Sex differences in control of blood pressure: role of oxidative stress in hypertension in females

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Lopez-Ruiz A, Sartori-Valinotti J, Yanes LL, Iliescu R, Reckelhoff JF. Sex differences in control of blood pressure: role of oxidative stress in hypertension in females. Am J Physiol Heart Circ Physiol 295: H466–H474, 2008. First published June 20, 2007; doi:10.1152/ajpheart.01232.2007.—In general, blood pressure is higher in normotensive men than in age-matched women, and the prevalence of hypertension in men is also higher until after menopause, when the prevalence of hypertension increases for women. It is likely then that the mechanisms by which blood pressure increases in men and women with aging may be different. Although clinical trials to reduce blood pressure with antioxidants have typically not been successful in human cohorts, studies in male rats suggest that oxidative stress plays an important role in mediating hypertension. The exact mechanisms by which oxidative stress increases blood pressure have not been completely elucidated. There may be several reasons for the discrepancies between clinical and animal studies. In this review, the data obtained in selected clinical and animal studies are discussed, and the hypothesis is put forward that oxidative stress may not be as important in mediating hypertension in females as has been shown previously in male rats. Furthermore, it is likely that differences in genetics, age, length of time with hypertension, endothelial dysfunction, and sex are all factored in to modulate the responses to antioxidants in humans. As such, future clinical trials should be designed and powered to evaluate the effects of oxidative stress on blood pressure separately in men and women.

F2-isoprostanes

WITH THE USE OF AMBULATORY blood pressure monitoring, it is now well accepted that blood pressure is higher in men than age-matched premenopausal women, and that, following menopause, this trend reverses, such that the incidence of hypertension in postmenopausal women is similar to or higher than in men (5, 69). A comparison of the data from the National Health and Nutrition Examination Survey (NHANES) III (1988–1994) with data from NHANES IV (1999–2002) revealed that the prevalence of hypertension is currently stable in men, and their blood pressures are well controlled with medication. In contrast, in women, the incidence of hypertension is progressively increasing, and blood pressure is often higher in women than in age-matched men (35). Furthermore, blood pressure in women is not well controlled with medication, despite typically higher levels of compliance compared with men (35). These disturbing statistics suggest that mechanisms responsible for hypertension in men and women may be different, and it is imperative that physicians and scientists begin to determine what these differences are to better treat hypertension in both men and women.

Oxidative Stress and Hypertension in Humans: Clinical Trials

The role that oxidative stress plays in mediating hypertension has been the focus of a significant amount of research in recent years. Several clinical trials have been done to determine whether treatment of hypertensive individuals with antioxidants will reduce their blood pressure. The data are inconsistent, with antioxidants having no effect, decreasing, or even increasing blood pressure in hypertensive individuals (9, 36, 42, 44, 60, 67). There have been concerns regarding many of these trials, however. For example, many of the studies have not been powered to consider the depressor response to antioxidants separately from other end points or even from other antihypertensive medications (42). Furthermore, data from men and women are not analyzed separately, nor are the studies powered to determine whether there are sex differences in response to antioxidants. Blood pressure is not always measured by 24-h ambulatory monitoring, which is essential to measure and delineate subtle differences in blood pressure between men and women and between control and antioxidant-treated individuals. Often the ages of the participants are not well controlled. For example, a small study (n = 24) from Japan was performed in which the cohorts were separated into “elderly” men and women, aged 67–84 yr, and “adult” individuals, aged 39–62 yr (58). The investigators found that ascorbic acid (vitamin C) treatment decreased blood pressure, measured by ambulatory monitoring, in the elderly group, but had no effect in the adult group. Furthermore, vitamin E can be a prooxidant or an antioxidant, depending on the dose (17). Other antioxidants, in addition to vitamins E and C, also give equivocal results on blood pressure lowering effects. For example, one study that employed N-acetyl cysteine as the antioxidant showed no effect on blood pressure (60).
Oxidative Stress and Blood Pressure in Animal Studies

Our laboratory has had a longstanding interest in the mechanisms responsible for sex differences in blood pressure control. As such, we have studied the role that oxidative stress plays in mediating the sex differences in hypertension in rats. In the next sections, we will discuss our own data, as well as data from other laboratories in various animal models in which oxidative stress and hypertension have been studied.

