E ffects of estrogen on the vascular wall: vasomotor function and inflammation

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1. Introduction

Far from being only an anatomic barrier to prevent the influx of circulating blood into the vessel wall, the endothelium is a metabolically active organ system that maintains vascular homeostasis. The endothelium modulates vascular tone, regulates local cellular growth and the deposition of the extracellular matrix, protects the vessel from the potentially harmful consequences of substances and cells circulating in the blood, mediates the hemostatic, inflammatory, and reparative responses to local injury. Nitric oxide (NO) produced by the endothelium plays a pivotal role in maintaining vascular health and protecting against vascular injury.

Conditions such as hypercholesterolemia [1], systemic hypertension [1], and estrogen deficiency [2] have been associated with impaired functions of the endothelium. As a result of impairment, the vessel wall may promote inflammation, oxidation of lipoproteins, smooth muscle cell proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich material, the activation of platelets (which promote clotting), and thrombus formation. All of these consequences of endothelial dysfunction may contribute to the development and clinical expression of atherosclerosis and coronary heart disease [3,4].

This hypothesis is supported by serial angiographic studies carried out before and after a myocardial infarction, which indicate that the underlying plaque responsible for unstable angina and myocardial infarction usually produces an arterial narrowing of less than 50% before the acute event [5,6]. Indeed, a recent angiographic study suggests that the positive correlation between the number of severely diseased arteries and coronary mortality may not just be related to the number of arteries with 70% or greater stenosis, but may also be tied to the amount of minor plaque disease in other vessels [7]. These observations have driven a search for other mechanisms, including the potential association of endothelial dysfunction and associated inflammation with plaque rupture, and the activation of platelets and coagulation with thrombus formation.

Advances in research in this area are particularly relevant to the health of older women, since cardiovascular disease (CVD) results in more deaths in women in the United States than any other disease. Several prospective, observational studies have suggested that postmenopausal women who do not have documented cardiovascular disease and who take estrogen replacement therapy (ERT) or hormone replacement therapy (HRT—estrogen plus a progestin) have a reduced risk of major coronary events compared with untreated women [8–10]. In contrast, the Heart and Estrogen/progestin Replacement Study (HERS) [11], a randomized controlled trial among older women with established heart disease, found an increased risk of cardiovascular events in the first year of treatment in this at-risk cohort. This result, in part, has led the American Heart Association [12] to advise against the initiation of HRT for the secondary prevention of CVD, or for the sole purpose of the primary prevention of CVD until the results of ongoing randomized trials are reported and analyzed.

The potential atheroprotective effects of estrogen have been attributed principally to the hormone’s effects on serum lipid concentrations [13,14]. However, estrogen-induced alterations in serum lipids account for only approximately one-third of the observed reduction in risk of mortality from CVD among ERT or HRT users [15].

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Based on the literature done by others and our group, this article reviews the influence of ERT or HRT on such aspects of endothelial function as vasomotor function and inflammation as plausible mechanisms in the prevention of atherosclerosis and coronary heart disease in post-menopausal women. We recently reviewed the effects of HRT on hemostasis and thus will not discuss this topic in this review [16].

2. Inflammation and atherosclerosis

Growing evidence over the past decade implicates chronic inflammation in the pathogenesis of atherosclerosis (Fig. 1) [3]. Human coronary, carotid, and aortic specimens at necropsy have shown infiltration of the intima and media by granulocytes, lymphocytes, monocytes, and macrophages [17–19]. The initiation of inflammation is probably multifactorial; more recently described risk factors include infectious pathogens [20], homocysteine [21], and lipoprotein(a) [Lp(a)] [22].

Once activated by injury, endothelial and smooth muscle cells of large arteries become transcriptionally active and synthesize proinflammatory proteins, including: (1) chemokines and cell adhesion molecules (CAMs), which attract circulating inflammatory cells to the arterial surface and facilitate their attachment and incorporation into the vessel wall; (2) cytokines that activate inflammatory cells and transform monocytes into macrophages; (3) growth factors that stimulate smooth muscle cell proliferation; (4) angiogenic peptides that increase the vascularity within the arterial wall; and (5) prothrombogenic substances such as tissue factor and thromboxane. Cytokine-activated macrophages and smooth muscle cells secrete matrix metalloproteinases, which, when activated, digest connective tissue elements within the vessel wall and thin the fibrous cap overlying vulnerable plaques, with the potential for plaque rupture and exposure of the thrombogenic contents of the plaque to blood [23].

