement Study (HERS) has spawned considerable comment and controversy [4,5]. The first prospective, double-blind, placebo-controlled clinical trial for secondary prevention, HERS determined that HRT with oral conjugated equine estrogens (CEE; 0.625 mg/day) and medroxyprogesterone acetate (MPA; 2.5 mg/day) conferred no cardioprotective benefit on 2763 postmenopausal women with CAD at baseline (mean age, 66.7 years) [6]. At an average follow-up of 4.1 years, there was no difference

*Corresponding author. Tel.: +82-32-460-3683; fax: +82-32-460-3117.

E-mail address: kwangk@ghil.com (K.K. Koh).

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between groups in the occurrence of CAD death or nonfatal myocardial infarction, the primary outcome. What has provoked concern is the fact that more women in the hormone-treated group than placebo recipients experienced cardiovascular events noted during the first year of HRT and furthermore, deep vein thrombosis (25 vs. 8; $P=0.004$) and pulmonary emboli (11 vs. 4; $P=0.08$). The risk of venous thromboemboli and pulmonary emboli in current users of HRT was three times higher than in non-users, an increase similar to the 2–4-fold rise reported in prior studies [7,8].

Women in the HERS trial may have been more prone to thrombotic events than the general population regardless of whether they received HRT, a phenomenon that to date has defied explanation. The incidence of venous thromboemboli in postmenopausal women with CAD randomized to placebo was 22 events per 10,000 women-years, a rate more than 10-fold higher than previously reported in observational studies [7,8]. Despite the possibility of an aberration in the study population, the results of the HERS have forced researchers and clinicians to reexamine the mechanisms of action of estrogens on the cardiovascular system of postmenopausal women over the long term.

The clinical evidence of the beneficial effect of estrogens on atherosclerosis progression has been noticeably contradicted by results of the recent Estrogen Replacement and Atherosclerosis (ERA) trial, which involved postmenopausal women with angiographic evidence of coronary atherosclerosis [9]. In this clinical trial, treatment with CEE or CEE/MPA significantly decreased LDL and significantly increased HDL cholesterol. That these changes were not accompanied by discernible improvements in angiographic parameters might be attributed, however, to the fact that HRT was initiated on average 23 years after the cessation of menses.

2. Observational and clinical trials

In contrast to HERS results, an updated Nurses' Health Study with 20 years follow-up confirmed that the risk for major coronary events was lower among current users of HRT, including short-term users, compared with never-users (relative risk, 0.61) [10]. Thus, the increase of rate of CAD for primary prevention may be less than that for secondary prevention. Furthermore, they found no strong evidence for a difference for unopposed estrogen versus estrogen and progestin. In the overall Nurses' Health Study cohort, the proportion of postmenopausal women using HRT increased by 175% during a 14-year follow-up. This increase explained 9% of the significant, age-adjusted 31% decline in the incidence of CAD during the same interval [11].

Clinical evidence also supports the hypothesis that treatment with ERT or HRT diminishes the expression of several proinflammatory cytokines and soluble adhesion molecules that mediate leukocyte-vascular wall interactions, which are in turn involved in the pathogenesis of atherosclerotic disease. For example, in a randomized, double-blind, crossover study [12,13], 6 or 8 weeks of treatment with CEE alone or combined with MPA significantly diminished E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression as compared with baseline. Expression of another pro-inflammatory mediator expressed by atheromatous lesions—monocyte chemoattractant protein-1—is also attenuated by HRT [13]. The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial confirmed the reduction of E-selectin by HRT [14].

3. 'Healthy endothelium' concept

Far from being only an anatomic barrier to prevent the extravasation of circulating blood into the vessel wall, the endothelium is a metabolically active organ system that maintains vascular homeostasis by (1) modulating vascular tone, (2) regulating local cellular growth and extracellular matrix deposition, and (3) regulating the hemostatic, inflammatory and reparative responses to local injury. Nitric oxide (NO) plays a pivotal role in maintaining vascular health and protecting from vascular injury. However, conditions including hypercholesterolemia, systemic hypertension, smoking, diabetes, and estrogen deficiency have been associated with impaired functions of the endothelium. As a result, the vessel wall in these conditions may promote inflammation, oxidation of lipoproteins, smooth muscle proliferation, extracellu-

lar matrix deposition or lysis, accumulation of lipid-rich material, platelet activation, and thrombus formation. All of these consequences of endothelial dysfunction may contribute to development and clinical expression of atherosclerosis [15].