Oxidative stress and animal models of angiotensin II-dependent hypertension. ANGIOTENSIN II INFUSION. Several years ago now, Rajagopalan and colleagues (46) reported that infusion of high doses (0.7 mg·kg⁻¹·day⁻¹) of angiotensin II (ANG II) resulted in an increase in blood pressure in male rats, accompanied by an increase in superoxide production in isolated aortae. The increase in oxidative stress was not caused by increasing the blood pressure alone, since increasing blood pressure with norepinephrine to similar levels as ANG II did not result in increases in superoxide (24). In additional studies, this team of investigators found that the increase in superoxide production produced by ANG II contributed to the development of hypertension, since treatment of rats with liposome-encapsulated superoxide dismutase to remove the superoxide anion protected against the ANG II-mediated increase in blood pressure (39).

Our laboratory recently reported studies in which ANG II infusion was given to male and female rats, which were given an ANG I-converting enzyme inhibitor (ACEI), enalapril, to block the endogenous production of ANG II (55). Blood pressure and excretion of 8-iso-prostaglandin F₂α (F₂-isoprostanes), an index of oxidative stress, were measured. By the end of 1 wk, ANG II infusion (150 ng·kg⁻¹·min⁻¹·sc) increased blood pressure to a higher level in females than males, but urinary F₂-isoprostanes were not increased in either group (see Fig. 1). Beginning the second week of ANG II, rats were switched to a high-salt diet, which continued for 2 additional wk. Blood pressure increased progressively in males with the increased salt eventually reaching a higher level than in females by week 3. Urinary F₂-isoprostanes increased in both control and ANG II-treated females, but not males, by the end of the second week of ANG II, suggesting the increase in F₂-isoprostanes was mediated by the increased salt rather than the blood pressure; salt has been shown previously to increase oxidative stress in male rats (37). Similarly, in males, urinary F₂-isoprostanes increased by the end of the third week of ANG II (second week of high-salt diet) in both ANG II and control rats. The fact that ANG II infusion in females increased blood pressure, but was independent of changes in F₂-isoprostanes, is consistent with previous studies by other investigators who found that ANG II infusion in females was not associated with either a change in the activity of NADPH oxidase (12), or on the expression of p67<sub>phox</sub>, a subunit of NADPH oxidase (64), both of which were increased in males receiving ANG II in these studies. In support of an increase in oxidative stress in male rats in response to ANG II, our laboratory showed previously that intravenous ANG II infusion (10 ng·kg⁻¹·min⁻¹) for 2 wk with the endogenous production of ANG blocked with ACEI and normal salt diet caused an increase in intrarenal F₂-isoprostanes that accompanied the increase in blood pressure (49). Thus the increase in oxidative stress in females was dissociated from ANG II or blood pressure in our studies.

SPONTANEOUSLY HYPERTENSIVE RATS. The spontaneously hypertensive rats (SHR) are a model of androgen- and ANG II-dependent hypertension that exhibit sex differences in blood pressure, with males having higher blood pressure than females (50). In addition, a dose-dependent increase in blood pressure...
occurs in ovariectomized females given testosterone (50). The sex difference in blood pressure can be abolished if SHR are treated with the ACEI, enalapril (48). Based on the studies of Rajagopalan and coworkers (46), cited above, that high-dose ANG II increased blood pressure in male rats in part by increasing oxidative stress, Schnakenberg and Wilcox (59) treated male SHR with a superoxide dismutase mimetic, tempol, and found that tempol reduced their blood pressure. These data suggested that the hypertension in male SHR was mediated in part by oxidative stress and superoxide anion, in particular. Based on these studies, we evaluated whether hypertension in female SHR was also mediated by oxidative stress. However, in contrast to males, we found that tempol had no effect on blood pressure in adult female SHR (57).

