Estrogen replacement therapy, atherosclerosis, and vascular function

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Abstract

There is strong evidence from both human and nonhuman primate studies supporting the conclusion that estrogen deficiency increases the progression of atherosclerosis. More controversial is the conclusion that postmenopausal estrogen replacement inhibits the progression of atherosclerosis. Estrogen treatment of older women (>65 years) with pre-existing coronary artery atherosclerosis had no beneficial effects. In contrast, estrogen treatment of younger postmenopausal women or monkeys in the early stages of atherosclerosis progression has marked beneficial effects. Whether progestogens attenuate the cardiovascular benefits of estrogen replacement therapy has been controversial for more than a decade. Current evidence from studies of both monkeys and women suggest little or no attenuation of estrogen benefits for coronary artery atherosclerosis. Lack of compliance with estrogen replacement therapy, usually because of fear of breast cancer, remains a major problem. Future regimens may overcome that fear by the co-administration of a breast cancer preventive agent (i.e., selective estrogen receptor modulators, phytoestrogens) with low dose estrogen. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

The cardiovascular diseases remain the largest cause of morbidity and mortality among postmenopausal women in westernized societies. The advantages/disadvantages of estrogen replacement following menopause are public health issues of major importance, given the rapidly increasing numbers of postmenopausal women and the fact that the average life span now extends to about 35 years beyond normal ovarian function. A decade ago, a consensus had emerged that postmenopausal estrogen replacement markedly reduced the burden and mortality associated with coronary heart disease. Today, many question that consensus because two randomized prospective trials with coronary heart disease patients failed to find benefits from estrogen replacement.

In this review, we attempt to place in context the comparative, experimental, and clinical data on estrogen replacement therapy and cardiovascular disease. Particularly, we make an effort to interpret the negative clinical trials in view of observational and experimental data. Further, we present our best thoughts about future directions.

2. What is the evidence that endogenous estrogen modulates cardiovascular disease?

Among premenopausal women, coronary heart disease is extremely rare, even in high-risk populations [1], and the overall incidence of cardiovascular complications is much lower in premenopausal women than in men of similar age [2]. After menopause, the reduced risk for coronary heart disease in women is gradually lost, and this female
protection is more accurately characterized as a delay in disease onset, with the incidence curve for women lagging behind that of men 5–10 years [3]. The most plausible explanation for these observations is the protective effect of endogenous female sex steroids, particularly estrogens, during premenopausal years. This is supported by the initial Framingham Study, which showed that the cardiovascular incidence rates were lower in premenopausal women [4]. Although the number of person–years during the 20 years of observation in this study was nearly the same for premenopausal and postmenopausal status, there were significantly more cardiovascular events among postmenopausal women compared to premenopausal women of the same age.

In Western populations, the average age at menopause is about 51 years [5]. Premature menopause, natural or surgical, with reduced lifetime exposure to endogenous estrogens, should result in increased risk of coronary heart disease based on the assumption that estrogen is cardioprotective. Most of the early studies in women who experienced an early menopause show increased risk of heart disease; nevertheless, some of the results are inconsistent and not statistically significant [2]. Potentially, this could be due to small sample size and variability in the study settings in some of the early investigations. The Framingham Study reported that women experiencing natural menopause between ages 45 and 49 years or a surgical menopause between ages 40 and 44 years were more likely to develop coronary heart disease than premenopausal women of the same age [4]. The Nurses’ Health Study found no significant association between time since natural menopause and cardiovascular disease, but found increased risk of cardiovascular disease in women with bilateral oophorectomy without estrogen replacement compared to oophorectomized women receiving estrogen replacement therapy [6]. However, a more recent evaluation of the Nurses’ Health Study, where analysis was restricted to naturally postmenopausal women who had never used estrogen replacement, found an overall significant association between younger age at menopause and higher risk of coronary heart disease [7]. Similar results were reported in a study with a 29-year follow-up of postmenopausal women, assessing the relationship between age at natural menopause and mortality from ischemic heart disease [8]. Another study investigating a cohort of postmenopausal women aged 50–65 years at enrollment and followed up to 20 years, showed that the risk of cardiovascular mortality was higher for women with early menopause than for those with late menopause [9].

Recently, in a population-based study, Joakimsen et al. found a significant inverse relationship between the age at menopause and both the prevalence and extent of carotid atherosclerosis assessed by ultrasound in 2588 postmenopausal women [10]. Therefore, a substantial body of evidence suggests that endogenous estrogen in premenopausal women provide protection against cardiovascular disease.