The concept that the loss of a healthy endothelium may prevent patients from deriving pronounced cardioprotective benefits from HRT is supported by observational and clinical studies. Thus, a new pathophysiologic model of the 'healthy endothelium' may shed some light on the findings of the HERS trial and guide future lines of clinical inquiry concerning HRT. This concept holds that many of the anti-atherogenic and other favorable vascular effects of estrogen are receptor-mediated and endothelium-dependent. Consequently, endothelial injury [16] or declines in vascular estrogen receptor (ER) populations [17] can diminish the anti-inflammatory, antithrombotic, and other cardioprotective benefits of this reproductive hormone.

In this regard, Holm and his co-workers [16] observed that in the undamaged aorta, cholesterol accumulation was almost completely inhibited by estrogen; in the balloon-injured aorta, the estrogen and placebo rabbits accumulated similar amounts of cholesterol in the reendothelialized areas; in the deendothelialized areas, the estrogen group surprisingly accumulated significantly more cholesterol than the placebo group. They reported that the direct antiatherogenic effect of estrogen was present, absent, or reversed, depending on the state of the arterial endothelium and pointed out the importance of 'healthy endothelium'. According to a histopathological trial involving 64 women who underwent hysterectomy, 9 out of 10 women with irregular menstrual function exhibited intimal thickening or sclerosis as compared with 14 of 33 (42%) women with regular menses ($P=0.0082$) [18]. In this study, both intimal thickening and sclerosis were accompanied by significant increases in levels of LDL cholesterol, as well as 2- and 10-fold increases, respectively, in free and esterified cholesterol in sclerotic intima. All eight postmenopausal women who were not on URT exhibited intimal thickening or sclerosis as compared with 7 of 13 (52%) of women treated with various HRT regimens. Also, in the Heart, Osteoporosis, Postmenopausal Estrogen (HOPE) study [19], focal plaque in carotid artery was evident in 49% of hysterectomized-only premenopausal women compared to 20% of their nonhysterectomized counterparts ($P=0.004$). Plaque was present in significantly fewer postmenopausal women on HRT regimens than in non-HRT users ($P=0.014$). Previous work had established a link between lack of expression of the ER gene and atherosclerosis [17]. Indeed, a recent ex vivo study conducted by Post and colleagues [20] demonstrated that the promoter region of the ERα gene exhibited age-related rises in methylation, and hence inactivation. In addition, endothelial cells explanted from coronary atheromata in patients displayed significant increases in ERα gene methylation as compared with grossly normal segments of the proximal aorta.

4. Biological effects of estrogen

4.1. Enhancement of endothelial function

4.1.1. LDL-oxidation and nitric oxide

Recent research suggests several mechanisms by which estrogen may help not only to preserve the vasculature of postmenopausal women but actually to enhance endothelial function. Mechanisms are antioxidant effects and the increase of NO bioavailability.

Experimental evidence strongly indicates that LDL, especially following oxidative modification within the vessel wall by free radical molecules [21], may importantly affect endothelial NO production and bioactivity [22]. Reduction in levels of lipoprotein(a) [Lp(a)] could also enhance NO bioavailability if concentrations of oxidized Lp(a) were reduced in the vessel wall [23]. HDL has been shown to protect LDL from oxidation [24]. 17β-Estradiol (E_2) has antioxidant effects in vitro [25] and in vivo [26]. Thus, in addition to reducing circulating LDL and Lp(a) and increasing HDL cholesterol levels, estrogen protects LDL from oxidation, which could enhance NO bioactivity.

