Review

Controversies regarding hormone therapy: Insights from inflammation and hemostasis

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Abstract

Many observational studies and experimental and animal studies have demonstrated that estrogen therapy (ET) or hormone therapy (HT) significantly reduces the risk of coronary heart disease. Nonetheless, recent randomized controlled trials and the Nurses’ Health Study in secondary prevention demonstrate trends toward an increased risk of cardiovascular events rather than a reduction of risk from HT. HT has both anti-inflammatory and pro-inflammatory effects, and it activates coagulation and improves fibrinolysis. These effects depend on the route of administration, doses of estrogen, age of women, and the presence of coronary artery disease or the coexistence of other risk factors for hypercoagulability. In this review, we discuss effects of HT on markers of inflammation, hemostasis, and fibrinolysis that may link endothelial dysfunction in cardiovascular diseases. We also briefly discuss effects of lower doses of HT and tibolone in postmenopausal women.

Keywords: Hormone therapy; Lower doses; Tibolone; Inflammation; Hemostasis

1. Introduction

Estrogen deficiency contributes to endothelial dysfunction, in part, by increasing inflammation in the vessel wall, lipoprotein oxidation, smooth muscle cell proliferation, extracellular matrix deposition, accumulation of lipid-rich material, activation of platelets, and thrombus formation [1]. These pathogenic features contribute to development of atherosclerosis and coronary heart disease [1–3]. The clinical manifestation of atherosclerotic disease hinges on thrombogenic as well as inflammatory cellular and molecular pathways.

Many observational studies and experimental and animal studies have demonstrated that estrogen therapy (ET) or hormone therapy (HT) significantly reduces the risk of coronary heart disease. Nonetheless, recent randomized controlled trials and the Nurses’ Health Study in secondary prevention demonstrate trends toward an increased risk of cardiovascular events rather than a reduction of risk [4–7]. HT has both anti-inflammatory and pro-inflammatory effects, and it activates coagulation and improves fibrinolysis [1,8]. These effects depend on the route of administration, doses of estrogen, age of women, and the presence of coronary artery disease or the coexistence of other risk factors for hypercoagulability. In this review, we discuss the effects of HT on inflammation, hemostasis, and fibrinolysis markers that may contribute to endothelial dysfunction in cardiovascular diseases. We also briefly discuss effects of lower doses of HT and tibolone in postmenopausal women.

2. Effects of hormone therapy on inflammation markers

2.1. C-reactive protein

Obesity and age are both positively associated with C-reactive protein (CRP) concentrations [9,10]. Messenger
cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF)-α released by adipose tissue contribute to increases in CRP. This cross-talk between inflammation and metabolic physiology may have a greater impact on coronary heart disease risk in women than in men [11]. Although IL-6 levels increase following natural or surgical menopause [12], CRP concentrations are not influenced by menopause per se [13]. Nested case–control analyses of the Women’s Health Study [14] and the Nurses’ Health Study [15] reported the predictive value of CRP in determining the risk of future cardiovascular events in women as well as men.

