Cardiovascular Actions of Estrogens in Men

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Cardiovascular disease is the major cause of death among both men and women in developed countries. Coronary artery disease, the single most important component of cardiovascular disease, is responsible for about one quarter of all deaths. It is well recognized that, at all ages, women are relatively protected against cardiovascular disease in comparison with men, and many observational studies have suggested that estrogen treatment of postmenopausal women significantly reduces cardiovascular risk (1). Further, the Postmenopausal Estrogen/Progestin Replacement Study (PEPSI) Study (2), which assessed the effect of hormonal therapy on cardiovascular risk factors over a 3-yr period, showed beneficial effects on lipoproteins in all treatment groups and concluded that the best regimen for women with an intact uterus was estrogen plus micronized progesterone, and the best regimen for women without a uterus was unopposed estrogen. However, the recently concluded Heart Estrogen/progestin Replacement Study (HERS) found no significant evidence of a clinical benefit of hormonal therapy in women with established coronary artery disease (3). The question of whether such therapy is useful in women as primary prevention against cardiovascular disease is being addressed by the Women’s Health Initiative, the results of which will not be known for several more years (4).

Although in men estrogens are produced in significant quantities by local tissue aromatization of androgenic precursors from the testes and adrenal glands (5), there has been relatively limited study of the biological role of these hormones or their clinical implications. An investigation conducted 25 years ago into the cardiovascular effects of estrogen administration in men after myocardial infarction, the “Coronary Drug Project” showed an excess of deaths and recurrent infarction in the treatment group. This trial, which employed high doses of conjugated equine estrogens, was subsequently abandoned, and the subject has not been studied in detail since (6). Data gathered over the last quarter century on the epidemiology of cardiovascular disease, the mechanisms of actions of estrogens in women and men, and the biological role of endogenously produced estrogens in men, suggest that it might be time to re-open the Coronary Drug Project file, and re-assess the potential for estrogen therapy in men. Newer approaches to treatment and an ability to identify more precisely individuals at risk of coronary heart disease (CHD) may lead to new clinical applications for this group of hormones.

Evidence of a role for endogenous estrogens in men

Endogenous estrogens, lipoproteins, and glucose metabolism. There is now compelling evidence that endogenous production of estrogens in men plays an important role in cardiovascular health and disease. Physiological levels of estrogen have been reported to play a role in influencing plasma lipoprotein concentrations in men. When selective estrogen deficiency was induced in young men by administration of combined drug therapy with a GnRH antagonist (to suppress endogenous steroid hormones), testosterone (to restore testosterone levels to baseline), and testolactone (an aromatase inhibitor that prevents conversion of testosterone to estrogens), plasma high density lipoprotein (HDL) and apolipoprotein A-1 decreased, while plasma low density lipoprotein (LDL) and triglyceride levels did not change (7). Shono et al. (8) investigated the relationships of plasma sex hormones to lipid and glucose metabolism in a cross-sectional study on 212 apparently healthy men ranging in age from 18 to 59 yr. They showed that the estradiol level was negatively related to both LDL cholesterol and fasting blood glucose, suggesting that the levels of estradiol within the physiological range for healthy men may help maintain a desirable profile of lipid and glucose metabolism.

Lessons from “experiments of nature”. Recent evidence from a 28-yr-old man with estrogen insensitivity caused by a disruptive mutation in the estrogen receptor (ER) gene suggests that estrogen may play an important role not only in bone metabolism but also in cardiovascular function. This individual presented with tall stature, normal masculinization, incomplete epiphyseal closure, and decreased bone mineral density. His serum estradiol and estrone, FSH, and LH concentrations were elevated, while testosterone was normal. Direct sequencing of exon 2 of his ER gene revealed a cytosine-to-thymine transition at codon 157 of both alleles, resulting in a premature stop codon and expression of a truncated nonfunctional ER protein (9). Peripheral vascular...
In females this estrogen deficiency results in virilization been described that give rise to complete estrogen deficiency. Recently, a number of mutations of the aromatase gene have corresponding phenolic A-ring characteristic of estrogens. aromatization of the delta 4–3-1 A-ring of the androgens to the corresponding estrogens, involving the geneic steroids to the corresponding estrogens, responsible for the conversion (aromatization) of C19 andro- genic steroids to the corresponding estrogens, involving the conversion of the delta 4–3-1 A-ring of the androgens to the corresponding phenolic A-ring characteristic of estrogens. Recently, a number of mutations of the aromatase gene have been described that give rise to complete estrogen deficiency. In females this estrogen deficiency results in virilization in utero and primary amenorrhea with hypergonadotropic hypogonadism at the time of puberty. In men the most striking feature is continued linear bone growth beyond the time of puberty, delayed bone age, and failure of epiphyseal closure, thus indicating an important role of estrogens in bone metabolism in men. Such patients also have low HDL and increased total and LDL cholesterol concentrations and triglycerides, and hyperinsulinemia. Thus, as has been suggested in menstruating women, it is possible that part of a putative protective role for endogenous estrogens in men is by maintenance of normal HDL concentrations and possibly by reducing LDL cholesterol.