Endothelial dysfunction and reduced NO could also stimulate the synthesis and release of endothelin, resulting in enhanced vasoconstrictor tone; promote the release and activity of growth factors, resulting in smooth muscle hyperplasia and migration into the intima; and enhance the synthesis and release of proinflammatory cytokines. Additionally, reduced NO could promote platelet attachment and release of growth factors in the vessel wall. All of these consequences of endothelial dysfunction and reduced NO bioactivity may be important in the initiation, progression, and clinical expression of atherosclerosis. In this regard, the transition from stable to unstable angina appears to be associated with a systemic inflammatory response [24], and markers of acute inflammation (including cytokines, C-reactive protein and white cell count) are all related to increased cardiovascular risk [24,25].

3. Mechanisms of estrogen’s effects on the vascular wall

Both endothelial cells and vascular smooth muscle cells possess estrogen receptors and are thus physiological targets for estrogen action [26,27]. Estrogen receptor $\alpha$ (ER$\alpha$) and $\beta$ have been identified and both of these receptors are expressed and functional in cardiovascular tissues, supporting a direct role for estrogen in cardiovascular physiology. Cellular responses elicited by estrogen may be achieved via both genomic and

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Fig. 1. Injury to the arterial surface activates endothelial cells and initiates synthesis of protein mediators of inflammation. Monocytes attracted into the vessel wall are transformed into macrophages, which incorporate oxidized lipoproteins, becoming lipid-laden foam cells.
nongenomic mechanisms. Estrogen regulates the transcription of numerous genes, and its cellular actions are mediated through the translation of specific mRNA transcripts and synthesis of proteins. Nongenomic responses to estrogen occur more rapidly and do not rely on the activation of specific gene products [28].

An important transcriptional target of estrogen is the nitric oxide synthase (NOS) gene. Estrogen stimulates the constitutive synthesis of NO in numerous tissues, including blood vessels, heart, uterus, and skeletal muscle. Both pregnancy and estrogen therapy enhance neuronal (NOS-I) and endothelial (NOS-III) NOS expression, whereas the inducible NOS isoform (NOS-II) is unaffected [29]. Furthermore, the 5'-flanking region of the gene for NOS-III contains transcription factor-binding sites for estrogen [30]. Thus, estrogen regulates the activity of the NOS-III isoform by genomic mechanisms.

Reduction in vessel wall concentration of oxidized LDL may improve NO synthesis, release, and biological activity. Reduction in levels of Lp(a) could also enhance NO bioavailability if concentrations of oxidized Lp(a), which can also inhibit NO bioactivity [31], were reduced in the vessel wall. HDL has been shown to protect LDL from oxidation [32], and, if in proximity to LDL within the vessel wall, could indirectly augment NO bioactivity via its antioxidant effect on LDL. In addition to reducing circulating LDL levels, estrogen protects LDL from oxidation by reducing the vessel wall concentration of oxidized LDL.

Meanwhile, estrogen may also augment the bioactivity of NO independent of lipoprotein effects. Estrogen may directly stimulate the release of NO as shown in endothelial cells in culture [34–36]. The rapid effects of estrogen on stimulation of NOS activity could be mediated by a known estrogen receptor, perhaps located in the plasma membrane [37] and able to activate NOS rapidly in a nongenomic manner. ERα can directly activate endothelial NOS (eNOS), perhaps through a tyrosine kinase pathway or the mitogen-activated protein kinase signaling pathway [38]. In this regard, one recent paper provided evidence that activation of eNOS in endothelial cells by ERα involves the phosphatidylinositol 3 (PI3)-kinase-Akt pathway [39]. They demonstrated inhibition of estrogen-induced NO release by a pharmacological inhibitor of PI3-kinase and showed that 17β-estradiol (E2) and E2-BSA increased the phosphorylation of both Akt and eNOS. Another study demonstrated Akt in the activation of eNOS by estrogen and provided substantial additional insight into the signal transduction pathway involved in ERα–eNOS activation [40]. They observed a direct interaction of ERα with the p85 regulatory subunit of PI3-kinase. Of interest, a recent study presented localization of the functional ERα–eNOS signaling complex to endothelial cell caveolae, the membrane microdomains well-known to be enriched in many signaling molecules [41]. These investigators provided immunological evidence that ERα was expressed in caveolae using both amino- and carboxy-terminal domain. Taken together, these three recent studies allow construction of a model of this interesting new signal transduction pathway that now can be tested and further refined [42] (Fig. 2). These rapid effects do not require changes in gene expression but may involve proteins that interact with the ER, such as heat-shock protein 90, which also binds to and