Orally administered estrogens increase blood CRP levels [16–22] and maintain sustained increases for up to 3 years [17]. CRP increases mediated by oral estrogen seem to be higher in heavier women [23]. By contrast, transdermal estradiol in healthy postmenopausal women significantly lowers CRP levels in some studies [24,25] while no change was noted in other studies [18,23]. In premenopausal women, CRP correlates inversely with blood estradiol concentrations during the menstrual cycle [26]. In 389 postmenopausal women with increased cardiovascular risk, CRP levels significantly increase after 6 months of HT when compared with baseline. By contrast, plasma levels of cell adhesion molecules (CAMs), IL-6, and s-thrombomodulin significantly decrease after HT. No significant changes in either CRP or vascular inflammatory marker are detected in women not taking HT [22]. These observations strongly suggest that increases in CRP following oral HT may be related to hepatic metabolism rather than a systemic pro-inflammatory response. Co-administered medroxyprogesterone acetate (MPA) for 3 months may attenuate conjugated equine estrogen (CEE) action in a dose-dependent manner in healthy postmenopausal women (mean age: 52 years, mean body mass index (BMI): 21.8 kg/m²) [27]. Compared with CEE alone, addition of MPA at 2.5 mg increases CRP significantly but to a lesser degree. At a dose of 5 mg, there is no increase in CRP. Other studies reproduce the findings with MPA at 2.5 mg dosing [17,28]. However, the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial (mean age: 56.1 years, mean BMI: around 26 kg/m²) reports that concomitant MPA at 10 mg administered sequentially does not influence the CEE effect on CRP levels measured at years 1 and 3 [17]. Differences in baseline age and BMI might account, at least in part, for different responses to higher dose of MPA. Effects of micronized progesterone (MP) have been studied less systemically. Koh et al. [29] demonstrate that MP 100 mg with CEE 0.625 mg daily for 2 months leads to significant increases in CRP in healthy postmenopausal women (mean age: 57 years, mean BMI: 24.8 kg/m²). Of note, MP 100 mg with CEE 0.3 mg daily does not increase CRP. This suggests a dose-dependency similar to that observed with MPA. According to the PEPI trial, there are no long-term differences in CRP levels between CEE+MPA and CEE+MP therapy [17]. However, controversy regarding the significance of the pro-inflammatory effects of estrogen persists [30,31]. The Women’s Health Initiative (WHI) study confirms that increases in CRP levels associated with oral HT does not link to an increase in the risk of coronary heart disease [32]. This result is consistent with reports that there is no relation between CRP and Framingham Coronary Heart Disease Risk Score among women taking HT [33]. Nonetheless, because CRP has several important atherogenic properties in addition to being a biomarker of inflammation [30,31], the oral estrogen-induced CRP increase over many years may contribute to progression of atherosclerosis.

2.2. Cell adhesion molecules, chemokines, and cytokines

The pathophysiological relevance of CAM in humans is suggested by its localization in atherosclerotic plaques. Transendothelial migration and differentiation of monocytes into macrophage in subendothelial space are controlled by monocyte chemoattractant protein-1 (MCP-1). In vitro and animal studies demonstrate that estrogens suppress expression of CAMs [34,35], MCP-1 [35,36], TNF-α [37], and IL-6 [35,38].

Blood levels of soluble CAMs, chemokines, and cytokines are potential candidates for markers of inflammation. At present, these assays remain research tools and are not recommended for routine use in clinical laboratory. Koh et al. [39] first showed that transdermal estradiol alone or combined with MPA decreases intercellular adhesion molecule (ICAM)-1 levels. CEE 0.625 mg daily decreases E-selectin [17,27,39–41], but its effects on soluble ICAM-1 and vascular cellular adhesion molecule (VCAM)-1 are inconsistent. Both ICAM-1 and VCAM-1 levels are reduced [28,40] or unchanged [27]. CEE+MPA consistently decreases E-selectin, ICAM-1, and VCAM-1 levels [17,27,28,41]. CEE+MP shows a comparable reduction, when compared with CEE+MPA [17,41].

There is a significant correlation between mean maximum intimal medial thickness and MCP-1 levels at baseline in postmenopausal women. ET significantly reduces MCP-1 levels [42]. MCP-1 concentrations are also lowered by both progestogens in combination with CEE by a similar degree [41,43]. We observe that CEE with MP or MPA significantly reduces TNF-α levels from baseline in hypertensive or overweight postmenopausal women [44]. Our observation is consistent with the findings of Walsh et al. [21]. The effects of ET or HT on soluble IL-6 levels in postmenopausal women are inconsistent. Some studies observe an increase in IL-6 levels [19,20], whereas our study and others found no significant changes [16,21].

In summary, the effect of HT on inflammation in postmenopausal women is complex. Orally administered HT increases levels of CRP while decreasing levels of the soluble CAMs, MCP-1, and TNF-α that may contribute to the risk of cardiovascular diseases (Table 1). It is likely that there is a first-pass effect of orally administered estrogen on
hepatic synthesis of CRP. Nevertheless, elevated CRP may have deleterious effects on vascular inflammation, as discussed previously. This may have contributed to the unexpected increase in myocardial infarction risk within the first year of treatment in randomized and observational clinical studies [4,5,7].