3. What is the evidence that estrogen replacement therapy modifies the progression of atherosclerosis?

3.1. Observational data

A large body of observational data suggests that women using postmenopausal estrogen replacement therapy (ERT) have lower risk for coronary heart disease [11,12]. The most comprehensive observational study is the Nurses’ Health Study published first in 1985 [13] and updated in 1991 [14], 1996 [15], and recently in 2000 [16]. The Nurses’ Health Study began in 1976 when 121,700 female nurses 30–55 years of age completed a mailed questionnaire about their postmenopausal hormone use and medical history. In the latest report, with 70,533 postmenopausal women followed for up to 20 years, were identified 953 nonfatal myocardial infarctions, 305 coronary deaths, 767 strokes (432 ischemic, 174 hemorrhagic, and 161 other or unspecified type), and 119 deaths due to stroke [16]. Overall, current use of hormone replacement therapy was associated with relative risk for major coronary event of 0.61 (confidence interval (CI), 0.52–0.71) when adjusted for age and the common cardiovascular risk factors (Fig. 1). Surprisingly, longer duration of hormone use was associated with less coronary benefit than short-term use; the relative risk increased from 0.4 for less than 1 year of current use to 0.7 for 10 or more years. As in previous reports from the Nurses’ Health Study, risk for ischemic stroke, but not hemorrhagic stroke, was increased slightly among hormone users compared with never-users (relative risk, 1.26; CI, 1.00–1.61).

Although the evidence from the observational studies indicating beneficial effect of ERT on coronary heart disease is consistent, there are several potential sources of bias that might account for these findings. Women who take estrogen tend to be more educated and of higher social class, and have more favorable lifestyles, reduced levels of several heart disease risk factors, and less diabetes than untreated women [12]. Matthews et al. reported that women who elect to take estrogen after the menopause had more favorable levels of multiple coronary risk factors before the menopause than women who chose not to take estrogen [17]. Thus, some of estrogen’s putative benefits might be spurious, reflecting ‘a healthy woman effect’. Women who take estrogen are also an unusually compliant subset of all women [18], and in randomized, double-blind, clinical trials, good compliance has been shown to reduce risk of coronary events 40–60%, even when the medication is placebo [19,20]. Obviously, adherence to placebo treatment is not causing this benefit but is a marker for unconsidered factors that result in better coronary outcomes. Thus, randomized, placebo-controlled
Fig. 1. Multivariate adjusted relative risk for coronary heart disease±95% confidence interval (CI). Adjusted for age, body mass index, history of diabetes, hypertension, high cholesterol level, age at menopause, cigarette smoking, and parental history of premature heart disease [16].

studies are needed to confirm the beneficial effects of estrogens on the cardiovascular system shown by the observational studies.

3.2. Randomized studies with non-human primates

To overcome the general problems faced with the observational human studies as described above, and the fact that a truly randomized, placebo-controlled, ERT trial in women is difficult to carry out in practice [21], the surgically menopausal female cynomolgus macaque monkey model is useful in evaluating the cardioprotective aspects of ERT [22]. This animal model is often used since these monkeys share with humans DNA greater than 90% homology, and their hormonal profile resemble those of women; distinct menarche, 28-day menstrual cycles, and menopause. Premenopausal macaques also have higher high-density lipoprotein (HDL) cholesterol levels than do male macaques, and females develop fewer coronary artery atherosclerotic lesions. Likewise, postmenopausal cynomolgus macaques have decreased HDL cholesterol concentrations compared to their premenopausal counterparts and increased progression of coronary artery atherosclerosis. Surgical menopause, combined with a moderately atherogenic diet, produces progressive atherosclerosis that is responsive to ovarian hormone replacement.

Direct evidence for a beneficial effect of ERT on progression of coronary artery atherosclerosis was found in the results of a study from Adams et al. [23]. In this study, ovariectomized monkeys that were treated for 30 months with parenterally administered 17β-estradiol showed approximately 50% reduction in coronary artery atherosclerosis compared to the control animals. These findings were confirmed in a subsequent 30-month study evaluating the effects of continuous oral conjugated equine estrogen (CEE) treatment on coronary artery atherosclerosis [24]. Oral CEE treatment caused a 72% reduction in coronary artery plaque size relative to untreated, estrogen-deficient controls. These studies strongly support the observational findings that estrogens are cardioprotective.

4. Are the cardiovascular benefits of estrogen replacement therapy altered by the stage of atherosclerosis?

Results from two randomized prospective secondary prevention trials are challenging the conviction that postmenopausal ERT provides cardiovascular benefits. The first, the Heart and Estrogen/Progestin Replacement Study (HERS), determined whether daily treatment with 0.625 mg CEE and 2.5 mg medroxyprogesterone acetate (MPA) would reduce coronary heart disease events in postmenopausal women [25]. This study recruited 2763 women who were generally beyond 65 years of age and had pre-existing coronary heart disease. No reduction in coronary events could be shown by the hormone treatment during an average of 4.1 years of follow-up. In fact, during the first year of the trial, there was an increase in events (relative risk, 1.52; CI, 1.0–2.29), mostly seen in the first 4 months. After 2 years of treatment, there was a tendency toward risk reduction, strongest in the 4th year (relative risk, 0.67; CI, 0.43–1.04). It remains to be seen whether continuation of the trial beyond the four years will reveal significant reduction of coronary events in the treatment group.