Aromatase inhibition in men: vascular effects. In a recent preliminary study, we have demonstrated a potential role for endogenous sex hormones in vascular reactivity in elderly men taking the aromatase inhibitor testolactone for benign conditions for prolonged periods. Cessation of testolactone therapy was associated with a fall in serum testosterone, a trend to an increase in estrogens, and significant improvement in forearm endothelium-dependent vasorelaxation. However, norepinephrine-induced vasoconstriction was enhanced, and arterial compliance decreased, suggesting a complex interaction between sex hormones and vascular function. In addition, because the presence of aromatase has been demonstrated in the vasculature, local changes in steroid concentrations resulting in vascular effects cannot be excluded.

Of note, variations in endogenous estrogen levels are observed in men in conditions such as obesity. The degree of obesity appears to have a direct effect on estradiol levels, probably through transformation of androgens in adipose tissue to estrogens. Moderate to high ethanol intake is also reported to influence sex hormone levels in men; in particular, plasma testosterone levels decrease, probably via alcohol-induced inhibition of testosterone. In addition, ethanol induces an increase in cytosolic ER in the human male liver, a possible explanation for feminization in chronic liver disease due to alcohol. However, the influence of such variations in endogenous sex hormone levels and ER on cardiovascular physiology in men with obesity or chronic liver disease is unknown.

Estrogens and vascular function in women

In women, estrogens directly influence vascular function. Estrogens act as vasodilators in the peripheral and coronary circulations through effects both on the endothelium and on smooth muscle cells. Several studies have recently shown that estrogen preserves endothelial function. Physiological levels of estrogen enhance acetylcholine-induced vasorelaxation in the forearm and coronary vascular beds in postmenopausal women. The beneficial effect of estrogens on endothelium dependent dilation are probably mediated by an increase in nitric oxide production. Postmenopausal women supplemented with transdermal estradiol have increased serum concentration of nitrites and nitrates, metabolites of nitric oxide, compared with women not on hormonal therapy. A recent study in perimenopausal women showed greater basal nitric oxide release in the forearm vasculature after 8 weeks of estrogen supplementation. Consistent with these observations, studies in human umbilical vein endothelial cells have shown up-regulation of endothelial nitric oxide synthase after exposure to estrogen.

Biological effects of estrogens in male tissues

As already argued, the biological effects of estrogens are less well defined in men than they are in women. Data from in vitro studies, however, suggest that estrogens may act directly on vascular cells in males in a physiologically important manner. Karas et al. have reported the presence of ER protein by immunoblotting in human vascular smooth muscle cells from both male and female sources. Dai-do et al. have reported that estradiol inhibits growth factor-induced proliferation and migration in smooth muscle cells, independent of gender. Further, in human endothelial cells, which reportedly have a high density of ER (20–80,000/cell), the intensity of immunostaining for ER is similar in male and female donor cells, and neither electrophoretic mobility shift assays nor ligand-binding studies show reproducible gender differences in ER expression.

Recently, Kuiper et al. described another ER, termed the beta receptor (ERβ), which probably mediates some of the biological effects of estrogens. There is evidence from studies of human vascular smooth muscle cells, as well as from the ER-α knock-out mouse that ER-β exists on the vasculature. In the ER-α knock-out mouse, ER-β in male vascular tissues appears to be induced after vascular injury, supporting a role for ER-β in the direct vascular effects of estrogen in males. Preliminary studies in human vascular smooth muscle cells suggest that, in cells from females, ER-α is more prevalent than ER-β, while approximately equal...
amounts of both receptors are present in males (32). The significance of this gender-associated difference in vascular ER subtypes remains to be determined. Cardiac myocytes from both male and female rats also express ER-α and ER-β, and both receptors appear to be up-regulated in the presence of estrogen (33); however, at present, the significance of cardiac ER in cardiovascular physiology is unclear.