![Fig. 2. Composite model of nongenomic, ER-mediated activation of eNOS, based on published data [39–41]. These recent studies show immunological localization of ERα to caveolae, direct interaction of ERα with the p85 subunit of PI3-kinase, and activation of the PI3-kinase-Akt-eNOS pathway. However, many issues remain unresolved, in particular, including whether the ERα hormone-binding domain is actually inside or outside the membrane, how this ERα is tethered to the caveolae, and the precise primary sequence of this membrane-associated ER. For details, see text. Used with permission from Dr Mendelsohn [42].](https://academic.oup.com/cardiovascres/article/55/4/714/306935)
activates eNOS [43]. Estrogen rapidly causes coronary vasodilatation ex vivo [27,44] and in vivo in cholesterol-fed ovariectomized primates [45] and other animals [46]. The short-term coronary vasodilatory effects of estrogen in humans are largely mediated by the increased production of NO [47].

Augmented bioactivity of NO by estrogen either indirectly by its effect on lipoprotein levels and protection of LDL from oxidation, or directly by effects on NO synthesis and release, might account not only for enhancement of endothelium-dependent vasodilation following estrogen administration to postmenopausal women [48–50] but also for much of the anti-atherogenic effects of estrogen by inhibition of platelet aggregation, platelet and inflammatory cell attachment to the endothelial surface of the vessel wall, and release of factors that stimulate growth and migration of smooth muscle cells within the vessel wall [51].

4. Biological effects of estrogen

4.1. Effects of estrogen on lipoproteins

Orally administered estrogens lower serum levels of low-density lipoprotein (LDL) cholesterol and raise levels of high-density lipoprotein (HDL) cholesterol each by approximately 15% and raise levels of triglyceride by approximately 20–25% in postmenopausal women [13]. The route of administration of estrogen influences its effects on serum lipids. Transdermally administered estrogens have less of an effect on serum lipid concentrations than do orally administered estrogens. Co-administration of a progestin can blunt the changes in serum lipids due to estrogen [13,52]. Human and animal studies show that a decrease in the total LDL to HDL cholesterol ratio enhances the clearance and metabolism of cholesterol esters. Indeed, autopsy examination of the hearts of 113 men with coronary disease who had died suddenly revealed that an elevated total LDL/HDL cholesterol ratio was associated with rupture of vulnerable plaques [53]. We demonstrated that 0.625 mg of conjugated equine estrogens (CEE) significantly reduced total LDL/HDL cholesterol ratio compared with pretreatment levels in postmenopausal women [54,55]. ERT has also been shown to reduce serum levels of Lp(a) [54–56], a lipoprotein that has structural features of LDL and plasminogen believed to be proatherogenic and anti-thrombolytic, and that increases in serum concentration following menopause [57]. These favorable changes were also seen in a recent randomized, double-blind, placebo-controlled trial [58] using lower doses of HRT than commonly prescribed. For example, the increase in HDL in women treated with 0.45 mg/day CEE plus 1.5 mg/day medroxyprogesterone acetate (MPA) after 1 year was similar to that seen with 0.625 mg/day plus 2.5 mg/day MPA. Lp(a) was also significantly reduced from baseline.

4.2. Effects of estrogen on LDL oxidation

Experimental evidence strongly indicates that LDL, especially following oxidative modification within the vessel wall by free radical molecules [59], may significantly affect endothelial NO production and bioactivity [60]. Therapeutic reduction in circulating LDL levels presumably reduces the vascular wall transit and extracellular matrix entrapment of these particles, which might reduce tissue concentrations of oxidized LDL.

E₂ has antioxidant effects in vitro [61]. However, it remains unclear whether in vitro antioxidant effects of estradiol are physiologically important, since in most studies very high concentrations of estrogen were used. Although Sack et al. [33] demonstrated that both long- and short-term administration of physiologic concentrations of E₂ decreased the oxidation of LDL cholesterol, in our recent study examining the effects of CEE, we did not find any protection of LDL from oxidation following 1 month of administration to 30 women [52]. A larger study in 56 women also found no change in LDL oxidation using CEE, among other forms of ERT and HRT [62]. In contrast, Wilcox et al. [63] found significant inhibition in vivo of LDL oxidation by estrone sulfate, equilin sulfate, and 17α-dihydroequilin sulfate in postmenopausal women. In vitro, CEE was effective in inhibiting both fatty acid and LDL oxidation [61].