One caveat to the use of tempol is that recent studies have shown that tempol does not act solely as a superoxide dismutase mimetic, but, in male SHR, it also affects peripheral sympathetic nervous activity and activates ATP-sensitive potassium channels (8), which can cause vasodilation that could impact blood pressure. In addition, in males with deoxycorticosterone acetate (DOCA)-salt hypertension, tempol can cause activation and increased synthesis of calcium-activated potassium channels, which also causes dilation of vascular smooth muscle (71). Both of these vascular effects of tempol were found in acute studies, however, and may not reflect chronic use of tempol, as in our studies. Furthermore, in terms of sympathetic effects of tempol, the sex difference in blood pressure in SHR is not mediated by differences in sympathetic activation, as our laboratory showed previously (29).

Since the source for superoxide anion production in the kidney is primarily due to NADPH oxidase (46), we determined whether an inhibitor of NADPH oxidase subunit synthesis and assembly (28), and thus activity, apocynin, would reduce blood pressure in male and female SHR. We found that chronic apocynin reduced blood pressure and expression of NADPH oxidase subunits in males (28) but had no effect in females (57). These data suggested that NADPH oxidase may play a role in mediating the hypertension in male SHR, but not females. Recently, in vitro studies showed that apocynin has antioxidant effects, independent of an inhibition of NADPH oxidase (23). In any case, the blood pressure response to apocynin was different in male and female SHR.

Finally, we determined whether generic antioxidants, vitamins E and C, would decrease blood pressure in male and female SHR. Although we found that vitamins E and C reduced oxidative stress and blood pressure in male SHR, in females there was no effect on renal F2-isoprostanes, but blood pressure was reduced with vitamins E and C (57). These data suggest that vitamins E and C reduced blood pressure by a mechanism independent of oxidative stress. These findings will be discussed in depth below.

In summary, of the antioxidants we used, tempol, apocynin, and ebselen, a glutathione peroxidase mimetic (J. Sartori-Valinotti and J. F. Reckelhoff, unpublished observations), failed to reduce blood pressure in young adult female SHR, whereas they all reduced blood pressure in males. Vitamins E and C reduced blood pressure in females, but failed to reduce oxidative stress. If oxidative stress played no role in maintenance of hypertension in females, we wondered whether development of hypertension in females would be mediated by oxidative stress. To address this question, we gave tempol to SHR dams from parturition until weaning and then to the pups until 15 wk of age when blood pressure was measured (14). We found that tempol from birth reduced renal F2-isoprostanes by 60% in male SHR, but only resulted in a reduction in blood pressure of 14% (14). In female SHR, tempol from birth reduced renal F2-isoprostanes by 50% and decreased blood pressure by 26% at 15 wk of age. These data demonstrate that there is not a linear correlation between the level of oxidative stress and the impact on blood pressure in either sex.

Sex Differences in Markers of Oxidative Stress in SHR

Since antioxidants had equivocal effects on blood pressure in adult female SHR, we hypothesized that perhaps females exhibited little oxidative stress compared with males; thus antioxidants would not reduce blood pressure. To test this hypothesis, F2-isoprostanes were measured by gas chromatography-mass spectroscopy in various fluids and tissues from the rats. In plasma samples, F2-isoprostanes were similar between age-matched (15 wk of age) male and female SHR (57). Consistent with plasma F2-isoprostanes, plasma total antioxidant status, the capacity of plasma to neutralize oxidants, measured with a commercially available kit (Calbiochem), was similar between males and females. In contrast, kidney F2-isoprostanes were 30% higher in males than females, although urinary excretion of F2-isoprostanes was 200% higher in females than males. Thus F2-isoprostane levels were not consistent between the sexes for the tissues assayed.

To further evaluate tissue oxidative stress, we performed lucigenin chemiluminescence studies. We found that the renal cortex of female SHR had higher levels of basal superoxide production, whereas the medulla had significantly higher levels of NADPH-stimulated superoxide production than did males (57). In contrast, we found that males exhibited ~200% higher levels of both basal and NADPH-stimulated superoxide production in aortae than did females. Consistent with our data in aortae, Dantas and colleagues (10) reported that oxidative stress in mesenteric arteries, as measured by hydroethidine microfluorography, was higher in male SHR than females. Similarly, Sullivan and colleagues (63) found that male SHR excreted higher levels of urinary hydrogen peroxide than did female SHR. Thus oxidative stress in SHR differs by sex and by tissue distribution. Despite the fact that blood pressure in female SHR does not respond to any of the specific antioxidants we tried, females likely have similar or higher levels of oxidative stress than do males.

