Testosterone supplementation in aging men and women: possible impact on cardiovascular-renal disease

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The use of testosterone supplements in aging men and women has increased in recent years. Aging men take testosterone supplements to improve muscle mass and bone strength, to improve libido and quality of life, to prevent and treat osteoporosis, and, with the phosphodiesterase-5 inhibitors, to treat erectile dysfunction. Postmenopausal women receive testosterone supplements mainly to improve their libido. In addition to physicians prescribing testosterone supplements, individuals are able to buy androgens, such as androstenedione, over the counter in health food stores. Serum testosterone levels that are achieved with supplements range from physiological to supra-physiological levels (sometimes as high as 3- to 16-fold higher in men and women than physiological levels) depending on the preparation, dose, and method of administration (9, 13, 42). While urologists are sounding the alarm for caution in using testosterone supplements because of their potential to increase the incidence of prostatic cancer (75), there have been few clinical trials to study the safety of testosterone supplements in aging men and/or women in terms of their effect on cardiovascular-renal disease. Earlier this year, the Food and Drug Administration refused to approve the testosterone “patch” for use in postmenopausal women, however, due to the possibility of cardiovascular side effects (79). In this review, research regarding the impact of androgens on cardiovascular and renal disease from human and animal studies will be discussed.

Changes in testosterone with age

Several cross-sectional (20, 83) and longitudinal (19, 28) studies documented a decline in total and bioavailable circulating testosterone levels with aging in men. More than 60% of healthy, elderly men over 65 yr of age have free testosterone levels below the normal values of men aged 30–35 yr. Because androgen receptors are upregulated in the presence of androgens and downregulated in its absence, it is likely that a reduction in androgen receptors may occur with aging as well. There have been studies in which the expression of androgen receptors in aging men was reduced in reproductive tissues, but there have been no studies in which the expression of androgen receptors has been studied in nonreproductive tissues in aging individuals. The decrease in androgen levels with aging in men appears to be the result of both gonadal and hypothalamic-pituitary failure and is independent of chronic illness, obesity, or medication (28). However, chronic illnesses (52), such as hypertension (81), diabetes (10), chronic kidney disease (33, 74), and end-stage renal disease (38, 39), are associated with a further reduction in serum testosterone levels in men of all ages. The fact that androgen levels decrease with age have led scientists to presume that androgens do not play a role in chronic cardiovascular and renal disease in aging men. However, growing evidence indicates that testosterone, even at levels observed in aging men, can have adverse consequences on cardiovascular-renal function.

Serum testosterone levels are significantly lower in women than in men, and whether serum testosterone levels decrease after menopause is not clear. The conventional wisdom is that
androgen levels, like estrogen, do decrease with age in women. However, Jiroutek and colleagues (37) reported that postmenopausal women who were followed for 10 yr after cessation of cycling and had serial measurements of sex hormones showed increases in serum testosterone and androstenedione and decreases in serum estradiol and dihydrotestosterone as they aged. Other investigators have observed that serum testosterone either does not change (30) or is decreased in postmenopausal women (57). In cross-sectional studies of women in the Rancho Bernardo cohort, Laughlin and colleagues (45) reported that serum testosterone decreased immediately following menopause, but thereafter increased with age, reaching premenopausal levels by 70–79 yr. In addition, these investigators found that women who had undergone surgical menopause did not show increases in testosterone with age and the levels of serum testosterone were 40–50% lower than in women who had undergone natural menopause. These data support the contention that the natural postmenopausal period in a woman’s life is a relatively hyperandrogenic state (2, 44, 60). Furthermore, Sluijmer and colleagues (77) found that the degree of ovarian stromal hyperplasia, a common characteristic of the ovaries in postmenopausal women, is not correlated with the level of estrone and estradiol measured in the ovarian vein but is positively correlated with the level of testosterone and androstenedione. For example, women with atrophic ovaries had little testosterone measured in the ovarian vein, whereas women with moderate to severe hyperplasia had higher levels of ovarian testosterone produced. In addition, the incidence and degree of stromal hyperplasia were quite variable among the women (77). Because the extent of ovarian stromal hyperplasia is rarely measured in most studies of postmenopausal women, this could explain the discrepancies in androgen levels found in various studies.

ANDROGENS AND CONTROL OF BLOOD PRESSURE

There is growing evidence that androgens can influence blood pressure regulation. Men have higher blood pressure than do women for most of their lives (41, 86). Following menopause, however, blood pressure increases in women to levels not different or even higher than in men (7). The prevalence of hypertension is also higher in men than women until after menopause (7). However, hypertensive men have been shown to have lower serum testosterone levels than normotensive men of the same age (81). These data have called into question the role that androgens could play in mediating or promoting hypertension.