**Acute vascular effects of estrogen administration in men**

There are conflicting data on the effect of estrogens on coronary vascular reactivity in men. Collins et al. (34) reported that short-term administration of 17-β estradiol did not attenuate acetylcholine-induced coronary vasoconstriction in men. However, more recent studies have shown that acute intravenous administration of conjugated estrogens improved coronary blood flow responses to acetylcholine (35) and abolished abnormal coronary vasoconstriction in response to an exogenous cold stimulus, both in men referred for routine coronary angiography (36) and in male cardiac allografts (37). In these latter studies, the improvement in response was seen after 15 min of estrogen administration, a likely nongenomic effect of estrogen. Like other steroid hormones, estrogens have classically been considered to act through binding to intracellular receptors, which act as ligand-dependent transcription factors to regulate protein synthesis. However, as with other steroid hormones (38), in recent years evidence has accumulated that estrogens may also act rapidly through nongenomic mechanisms (39). In the dog coronary circulation, Sudhir et al. (40) have shown that intracoronary administration of 17-β estradiol induces rapid coronary vasodilation of similar magnitude in both males and females, a response that is not blocked by pretreatment with the ER antagonist ICI 182,780. Komesaroff et al. (41) investigated the effects of estrogen on the cutaneous vasculature in young men, and found that at plasma estradiol concentrations within the physiological range for premenopausal women estrogens induced rapid onset, rapid offset, nongenomic effects specific to the endothelium. Following sublingual estradiol or intravenous conjugated equine estrogens, the vasodilator response to acetylcholine, but not sodium nitroprusside, was accentuated in these young male subjects.

**Chronic vascular effects of estrogen administration in men**

Estrogen supplementation in older men reportedly does not augment flow-mediated dilation in the brachial artery or influence serum levels of metabolites of nitric oxide (42). However, studies in male-to-female transsexuals have shown that both flow-mediated and nitroglycerin-induced vasodilation in the brachial artery are enhanced, compared to control men, suggesting that high dose estrogen treatment enhances vascular reactivity in genetic males (43, 44). In a recent study in hypogonadal men, we showed that 6 weeks of low dose estrogen supplementation had no effect on acetylcholine- or nitroprusside-induced vasodilation in forearm resistance arteries, but attenuated vasoconstrictor responses to norepinephrine and angiotensin II (45). We also demonstrated an accentuated forearm vasoconstrictor response to L-NMMA after estrogen supplementation, suggesting an estrogen-induced increase in basal nitric oxide release. With the dose administered (1 mg estradiol valerate daily for 8 weeks) minimal adverse effects were observed (45). In that study, we also observed a drop in baseline and stress-induced increases in blood pressure after estrogen supplementation, consistent with previous reports from our group in perimenopausal women (46, 47). Of note, estrogens increase sex hormone-binding globulin (SHBG), which may bind testosterone to a greater degree than estradiol, and thus potentially decrease bioavailability of testosterone in relation to that of estrogen. However, such an alteration in the hormonal milieu does not appear to result from short-term increases in circulating estradiol (48). Nevertheless, some of the consequences of long-term estrogen administration in men might be related to androgen withdrawal.

**Effect of estrogens on homocysteine, clotting factors, and platelets in men**

It has been recognized that an elevated plasma homocysteine level is an independent risk factor for coronary heart disease. There is evidence that endogenous estrogens play a role in controlling circulating levels of homocysteine. Plasma total homocysteine levels decrease after estrogen and androgen administration to male-to-female transsexual subjects, and increase after androgen administration to female-to-male transsexual subjects (49). A recent short-term (9 week) study in healthy elderly men showed that estradiol therapy reduced homocysteine, fibrinogen, and PAI-1 concentrations, and favorably influenced lipoprotein levels, without increasing markers of thrombotic risk (50). These observations are consistent with animal experiments showing that administration of both estrogens and glucocorticoids decrease plasma homocysteine levels, presumably by enhancing metabolism (51).

Estradiol may also play a role in the control of the membrane protein P-selectin, which tethers leukocytes to endothelial cells and activated platelets and may thus play a role in atherosclerosis. Lower plasma levels of a soluble form of P-selectin have been observed in premenopausal women compared to men, and it has been shown that a single dose of estradiol valerate (10 mg, intramuscular injection) in healthy male volunteers significantly decreases P-selectin levels (52), suggesting that an additional antiatherogenic mechanism for estradiol may be important in normal men. Estrogen may also control activity of the plasma platelet-activating factor (PAF)-acetylhydrolase activity, as administration of the synthetic estrogen mestranol in healthy men appears to lead to significant decreases in plasma PAF-acetylhydrolase activity (53).