### 3. Effects of hormone therapy on markers of hemostasis and fibrinolysis

Following menopause, the levels of several coagulation factors increase, including factors VII, VIII, and fibrinogen. However, effects of aging, independent of estrogen status, also likely contribute to these changes [45,46]. Further, levels of a critical inhibitor of fibrinolysis, plasminogen activator inhibitor type-1 (PAI-1) antigen, are higher in postmenopausal women than in premenopausal women. Indeed, they approach PAI-1 levels present in men of any age [47]. However, menopause-induced effects associated with the reaction products of thrombin activity are not observed in a longitudinal study [48].

According to the PEPI trial, orally administered CEE 0.625 mg results in a small (<2%), but significant reduction in plasma fibrinogen levels in healthy postmenopausal women [49]. Coadministration of MPA cyclic 5.0 mg or continuous 2.5 mg or MP cyclic 200 mg does not alter the CEE effect on fibrinogen. Koh et al. [50] demonstrate that oral CEE 0.625 mg therapy for 1 month decreases PAI-1 antigen levels by 50% from pretreatment values, with the greatest reduction occurring in women with the highest baseline PAI-1 levels. In addition, MPA 2.5 mg combined with CEE causes a similar decrease in PAI-1 levels (Fig. 1). These findings are surprising because MPA stimulates PAI-1 release from bovine aortic [51] and human umbilical endothelial cells [52]. In addition, the percent change in D-dimer levels exhibits a significant inverse correlation with the percent change in PAI-1 levels, suggesting enhanced fibrinolysis potential (Fig. 2). Six months of HT with oral cyclic 17β-estradiol combined with MP also increases global fibrinolytic capacity by 63% vs. baseline and reduces both PAI-1 antigen and PAI activity in 45 healthy postmenopausal women [53]. When administered orally with CEE, MP (cyclic 200 mg) reduces PAI-1 levels to a similar extent as MPA (cyclic 10 mg) [41]. Effects of transdermal estradiol may differ according to duration of therapy. No change in PAI-1 is observed after 1 month of 100 µg patch [50], whereas reduction is induced after 1 year of 50 µg patch [54]. Factor VII levels increase with CEE, and MPA attenuates CEE effects [55]. However, transfer-

![Fig. 1. Changes in the plasma levels of plasminogen activator inhibitor (PAI-1) before and after therapy with oral conjugated equine estrogen (CEE) 0.625 mg daily for 1 month, or a combination of CEE 0.625 mg with medroxyprogesterone acetate (MPA) 2.5 mg daily for 1 month by 30 healthy postmenopausal women. Mean values are identified by open circles. Used with permission from Dr. Koh [50].](image-url)
Mal estradiol therapy, does not affect factor VII [18,54]. Differences in fibrinogen, PAI-1, and factor VII responses caused by the route of estrogen administration strongly suggest that with oral estrogen, a first-pass effect on hepatic synthesis of thrombosis markers exists.

There is evidence for activation of the coagulation system even with low estrogen doses used orally in HT. Caine et al. [56] show that administration of CEE 0.625 mg or 1.25 mg daily increases indices of thrombin generation [prothrombin fragment 1 +2 (F1+2) and fibrinopeptide A] in a dose-dependent manner. Moreover, CEE decreases levels of inhibitors of thrombin generation (antithrombin III and protein S). In this regard, observational studies report an increased risk of venous thromboembolism, or pulmonary embolism, in postmenopausal women treated with HT when compared with non-users [57]. Interestingly, increasing daily dose is associated with increased risk of venous thromboembolism [57,58] or stroke [59]. These observations raise the question of whether potentiation of fibrinolysis is a consequence of activation of the coagulation pathway and a primary response to estrogen administration. Winkler speculates that HT at conventional dosages may affect fibrinolytic activity to a greater extent than coagulation activity mainly by reducing PAI-1 antigen levels, whereas converse trends may occur at higher estrogen doses [60]. Koh et al. [61] found that the increase in fibrinolytic potential is independent of any effect of CEE (at conventional dosages) on coagulation. Other groups also report no correlations between fibrinolytic potential and coagulation activation using HT regimens [53,62]. Cushman et al. [20] observe that markers of hemostasis and evidence of procoagulation are not associated with increased fibrinolytic potential. These studies support the concept that the increase in fibrinolytic potential may be independent of the increase in coagulation with HT at conventional dosage in healthy postmenopausal women. However, activation of coagulation following ET or HT may not be balanced by activation of fibrinolysis in some postmenopausal women [8,50,63]. Thus, ET or HT should not be initiated in women with coronary artery disease or the coexistence of other risk factors for hypercoagulability. Thrombogenic events are considered more likely in patients with certain heritable conditions, such as platelet antigen-2 (PIA-2) polymorphisms [64]. Homozygosity for factor V Leiden leads to enhanced arterial thrombosis and atherosclerosis in mice [65]. ET and HT may decrease or increase atherothrombosis risk depending on the presence or absence of the Factor V Leiden mutation [66,67]. Koh et al. [63] report that CEE 0.625 mg combined with MP 100 mg or MPA 2.5 mg, both enhance activation of coagulation measured as tissue factor activity, F1+2, and antithrombin III while they do not decrease PAI-1 antigen levels in hypertensive and/or overweight postmenopausal women. HT may increase the thrombin formation by an enhanced activation of coagulation in these postmenopausal women.