Although there have been few studies in men in which the role of androgens in mediating higher blood pressure has been examined, there are several animal models in which a sex difference in blood pressure has been described and the role of androgens has been studied. For example, the spontaneously hypertensive rat (SHR) is a model of hypertension that is androgen mediated (62, 68). Following puberty, males have higher blood pressure than females (68) (see Fig. 1). Castration of males is associated with reductions in blood pressure to the levels found in females. Furthermore, testosterone treatment of ovariectomized females increases their blood pressure in a dose-dependent manner (68, 69). Sex differences in blood pressure have also been found in Dahl salt-sensitive (DS) rats on a high-salt diet (32). The DS males have higher blood pressure than females when placed on 4% salt diets despite the fact that high-salt diet for 3 wk reduces serum testosterone levels in the males by 50% (n = 5/group) (Yanes LL, Iliescu R, and Reckelhoff JF, unpublished observations). Early studies failed to show a reduction in blood pressure with castration in the male DS on a high-salt diet (73). However, as shown in Fig. 2, we have found that castration attenuates the hypertensive response to high salt in male DS. These data support the contention that despite reductions in serum testosterone in DS males with high salt, there is still enough androgen present to play a role in mediating the salt-induced hypertension. It should be noted, however, that testosterone replacement studies have not been performed yet. Thus it is possible that other factors may play a role in the lower blood pressure with castration, although we doubt this will be the case. Nongenetic models of hypertension also exhibit sex differences, such as DOCA-salt-treated rats, in which the hypertension is more...
severe in males than females (11), but to our knowledge, serum testosterone levels have not been reported for rats receiving DOCA and salt.

**ANDROGENS AND RENAL DISEASE**

**Androgens in the Kidney**

To date, there is a paucity of data regarding the role of androgens and the androgen receptor in regulating renal function. However, the kidney expresses the enzymes capable of producing testosterone and the androgen receptor. For example, Quinkler and colleagues (60) found that kidney tissue obtained from tumor nephrectomy samples from postmenopausal women express 5α-reductase type 1, 3β-hydroxysteroid dehydrogenase type 2, and 17α-hydroxylase/17,20 lyase (P450c17) (see Fig. 3). They also found that radiolabeled pregnenolone, produced from cholesterol, was converted effectively to dehydroepiandrosterone (DHEA), and radiolabeled DHEA was converted via androstenedione to testosterone and dihydrotestosterone. Whether androgens can be produced in kidneys of men via these enzymes was not determined in these studies but is highly likely. In support of this hypothesis, in male rats, treatment of inner medullary collecting duct primary cultures with radiolabeled testosterone or androstenedione resulted in production of 5α-dihydrotestosterone and 5α-androstenediol (47). Androgen receptor expression has been found in the proximal tubule and in the cortical collecting ducts of human kidneys (43).

**Androgens and Proximal Tubule Reabsorption**

One of the most important recent findings regarding the effect of androgens in the kidney is the work of Quan and colleagues (59), who found by micropuncture studies that chronic (10 days) dihydrotestosterone (DHT) injections in Sprague-Dawley rats caused an increase in the proximal tubule volume reabsorption, which could be reduced with blockade of the renin-angiotensin system (RAS). Blood pressure was higher in the DHT-treated rats, but the glomerular filtration rate (GFR) was not affected nor was AT1 receptor binding. This is in contrast to acute infusion of testosterone, which causes renal vasodilation with increases in GFR and renal plasma flow and reductions in renal vascular resistance (Reckelhoff JF, unpublished observations). The data of Quan and colleagues (59) suggest that, in the chronic situation, androgens may upregulate ANG II and the Na/H exchanger, leading to increases in sodium and water reabsorption and elevations in blood pressure.

Androgens and Chronic Renal Disease

As in other chronic diseases, serum androgen levels are decreased in men with renal insufficiency (33, 74). Despite the reduction in androgens that occurs with aging, men progress to chronic renal failure at a more rapid rate than do women, even for similar levels of blood pressure (53). For example, Neugarten and colleagues (53) performed a meta-analysis of studies including 11,345 subjects and found that renal disease independent of diabetes progresses at a more rapid rate in men. Several renal diseases are also more common in men than women. Polycystic kidney disease (24, 80) and IgA nephropathy (27) are more common in men and progress more rapidly in men than women. Age-related reductions in renal function also progress at a more rapid rate in men than women (76). Despite the gender differences in the progression of chronic renal disease, Neugarten and colleagues (54) reported that there are few sex differences in normal renal structure that could account for these observations. For example, glomerular number was similar in men and women, and the glomerular volume and kidney weight were similar when corrected for body weight of the individuals.

