The roles that both male and female sex steroids play in mediating or protecting against cardiovascular disease (CVD) and hypertension are controversial. For example, whereas animal studies have strongly implicated androgens as being mediators of CVD and hypertension, human epidemiological studies have shown that with chronic disease, including hypertension, serum testosterone levels are actually reduced. Thus, whether androgens are truly causative of CVD is not clear. However, premenopausal women are typically protected from CVD and hypertension compared with men, and this has been hypothesized to be because of the protective effects of estrogens. The negative findings of Heart and Estrogen/progestin Replacement Study (HERS) I and II and Women’s Health Initiative (WHI) studies on hormone replacement therapy (HRT) in postmenopausal women have shaken our previous ideas that HRT was protective against CVD. In this short review, the latest findings regarding the roles of sex steroids in hypertension and CVD are discussed, questions yet to be answered are suggested, and some speculations are made.

Sex Steroids and Receptors

In both males and females, the hypothalamus secretes gonadotropin-releasing hormone, which stimulates the anterior pituitary to release both luteinizing hormone and follicular-stimulating hormone. Luteinizing hormone binds to receptors on thecal cells in ovaries of females and Leydig cells in testes of males to cause testosterone to be synthesized. Follicular-stimulating hormone, however, binds to receptors on granulosa cells in females or Sertoli cells in males and stimulates the synthesis of aromatase, which converts testosterone to estradiol.

There are 2 main types of estrogen receptors, ERα and ERβ, but several variants of both have also been identified. ERβ is present in a greater number of tissues than is ERα, but both isoforms are present in kidneys and the vasculature. ERβ has been found to be the predominant isoform in human vascular smooth muscle cells. The androgen receptor is also found in kidneys and the vasculature, as well as other organs. Both estrogen and androgen receptors are transcription factors that bind upstream in the promoter regions of genes to increase or decrease synthesis. In addition to the genomic effects of estrogens and androgens, both sex steroids also have nongenomic effects, particularly in the vasculature to cause acute vasodilation. The reader is referred to several excellent reviews on sex hormones and vascular responses.

Estrogens and Cardiovascular Disease

The goal of the WHI studies was to determine whether HRT could protect against primary events associated with CVD. Women, aged 50 to 79 years, were given conjugated equine estrogen (0.625 mg/d) and medroxyprogesterone acetate (2.5 mg/d). There was also an “estrogen only” arm in which women received only conjugated equine estrogen. The HERS I and II studies had shown previously that HRT was not effective in preventing against secondary cardiovascular events. The women in the HERS studies had previous cardiovascular disease, including myocardial infarction, and HRT was found not to be efficacious against subsequent cardiovascular events. Proponents of HRT reasoned that it was likely that once cardiovascular disease was started in postmenopausal women, it was difficult to attenuate it, much less arrest it, even with HRT. Unfortunately, the WHI studies showed that HRT was not protective against CVD and that HRT caused a higher incidence of ischemic stroke, pulmonary emboli, myocardial infarction, and dementia than placebo. These adverse effects were most prevalent within the first year of HRT. There was concern at the time that the progestin used may have offset any potential benefit of the estradiol. However, the “estrogen only” arm of WHI was also recently stopped because of the lack of any beneficial CVD effects. Some investigators questioned the use of the conjugated equine estrogen preparation because it is isolated from horse urine and is not purely estradiol. Whether different results would occur if a pure estradiol product were to be given to postmenopausal women must be determined in future studies.

Several things may explain the discrepancies between the HERS and WHI data and previous studies. Many of the women of the HERS and WHI cohorts had undergone

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menopause years before the start of the HRT. Thus, it is possible that starting HRT in women during perimenopause may prove more beneficial.\(^{11}\) In addition, the many previous studies that showed positive effects of HRT on CVD in postmenopausal women were usually small cohorts of women who were mostly healthy and active, were of middle socioeconomic status or higher, followed good dietary regimens, and did not have diabetes. In contrast, the cohort in WHI, although excluding women who had previous CV events, included all types of postmenopausal women independent of body weight and activity. Thus, the WHI cohort was more reflective of the general population of postmenopausal women in the United States.