In the second study, The Estrogen Replacement Atherosclerosis (ERA) Trial, Herrington and colleagues examined
the effect of postmenopausal hormone therapy on the progression of existing coronary artery atherosclerosis as assessed by angiography [26]. The average age of the 309 women in the study was 65 years, and half had experienced a previous myocardial infarction. After 3.5 years of daily treatment with 0.625 mg CEE alone, CEE combined with 2.5 mg MPA, or placebo, repeated angiography did not detect any differences in disease progression between any of the treatment groups.

The Women's Health Initiative (WHI) is a large, ongoing, randomized study evaluating the effects of hormone replacement therapy in postmenopausal women [27]; this study is scheduled for completion in 2005. Although WHI recruited generally healthy postmenopausal women with the intention to evaluate primary prevention, many of the subjects are relatively older women who began treatment years after menopause. Recently, the women in WHI were informed by the Data and Safety Monitoring Board that “during the first 2 years there was a small increase in the number of heart attacks, strokes, and blood clots in women taking active study pills compared to inactive placebo pills”, but recommended that the study continue. Thus, the preliminary data from the first years of WHI are similar as seen in the 1st year of HERS [25].

In attempting to understand the incongruity between some of the observational data on human subjects, the results of HERS and ERA, the preliminary data of WHI, and the data on non-human primates, one must sharpen the definition of ‘primary prevention’ and ‘secondary prevention’. These terms have different meanings for vascular biologists and cardiologists. To the vascular biologists, primary prevention of atherosclerosis means to prevent the progression of artery wall fatty streaks to atherosclerotic plaques. As cardiologists have used the term, primary prevention means to interfere with the progression of complicated atherosclerotic plaques to clinical coronary heart disease events. Clinical trials with coronary event endpoints can include subjects that vary markedly at the vascular wall level. Thus, it is possible that the 1st year results of HERS, as well as the preliminary data of WHI, are due to subgroups of women that have more complicated coronary atherosclerosis, perhaps susceptible to some yet to be defined effect of estrogen on plaque remodeling and destabilization, leading to propensity of plaque rupture and thrombosis.

In reconsidering several of the studies with cynomolgus monkeys, we arbitrarily divided them into three life stages (Fig. 2). In life stage 1 the monkeys had little or no atherosclerosis before being made surgically menopausal. Immediately after ovariectomy, CEE treatment was given together with the atherogenic diet. In studies that were carried out with monkeys at life stage 1, there was an average inhibition of coronary artery atherosclerosis of about 70% [24,28,29]. In life stage 2, the monkeys were allowed to develop a moderate amount of atherosclerosis premenopausally before they were made surgically menopausal. CEE treatment was given immediately after ovariectomy and the atherogenic diet continued. In that situation the degree of inhibition of coronary artery atherosclerosis was reduced from 70 to 50% [30]. Finally, in the life stage 3, the animals had little or no atherosclerosis premenopausally, were ovariectomized to make them surgically menopausal, were given an atherogenic diet, but there was a delay of 2 years (equivalent to approximately six years in human life) before CEE treatment was started. Given that postmenopausal delay in initiating the estrogen replacement therapy, no inhibition in the extensiveness of coronary artery atherosclerosis was observed [31]. Taken together, these studies would suggest that estrogens have beneficial effects in the early stages of

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**Fig. 2.** The relation of pre- and post-menopausal conditions to the degree of, or lack of, inhibition of coronary artery atherosclerosis [24,28,30,31]. OVX, ovariectomy; and CEE, conjugated equine estrogen.
atherogenesis but have little or no beneficial effects in the final stages of plaque complications, instability and coronary heart disease events.

5. Are the cardiovascular benefits of estrogen replacement therapy altered by progestogens?

Coadministration of a progestogen with ERT is necessary for postmenopausal women with an intact uterus to prevent endometrial hyperplasia and cancer [32]. The influence of added progestogens on the risk for cardiovascular disease is still subject to debate. The relatively few observational studies that evaluate treatment with estrogen combined with progestogen on the risk for coronary heart disease show comparable protective effects as with unopposed estrogen [15,16,33,34]. In most of these studies CEE was combined with MPA in sequential regimens, but also estradiol combined with levonorgestrel was shown to reduce the risk of acute myocardial infarction as effectively as CEE alone [33]. The recent update on The Nurses’ Health Study shows similar risk reduction for coronary heart disease among women taking CEE alone (relative risk, 0.55; CI, 0.45–0.68) and those taking CEE plus MPA (relative risk, 0.64; CI, 0.49–0.85) [16]. These results are consistent with those in the previous report from this study [15]. However, there was a 45% higher risk for stroke among women taking CEE plus MPA than in those who had never taken hormone therapy (relative risk, 1.45; CI, 1.10–1.92), while little association was demonstrated between stroke and the use of CEE alone (relative risk, 1.18; CI, 0.95–1.46) [16]. Because of the rather wide confidence intervals it is unclear whether this is a real difference.