Against these potentially beneficial roles for estrogen, a potentially harmful effect of estrogen in promoting thrombosis needs to be considered. Estrogens may increase the risk of thromboembolic events, as the HERS study in postmenopausal women (3) and studies of the effects of oral contraceptives in premenopausal women (54) have suggested. Further, female smokers over the age of 35 yr who use oral contraceptives have an unacceptably high incidence rate of myocardial infarction and stroke (55), and it is unclear
whether such a smoking-hormone interaction would be observed with administration of estrogenic compounds in men.

There is limited evidence for a deleterious effect of endogenous estrogens in men. Phillips et al. (56) have shown, in a case control study, that the mean serum estradiol level in men who had suffered myocardial infarction was significantly higher than in men with no history of infarction but with comparable established coronary artery disease, suggesting that higher estrogen levels may predispose to coronary thrombosis in susceptible individuals. Venous thromboembolism is a major complication in male-to-female transsexuals treated with oral estrogens and anti-androgens, but the use of transdermal estradiol in transsexual subjects over 40 yr of age appears to attenuate this risk (57). Clearly such data are important when re-assessing a potential role for estrogens in cardiovascular disease in men.

“Designer estrogen” therapy in men

The recognition that high doses of estrogen may produce effects distinct from those obtaining at physiological levels, and the developing appreciation of the complexity of the relationship between sex steroids and the cardiovascular system in women, suggest a need for further studies to clarify the roles of these hormones in men. The effects of newer agents with estrogenic activity, such as the so-called “selective ER modulators” or “designer estrogens” (58), and recently identified equine estrogens such as delta 8-estrone (59) and 17 α-dihydroequilenin (60), which may have fewer trophic effects on reproductive organs or mammary glands in males and females, are yet to be studied in men.Raloxifene, a designer estrogen that has been examined in postmenopausal women, appears to favorably alter markers of cardiovascular risk by lowering total and LDL cholesterol (61), fibrinogen, and lipoprotein(a), and by increasing HDL cholesterol, without raising triglycerides (62); its effects in men are unknown. Differences between the ER subtypes in relative ligand binding affinity and tissue distribution could be exploited to achieve selective action of ER agonists and antagonists in different tissues (63). Thus, the potential exists to develop an agent that has minimal adverse effects, but beneficial actions on the cardiovascular and other systems. Such agents might then bridge the current “gender gap” that exists in the context of estrogen therapy.

Potential role of phytoestrogens and other dietary factors

A significant number of structurally diverse plant and fungal secondary metabolites exist in nature that may contribute to the total estrogen exposure of the human population (64). It is now recognized that these phytoestrogens may act in beneficial ways on the cardiovascular system (65), and recent research has emphasized the widespread potential health benefits of dietary phytoestrogens (66). It is likely that the cardiovascular benefits of these substances would apply to men as much as to women, but to date little study of their effects in men has been undertaken. It is also known that other dietary factors may affect sex hormone metabolism. For example, it has been shown that in healthy men a high fat, low fiber diet is known to reduce urinary excretion of estradiol and estrone and their metabolites while increasing mean plasma concentrations of testosterone (67). It is therefore possible that dietary modification could also be used to control endogenous sex hormone levels in men in a manner potentially beneficial to the cardiovascular system.

Conclusions

We conclude that recent evidence shows that: 1) it is likely that estrogens act on the male cardiovascular system in a manner similar to that in women; 2) endogenous production of estrogens in men plays a significant role in cardiovascular health; 3) at least some of the gender differences in cardiovascular risk are likely to be related to differing levels of circulating estrogen levels between men and women; and 4) low dose estrogen supplementation in men may induce beneficial cardiovascular effects. Against this is the relative lack of clinical data from randomized placebo controlled trials in women showing improved clinical outcomes with estrogen-containing postmenopausal hormonal therapies and the possibility of adverse outcomes as a result of a possible increased tendency to thromboembolic events. These caveats notwithstanding, we believe that it is appropriate to reconsider the adverse outcomes of the Coronary Drug Project in the light of more recent knowledge, and to consider rigorous clinical testing of therapeutic estrogen administration in selected groups of men.

References

14. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin KJ. 1995 Aro-


