

# Estrogen replacement therapy and cardioprotection: mechanisms and controversies

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## Abstract

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Epidemiological and case-controlled studies suggest that estrogen replacement therapy might be beneficial in terms of primary prevention of coronary heart disease (CHD). This beneficial effect of estrogens was initially considered to be due to the reduction of low density lipoproteins (LDL) and to increases in high density lipoproteins (HDL). Recent studies have shown that estrogens protect against oxidative stress and decrease LDL oxidation. Estrogens have direct effects on the arterial tissue and modulate vascular reactivity through nitric oxide and prostaglandin synthesis. While many of the effects of estrogen on vascular tissue are believed to be mediated by estrogen receptors  $\alpha$  and  $\beta$ , there is evidence for 'immediate non-genomic' effects. The role of HDL in interacting with 17 $\beta$ -estradiol including its esterification and transfer of esterified estrogens to LDL is beginning to be elucidated. Despite the suggested positive effects of estrogens, two recent placebo-controlled clinical trials in women with CHD did not detect any beneficial effects on overall coronary events with estrogen therapy. In fact, there was an increase in CHD events in some women. Mutations in thrombogenic genes (factor V Leiden, prothrombin mutation, etc.) in a subset of women may play a role in this unexpected finding. Thus, the cardioprotective effect of estrogens appears to be more complicated than originally thought and requires more research.

### Key words

- Estrogen therapy
- Postmenopausal women
- Cardiovascular disease
- Plasma lipoproteins
- Lipoprotein oxidation
- Estrogen receptors
- Thrombosis
- Nitric oxide synthesis

## Introduction

Epidemiological studies have shown a 50% reduction in coronary heart disease (CHD) after estrogen replacement therapy (ERT) in postmenopausal women (1-2). This protective effect of estrogen is presumably due to its ability to favorably alter low/high density lipoprotein (LDL/HDL) ratios and decrease vascular reactivity and oxidative stress (3). Three major placebo-controlled trials designed to study whether ERT re-

duces CHD have questioned this protective effect of estrogen. The Heart and Estrogen/Progestin Replacement Study (HERS) was the first placebo-controlled trial. HERS noted that ERT not only failed to reduce the overall rate of coronary events, but actually increased CHD in the first year with a 89% increase in thromboembolic events (4). In the Estrogen and Atherosclerosis (ERA) Trial (5) there was no decrease in coronary artery disease progression with ERT in postmenopausal women with at least one coronary artery

stenosis. In the Women's Health Initiative Hormone Replacement Trial (WHI-HRT) which includes postmenopausal women with an intact uterus taking ERT plus progestin and those without a uterus taking only ERT (6,7), a small increase in the number of myocardial infarctions, strokes and thromboembolism was noted in women taking active hormones compared to the placebo group. Along with HERS and ERA, WHI-HRT was the third trial to suggest that ERT is not cardioprotective in postmenopausal women with CHD and may actually increase thromboembolism and CHD events. Several hypotheses have been advanced to explain these unexpected findings. The most widely accepted notion is that thrombosis may be limited to a subset of women on estrogen who may be increasingly susceptible to thrombosis because of confounding risk factors, i.e., genetic mutations (8-11) in thrombogenic factors (factor V Leiden, prothrombin and plasminogen activator inhibitor gene mutations). Premarin (a brand of conjugated equine estrogen mixture marketed by Wyeth/Ayerst Labs, Radnor, PA, USA) is the estrogen used in all major clinical trials. To date, the exact composition of Premarin is not known. Recently, another brand of conjugated estrogen called Cenestin (containing 10 estrogens in a known composition marketed by Duramed Pharmaceuticals) has received approval for the treatment of menopausal symptoms (12). Despite these concerns about thrombosis, a significant portion of postmenopausal women in the United States continue to take ERT for relief of menopausal symptoms and potential benefits in terms of osteoporosis and cardiovascular diseases. In terms of cardioprotection, studies continue to document improvements in risk factors related to heart disease (2,3,13). Some of these studies are discussed below.

### **Estrogen and plasma lipids**

Considerable data are available that docu-

ment an increase in HDL and a reduction of LDL cholesterol following estrogen therapy (14). Studies have clearly established that estrogen decreases total plasma cholesterol (15) and increases or maintains plasma triglyceride levels (15-17). With the addition of progestin, plasma total cholesterol, LDL cholesterol and triglyceride levels decrease (16-18). The addition of progestin, however, slightly blunts the increase in HDL levels (16-18). HDL2 levels are increased with estrogen, but changes in HDL3 have been inconsistent (17,18). Estrogen with or without progestin significantly lowered plasma lipoprotein(a) levels (19).