After the null effect of CEE plus MPA on the secondary prevention of coronary heart disease in HERS [25], it was further suggested that continuous MPA could attenuate the favorable effects of estrogen on atherosclerosis. The possibility of distinction between different types of progestogens, and sequential versus continuous progestogen treatment, is intriguing. In a study with cynomolgus monkeys receiving no treatment, continuous estradiol, or continuous estradiol plus cyclically administered progestrone (28 days on, 28 days off) for 30 months, animals in both treatment groups exhibited approximately one half of the amount of coronary artery atherosclerosis as compared with control animals [23]. In this study, the degree of atherosclerosis with combined treatment was not significantly different from that with estradiol alone, suggesting that progesterone did not attenuate the atheroprotective effects of estradiol (Fig. 3a). Another study with monkeys investigated the effect of continuous oral CEE (human equivalent to 0.625 mg/day), MPA (human equivalent to 2.5 mg/day), or CEE continuously combined with MPA, as opposed to no treatment [24]. After 30 months, opposed CEE treatment resulted in a 72% reduction in coronary artery atherosclerosis compared to controls, while animals that received MPA, or CEE plus MPA did not differ significantly from controls (Fig. 3b). Plasma lipoprotein concentrations were affected by treatments; however, this did not account for the differential effects of CEE as opposed to CEE plus MPA on coronary artery atherosclerosis. In a recent study by Clarkson et al. [29] where monkeys were treated for 24 months with CEE or CEE plus MPA in a similar manner as in the previous study [24], there was approximately 62% reduction in coronary artery atherosclerosis in both treatment groups compared to controls (Fig. 3c). Thus, in this study, MPA did not attenuate the atheroprotective effects of CEE. A possible explanation for the differences in the outcome of these two studies may be that in the study by Adams et al. [24], the treatments were given to the animals as a part of the diet once a day, while in the more recent study, the treatments were given in divided doses twice a day [29]. By studying the endometrium of the animals in these two studies by Ki67 labeling, a marker of cell proliferation, it appeared that giving the MPA as a single dose was more effective in inhibiting endometrial proliferation than giving the MPA in two divided doses [35]. Therefore, it seems the estrogenic stimulus was higher and the progestogenic effect reduced in the study by Clarkson et al. compared to that by Adams et al. The differences between the two studies might suggest that when MPA is given at a dose that has the desired antagonistic effect on the endometrium, it attenuates the cardiovascular benefits of CEE, but if the progestogenic potency is reduced, the undesirable effects on atherogenesis are also reduced. Taken together, it is possible that all progestogens are not alike with regard to their influence on estrogen-induced cardioprotection, which perhaps more importantly is affected by the dose of the progestogen. However, further studies are needed to determine how progestogens alter the cardiovascular benefits of ERT.

6. What are the lipid-dependent and -independent effects of estrogen replacement therapy on the cardiovascular protection?

6.1. Plasma lipids and lipoproteins

Dyslipidemia is associated with increased risk of atherosclerosis in both men and women. Lipoprotein concentrations among premenopausal women differ from men; total and low-density lipoprotein (LDL) cholesterol are lower while HDL cholesterol is higher [36,37]. After menopause, women lose this beneficial lipid profile when total and LDL cholesterol become higher and HDL cholesterol falls [36–39]. Estrogen replacement therapy has, however, the potential to restore the premenopausal lipid
profile in women. The effects of estrogen on lipids and lipoproteins depend on the type and dose of estrogen used, and its route of administration.

Oral estrogen regimens have been shown to increase plasma triglycerides, HDL cholesterol and apoA1 levels, while decreasing LDL cholesterol and lipoprotein (a) [Lp(a)] levels [40–43]. Estrogen therapy increases triglyceride levels primarily by increasing the production of large, very-low-density lipoprotein particles, most of which are cleared by the liver rather than being converted to small and more atherogenic very-low-density lipoprotein particles or LDL [40]. Thus, the atherogenic potential of estrogen-induced hypertriglyceridemia may be of little concern. In the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, 875 healthy postmenopausal women aged 45–65 years were studied for 3 years using a randomized, double-blind protocol [43]. The group receiving oral CEE (0.625 mg/day) had a 10–12% decrease in LDL cholesterol levels, and a similar increase in HDL cholesterol levels during the study. In the PEPI trial, Lp(a) concentrations, shown to be an independent predictor of the risk of coronary heart disease in women [44], were reduced by 25% on average among women treated with CEE, with or without the addition of a progestogen [45]. Neither the addition of MPA nor micronized progesterone to CEE affected the decreases in LDL levels, but MPA diminished the beneficial increases in HDL, while micronized progesterone did not attenuate HDL increases associated with CEE [43]. In healthy postmenopausal women, treatment with continuous oral combined 17β-estradiol plus norethisterone acetate or continuous oral 17β-estradiol plus intermittent norgestimate show favorable effects on both lipid and lipoprotein concentrations [46]. In another study where postmenopausal women were treated for 3 months with 0.625 or 1.25 mg/day oral CEE, LDL concentrations were decreased by 15 and 19%, respectively, while HDL concentrations were increased by 16 and 18%, respectively [40]. In this study also, oral micronized estradiol (2 mg/day) was investigated revealing similar results, decreasing LDL concentrations by 14% and increasing HDL concentrations by 15% [40]. Transdermal route of estrogen delivery has been reported to result in trivial beneficial changes [47,48] or no changes [40,49] in plasma LDL and HDL concentrations. The fact that transdermally-administered estrogen has less of an effect on serum lipids and lipoproteins is due to the bypass of the portal circulation and thus, minimal effect on hepatic metabolism [50].