Renal Expression of Antioxidant Enzymes in SHR

In addition to measuring the tissue levels of oxidative stress, we determined the expression of enzymes important in control of oxidative metabolites. We found that males exhibited significantly higher levels of Mn- and CuZn-SOD, glutathione peroxidase, and catalase than did females (57). These data may explain why we measured higher basal levels of reactive oxygen species (ROS) by lucigenin chemiluminescence in the renal cortex of females. Our data are supported by studies by Sullivan and coworkers (63), who found that total renal superoxide dismutase and catalase activities were higher in male SHR than females.
Sex differences in pressor response to a prooxidant in SHR. To evaluate whether blood pressure in female SHR would respond to an increase in oxidative stress, rats were given molsidomine (also known as SIN-1) in an attempt to further increase blood pressure. Male and female SHR and Wistar-Kyoto (WKY) were treated for 1 wk with molsidomine (13, 57). Molsidomine is a drug that releases both nitric oxide and superoxide. We found that male WKY had a 5% reduction in blood pressure with molsidomine that was accompanied by increases in renal expression of Mn- and CuZn-SOD, catalase, and glutathione peroxidase, suggesting that these normotensive animals increase antioxidant defenses in response to increased oxidative stress. In contrast, male SHR exhibited a 14% increase in blood pressure in response to molsidomine that was not accompanied by changes in expression of the antioxidant enzymes in the kidney (13), suggesting that the failure of male SHR to upregulate intrarenal expression of antioxidant enzymes contributed to their increased blood pressure with molsidomine. In females, although oxidative stress was increased as found in males, molsidomine failed to affect blood pressure in either WKY or SHR (57). Thus, unlike in male SHR, females do not increase blood pressure in response to increases in oxidative stress, further calling into question the role that oxidative stress plays in mediating the hypertension in female SHR.

Oxidative Stress in Other Animal Models of Hypertension

A survey of the literature shows that there is a paucity of data from other laboratories in which the role of oxidative stress in mediating hypertension has been evaluated in female animals. Below is a discussion of the relevant studies.

mREN2.Lewis rats. The mREN2.Lewis rat is a model of hypertension that is mediated by activation of the renin-ANG system. Despite the fact that the hypertension in these rats is mediated by ANG II, Chappell (7) recently reported that tempol had no effect on the blood pressure of female rats (Fig. 2), tempol failed to affect blood pressure in either male or female SHR and molsidomine (also known as SIN-1) in an attempt to further increase blood pressure. Male and female SHR were treated for 1 wk with molsidomine (13, 57). Molsidomine is a drug that releases both nitric oxide and superoxide. We found that male WKY had a 5% reduction in blood pressure with molsidomine that was accompanied by increases in renal expression of Mn- and CuZn-SOD, catalase, and glutathione peroxidase, suggesting that these normotensive animals increase antioxidant defenses in response to increased oxidative stress. In contrast, male SHR exhibited a 14% increase in blood pressure in response to molsidomine that was not accompanied by changes in expression of the antioxidant enzymes in the kidney (13), suggesting that the failure of male SHR to upregulate intrarenal expression of antioxidant enzymes contributed to their increased blood pressure with molsidomine. In females, although oxidative stress was increased as found in males, molsidomine failed to affect blood pressure in either WKY or SHR (57). Thus, unlike in male SHR, females do not increase blood pressure in response to increases in oxidative stress, further calling into question the role that oxidative stress plays in mediating the hypertension in female SHR.