Koh et al. [29] report that compared with conventional dose CEE 0.625 mg combined with MP 100 mg, CEE 0.3 mg combined with MP 100 mg does not increase F1+2 (Fig. 3) and decreases antithrombin III to a lesser extent, whereas it decreases PAI-1 antigen levels to a similar degree. This observation is also in line with the concept of independent activation of fibrinolysis by CEE.

4. Tibolone

Tibolone, a synthetic steroid with estrogenic, androgenic, and progestogenic properties, has long been used in Europe to relieve climacteric symptoms and prevent postmenopausal bone loss. However, the impact of tibolone on cardiovascular disease risk remains unclear. Although it lowers
high-density lipoprotein (HDL) cholesterol, tibolone improves overall lipid profile and flow-mediated brachial artery dilator response to hyperemia. Koh et al. [68] investigated effects of tibolone on markers of inflammation and thrombosis. Compared with CEE 0.625 mg combined with MP 100 mg, tibolone shows different effects on CRP, antithrombin III, and F\textsubscript{1+2}. CRP and antithrombin III do not change (Fig. 4) and F\textsubscript{1+2} increases less. Meanwhile, tibolone does not affect fibrinogen like HT, but decreases PAI-1 antigen to a similar degree. In another study, when compared with CEE 0.3 mg combined with MP 100 mg [lower-dose (L-HT)], tibolone significantly reduces total cholesterol, triglyceride, HDL cholesterol levels, and triglyceride/HDL cholesterol ratios but not total cholesterol/HDL cholesterol ratios [69]. Tibolone improves flow-mediated response to hyperemia from baseline values by a similar magnitude to L-HT. L-HT and tibolone do not increase CRP relative to baseline values. L-HT reduces antithrombin III from baseline values, when compared with tibolone that does not cause these changes. By contrast, tibolone increases F\textsubscript{1+2} from baseline values when compared with L-HT that did not cause these changes. Tibolone significantly reduces PAI-1 antigen levels from baseline values when compared with L-HT that did not cause these changes. Tibolone decreases factor VII activity [70]. Further, in vitro studies demonstrate that tibolone suppresses expression of VCAM-1 and E-selectin in human vascular endothelial cells [71]. These findings suggest that tibolone causes less activation of coagulation, independent activation of fibrinolysis, and has anti-inflammatory actions.

5. Clinical implications and future prospects

The fact that ET and HT do not confer cardioprotective effects in the recent randomized controlled trials can be assimilated readily according to the “healthy endothelium” concept [1,72,73]. 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 estrogen receptor populations in endothelial cells and vascular smooth muscle cells [74]. These conditions were probably not met by most women in these trials because of their advanced age, multiple risk factors, and coronary atherosclerosis. Optimization of estrogen’s cardioprotective properties may depend on maintenance of a healthy endothelium. Miller et al. [35] demonstrate that 17\textbeta-estradiol inhibits expression of CAMs, MCP-1, and proinflammatory cytokines (IL-1, IL-6) in rat carotid arteries after balloon injury and thus attenuates the stimulus for leukocyte entry and negatively modulates the injury response. Quite interestingly, in the same model this group observes aged ovariectomized rats lose the vasoprotective and anti-inflammatory responses to exogenous 17\textbeta-estradiol seen in injured arteries of younger ovariectomized rats [75]. In healthy postmenopausal women, we demonstrate that CEE 0.625 mg reduces CAMs levels [40]. However, we observe that CEE 0.625 mg does not reduce CAMs levels in type 2 diabetic postmenopausal women [76].