Meanwhile, estrogen may also augment the bioactivity of NO independent of lipoprotein effects. Estrogen has been shown in endothelial cell culture studies to increase transcription and activity of NO synthase [27]. The rapid effects of estrogen on stimulation of NO synthase activity could be me-

diated by ER rapidly in a nongenomic manner [2]. ERα can directly activate endothelial NO synthase [28]. The short-term coronary vasodilatory effects of estrogen in humans are largely mediated by the increased production of NO [29].

4.1.2. Endothelium-dependent vasodilation

Flow-mediated dilation (FMD) is one prominent marker of NO bioactivity and overall endothelial function. According to findings from clinical trials, ERT and HRT significantly enhanced FMD [12,13,30]. Lieberman et al. [30] reported that oral E_2 resulted in significant improvement in flow-mediated brachial artery dilation compared with placebo. Furthermore, in one trial [31], vasodilation in response to intrabrachial acetylcholine was blunted by ovariectomy, with forearm blood flow (FBF) increasing nearly fourfold after ovariectomy, as compared with a change of more than seven-fold at baseline ($P<0.05$). Three months of ERT restored to preovariectomy values the amplitude of the FBF response to acetylcholine.

Parallel finding concerning the benefits of HRT on forearm vascular reactivity in postmenopausal women has been reported. Treatment with either a CEE/micronized progesterone (MP) or CEE/MPA regimen for 8 weeks increased brachial-artery FMD by more than 60% as compared with baseline (Fig. 1A) [13]. However, some studies observed no effect of estrogen on endothelium-dependent vasodilation as well as on lipoproteins [32,33]. In this regard, Vehkavaara et al. [34] reported that oral estradiol-induced increase in endothelium-dependent vasodilation could be explained not by acute estradiol effects but by several antiatherogenic changes in lipoproteins in contrast to transdermal estradiol showing no effects on both endothelium-dependent vasodilation and lipoproteins. Recently, we observed that compared with placebo, CEE 0.625 mg daily for 8 weeks did not significantly improve the percent flow-mediated dilator response to hyperemia in type 2 diabetic postmenopausal women (Fig. 1B) [35]. Of interest, Williams and his co-workers [36] observed that aging inhibited E_2 effects on both smooth muscle- and endothelium-mediated vascular reactivity and furthermore, impaired E_2-mediated vascular reactivity may be associated with aging effects on ER-β function. This observation was confirmed by another prelimin-