CEE may conceivably lack an antioxidant effect because it is comprised primarily of equine estrogens: the one human-like estrogen contained in this preparation (estrone) is a weaker antioxidant than E₂ [61]. Although some estrone is converted to E₂, the serum levels of this antioxidant form of estrogen are approximately one-third the levels achievable by direct administration of E₂.

Of related interest, ERT had no effect on LDL oxidizability in postmenopausal women with type II diabetes mellitus [64]; however, plasma levels of HDL cholesterol, apolipoprotein (apo) A-I, LDL cholesterol, apoB, and glycated hemoglobin were improved, indicative of a better metabolic control. Since 19% of participants in the Heart and Estrogen/progestin Replacement Study (HERS) were diabetic, the lack of an inhibition of LDL oxidation may be one reason why HRT users did not benefit in the first year of treatment [11]. Indeed, we observed that the effects of ERT on endothelial function-vascular dilator and other homeostatic functions were less apparent in type II diabetic postmenopausal women, despite beneficial effects of estrogen on lipoprotein levels [65].

4.3. Effects of estrogen on vasomotion

Endothelium-dependent vasodilatation has been studied in both the coronary and peripheral circulation. Williams and
colleagues [66] have demonstrated that when oophorectomized monkeys are fed a high-lipid diet, acetylcholine causes coronary vasoconstriction when infused into the coronary vessels, suggesting loss of endothelial cell function. When these monkeys received ERT, however, the infusion of acetylcholine caused vasodilation, as it does in normal animals. Indeed, E2 infused into the left coronary arteries of 20 postmenopausal women enhanced acetylcholine-stimulated increases in coronary flow [48]. This effect of estrogen on improvement of vasomotion may be due to potentiation of the activity of NO [47] and prostacyclin synthase [28] and decrease in potent vasoconstrictor endothelin-1 levels [46,67,68]. Finally, E2 infused into the brachial arteries of postmenopausal women enhanced acetylcholine-stimulated forearm blood flow [49].

We found that CEE 0.625 mg administered to 28 hypercholesterolemic postmenopausal women improved flow-mediated dilation comparable to the effect of simvastatin 10 mg daily, despite greater reduction in LDL cholesterol levels with simvastatin [54]. Lieberman et al. [50] reported that oral E resulted in significant improvement in flow-mediated brachial artery dilation compared with placebo. Hashimoto et al. [69] observed that flow-mediated brachial artery diameter was greater during the follicular or luteal phase (when serum E2 level was high) than during the menstrual phase (when serum E2 level was low) in 17 premenopausal women. Gerhard et al. [70] observed that intravaginal MP added to E2 did not significantly attenuate the improvement in flow-mediated dilation that was observed with E2 alone. In contrast, Sorensen and coworkers [71] reported that cyclical E2 and norethisterone did not improve endothelial function. We observed that CEE 0.625 mg combined with MP or MPA improved flow-mediated brachial artery dilator response to hyperemia in 20 healthy postmenopausal women [72]. Of interest, Vehkavaara et al. [73] reported that oral E2-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.

In summary, although few studies observed no effect of estrogen on endothelium-dependent vasodilation, most studies reported that oral estrogen increased endothelium-dependent vasodilation, which contributes to the prevention of CVD in postmenopausal women.

### 4.4. Effects of estrogen on inflammation

The gene transcription of many adhesion molecules, including vascular CAM-1 (VCAM-1) and intercellular CAM-1 (ICAM-1), is regulated by a nuclear transcription factor, NF-kB, normally maintained in an inactive state [74]. In addition to VCAM-1, ICAM-1, and E-selectin, NF-kB also activates transcription of genes encoding chemoattractant factors, such as monocyte chemotactic peptide and macrophage stimulatory factor, that attract monocytes into the vessel wall (Fig. 3). NF-kB additionally increases the synthesis and release of cytokines such as interleukin (IL)-1, IL-2, and IL-6, which activate inflammatory cells, enhancing their attachment to the vessel wall [75]. Harnish et al. found that E2 bound to the ERα antagonizes NF-kB activity in human hepatoma HepG2 cells [76].
4.4.1. Cell adhesion molecules