Obesity and subsequent type II diabetes is becoming an epidemic in the United States, and weight gain with aging is commonly found in postmenopausal women. To our knowledge, there is no information that female gender or estradiol supplements protect against the complications of diabetes. In type I diabetic subjects, women have complications such as diabetic nephropathy, retinopathy, and cardiovascular disease at a similar rate as men. In fact, in one study, type I diabetic women younger than age 40 years had a higher incidence of ischemic heart disease than did men.\(^{12}\) Women with type 2 diabetes also have a greater incidence of aortic stiffening\(^ {13}\) and left ventricular hypertrophy,\(^ {14}\) and similar\(^ {15}\) or higher risk of cardiovascular mortality than men.\(^ {16}\) It is not clear why premenopausal women are not protected from the complications of diabetes. However, one possibility is that estradiol biosynthesis may be compromised somewhat in chronic diseases, such as diabetes, just as testosterone biosynthesis is in men. To our knowledge, studies to evaluate serum estradiol levels in premenopausal diabetic women, especially women who do not have strict glycemic control, have not been performed. So, it is possible that in premenopausal women with type I or II diabetes, the reason that they are not protected from CVD is that their bioavailable estradiol levels are reduced. This hypothesis remains to be evaluated in clinical studies.

**Estrogen: “Good Girl”**

Many in vitro studies have shown that estradiol should be cardiovascular-protective. For example, several investigators have shown that estradiol can downregulate components of the renin-angiotensin system (RAS). Ovariectomy of rats increases and estradiol repletion decreases the expression of AT\(_1\) receptors in vasculature and kidneys.\(^ {17,18}\) In addition, estradiol reduces the expression and activity of angiotensin I-converting enzyme.\(^ {7,19}\) The reductions in both AT\(_1\) receptors and angiotensin-converting enzyme should protect against an activation of the RAS. However, estradiol also causes release of angiotensinogen substrate from the liver,\(^ {20}\) and this could offset any potential protective effect if the renin enzyme is not working at maximal rate of reaction (V\(_{\text{max}}\)), and thus renin activity is increased by increased angiotensinogen substrate leading to increased angiotensin II.

Endothelin is another vasoconstrictor that has been shown to be affected by estradiol. For example, in male-to-female transsexuals, estradiol therapy caused a significant reduction in serum endothelin.\(^ {21}\) In premenopausal women, a differential ratio of ET\(_A\) to ET\(_B\) receptors has also been found, favoring a vasodilator effect of endothelin via ET\(_B\) receptors.\(^ {22}\) In recent animal studies, estradiol\(^ {23}\) or its metabolites\(^ {24}\) have been shown to inhibit endothelin synthesis and improve endothelial dysfunction in ovariectomized female spontaneously hypertensive rats\(^ {25}\) and DOCA-salt hypertensive rats.\(^ {23}\)

Therefore, estradiol should be cardiovascular protective because of their positive effect on endothelin inhibition.

Estradiol should also be protective because of its antiinflammatory effects. Mori et al reported that the estradiol was protective against neointimal proliferation in female rats subjected to balloon dilatation of the carotids.\(^ {26}\) In addition, Dubey et al have reported that estradiol and some of its metabolites are antimigrogenic in human aortic smooth muscle cells,\(^ {27}\) mesangial cells,\(^ {28}\) and cardiac fibroblasts.\(^ {29}\) Estradiol-eluting stents are now available and are being used in clinical trials in humans\(^ {30}\) to determine whether the estradiol-eluting stents are successful in protecting against neointimal proliferation.

Estradiol is also an antioxidant and protects against oxidative stress,\(^ {31}\) which is thought to be a causative factor in endothelial dysfunction associated with hypertension.\(^ {32}\) For example, Lacy et al reported that women with essential hypertension had lower levels of plasma hydrogen peroxide than did men.\(^ {33}\) Premenopausal women have also been shown to have lower levels of oxidative stress, as measured by F\(_2\)-isoprostanes, than do men\(^ {34}\) or postmenopausal women,\(^ {35}\) which suggests a role for estrogens in the lower levels of oxidative stress. In animal studies, Streulow et al found that 17\(\beta\)-estradiol not only reduced Ang II–induced oxidative stress but also increased expression of some of the isoforms of superoxide dismutase,\(^ {36}\) an important antioxidant enzyme. These data were also confirmed by Gragas et al in cultured endothelial cells in which estradiol treatment reduced Ang II–mediated expression of NOSPH oxidase and peroxynitrite production.\(^ {37}\) The effect of estrogen was independent of the estrogen receptor, however.