Dahl salt-sensitive rats. Sex steroids influence the blood pressure in Dahl salt-sensitive (DS) rats. When maintained on a low-salt diet, Hinojosa-Laborde and colleagues (24, 25) reported that blood pressure was higher in males than females, that ovariectomy increased the blood pressure to levels higher than in intact males, and that castration of the males attenuated the blood pressure to levels similar to females. These investigators also showed that, when DS rats are placed on a high-salt diet, the sex differences in blood pressure remain. The increase in blood pressure in male DS rats with increased salt intake is mediated by oxidative stress, since treatment with diphenyl iodinium, tempol, vitamins E and C, or apocynin reduces their blood pressure (2, 65, 66). Similar studies have not been done to evaluate whether antioxidants will reduce the salt-sensitive blood pressure in female DS rats.

To understand the mechanisms by which ovariectomy alone increases blood pressure in DS females independent of the salt effect, we addressed whether the increase in blood pressure with ovariectomy was mediated by oxidative stress. Intact female and ovariectomized DS rats were given tempol (30 mg·kg⁻¹·day⁻¹) in drinking water for 2 wk while being maintained on a low-salt (0.3%) diet, and blood pressure was measured in conscious, chronically catheterized rats. As shown in Fig. 2, blood pressure was significantly higher in ovariectomized rats than in intact females. However, urinary F₂-isoprostanes were significantly higher in intact DS rats than in ovariectomized females (Fig. 2). While tempol reduced urinary F₂-isoprostane excretion in both intact and ovariectomized DS rats (Fig. 2), tempol failed to affect blood pressure in either ovariectomized or intact female DS rats.

DOCA-salt model of hypertension. Sex differences in blood pressure also occur in rats treated with DOCA-salt, with males exhibiting higher blood pressure than females. Kawanishi and colleagues (32) investigated the role of endothelin B (ETB) receptor in mediating the sex difference in blood pressure in DOCA-salt-treated wild-type (WT) rats or rats containing the spotted lethal (sl/sl) (SL) mutation that carries a naturally occurring deletion in the ETB receptor gene. DOCA-salt increased blood pressure in both male and female WT rats, but to a higher level in males than females. Oxidative stress, as measured by lucigenin chemiluminescence, was only increased in WT males, not females. In male and female SL rats lacking ETB receptor, DOCA-salt increased blood pressure to similar levels. Despite similar blood pressures, DOCA-salt significantly increased oxidative stress in aortae from SL males, but not females. Thus there was a dissociation between the increase in oxidative stress and the development of hypertension with DOCA-salt in both WT and SL female rats, compared with males.

The “renal wrap” model of hypertension. The “renal wrap” is a model of hypertension produced by uninephrectomy and placement of a snare on the remaining kidney in the form of a...
“figure 8”. Renal wrap hypertension is associated with renal injury and fibrosis, with males developing more renal injury after the procedure than females (19). Males are also hypertensive as a result of the procedure, whereas females are not (19). Whether there is a sex difference in the blood pressure in response to high-salt diet is controversial, however, since some investigators find there is an increase in blood pressure with the “renal wrap” maneuver, whereas others do not. For example, Haywood and Hinojosa-Laborde (19) reported that blood pressure is higher in male renal wrap rats on high-salt diet than in females. This group has not determined whether there are sex differences in oxidative stress in their model, however. In contrast, Ji and colleagues (30) reported that, in rats with “renal wrap hypertension” and high-salt diet, there was no difference in blood pressure between males and females. Despite the lack of a difference in blood pressure in this latter study, the males exhibited higher levels of oxidative stress, as determined by measuring urinary malondialdehyde, renal cortical NADPH oxidase activity, and expression of NADPH oxidase subunit p22\textsuperscript{phox}. These investigators did not evaluate the blood pressure response to antioxidants, however. Thus the mechanisms responsible for the increase in blood pressure in male and female rats subjected to renal wrap hypertension may be different, with an increase in oxidative stress perhaps contributing to the increase in blood pressure in males, but not females.