### **Estrogens as antioxidants**

Recent studies have documented that estrogens are potent antioxidants and decrease LDL oxidation *in vitro* and *in vivo* (3,20). Although earlier studies have used pharmacological concentrations of estrogens to document antioxidant activity, recently it has been shown that 17 $\beta$ -estradiol is active even at physiological concentrations (21). Furthermore, the potency of catechol estrogens is far greater than that of parent estrogens (22). Studies on the mechanism of estrogen antioxidant effects have shown that estrogens strongly inhibit superoxide formation with minor effects on hydrogen peroxide and hydroxyl radical formation (23). While estrogens decrease lipid peroxidation and formation of reactive oxygen species (23), androgens and progestins increase oxidative stress parameters (24). Clinical studies on humans using E2-based preparations have clearly shown decreased LDL oxidation (25,26), while other studies using conjugated estrogens have yielded conflicting results (27,28). Whether these differences are due to different estrogen preparations or time frames is not clear at this time.

### **Estrogen and vascular tone**

Currently, there is a strong interest in the

role of estrogens in mediating vascular tone and response to vasoactive agents. Studies have documented that E2 can induce relaxation of coronary arteries, reverse acetylcholine-induced vasoconstriction and improve exercise-induced myocardial ischemia in women with coronary artery disease (29-32). Collins et al. (33) showed that E2 decreases acetylcholine-induced coronary artery responses only in women, but not in men. These vasodilatory effects of estrogen are largely believed to be mediated by increased synthesis and release of nitric oxide, a potent relaxant of vascular smooth muscle (34). Short-term E2 treatment significantly increased plasma nitric oxide levels in postmenopausal women (35). Synthesis and release of nitric oxide in cultured endothelial cells are increased significantly by estrogens (36,37) and inhibited by androgens (37). Some investigators have been able to demonstrate (38) increased expression of endothelial nitric oxide synthase in women treated with estrogens. The effects of estrogens on nitric oxide synthesis is believed to be manifested by rapid non-genomic (without changes in gene expression) effects (38,39). Elucidation of this phenomenon has indicated that the non-genomic effects may still be modulated by estrogen receptors and the readers are referred to an excellent review of this topic by Mendelsohn and Karas (39).

### **Other mechanisms of estrogen action**

Some of the other mechanisms responsible for estrogen-mediated cardioprotection include increases in vascular prostacyclin synthesis (40), inhibition of aortic smooth muscle cell proliferation (41) and decreases in hemostatic factors (42,43) like fibrinogen and plasminogen activator inhibitor-1. The expression of vascular cell adhesion molecule, a chemotactic factor produced by endothelial cells that attracts monocytes (44),

is also inhibited by estrogens (45) and stimulated by androgens and progestins (46). The readers are referred to a critical review by Farhat et al. (47) on some of these mechanisms.

### **Role of estrogen receptors**

Recent research has provided a great deal of information on the mechanisms involved in the intracellular binding of estrogens to estrogen receptors ( $\alpha$  and  $\beta$ ), translocation to the nucleus and the occurrence of genomic effects upon binding to estrogen response elements (48-50). However, the significance of these two receptors in the manifestation of the cardioprotective effect of estrogen is still open to question since mice lacking both of these receptors continue to demonstrate inhibition of intimal proliferation after vascular injury (51). The distribution of these receptors in vascular and other tissues and their interactions with estrogens and antiestrogens are beyond the scope of this article and have been covered in some excellent recent reviews (52). The carcinogenic effect of exogenous estrogens either by estrogen receptor activation and cell proliferation (53) or by DNA adduct formation by metabolites of catechol estrogens (54) in breast tissues has been of much concern in postmenopausal women. There is strong evidence suggesting that long-term estrogen use increases the risk for endometrial and breast cancer in women on estrogen therapy (55,56). Consequently there is a lot of interest in developing "designer estrogens" that do not have adverse effects on breast and endometrium, yet retain their beneficial effects on the bone and cardiovascular system.

Recently discovered selective estrogen receptor modulators seem to have no estrogen agonistic effects on breast and endometrial tissue (57), but their long-term cardiovascular benefits are still being assessed.

### Significance of differential delivery of estrogens

Therefore, considerable attention has been focused on targeting estrogens to desired tissues (ex: vascular tissue or site of atherosclerosis, bone, etc.). Our laboratory has been interested in achieving differential effects of estrogens by differential delivery to cells. During studies exploring this possibility, we have shown (58) that a significant fraction of  $17\beta$ -estradiol (<10%) is associated with lipoproteins (predominantly with HDL), where it can be subsequently esterified and transferred to LDL (59,60). These steps also appear to be important in the

manifestation of the antioxidant effect of estrogens (61). Interestingly, increasing hydrophobicity of the estrogen molecule (by esterification) increases its association with LDL (61,62), providing an opportunity to target estrogen derivatives complexed with native or modified LDL to vascular tissues. Future research should be directed at targeting estrogens to specific tissues without undesirable side effects.

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