In aging rats, serum testosterone levels also decrease with age as found in men. Several investigators have reported that normotensive and hypertensive males experience a more rapid reduction in GFR and more renal injury with age than do females (6, 22, 64–66, 68). However, the reduction in GFR cannot be fully accounted for by the level of glomerular injury found in the kidneys (22, 68). For example, in old male Sprague-Dawley rats aged 20–22 mo, a model of accelerated renal aging, GFR was reduced by ~50% compared with young males and yet only 20% of their glomeruli exhibited any injury (68). These data suggest that aging is a state of renal vasocstriction. We have made similar findings in the aging SHR. In old male SHR, renal vascular resistance was increased by 30%, whereas blood pressure was only increased by 10% compared with young SHR (22). As in normotensive rats, the reductions in GFR in old male SHR cannot be explained by glomerular injury because <10% of glomeruli are injured in these rats at 18 mo of age, whereas GFR was reduced by ~30% (22). In more recent studies in SHR, we found that castration of aging males completely prevents reductions in GFR and glomerular injury (22). In addition, castration prevents the increase in renal vascular resistance found in aging male SHR despite a significant age-related reduction in serum androgens in the intact male. Taken together, these data suggest that remnant androgens mediate the reductions in renal function in the aging animal.

In another model of hypertension and renal injury that is not genetically mediated, the renal wrap hypertension model, Ji and colleagues (36) recently reported that castration of male rats attenuated the glomerular injury and proteinuria. When castrated rats were treated with DHT, the glomerular injury was exacerbated. Similarly, in renal wrap female rats that were ovarioctomized (a model of ovarian hormone deficiency), testosterone treatment also exacerbated renal injury and proteinuria. Thus there is compelling evidence that indicates that testosterone promotes renal injury and declines in renal function.

Androgens and the RAS in the Kidney

Androgens play a role in modulating the RAS. Several groups have shown that androgens can stimulate the upregu-
lotion of angiotensinogen in the kidneys of normotensive and hypertensive rats (8, 18). In addition, Chen and colleagues (8) reported that renin mRNA was upregulated by androgens in kidneys of SHR. These data suggest that androgens can stimulate the intrarenal RAS. Furthermore, Baltatu and colleagues (4) also reported that renal injury could be abolished by androgen receptor antagonism in a Ren-2 rat model of hypertension that has an overactive RAS.

How androgens affect the systemic RAS is not clear. In humans, plasma renin activity (PRA) is higher in men than in age-matched premenopausal women (35, 58). We have found previously that testosterone repletion in castrated normotensive rats leads to a dose-dependent increase in PRA (62), which is consistent with the data from human studies. In contrast, Quan et al. (59) found that chronic DHT treatment reduced serum ANG II levels. While estradiol has been shown to modulate the synthesis of AT1 receptors in various tissues, including kidneys and vasculature (29, 55), androgens have only been shown to increase AT1 receptor expression in male reproductive tissues (46). Therefore, the effect of androgens on AT1 receptor expression in kidneys should be examined.

There are gender differences in the renal response to infusion of ANG II as well. When graded doses of ANG II were infused into healthy young adults, there was a similar increase in blood pressure and reduction in effective renal plasma flow (ERPF) in men and women, but GFR was maintained in men only, leading to an increase in the filtration fraction (FF), which suggests an increase in glomerular capillary pressure (48). In women, the reduction in ERPF was associated with a concomitant reduction in GFR, resulting in no change in FF. These studies were performed without blockade of the endogenous RAS. Therefore, men may have been more responsive because they have higher basal levels of endogenous renal ANG II than women. In any case, these data further support the notion that the combination of androgens and ANG II is important in modulating renal function.

In SHR, blockade of the RAS with converting enzyme inhibitors (CEI) reduces blood pressure to the same level in both males and females (70), supporting the important role of the RAS in mediating the hypertension in SHR independently of the sex steroids. In addition, CEI also prevents increases in blood pressure in ovariectomized female SHR receiving chronic testosterone supplementation (70). These data suggest that an intact RAS is necessary for androgens to increase blood pressure in SHR. In other words, the mechanism by which androgens are capable of increasing blood pressure in SHR is mediated by their effects on the RAS.