**Estrogen: “Bad Girl”**

With all the positive effects of estradiol that have been found in both in vitro and in vivo studies, the question still remains, “What changes occur after menopause that make estrogens not protective, but actually causative of CVD?” This question is predicated in part on the hypothesis that it is estrogens that protect premenopausal women from CVD. With regard to postmenopausal hypertension, the increase in blood pressure does not occur as soon as a woman passes through menopause, but occurs 5 to 10 years after menopause.\(^ {38}\) Not only does this suggest that loss of estrogen is not the primary mediator of postmenopausal hypertension but also suggests that other secondary factors may play causative roles. Perhaps there are age-related changes that occur independent of estrogen loss that promote CVD in postmenopausal women independent of estrogens. This is supported by the numerous epidemiological studies in which outcomes for men and women undergoing treatment for coronary disease were evaluated. In most of these studies, women fare the same or even poorer than men. However, the women in most of these studies were considerably older than the men, which probably...
also contributes to their poorer outcomes.\textsuperscript{39,40} Perhaps pre-menopausal women are protected from CVD because they lack high levels of testosterone rather than that they have high levels of estradiol. This is the situation in the female spontaneously hypertensive rats in which hypertension develops at a lower level than it does in males.\textsuperscript{41} When ovariectomized, there is no effect on their blood pressure, but when given testosterone supplements, their blood pressure increases to the same level as in males.

If estradiol is the important protective factor against CVD in premenopausal women, then something must change after menopause that affects their response to estradiol. One possibility is that after menopause, changes occur in the estrogen receptors or in the intracellular and intranuclear signaling associated with the receptors, which changes the phenotype to promote CVD. For example, Hestiantoro and Swaab reported that the hypothalamus of young women exhibited more nuclear staining of ER-\(\alpha\) whereas in post-menopausal women the staining for ER-\(\alpha\) was more pronounced in the cytosol of the hypothalamic cells.\textsuperscript{42} In contrast, there was no such change in ER cellular localization with age in men. In agreement with these data, Wynne et al reported that aging female rats exhibited an attenuated inhibitory effect of estradiol on the vasoconstriction of vascular smooth muscle that was estrogen receptor-mediated.\textsuperscript{43}

Studies to dissect the effect of menopause on CVD and hypertension have been hampered in part by the lack of a suitable animal model. Typically, ovariectomized female animals have been used as models to study CVD in post-menopausal women. This is a brutal technique that not only reduces the production of sex hormones but also is somewhat naive in that it ignores the contribution of aging to menopausal changes. The other drawback to most of the menopausal studies in animals is that ovariectomy does not cause hypertension and increased CVD. Two contradictions to this statement are the SHR female that, when ovariectomized at middle age and placed on high-salt diet, becomes more hypertensive\textsuperscript{44} and the young ovariectomized Dahl salt-sensitive rat that exhibits increased blood pressure compared with intact females independent of salt diet.\textsuperscript{45}

There are now 3 animal models that have been described that develop progressive hypertension and CVD with aging that can be used to better determine the changes that occur after menopause. The female SHR that, before cessation of cycling, has a lower blood pressure than males exhibits increased blood pressure after cessation of cycling.\textsuperscript{46} and the hypertension is mediated in part by endothelin and oxidative stress.\textsuperscript{46,47} The Dahl salt-sensitive female rat, when kept on a low-salt diet, has progressive increases in blood pressure with aging that is exacerbated by ovariectomy and is associated with increased renal injury and vascular disease and increased AT\(_1\) receptor number.\textsuperscript{48,49} In addition, the folliclitrin receptor knockout mouse was characterized by Touyz et al and was found also to be hypertensive with increased vascular disease changes.\textsuperscript{50} However, to date, to our knowledge, there have been no aging studies performed on this mouse. Hopefully, the use of these animal models will shed light on the mechanisms by which estradiol changes from a “good girl” to a “bad girl” with aging, studies that could not be performed in humans for ethical reasons.

### Androgens and CVD

Men have higher blood pressure than women throughout most of their lives, and CVD develops at an earlier age in men than it does in women.\textsuperscript{38} These data support a role for androgens in mediating CVD in men. However, in epidemiological studies in which serum testosterone levels were measured in men with chronic CVD, such as hypertension, the levels are lower than in healthy age-matched men.\textsuperscript{1} These findings have led investigators to presume that androgens could not be responsible for initiating and/or mediating CVD. However, it is also possible that the downregulation of androgen synthesis is a protective compensatory mechanism that occurs once the diseases are initiated.