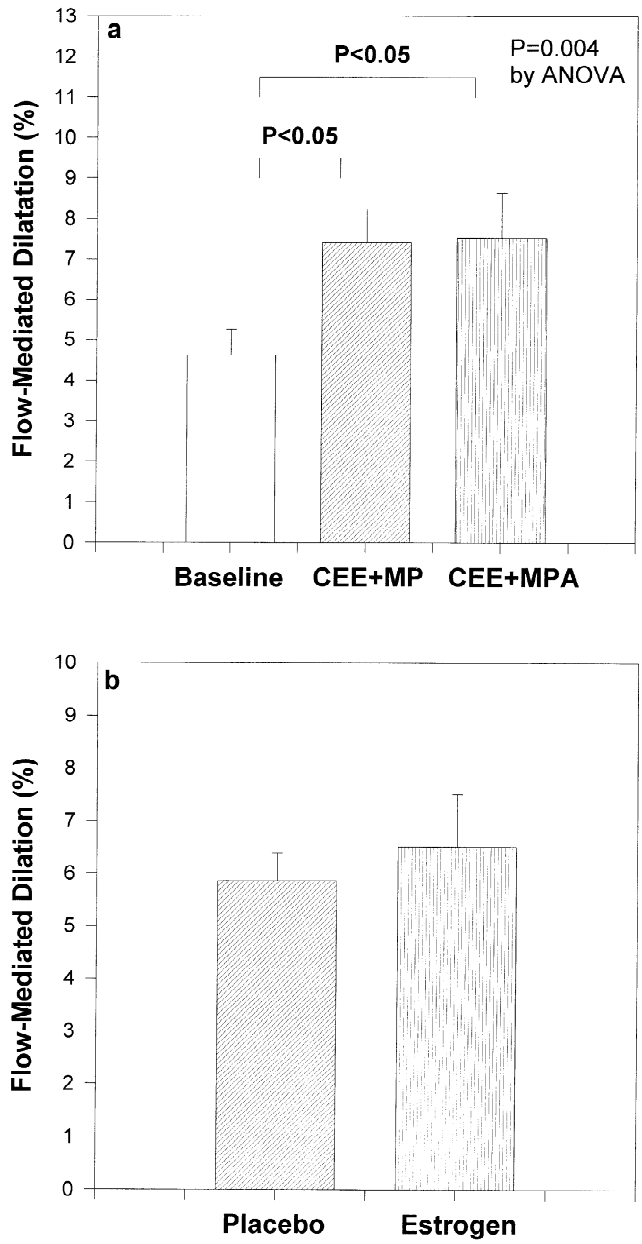

Fig. 1. A) Flow-mediated dilatation before therapy (Baseline) and following micronized progesterone (CEE+MP) or medroxyprogesterone acetate (CEE+MPA) combined with conjugated equine estrogen (CEE). CEE+MP or CEE+MPA significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements ($P=0.004$ by ANOVA) by a similar degree. Standard error of the mean is identified by the bars. Reproduced with permission from Koh et al. [13]. B) Flow-mediated dilation following placebo and conjugated equine estrogen. Estrogen improved, but not significantly, the percent flow-mediated dilator response to hyperemia compared with placebo ($P=0.501$). Standard error of the mean is identified by the bars. Reproduced with permission from Koh et al. [35].

ary study [37]. Indeed, Celermajer et al. [38] documented a marked age-related decline in endothelium-dependent vasodilation.

4.1.3. Arterial compliance and stiffness

Twenty-six postmenopausal women taking HRT with no evidence of CAD at baseline had a significantly increased total systemic arterial compliance and lower pulse wave velocity than those not taking HRT [39]. Of interest, 11 postmenopausal women had HRT withdrawn for 4 weeks, resulting in a significant decrease in total systemic arterial compliance and significant increase in pulse wave velocity. Another study observed that the carotid arterial stiffness index was similar in both ERT users with and without MPA, who had no evidence of CAD and significantly lower than in nonusers [40].

4.2. Thrombosis, fibrinolysis, and coagulation

The clinical manifestation of atherosclerotic disease hinges on thrombogenic as well as inflammatory cellular and molecular pathways. Following plaque disruption, platelets and circulating factors that mediate thrombosis are exposed to the lesional lipid core, which is thrombogenic. Therefore, the effects on thrombosis, fibrinolysis, and overall coagulation status of endogenous estrogen in women of childbearing status, as well as ERT and HRT in postmenopausal women, bear directly on endothelial function.

The relationship between thrombosis, estrogen status, and endothelial dysfunction is supported by the fact that soluble thrombomodulin—a key regulator of activated thrombin—and tissue-plasminogen activator (t-PA), which promotes fibrinolysis, are considered markers of endothelial damage and were elevated in a study of prematurely menopausal women [41]. Six weeks of HRT resulted in a significant reduction in mean soluble thrombomodulin, t-PA, and von Willebrand factor (vWF), compared to premenopausal levels, suggesting beneficial effects on endothelial injury and hemostasis [41]. Indeed, findings on coagulation status reported in the PEPI trial may partly explain the higher risk of thromboemboli in the HERS placebo group [42]. Among control patients in the PEPI trial, factor VIIIc and fibrinogen increased over time. The vWF antigen concentration also increased to 34% after 12 months, then returned to baseline at month 36.