Obese Zucker (fa/fa) rats. Male and female obese Zucker rats (OZR) exhibit hyperphagia-mediated obesity. Riazi and colleagues (53) studied salt sensitivity of blood pressure in OZR and the levels of NADPH oxidase activity and subunit expression. They found that male OZR and their lean controls (LZR) developed higher blood pressures with elevated salt diet than did their female counterparts. Female OZR exhibited higher blood pressures with high-salt diet than did female LZR, which did not develop salt-sensitive hypertension. Despite female LZR having the lowest blood pressure of the four groups of rats, they expressed the highest levels of protein expression of renal medullary NADPH oxidase subunits (gp91\textsuperscript{phox}, p47\textsuperscript{phox}, and p67\textsuperscript{phox}). However, NADPH oxidase enzyme activity was not elevated in female LZR compared with male LZR or OZR or female OZR. In contrast, in OZR, NADPH oxidase activity was similar in males and females, despite the greater hypertension developed with salt diet in males than females. These data demonstrate that there is a dissociation between oxidative stress and development of hypertension with high salt in OZR females compared with males.

In summary, although oxidative stress is present in female animal models of hypertension other than SHR, whether an increase in oxidative stress plays a role in mediating the increased blood pressure in females is not as clear as it is in males, and there is a dissociation between the levels of oxidative stress and the hypertension developed in females compared with males.

**Oxidative Stress in Aging SHR**

Aging in female SHR is associated with an increase in blood pressure to levels that are similar to or higher than in males (15). To determine whether aging-associated increases in oxidative stress mediated the increase in blood pressure in aging female SHR, male and female SHR were treated with tempol or vitamins E and C for 8 mo, starting at 8 mo of age (15). We found that, in aging males, tempol reduced urinary F\textsubscript{2}-isoprostanes and their blood pressure, just as we found previously in young male SHR (described above). However, in contrast to young adult males, vitamins E and C failed to reduce blood pressure significantly in old male SHR, although urinary F\textsubscript{2}-isoprostanes were reduced. As mentioned previously, in young adult female SHR, 6-wk treatment with vitamins E and C failed to have an effect on oxidative stress but reduced blood pressure (56). Tempol had no effect on either oxidative stress or blood pressure in young females. In old female SHR, tempol reduced urinary excretion of F\textsubscript{2}-isoprostanes slightly, but failed to reduce their blood pressure. In contrast, in old female SHR, chronic vitamin E and C reduced oxidative stress and blood pressure slightly (10%) (15).

Why there was a difference in the depressor and antioxidant effects of vitamins E and C in male and female SHR was not immediately apparent. Recently, Barella and colleagues (1) found that chronic treatment (1.2 yr) of male Sprague-Dawley rats with vitamin E decreased plasma levels of cholesterol by suppressing the expression of low-density lipoprotein receptor and several of the enzymes necessary for cholesterol synthesis. They hypothesized that the vitamin E effect on cholesterol synthesis would reduce androgen synthesis, since cholesterol is the necessary substrate for steroidogenesis. Therefore, we measured plasma testosterone levels in aging male and female rats that were treated with either vitamins E and C or tempol. Indeed, we found that vitamins E and C, but not tempol, reduced plasma testosterone in both male and female SHR (Fig. 3).

The reduction in plasma testosterone in response to vitamins E and C shed light on the blood pressure responses we measured in aging female SHR. Estradiol plays no role in hypertension in young female SHR (aged 4 mo), since ovariectomy does not impact blood pressure (50). In contrast, when young ovariectomized female SHR are given testosterone supplements, their blood pressure increases in a dose-dependent manner. Plasma testosterone levels are low in young female SHR, but increased by fourfold in old females (aged 16 mo) compared with young rats. Therefore, the fact that vitamins E and C, but not tempol, reduced plasma testosterone in aging females suggests that it was the reduction in testosterone, not the effect on oxidative stress, that mediated the reduction in blood pressure in the old females. This hypothesis is supported by the fact that tempol had no effect on blood pressure in old females, and we have preliminary data that the androgen receptor antagonist, flutamide, reduces blood pressure in aging female SHR. Although we did not measure plasma testosterone in young females given vitamins E and C, it is possible that there was also a reduction in testosterone that impacted their blood pressure, since vitamins E and C failed to affect F\textsubscript{2}-isoprostanes in the young females.