Oxidized LDL and its membrane components have been shown to induce the expression of inflammatory CAMs on the endothelial cell surface [77]. Serum concentrations of vascular CAM-1 (VCAM-1), intercellular CAM-1 (ICAM-1), and L-selectin, also an adhesion molecule, have been reported to be higher in patients with coronary artery disease than in healthy controls [78,79]. Moreover, men in the Physician’s Health Study with the highest quartile of ICAM-1 levels were found to be at greater cardiovascular risk than men in the lowest quartile [80]. Plasma concentrations of ICAM-1 increased with increasing prevalence of usual cardiovascular risk factors in healthy men [81].

The selectin family of adhesion molecules, which includes L-selectin and E-selectin, binds to carbohydrate ligands on leukocytes and promotes ‘rolling’ of these cells—the first step in adhesion—on activated endothelium prior to the firm adherence of ICAM-1 and VCAM-1, with subsequent incorporation into the vessel wall. The pathophysiological relevance of E-selectin in humans has been suggested by its localization in atherosclerotic plaques [82], higher levels of E-selectin in patients with coronary artery disease or carotid artery atherosclerosis relative to controls, and correlation of E-selectin levels with carotid artery wall thickness by ultrasound [83].

Conflicting findings have been reported from cell culture studies regarding the effect of estrogen on CAM expression. E₂ pretreatment for 48 h was found to inhibit interleukin-1-induced expression of CAMs in endothelial cell cultures by Caulin-Glaser and colleagues [84]. Cid et al. [85], however, found E₂ increased the expression of CAMs on endothelial cells in culture during simultaneous stimulation by tumor necrosis factor (TNF)-α, with increased adherence to mononuclear cells.

Studies of the effects of HRT on CAMs in postmenopausal women have been promising. Koh et al. [86] first reported the effects of either transdermal E₂ or oral E₂, or transdermal E₂ and oral MPA on inflammatory CAMs in postmenopausal women, which lowered CAM levels from the baseline. In a randomized, double-blind, crossover study, 6 or 8 weeks of treatment with CEE alone or combined with micronized progesterone (MP) or MPA significantly diminished E-selectin, ICAM-1, and VCAM-1 expression compared with baseline (Fig. 4) [54,55,72]. The Postmenopausal Estrogen/Progesterin Interventions (PEPI) trial confirmed the reduction of E-selectin by HRT [87]. Serum concentrations of E-selectin, ICAM-1, and VCAM-1 have been reported to be higher in postmenopausal women with coronary artery disease not taking HRT than postmenopausal women with coronary artery disease taking HRT at the time of cardiac catheterization [88]. Several recent papers reported the same observations [89,90].

In order to identify a mechanism for the CEE treatment effects on CAMs, Koh et al. [54,55] assessed correlations between changes in levels of E-selectin and changes in LDL cholesterol, Lp(a), and HDL cholesterol levels based on experimental studies showing stimulatory (LDL [91], Lp(a) [92]) or inhibitory (HDL) [93] effects of these lipoproteins on CAM expression. However, no significant or consistent correlations were determined. We speculate that the inhibitory mechanisms of estrogen on expression of CAMs are multifactorial: increase in NO and HDL cholesterol, decrease in LDL cholesterol and Lp(a), protection of LDL from oxidation, and direct inhibition of NFκB activation (Fig. 3).

4.4.2. Monocyte chemoattractant protein-1

Some cell culture and animal studies observed that E₂ inhibited the increase in monocyte chemoattractant protein (MCP)-1 mRNA expression [94,95]. We recently observed that CEE with MP or MPA significantly decreased MCP-1 levels from baseline values in healthy postmenopausal women (P<0.005 by ANOVA) (Fig. 4) [72,96], which were consistent with a preliminary study by Stork et al. [97]. As to its clinical significance, restenotic patients had statistically significant (P<0.0001) elevated levels of MCP-1 compared with nonrestenotic patients after coronary angioplasty [98]. Stable and unstable angina patients had statistically significant (P<0.001) elevated levels of MCP-1 compared with controls, particularly higher levels in unstable angina than in stable angina [99]. Moreover, increased MCP-1 was significantly correlated with increased monocyte activity, as reflected by enhanced O₂ generation [98,99].