Clinical trials have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are effective in reducing cardiovascular-related morbidity and mortality [51,52]. Statins lower LDL cholesterol to a
greater degree than estrogen therapy in postmenopausal women, but statins have a lesser effect on HDL cholesterol and Lp(a) levels than estrogen treatment [53]. In a study where CEE (0.625 mg/day) and simvastatin (10 mg/day) therapies were combined, LDL cholesterol was lowered to a greater degree than either therapy alone, with elevation of HDL cholesterol similar to that of estrogen alone [54]. Lp(a) levels were reduced with therapies including CEE but not with simvastatin alone. In another study, CEE (0.625 mg/day) and simvastatin (5 mg/day) combined therapy had an additive effect on reducing the plasma total cholesterol concentration, and concentrations of large and small LDL cholesterol particles [55] (Fig. 4). HDL cholesterol concentrations were elevated to the same extent in all treatment groups, and combination therapy prevented an estrogen-induced increase in triglyceride concentrations. A randomized, double-blind, cross-over study compared, in 6-week treatment periods, CEE plus MPA, lovastatin (20 mg/day), or a combination of these two, and concluded that the combination therapy resulted in a greater decrease in LDL cholesterol levels and a greater increase in HDL cholesterol, levels than either treatment alone [56]. Clinical trials are needed to determine whether estrogen combined with statins also may have an additive effect in reducing coronary artery atherosclerosis.

6.2. Lipid-independent effects

Changes in plasma lipid levels are thought to account for only 25–50% of the overall estrogen-mediated cardiovascular protection [57,58]. Thus, lipid-independent direct actions of estrogen on the blood vessel wall may contribute substantially to the cardioprotective effects of estrogen. Steroid actions are generally mediated by specific receptors, which act as transcription factors, regulating gene expression when activated by steroid binding. Two subtypes of the estrogen receptor (ER) have been identified, the classical ER-α [59] and the more recently discovered ER-β [60]. Both receptors are expressed in the human [61–64] and non-human primate [65] vascular cells, although the function of these two receptor subtypes at the vascular level has not yet been defined fully. In fact, both ER-α [66] and ER-β-deficient mice [67] respond to estrogen-mediated vasoprotection after vascular injury, suggesting that there may be an overlap between the actions of the two receptors, or that an undefined receptor for estrogen exists that mediates the vasoprotective effects of estrogen. A recent study with apoE-deficient female mice with or without ER-α demonstrated that ER-α is a major mediator of the atheroprotective effect of 17β-estradiol, although existence of ER-α-independent atherosprotection was also observed [68]. Furthermore, consistent with this, a disruptive mutation of ER-α in a young man was associated with endothelial dysfunction [69] and premature coronary artery atherosclerosis [70].

Estrogen treatment may act directly at the vascular wall level by affecting endothelial function and vascular tone [58,71]. The effect of estrogen on coronary artery reactivity has been evaluated by repeated quantitative angiography in ovariectomized cynomolgus monkeys [72,73]. Changes in the coronary artery diameter were measured after intracoronary infusion of acetylcholine, which induces endothelium-mediated vasodilatation in normal arteries and constriction in atherosclerotic arteries. After long-term [72]

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**Fig. 4.** Effect of ERT Alone, Simvastatin Alone and ERT and Simvastatin in Combination on Plasma Lipoprotein Cholesterol Concentrations. ERT 0.625 mg CEE/day; Simvastatin 5 mg/day. * P<0.05, ** P<0.01, *** P<0.001 [55]. ERT, estrogen replacement therapy; CEE, conjugated equine estrogen; Total-C, Total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and LDL2-C, low-density lipoprotein₂ cholesterol.
and short-term [73] estrogen treatment, the animals exhibited vasodilatation in response to acetylcholine, whereas estrogen-deficient controls exhibited vasoconstriction. Similar effects have been shown later in both atherosclerotic [74–76] and healthy [76] coronary arteries from estrogen-treated postmenopausal women. In addition to the studies with acetylcholine-induced vasomotion, estrogen treatment induces an increase in flow-mediated vasodilatation of the brachial artery in postmenopausal women [77,78], and this increase may be even greater in women who are hypertensive [79]. Furthermore, a randomized clinical trial comparing oral and transdermal hormone replacement therapy in postmenopausal women evaluated blood flow velocity and peripheral vascular impedance by Doppler [80]. Both regimens reduced impedance to flow in the carotid and uterine arteries, while this reduction became detectable within the first 2 months of treatment and increased further over 6–12 months.