The importance of the timing of intervention on the effect of estrogens on atherogenesis has been previously observed.

Fig. 3. Plasma prothrombin fragment 1+2 (F\textsubscript{1+2}) levels before treatment (Baseline) and following micronized progesterone combined with conjugated equine estrogen 0.625 mg (C-HT) or 0.3 mg (L-HT) treatment. C-HT significantly increased F\textsubscript{1+2} from baseline values (p<0.001), however, L-HT did not significantly change F\textsubscript{1+2} (p=0.558). Of interest, the effects of C-HT and L-HT on F\textsubscript{1+2} were significantly different (p<0.001). Median values are identified by open circles. Used with permission from Dr. Koh [29].
in nonhuman primates [73,74]. The possible protective role of estrogen in preventing development of atherosclerotic lesions in perimenopausal and postmenopausal mice has recently been investigated [77]. This study examines development of atherosclerotic lesion in ovariectomized versus follicle-depleted ovary-intact cholesterol-fed female low-density lipoprotein receptor-deficient mice. Replacement with 17\(\alpha\)-estradiol causes reduction of atherosclerotic lesions. Of note, this is most efficacious in suppressing atherosclerotic lesions in perimenopausal mice when compared with postmenopausal mice. Indeed, a recent report from the WHI study finds that CEE+MPA reduce risk of coronary heart disease differentially according to the year since menopause and the presence of hot flashes [78]. Of great interest, the ET arm of the WHI study also shows that the subgroup of women in the youngest decade appears to respond to estrogen more favorably than older women for many of the outcomes, including the coronary heart disease and global index [6].

On the other hand, procoagulant effects of estrogen in a susceptible cohort may have precipitated the early increment in coronary event rates in the recent randomized controlled trials and observational study in women with known coronary heart disease [4–7]. The discrepancy in coronary heart disease outcomes between randomized controlled trials and observational studies may be explained by age of women, the time from menopause when given HT, and the presence of coronary heart disease. Indeed, no early harm is observed in younger women given HT at the onset of menopause in prospective studies including over 7000 women [79]. In a hospital-based case–control study of 597 postmenopausal women, the impact of oral HT on venous thromboembolism risk when compared with HT non-users differs significantly according to age at menopause (OR=39 in women with late menopause and OR=3 in those with early menopause) [80].

L-HT may prevent coronary heart disease without increasing risk of stroke or thromboembolism [57–59,81]. Beneficial effects of CEE 0.3 mg combined with MP 100 mg daily on vasomotor function, inflammation, and hemostasis may pave the way for a new clinical trial [29]. Indeed, Kronos Early Estrogen Prevention Study (KEEPS) was launched in 2005 [82]. KEEPS is a 5-year clinical trial that will evaluate the effectiveness of CEE 0.45 mg, transdermal estradiol 50 \(\mu\)g (both in combination with cyclic MP 200 mg), and placebo in preventing progression of carotid atherosclerosis and coronary artery calcification in early postmenopausal women. The Early versus Late Intervention Trial with Estradiol (ELITE) study will compare the effects of HT in both early and late postmenopausal women. These studies may answer remaining questions regarding L-HT effects and the healthy endothelium concept.

In conclusion, HT has both anti-inflammatory and pro-inflammatory effects and it activates coagulation and improves fibrinolysis. These effects depend on the route of administration, doses of estrogen, age of women, and the presence of coronary artery disease or the coexistence of other risk factors for hypercoagulability. Considering these points, the most effective approach may be to initiate HT at early menopause. As alternatives, lower doses of HT or tibolone may prevent coronary heart disease without
increasing stroke or thromboembolism risk. However, clinical recommendations regarding the effects of lower doses of HT or tibolone on cardiovascular outcomes await additional studies with clinical endpoints.

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