4.4.3. C-reactive protein

 Messenger cytokines such as IL-1β, IL-6 and TNF-α, which are released from macrophages and other activated cells within the vessel wall during an inflammatory response, enter the circulation and have biological effects at a distance from the site of inflammation, including the activation of genes within hepatocytes that encode specific proteins (Fig. 5). These acute phase reactants include fibrinogen, C-reactive protein (CRP), and serum amyloid A [100]. CRP may induce the synthesis of cytokines, CAMs, and tissue factor in monocytes and endothelial cells [100–102]. Tissue factor activates the extrinsic coagulation cascade, providing a link between inflammation and thrombosis. In addition, CRP may contribute to atherogenesis by facilitating uptake of LDL by macrophages, thus accelerating foam cell formation [103].

Ridker et al. [104] reported the predictive value of CRP in determining the risk of future cardiovascular events in 122 apparently healthy participants in the Women’s Health Study who subsequently suffered a first cardiovascular event during a 3-year follow-up period. They found that women who developed cardiovascular events had higher baseline CRP levels than control subjects. In another study [105], median CRP levels were twice as high among women taking HRT than among women not taking HRT (0.27 vs. 0.14 mg/dl; P=0.001) in 493 healthy post-
menopausal women. The PEPI Study showed that HRT in response to both transient myocardial ischemia and reperfusion [113], and persistent overexpression of TNF-α after ischemia might lead to adverse coronary outcomes [114]. TNF-α levels increased acutely with coronary ischemia [115], and plasma levels of TNF-α were persistently elevated among post-myocardial infarction patients at increased risk for recurrent coronary events [116]. TNF-α was expressed in donor heart cardiac myocytes and predicted right ventricular failure early after human heart transplantation [117].

4.4.4. Tumor necrosis factor-α

TNF-α is a multifunctional circulating cytokine derived from endothelial and smooth muscle cells as well as macrophages associated with coronary atheroma (Fig. 5). Further, TNF-α enhances the rate of monocyte recruitment into developing atherosclerotic lesions [111]. TNF-α is involved in several cardiovascular processes. The association of accumulation of LDL in rat arteries and activation of TNF-α expression suggests a possible mechanism for the inflammatory response in the early stages of atherosclerosis [112]. TNF-α is upregulated in the myocardium in response to both transient myocardial ischemia and reperfusion [113], and persistent overexpression of TNF-α after ischemia might lead to adverse coronary outcomes [114]. TNF-α levels increased acutely with coronary ischemia [115], and plasma levels of TNF-α were persistently elevated among post-myocardial infarction patients at increased risk for recurrent coronary events [116]. TNF-α was expressed in donor heart cardiac myocytes and predicted right ventricular failure early after human heart transplantation [117].

Experimentally, estrogen blocks monocyte/macrophage production of TNF-α by decreasing the activity of Jun NH₂-terminal kinase [118]. We observed that CEE with MP or MPA significantly reduced TNF-α levels from the baseline in hypertensive or overweight postmenopausal women and, furthermore, patients with the highest baseline of TNF-α levels showed the greatest extent of reductions [119]. Our observation was consistent with the findings of Walsh et al. [108].

Fig. 4. Soluble E-selectin (A), vascular cell adhesion molecule (VCAM-1) (B), intercellular adhesion molecule (ICAM-1) levels (C), and monocyte chemoattractant protein (MCP)-1 levels before therapy (Baseline) and following micronized progesterone (CEE+MP) or medroxyprogesterone acetate (CEE+MP A) combined with conjugated equine estrogen (CEE). Both therapies significantly decreased E-selectin, VCAM-1, ICAM-1, and MCP-1 levels from baseline values (P<0.001, P=0.016, P=0.048, and P<0.005 by ANOVA, respectively) by a similar degree. Used with permission from Koh et al. [72].
Fig. 5. Cytokines released from the injured artery initiate hepatic synthesis of several proteins (acute phase reactants), and inhibit the synthesis of others. Some acute phase reactants may have effects on the arterial segment and contribute to the inflammatory response.