Whether PRA and RAS activity decrease with age in men and women is somewhat controversial. However, James and colleagues (35) reported from serial analyses that PRA was higher in men than in age-matched women, that PRA was higher in postmenopausal women than in premenopausal ones, and that in white men, PRA did not decrease with age. Blood pressure becomes more salt sensitive with aging in both men and women (85), which suggests that RAS activity and ANG II do not respond appropriately in the presence of salt in aging individuals. Therefore, androgen supplements in aging individuals could be expected to both stimulate reabsorption in the proximal tubule and stimulate PRA, which would further aggravate hypertension and renal injury.

**Androgens and Endothelin**

Endothelin is a potent renal vasoconstrictor and mitogen that plays a role in renal injury associated with aging (5, 25, 26). Goddard and colleagues (26) reported that ETA receptor antagonism in individuals with chronic renal failure caused an increase in renal blood flow and a reduction in blood pressure mainly due to the activation of the ETB receptors, because combined ETA/ETB receptor antagonism reduced blood pressure but had no effect on renal function. In addition, Eliovich and colleagues (17) reported that hypertensive individuals with nephrosclerosis exhibited increased plasma endothelin that was independent of aldosterone:renin ratios but was positively correlated with the level of proteinuria. In aging male Wistar rats, Ortmann and colleagues (56) recently reported that glomerulosclerosis and proteinuria could be reversed when rats were given darusentan, an ETA receptor antagonist, independently of reductions in blood pressure, changes in renal function, or tubulointerstitial renal injury. In addition, endothelin-1 is secreted from mesangial cells in response to a variety of cytokines, hormones, and oxidative stress (78). There is also evidence that endothelin may play a role in the gender difference in the death rate of rats in response to renal ischemia-reperfusion (51). Muller and colleagues (51) found that 50 min of left vascular pedicle clamping resulted in death in 92% of male rats by day 7, but only 25% of females died during this time period. Castration reduced the number of deaths in males with ischemia-reperfusion to 33%, and pretreatment of males with an endothelin ETA receptor antagonist totally protected males from death (51).

Androgens can upregulate the production of endothelin. In female-to-male transsexuals who receive testosterone supplements chronically, plasma endothelin levels are elevated (82). In addition, in women who suffer from polycystic ovary syndrome, in which serum testosterone is elevated, endothelin is also elevated (15). Whether the effects of androgens on endothelin production are direct or mediated by the effects of androgens on the RAS, which, in turn, increases endothelin production (3), is not clear. In any case, androgen supplementation in both aging men and women could promote renal injury mediated via endothelin. This is especially important following menopause, because estradiol has been shown to reduce expression of endothelin (82), and thus this protection would be lost in postmenopausal women. In support of this hypothesis, we have found that the postmenopausal increases in blood pressure found in aging female SHR are mediated, in part, by endothelin (87). In contrast, endothelin plays no role in the hypertension of young female SHR (87).

**Androgens and Oxidative Stress**

The role of oxidative stress in acute renal failure and ischemia-reperfusion is widely accepted. However, oxidative stress also plays a role in chronic renal disease (1, 49). Men have higher levels of oxidative stress than do age-matched women as measured by F2-isoprostanes or thiobarbituric acid-reactive substances in plasma (34), despite the reduction in androgen levels in aging men. Postmenopausal women also exhibit higher levels of oxidative stress than premenopausal women (31). Oxidative stress is increased in the kidney with normal aging (72), and we have been able to protect against...
age-related renal injury in Sprague-Dawley rats by treating them chronically with vitamin E (65).

The major reactive oxygen species in the kidney is thought to be superoxide, which can quench nitric oxide (NO) (67), leading to a reduction in the NO bioavailability for dilation and thereby causing renal vasoconstriction. It is possible then that a reduction in vasodilator substances could play a role in the age-related renal vasoconstriction in males. We have found previously that there are sex differences in the renal vasculature response to NO. Young male normotensive rats without deficiencies in androgen synthesis are more dependent on the NO system for maintenance of renal hemodynamics than are age-matched females (63). Males had 80% lower renal expression of endothelial NO synthase compared with females, yet when NO synthase was blocked with nitro-L-arginine methyl ester, despite similar blood pressure increases in males and females, renal plasma flow (RPF) decreased by 40% and renal vascular resistance (RVR) increased by 23% in males compared with 20% and 60% for RPF and RVR, respectively, in females. The importance of the NO system in preserving renal hemodynamics is even more striking in aging males. When treated with NO synthase inhibitors, GFR and RPF decreased to a much greater extent and glomerular capillary pressure almost doubled in aging males compared with young males (66).