The effects exerted by estrogens on the vascular wall tone may be produced, after long-term treatment, by typical transcriptional ‘genomic’ regulation, but also rapidly after short-term exposure to estrogens, generally described as a ‘non-genomic’ mechanism [58,71]. Long-term estrogen treatment may direct the production of endothelium-derived vasoactive agents, nitric oxide, prostacyclin and endothelin-1 toward vasodilatory direction [81]. Although the relevance of plasma measures of these locally acting vasoactive agents has been questioned, ERT in postmenopausal women has been shown to increase plasma levels of nitric oxide and decrease endothelin-1 levels, in some [82–84], but not in all studies [85]. In vitro estrogen increases nitric oxide production in endothelial cells [86], mRNA for nitric oxide synthase [87] and prostacyclin production [88,89], while estrogen decreases [90] or has no effect [88] on endothelin-1 production. The rapid non-genomic estrogen-mediated vasodilatory effects are thought to be mediated mainly via ion-channel function and nitric oxide. In endothelial cells, estrogen has been shown to regulate nitric oxide release by rapid activation of endothelial nitric oxide synthase [91], whereas in smooth muscle cells, estrogen opens calcium-activated potassium channels [92], thus producing vasodilatation. Recently it was shown that 17β-estradiol interacts directly with the regulatory β subunit of Maxi-K potassium channels on vascular smooth muscle cells [93], thus demonstrating a novel way for estrogen to modulate rapidly vascular wall function.

Another potential site of an estrogen direct vascular action is the uptake and metabolism of LDL cholesterol by cells of the artery wall since increased arterial LDL cholesterol accumulation and degradation are shown to be early events in the atherogenic process [94,95] (Fig. 5). In a study with ovariectomized cynomolgus monkeys, Wagner et al. [96] conducted an 18-week study wherein the animals were given parenterally physiologic doses of 17β-estradiol and cyclic progesterone. The relatively short duration of the experiment was designed to allow investigation of the effects of the treatment on arterial LDL cholesterol metabolism in the earliest stages of the pathogenesis of atherosclerotic lesions. The degradation and accumulation of LDL cholesterol in coronary arteries were measured using radiolabeled LDL particles. Animals receiving hormone treatment had about a 70% reduction, compared to control animals, in degradation products following accumulation of LDL in the coronary arteries, while the plasma lipid, lipoprotein and apoprotein con-

![Fig. 5. In experiments using surgically postmenopausal cynomolgus monkeys, hormone replacement therapy significantly decreased LDL (low-density lipoprotein) uptake in the coronary arteries [96].](image-url)
centrations were not affected. A subsequent 16-week study, using the same techniques, determined the effects of oral esterified estrogens with and without methyltestosterone on LDL cholesterol metabolism [97]. Again, there was about a 70% reduction in coronary artery LDL cholesterol accumulation associated with the hormone treatments. In contrast to the previous study where parenteral hormone treatment did not affect lipoprotein concentrations [96], the use of oral estrogens resulted in reduced apolipoprotein B-containing lipoprotein concentrations and smaller LDL particle size [97]. In a third study using oral contraceptives, regardless of the adverse effects on plasma lipoprotein concentrations, arterial LDL accumulation was also reduced by approximately 70% [98]. Thus, these studies suggest that the estrogen-induced reduction in arterial LDL cholesterol accumulation is independent of plasma lipoprotein concentration. Although estrogens appear to limit retention of lipoproteins in the arterial wall, studies on the effect of estrogens on the vascular wall permeability are controversial [99,100]. Thus, the exact mechanism behind this estrogen-induced reduction in arterial LDL degradation and accumulation is unclear.