4.4.5. Interleukin-6

Sukovich et al. [120] demonstrated that IL-6 mRNA and protein were expressed in the atherosclerotic plaques of apoE-knockout mice aortas; treatment of male apoE-knockout mice with E₂ for 3 weeks resulted in a statistically significant 50% reduction (P<0.01) in IL-6 secretion from ex vivo aortic tissue segments, suggesting the antiatherosclerotic effects of E₂. The effects of ERT or HRT on soluble IL-6 levels in postmenopausal women are inconsistent. Some studies observed the increase in IL-6 levels [90,107], whereas our study and others observed no significant changes [106,108].

In summary, the effect of HRT on inflammation in postmenopausal women varies because orally administered HRT increases the levels of CRP, and, on the other hand, decreases levels of the soluble CAMs, MCP-1, TNF-α that may contribute to the risk of CVD (Table 1). Although likely a first pass effect of orally administered estrogen on the hepatic synthesis of CRP, elevated CRP could have deleterious effects on vascular inflammation, as discussed previously, and may have contributed to the unexpected increase in myocardial infarction risk within the first year of treatment in the HERS [11]. The response of CRP only to oral route preparation is similar to the response of plasminogen activator inhibitor (PAI)-1, which decreased only with oral estrogen compared with transdermal estrogen, as reported by Koh et al. [52] and others [89,121].

4.5. Clinical implications

The determination of endothelial dysfunction has been found to be a sensitive and specific screening test to predict the presence of CVD [122–124]. Gaeta et al. [123] reported that the offspring of patients with premature myocardial infarction had lower flow-mediated dilation compared with the control subjects. Vascular inflammation plays an important role in the pathogenesis of atherosclerosis and may contribute to increase the risk of myocardial infarction and stroke. Plasma levels of inflammatory markers were increased and correlated with the extent of disease in patients with atherosclerosis of the coronary and peripheral arteries [125]. Hingorani et al. [126] demonstrated that acute systemic inflammation with Salmonella typhi vaccine impaired endothelium-dependent dilation in humans. Also of interest, Raza et al. [127] reported that flow-mediated dilation was significantly impaired in adults with primary systemic necrotizing vasculitis. Furthermore, suppression of inflammation re-
Table 1

Effects of estrogen on inflammation

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<tr>
<th>Cell Adhesion Molecules (CAMs)</th>
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<tr>
<td>Inhibit IL-1 induced expression of CAMs</td>
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<td>Increase TNF-α-induced expression of CAMs</td>
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<td>Not change soluble IL-6 in healthy women</td>
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* Transdermal estrogen.

stored and normalized impaired endothelial function in these patients.

Accordingly, therapies that improve endothelium-dependent dilation and reduce vascular inflammation may reduce cardiovascular risk. ERT or HRT improves endothelium-dependent dilation in postmenopausal women. However, the effects of ERT or HRT on inflammation in postmenopausal women are divergent: orally administered estrogen increases the levels of CRP, but decreases levels of the soluble CAMs, MCP-1, and TNF-α. Reduction of soluble cell adhesion molecules, chemokines, and cytokines might protect against atherosclerotic development and progression in women.

So, where are we left at present with regard to HRT and CVD in postmenopausal women? Perhaps the most important current need is greater appreciation of the complexities associated with understanding the cardiovascular effects of steroid hormones. Although we await the results of large, prospective, randomized trials, such as the Women’s Health Initiative (WHI) and Women’s International Study of Long-Duration Oestrogen After the menopause (WISDOM), many observational studies support the conclusion that the use of HRT has cardiovascular benefit in healthy postmenopausal women (primary prevention) [128]. 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, vasomotion, and hemostasis 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 [129,130]. 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.

Other randomized, placebo-controlled trials are under way and hopefully will answer many of the questions and concerns raised regarding HRT in postmenopausal women with coronary artery disease. The Women’s Lipid Lowering Heart Atherosclerosis Trial (WELL-HART) and Women’s Atherosclerosis Vitamin / Estrogen Trial (WAVE) are secondary prevention trials and will be completed soon. Until the results of these trials are available, elderly women with coronary artery disease should not be started on HRT, but may be left on previously prescribed HRT, as adverse events seemed to occur relatively soon after initiation of therapy [12]. Instead, lipid-lowering therapy with statins, aspirin, or angiotensin-converting enzyme inhibitors may provide morbidity and survival benefit, as suggested by the clinical trials [131,132]. If women want to take HRT, the combination of statin with estrogen may attenuate the potential harmful effects of estrogen therapy in postmenopausal women, and maximize any benefit to cardiovascular risk [54,106,133].
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