Significantly enhanced systemic fibrinolysis resulted from 1 month of treatment with oral CEE, either alone or combined with MPA, in 30 postmenopausal women in a randomized crossover trial [43]. Both CEE and CEE/MPA decreased plasma plasminogen activator inhibitor-1 (PAI-1) levels from baseline by more than 50%. These effects were more pronounced in women with higher levels of PAI-1 at baseline. In addition, levels of D-dimer exhibited a significant inverse correlation with PAI-1 levels, suggesting enhanced fibrinolysis potential (Fig. 2). Six months of HRT with oral cyclic E_2 combined with MP also increased global fibrinolytic capacity by 63% vs. baseline ($P=0.001$) and reduced both PAI-1 antigen (24%; $P=0.02$) and PAI activity (54%; $P=0.004$) in 45 healthy postmenopausal women [44]. However, such treatment was also associated with an activation in coagulant function. This hypercoagulable state was reflected by significant increases in prothrombin fragment (F_{1+2}) and decreased antithrombin activity among HRT users as compared with women who received no HRT.

On the other hand, because activation of coagulation pathways has been detected dose-dependently in postmenopausal women treated with CEE 0.625 and 1.25 mg [45], potentiation of fibrinolysis could be a consequence of activation of coagulation pathways as a primary response to estrogen administration. However, Winkler et al. [46] speculated that small doses of estrogen/progestogen induce increases in fibrinolytic capacity via a marked reduction of PAI-1. In other words, HRT at conventional dosages may affect fibrinolytic activity to a greater extent than coagulation activity, whereas the converse trend holds at higher estrogen doses. To evaluate this hypothesis, Koh and co-workers [47] measured indicators of coagulation-system activation and fibrinolysis before and after 1 month of CEE (0.625 mg/day). After CEE treatment, the t-PA activity: PAI-1 activity ratio (t-PA:PAI-1), which is more reflective of fibrinolytic potential than either activity individually, increased by approximately five-fold ($P=0.008$ vs. baseline). These increases correlated with in vitro evidence of heightened fibrinolytic activity: D-dimer concentrations in clotted blood incubation for 4 hours. Consistent with our previous study [43], D-dimer con-