Whether androgens can directly produce oxidative stress has not been fully elucidated. In preliminary studies, we have found that physiological concentrations of dihydrotestosterone are capable of increasing dihydroethidium fluorescence in cultured SHR mesangial cells (Cucciarelli V, Iliescu R, and Reckelhoff JF, unpublished observations). We have also found that castration reduces superoxide production in the kidneys of male SHR. In addition, tempol, a superoxide scavenger, reduces blood pressure and oxidative stress in young and aging male SHR but has little or no effect in females (21, 23).

Regardless of whether androgen supplementation directly causes oxidative stress, androgens can stimulate the RAS and endothelin production, which have been shown to increase reactive oxygen species. ANG II, at both supraphysiological and physiological levels, can increase oxidative stress (61, 71), mainly via upregulation of the subunits of NADPH oxidase (50). In addition, ANG II can stimulate the production of endothelin (3), which also causes oxidative stress by upregulating NADPH oxidase (16). Furthermore, while endothelin can cause oxidative stress, oxidative stress can also upregulate endothelin synthesis (40), setting up a vicious cycle.

Therefore, because aging is associated with increased oxidative stress, men at all ages have elevated levels of oxidative stress compared with women, and after menopause oxidative stress increases in women, androgen supplements could cause a further increase in oxidative stress in both men and women, leading to reductions in renal function and renal injury. The renal changes could be caused by the direct effect of androgens on oxidative stress or indirectly via their effect on the RAS or endothelin system. Furthermore, because estradiol is a mild antioxidant and has been shown to inhibit synthesis of NADPH oxidase (84), postmenopausal women would be at increased risk for androgen supplement-induced oxidative stress.

**Androgens and Cytokines**

Aging renal disease is associated with increases in inflammation and cytokine release. Both TNF-α and IL-6 have been shown to upregulate or activate the androgen receptor (12, 14). In addition, upregulation of many inflammatory mediators involve the transcription factor, NF-κB, and the androgen receptor promoter contains several NF-κB enhancer elements. Although the role of the androgen receptor in the inflammation associated with aging renal disease has not been studied, it is attractive to propose that the androgen receptor could be upregulated in renal tissue in response to inflammation. Androgens have also been shown to activate a Fas/Fas ligand-dependent apoptotic pathway in proximal tubule cells, which is characteristic of chronic renal diseases (24). Androgens could, therefore, activate an apoptotic mechanism, leading to renal tubular loss and interstitial fibrosis in the elderly.

**SUMMARY**

Aging men and women frequently receive androgen supplements. Aging men are already at greater risk for cardiovascular and renal disease than women, despite the fact that serum testosterone levels decrease significantly with age and with chronic diseases. We hypothesize that the reduction in testosterone with age and chronic disease is a protective mechanism against even greater cardiovascular-renal disease incidence and poorer outcomes. Therefore, androgen supplements in aging men could offset this natural protective mechanism.

In women, menopause increases a woman’s risk for cardiovascular and renal diseases. However, women are somewhat protected compared with men, but androgen supplements may increase the risk of renal injury and cardiovascular disease in postmenopausal women to levels similar to those in men. It is also possible that androgen levels may increase naturally with age in some women and may further impact disease risk factors naturally.

As with estradiol-progesterone replacement therapy in postmenopausal women, it is likely that some healthy, active, aging men and women would not have adverse cardiovascular consequences with androgen supplementation. However, in view

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**Fig. 4. Schematic diagram of the influence of androgens on cardiovascular and renal disease.** R\textsubscript{a}, afferent arteriolar resistance; R\textsubscript{e}, efferent arteriolar resistance, P\textsubscript{GC}, glomerular capillary pressure.
of the increasing experimental evidence that androgens promote cardiovascular and renal disease, even when the serum levels are decreased, aging men and women who have the predisposition to cardiovascular-renal diseases should take androgen supplements with caution until rigorous clinical trials have been performed.

As shown in Fig. 4, we hypothesize that androgens can stimulate proximal reabsorption of sodium and water, causing a reduction in the sodium delivery to the macula densa, and, by tubuloglomerular feedback, cause a reduction in afferent resistance that could lead to an increase in glomerular capillary pressure. Androgens could also activate the RAS, leading to further increases in tubular sodium reabsorption, but also increases in efferent resistance and glomerular capillary pressure. ANG II has been shown to increase endothelin synthesis increases in efferent resistance and glomerular capillary pressure that could lead to an increase in glomerular capillary resistance. Furthermore, androgens may directly increase both endothelin and upregulate NADPH oxidase to increase oxidative stress. Endothelin has also been shown to increase oxidative stress. Endothelin synthesis. ANG II has been shown to increase endothelin synthesis, further increases in tubular sodium reabsorption, but also increases in efferent resistance and glomerular capillary pressure. ANG II has been shown to increase endothelin synthesis.

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GRANTS

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