7. Are the cardiovascular benefits of estrogen replacement therapy modulated by inflammation?

Atherosclerosis is a chronic inflammatory condition of the vascular wall that is converted to an acute clinical event by the induction of plaque rupture leading to thrombosis [101]. Several plasma markers of inflammation, such as high-sensitivity C-reactive protein (CRP), cytokines and different soluble adhesion molecules, have been evaluated as potential tools for predicting the risk of cardiovascular disease in women [102]. The association of CRP and ERT was first reported from a study comparing levels of inflammation variables among elderly women (mean age over 72 years) using unopposed estrogen (n = 230, years of use 18.4±11.6) or estrogen/progestin (n = 60, years of use 12.0±9.0), with nonusers (n = 196) [103]. In this cross-sectional study, CRP levels, compared to controls, were about 60% higher among women using unopposed estrogen, without a change in women using estrogen/progestin. However, use of unopposed estrogen was associated with lower levels of two other inflammation markers, fibrinogen and alpha-1 acid glycoprotein [103]. These results were confirmed from another cross-sectional survey of 493 healthy postmenopausal women (mean age 51 years) showing median CRP levels two times higher among women using unopposed estrogen or estrogen plus progesterone compared to nonusers [104]. Furthermore, similar findings were reported from the PEPI trial, where relative to placebo, when combining the active treatment arms, final concentrations of CRP were 85% higher, while the adhesion molecule E-selectin was 18% lower compared with baseline [105]. In contrast, a later report from a double-blind trial of 33 women with type 2 diabetes, a combination of transdermal estradiol (80 µg) and continuous oral norethisterone (1 mg) significantly reduced CRP concentrations relative to placebo after 6 months of therapy [106]. Synthesis of CRP in the liver is primarily regulated by interleukin-6, and the plasma levels of interleukin-6 were shown to be lower among women using hormone replacement therapy compared to nonusers [107]. Although the clinical relevance of the effect of ERT on CRP remains unclear, it is possible that the CRP-raising effect of oral ERT is related to first-pass liver effects on CRP production, rather than cytokine-dependent changes in CRP.

Estrogen replacement therapy has been shown to reduce plasma concentrations of the adhesion molecules, E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [54,105,108–110]. Reduction of these vascular adhesion molecules could be potentially atheroprotective by reducing the attachment of white blood cells to the vessel wall. However, one study which reported reduction in the E-selectin, VCAM-1 and ICAM-1 levels, showed at the same time that estrogen may increase the expression of matrix metalloproteinase-9 [110], activation of which could digest fibrous caps of vulnerable plaques, resulting in thrombosis. A recent study on the histologic features of coronary plaques in pre- and postmenopausal women who died from noncoronary causes implies that the anti-inflammatory effect of estrogen results in plaque stabilization, but does not appear to inhibit plaque erosion [111]. Furthermore, in vitro studies investigating the effects of 17β-estradiol on the cytokine-activated endothelial cell adhesion molecules show opposite results [112,113]. Taken together, it seems that several markers of inflammation can be modulated by ERT, but the meaning of these divergent findings remains to be determined.

8. Are there any cardiovascular benefits to be derived from synthetic or natural selective estrogen receptor modulators (i.e. raloxifene or phytoestrogens), or compounds with selective steroid actions (i.e. tibolone)?

The majority of postmenopausal women choose not to undergo traditional ERT [114], primarily due to the fact that both CEE and 17β-estradiol are estrogen agonist for all target organs and thus, can pose a potential cancer risk for the breast and endometrium. Current research seeks to identify estrogen substitutes exerting estrogen agonist or antagonist actions on different tissues, compounds that are referred to as selective estrogen receptor modulators (SERMs) [115]. Some compounds, such as tamoxifen, have been in use for many years. Tamoxifen is used in the
treatment of breast cancer since it has estrogen antagonist effects on breast, while acting as an estrogen agonist for endometrium, arteries and bone. In the Scottish breast cancer trial, the incidence of fatal myocardial infarction was 63% less in patients who received tamoxifen compared to those who did not [116]. The follow-up of this study, where the women were up to 14 years on tamoxifen show similar results [117]. However, the Breast Cancer Prevention Trial, the only one carried out in women who share structural similarities with 17β-estradiol and exert both estrogen agonist and antagonist properties [123], thus phytoestrogens could be considered as natural SERMs. This could be partly explained by the fact that phytoestrogens are shown to have different binding affinities to the estrogen receptors [124]. For example, the binding affinity of the soy-derived phytoestrogen genistein for ER-α is only 4%, whereas its affinity for ER-β was 87% compared to 17β-estradiol. Given the different tissue distribution of the ER-α and ER-β [63,125], there is a basis for tissue-selective effects of the phytoestrogens.

There is growing evidence from epidemiological [126] and experimental [30,127] studies that consumption of phytoestrogens has a beneficial effect on the risk of coronary heart disease. Soy protein intake is associated with approximately 10–13% reduction in plasma triglyceride and LDL cholesterol, and a slight increase in HDL cholesterol [128]. Similar results on LDL and HDL cholesterol were shown in a study with postmenopausal cynomolgus monkeys fed a moderately atherogenic diet where the primary source of protein was casein or soy protein [127]. In this study the soy protein-fed animals had 90% less atherosclerosis compared to the casein-fed animals. In another more recent study of 36 months duration, cynomolgus monkeys were fed soy protein, or soy protein that had been alcohol-washed to remove the soy phytoestrogens (control), and these groups were compared to CEE-treated animals [30]. The effect of soy phytoestrogens on LDL and HDL cholesterol was similar to results shown before [127]. CEE treatment reduced coronary artery atherosclerosis about 50%, while in animals treated with soy phytoestrogens, the reduction was intermediate between the control and CEE treatment [30]. Interestingly, the treatment with soy phytoestrogens was comparable to CEE in reducing common carotid and internal carotid artery atherosclerosis [30] (Fig. 6). Soy protein isolate, containing its phytoestrogens, has been shown to also lower diastolic blood pressure in women [129,130], and improve vascular reactivity and endothelial function in humans and non-human primates [131,132].