Animal studies refute the hypothesis that CVD is not mediated by androgens in males. For example, in SHR, males have higher blood pressure than do females.\textsuperscript{41,51} Removal of the testes in male SHR reduces blood pressure in the rats. Similar observations have been made in models of nongenetic hypertension, such as DOCA-salt treated rats.\textsuperscript{52}

The mechanisms by which androgens could initiate and/or mediate CVD and hypertension have not been elucidated. One of the most important recent findings are the studies of Quan et al who reported that testosterone is able to directly stimulate sodium reabsorption via the proximal tubule of the kidney.\textsuperscript{53} which they proved using standard micropuncture techniques. Investigators had shown previously that androgen receptors were localized to the proximal tubule of the kidney,\textsuperscript{49} and because androgens could affect the synthesis of components of the RAS,\textsuperscript{55,56} it had been hypothesized that androgens could mediate sodium reabsorption indirectly via the RAS. This new information is particularly important because it provides evidence that androgens can affect proximal sodium reabsorption \textit{directly} and thereby influence blood pressure by a variety of mechanisms.

As mentioned, one mechanism by which androgens could cause CVD and hypertension is via its effects on production of vasoconstrictors. Plasma renin activity is typically higher in men than in premenopausal women.\textsuperscript{57,58} Castration attenuates and testosterone repletion increases mRNA for angiotensinogen and renin in kidneys of SHR.\textsuperscript{55,56} Taken together, these data suggest that androgens could cause an increase in Ang II production in the kidney. Androgens have also been shown to cause an increase in endothelin in humans. Female-to-male transsexuals receiving large doses of testosterone have elevated levels of serum endothelin compared with untreated females.\textsuperscript{21} Thus, androgens could cause increases in blood pressure and endothelial dysfunction leading to CVD by increasing vasoconstrictors.

There is also evidence that androgens may stimulate oxidative stress. Oxidative stress produced by renal intramedullary inhibition of superoxide dismutase has been shown to cause an increase in blood pressure in normotensive rats.\textsuperscript{59} We have preliminary data that treatment of mesangial cells from SHR with dihydrotestosterone at physiological doses increases oxidative stress and upregulates expression of p47\textsuperscript{phox}, a subunit of NADPH oxidase. The p47\textsuperscript{phox} expression
is also attenuated in SHR subjected to castration, and NADPH oxidase activity and superoxide production are higher in intact males than in castrated males. Furthermore, apocynin, the inhibitor of NADPH oxidase, reduces blood pressure in SHR males but has no effect on castrated males (V. Cucchiarelli, R. Iliescu, J.F. Reckelhoff, unpublished results, 2004).

Several recent reviews have illustrated how oxidative stress can promote CVD and hypertension. With regard to mechanisms involving androgens, increases in superoxide caused by androgens could quench the bioactivity/bioavailability of nitric oxide, leading to an increase in peroxynitrite, which could lead to reductions in prostacyclin synthase and the vasodilator prostacyclin and increases in thromboxane A2.60 Therefore, increases in oxidative stress caused by androgens could play a role in the CVD risk in men.

Recently, men have been using androgen supplements to combat some of the symptoms of aging, such as osteoporosis, depressed mood, and erectile dysfunction (along with phosphodiesterase-5 inhibitors), despite the lack of safety studies. Androgens can also be obtained over-the-counter from health food stores in the form of androstenedione. There is little information regarding cardiovascular complications with the use of androgen supplements. Increases in blood pressure in both young and older men have been documented with low-dose or high-dose androgen therapy, however.65–66 With aging, serum testosterone, DHT, and other androgens decrease. If this is a compensatory mechanism to protect from increased CVD associated with aging, then the use of androgen supplements defeats this purpose. With all the information now regarding the physiological and pathophysiological effects of androgens on CVD, lipid profiles, oxidative stress, and hypertension, it will be important that future clinical studies be undertaken to evaluate the safety of androgen supplements in men.

**Androgen Supplements in Aging Women and CVD**

In recent years, androgens have been widely prescribed to postmenopausal women to improve libido. Just as in men, there are no studies regarding the safety of androgen supplements and CVD and hypertension in women. Whether androgens are safe is a particularly important issue in this population, because androgen therapy is given right at the time in a woman’s life when CVD, obesity, diabetes, and hypertension are increasing dramatically, i.e., after menopause. As mentioned, androgens can upregulate vasocostricators, cause oxidative stress, and directly increase sodium reabsorption by the proximal tubule of the kidney, all of which increase the likelihood of developing and promoting CVD. Therefore, it is imperative that safety studies be performed to evaluate the effect of androgen supplements on CVD and hypertension in postmenopausal women.

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Sex Steroids, Cardiovascular Disease, and Hypertension: Unanswered Questions and Some Speculations

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