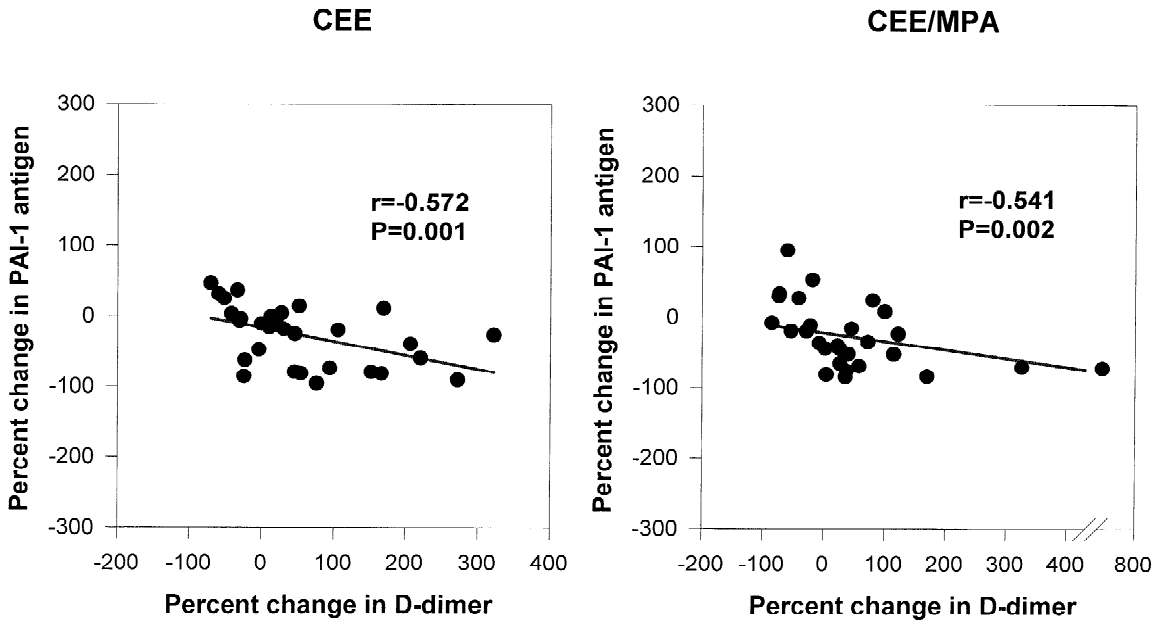

Fig. 2. Scatter plots showing the correlation between the percent change in plasminogen activator inhibitor type-1 (PAI-1) antigen and percent change in D-dimer after oral conjugated estrogen (CEE) alone and after combined treatment of conjugated estrogen and medroxyprogesterone acetate (CEE/MPA). Reproduced with permission from Koh et al. [43].

centrations were significantly correlated (positively) with an increase in the t-PA:PAI-1 ratio [47]. However, the increases in fibrinolytic potential (t-PA:PAI-l) did not correlate with the minimal changes observed in F_{1+2} or thrombin-antithrombin (TAT) levels after CEE. The increase in fibrinolytic potential was independent of any effect on coagulation of CEE at conventional dosages. Scarabin et al. [44] also reported no correlation between fibrinolytic potential and coagulation activation using ERT regimens. Cushman and colleagues [48] found that hemostasis markers and evidence of procoagulation were not associated and fibrinolytic potential increased. However, in contrast to healthy postmenopausal women [13,43,44,47], we recently reported that HRT did not significantly decrease PAI-1 antigen levels and rather, tended to increase F_{1+2} levels from baseline in hypertensive or overweight postmenopausal women [49], consistent with the HERS.

Although Lp(a) is usually construed as an independent risk factor for CAD, it is structurally homologous with plasminogen. Through competition with this molecule as a substrate for fibrinolytic enzymes, Lp(a) can exert prothrombotic effects. In this regard, both ERT and HRT significantly decreased Lp(a) levels [50]. Indeed, a recent study from HERS reported that CEE+MPA appeared to have a more favorable effect in women with high initial Lp(a) levels than in women with low levels [51]. However, activation of coagulation following ERT or HRT may not be balanced by activation of fibrinolysis in some postmenopausal women [43,47,49]. Thus, ERT or HRT should not be initiated in women with CAD or the coexistence of other risk factors for hypercoagulability.

5. Clinical implications

The fact that HRT did not confer cardioprotective effects in the HERS can be assimilated readily according to the 'healthy endothelium' concept. In short, the favorable vascular effects of estrogen on atherosclerosis, inflammation, hemostasis, and coronary flow reserve are dependent on the integrity of both the endothelium and ER populations in endothelial cells and vascular smooth muscle cells, and these conditions were probably not met by most women in the HERS trial because of their advanced age and coronary atherosclerosis. Optimization of

estrogen's cardioprotective properties may depend on maintenance of a healthy endothelium. This includes a prudent, low-fat diet, smoking cessation, more physical activity, and enhanced efforts by clinicians to diagnose and treat hypertension or diabetes.

Commenting on the controversy surrounding results from the HERS trial, authorities have sought other plausible explanation 'beyond the null hypothesis' [4] to accommodate the neutral effects of HRT on coronary event rates in women with baseline CAD. First, the HERS trial had certain methodological pitfalls, including insufficient statistical power, a high crossover rate between treatment arms, and other medications effect. Second, the early increment in coronary event rates might have been precipitated by procoagulant effects of HRT in a susceptible cohort. Thrombogenic events are considered more likely in patients with certain heritable conditions, such as platelet antigen-2 (PIA-2) polymorphisms [52]. Further, Factor V Leiden mutation increases the risk of primary and recurrent venous thromboembolic events by three- to six-fold [53] and the risk of myocardial infarction [54]. Indeed, HRT may decrease or increase atherothrombosis risk depending on the presence of Factor V Leiden mutation [55].

The controversy occasioned by the HERS trial can be resolved only through sufficiently powered, randomized controlled trials. Of these, the Women's Health Initiative, which completed enrollment of 27,000 women to evaluate the effects of HRT and dietary intervention on coronary events, is perhaps the most promising.

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