Hypertension in male SHR, as mentioned previously, is mediated by androgens, as well as, since castration and androgen receptor antagonists attenuate the hypertension (47, 52). Although we did not measure the plasma testosterone levels in young male SHR in response to vitamins E and C, we would expect that vitamin E and C treatment would reduce blood pressure, if the treatment also reduced testosterone levels and reduced oxidative stress. In contrast, in aging male SHR, 16 mo, plasma testosterone levels are ~40% lower compared with...
that in young males, aged 4 mo. Thus a further reduction in plasma testosterone with vitamins E and C is not likely to have a profound effect on blood pressure in aging male SHR, which is what we found. However, despite the lack of a depressor response to vitamins E and C, and in contrast to female SHR that were unresponsive to both anti- and prooxidants, it is likely that oxidative stress contributes to the hypertension in male SHR, regardless of age, since tempol reduces blood pressure in young and old males (14, 15, 59), apocynin reduces blood pressure in young males (28), and finally, increasing oxidative stress with molsidomine increases blood pressure in aging male SHR, regardless of age, since tempol reduces blood pressure in young and old males (14, 15, 59), apocynin reduces blood pressure in young males (28), and finally, increasing oxidative stress with molsidomine increases blood pressure in young males (13).

Bearing in mind the data regarding vitamin E and androgen synthesis, there are two clinical studies that are worth mentioning. Schutte and colleagues (61) found that blood pressure was reduced in young normotensive men treated with vitamins E and C and folate for 12 wk. In addition, Rodrigo and colleagues (54) reported that men, aged 35–60 yr, with essential hypertension, had reductions in blood pressure with treatment of vitamins E and C for 8 wk. These latter individuals were without obesity, dyslipidemias, diabetes mellitus, smoking, vigorous physical exercise, or any medication. It is tempting to speculate that a reduction in plasma testosterone levels, which were not reported, and likely were not measured, in either study, could have impacted the blood pressure results of these studies rather than the antioxidant effect of the vitamins.

**Estradiol and Oxidative Stress**

As mentioned previously, estradiol is thought to have antioxidant properties, since estradiol increases Mn-superoxide dismutase and glutathione peroxidase expression and decreases NADPH oxidase enzyme activity and superoxide production (31, 43). Estradiol also plays a role in mediating the lower blood pressure in some female animal models with hypertension (25, 70). However, as mentioned above for the SHR and DS rats, despite the antioxidant properties attributed to estradiol, the lower blood pressure in females is often independent of the levels of oxidative stress. In addition, the levels of oxidative stress in female animals and women are independent of the levels of estradiol in many studies. For example, although hormone replacement therapy reduced blood pressure, hormone replacement therapy had no effect on levels of F2-isoprostane in a study of postmenopausal women (41). In the Study of Women’s Health Across the Nation, Sowers and colleagues (62) also found that endogenous estrogen in perimenopausal women, aged 47–57 yr, was independent of F2-isoprostane excretion. In female stroke-prone SHR or WKY rats, estrogen replacement had no effect on superoxide production in either strain, but increased expression of endothelial NO synthase in WKY, but not stroke-prone SHR females (18). Thus it is not clear whether exogenous or endogenous estradiol protects against oxidative stress and hypertension.

**Markers of oxidative stress in humans.** Whether men or women have higher levels of oxidative stress has been a question that is difficult to answer, partly due to the inconsistencies in measures of oxidative stress. In many studies, men have been shown to exhibit higher levels of markers of oxidative stress than women, as measured by higher levels of 15-F2-isoprostanes and thiobarbituric acid reactive substances (such as malondialdehyde) in the plasma (21, 26, 34). One of the major F2-isoprostanes is 8-iso-prostaglandin F2α, which is often measured in clinical studies, although recently the metabolite of 8-iso-prostaglandin F2α has been measured in urine samples instead (11). Postmenopausal women have higher urinary levels of 8-iso-prostaglandin F2α than their premenopausal counterparts (21). However, in a study of 121 men and 177 women, aged 19–78 yr, plasma malondialdehyde and F2-isoprostanes were found to be significantly higher in women than men. In addition, in the Insulin Resistance Atherosclerosis Study, in which one-half of the individuals developed Type 2 diabetes, while the other one-half did not, metabolite of 8-iso-prostaglandin F2α in the urine was significantly higher in women than men (27).