Tibolone is a synthetic steroid possessing estrogenic, progestogenic, and androgenic action [133]. Clinically, tibolone has been shown to be effective for the treatment of climacteric symptoms [134] and for the prevention of postmenopausal osteoporosis [135], without proliferative effects on the endometrium [136]. The selective steroid actions of tibolone are due to metabolites of tibolone;
3α-OH and 3β-OH metabolites having estrogenic effects, and the Δ⁴-isomer having progestogenic and androgenic effects. The Δ⁴-isomer, produced primarily within the endometrium, protects the endometrium from the agonist effects of the two estrogenic metabolites [136]. Tibolone treatment in postmenopausal women reduces plasma triglyceride [137] and Lp(a) [138] concentrations, with little effect on LDL. However, concern that tibolone may be atherogenic has arisen because tibolone also reduces plasma HDL cholesterol concentrations by approximately 30% [139]. In postmenopausal women the long-term vascular effects of tibolone has not been evaluated, but recently Clarkson, et al. [29] concluded a study in postmenopausal cynomolgus monkeys comparing the effect of tibolone and ERT on coronary artery atherosclerosis. In this study, the animals were treated for 2 years with CEE alone or combined with MPA, or tibolone at two doses comparable to women taking 1.25–2.5 mg/day, or no treatment. Despite the fact that tibolone reduced plasma HDL cholesterol concentrations similar to or greater than that seen in women, there was no increase in coronary artery atherosclerosis. Furthermore, with a 30% reduction in HDL cholesterol concentrations there was no reduction in the serum cholesterol efflux potential, and thus, no indication for adverse effects of tibolone treatment on reverse cholesterol transport [140]. Our results imply that although tibolone does not share CEE’s benefits on coronary artery atherosclerosis, there is no indication that the effects of tibolone on plasma lipoprotein concentrations are associated with an increase in atherosclerosis.

9. What are the future directions in estrogen replacement therapy?

9.1. Early initiation of ERT

As reviewed above, there is a substantial body of observational and experimental data to support the beneficial effects of ERT on prevention of cardiovascular disease. Whether WHI will confirm these findings remains to be seen in 2005 when this study with large expectations is completed. However, it is possible that the ERT-mediated cardiovascular protection does not apply at later stages of atherosclerosis, as shown by the randomized human [25,26] and non-human primate [31] studies. The preliminary data from WHI may indicate that the age (stage of atherosclerosis) and complexity of atherosclerotic lesions will be nonresponsive to estrogen treatment. This would imply that it is important for healthy women to start ERT immediately after menopause in order to maintain the estrogen-mediated cardiovascular benefits. Since conventional ERT is not acceptable to the majority of postmenopausal women, mainly because of the fear of cancer, the challenge is to search for new regimens or modify conventional ERT to obtain a better overall risk profile.

9.2. Lower doses of estrogens

The most recent update on the Nurses’ Health Study shows that women taking oral CEE, either 0.625 or 0.3 mg, were both associated with a reduced risk for heart
disease [16]. However, there was an increase in risk for
stroke among women taking CEE 0.625 mg (relative risk, 1.35; CI, 1.08–1.68), or 1.25 mg or more (relative risk, 1.63; CI, 1.18–2.26). These findings would strongly
recommend the use of a lower CEE dose, which could theoretically also reduce the ERT-associated cancer risk.

9.3. Combinations of ERT with SERMs

Since the SERMs so far have not fulfilled the expecta-
tions as the ‘ideal’ hormone replacement, an intriguing
possibility is to combine SERMs with low-dose estrogen. Support for this comes from animal studies where soy phytoestrogens are combined with estrogens. In cynomol-
gus monkeys, soy phytoestrogen treatment showed anti-
proliferative effects in the endometrium and mammary
gland when combined with 17β-estradiol [141]. Similar
results on mammary gland were shown in a more recent
study with ovariectomized rats when treated with soy
phytoestrogens combined with CEE [142]. Furthermore, in
cynomolgus monkeys, soy phytoestrogens combined with
17β-estradiol showed additive effects in reducing arterial cholesterol content and improvement in vascular reactivity
[143]. Taken together, these results imply that combining
soy phytoestrogens with estrogens may protect breast and
dometrial tissues while having additive beneficial effect
on cardiovascular protection. These findings provide en-
couragement for further studies with other combined
therapies.

10. Conclusions

Despite disappointing outcomes from trials to evaluate
estrogen treatment on postmenopausal cardiovascular dis-
ease, there is considerable evidence to support the benefi-
cial effects of estrogens in the early stages of atherogenesis
(during the menopausal transition and the early years of
postmenopause). Lack of compliance with ERT, usually
because of fear of breast cancer, remains a major problem.
Future regimens may overcome that fear by the co-ad-
minstration of a breast cancer preventive agent (i.e.
SERMs, phytoestrogens) with low dose estrogen.

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