Furthermore, in pathological conditions in which we assume oxidative stress to be elevated, often the markers of oxidative stress are not increased. For example, in the study of Block and colleagues (3), while there was a correlation between markers of oxidative stress and body mass index, there was no correlation with aging. These data are in contrast to another study in which there was a strong, positive association between plasma F2-isoprostanes and aging (68). In both studies, F2-isoprostanes were measured by gas chromatography/mass spectroscopy by the same laboratory, which rules out the method of measurement as the source of the discrepancy. To complicate matters further, in the study by Block and colleagues, African-American individuals had significantly higher malondialdehyde levels, but significantly lower F2-isoprostane levels than did the
other individuals in the cohorts (3). Finally, in another study, there was an inverse correlation between the plasma levels of the antioxidant β-carotene and blood pressure in men that was not present in women, although neither men nor women responded to antioxidant vitamins with reductions in blood pressure (36).

The reasons for the discrepancies in the results of oxidative stress measurements in clinical studies are complex. One problem has been the choice of the indicator of oxidative stress. While the measurement of F2-isoprostanes has been heralded frequently as the most sensitive measure of oxidative stress, Helmersson and Basu (20) found that there were significant (42%) day-to-day variations in plasma and urinary F2-isoprostane levels in 13 healthy men and women on controlled diets in a research center for 10 consecutive days. These data suggest that, to measure differences in oxidative stress markers, the cohorts must be sufficiently large and that the differences in levels of oxidative stress between groups must be substantial.

Other Considerations in Human Studies

Genetics. While the measurements of oxidative stress are not consistent among groups and thus may explain why many of the clinical trials with antioxidant therapies have not been successful in showing protection against cardiovascular outcomes, including lowering blood pressure, another aspect of the human studies should be taken into consideration. It is very likely that there may be a genetic component to the relative contribution that oxidative stress may play in mediating cardiovascular disease and hypertension, and thus the response to antioxidants may reflect those genetic differences. For example, in the Women’s Angiographic Vitamin and Estrogen trial, pre- and postmenopausal women underwent baseline and follow-up coronary angiography after vitamins E and C therapy for up to 36 mo (40). There was a significant improvement with antioxidant vitamins in coronary minimum luminal diameter, as measured with angiography in women who exhibited the Hp 1-1 allele of haptoglobin. However, this was not found in women with Hp 2-2 allele. In contrast, neither flow-mediated, endothelium-dependent dilation, nor nitroglycerin-induced vasodilation was improved in any of the women taking vitamins E and C in this trial (33). These data suggest that there may be a genetic component involved in the mechanisms by which oxidative stress mediates cardiovascular disease.

Endothelial dysfunction and nitric oxide deficiency. There is another consideration for why antioxidants may have little effect in lowering blood pressure in humans. In young male SHR, we found that, if the nitric oxide system is blocked with nitro-l-arginine methyl ester, tempol can no longer reduce their blood pressure, despite the fact that oxidative stress, as measured by F2-isoprostane levels in the kidney, was significantly reduced (72). These data suggest that, in aging individuals who suffer endothelial dysfunction, as is common with individuals with longstanding essential hypertension, antioxidants are not able to reduce blood pressure because of the deficiency of nitric oxide, a common finding associated with endothelial dysfunction.

Summary

Thus whether oxidative stress contributes differently to hypertension in men and women remains to be determined. While oxidative stress likely impacts blood pressure in males, most animal studies do not support a role for oxidative stress in mediating hypertension in female animals. Furthermore, the lack of antioxidants that are specific make interpretation of studies difficult. Future studies will require more specific scavengers or inhibitors of ROS, and better and more consistent methods of detection of ROS in both humans and animal models of hypertension.

However, the lack of consistency between blood pressure and oxidative stress levels and the fact that rat studies using different hypertensive models fail to show a definitive role for oxidative stress in mediating hypertension in females suggest that hypertension in women may have different etiologies and contributing factors than in men, and that different antihypertensive treatment regimens need to be developed for the sexes. In the future, investigators may need to consider not only the sex of the individual, but age, length of time with hypertension, level of endothelial dysfunction, and genetics, when determining the possible mechanisms responsible for hypertension in their cohorts.

REFERENCES


