Sex Steroids and Renal Disease
Lessons From Animal Studies

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Epidemiological studies have shown that male sex is an independent risk factor for the development and progression of renal disease, and men progress to end stage renal disease (ESRD) faster than premenopausal women in such diseases as autoimmune glomerulonephritis, hypertensive glomerulosclerosis, and polycystic kidney disease.\textsuperscript{1–3} Although the age-related decline in renal function is also faster in men, women become more susceptible to renal diseases after menopause.\textsuperscript{4} Insight from in vitro studies and animal models suggest that sex steroids play pivotal roles in modifying the progression to ESRD. Because there is a paucity of data in humans showing the mechanisms by which sex steroids impact renal disease, most of the studies discussed in this review will be in animals, mainly rats. Furthermore, determining the roles of sex steroids in various diseases has been done using gonadectomized animals; however, although gonadectomy removes more than sex steroids and most studies only replace the sex steroid of interest, these are still the best studies in which to evaluate mechanisms responsible for sex differences in renal disease. Studies in postmenopausal women will also be kept to a minimum in this review because it is likely that sex steroids change action with aging, as suggested by the Women’s Health Initiative study.

Estradiol and the Kidney

Estrogen receptors (ER) are present in the kidney, although their localization in nephron segments has not been fully elucidated. Mesangial cells contain both ER\(\alpha\) and ER\(\beta\),\textsuperscript{5–7} as do endothelium and vascular smooth muscle cells.\textsuperscript{5–7} Whether the newly described transmembrane estrogen receptor, GPR30,\textsuperscript{8} is present in kidneys has not been determined. Ovariectomy (ovx) of Dahl salt sensitive rats (DS) caused decreases in ER\(\alpha\) but increases in ER\(\beta\) expression in the renal cortex and medulla,\textsuperscript{9} whereas ovx in salt resistant (DR) rats caused decreases in cortical and increases in medullary ER\(\alpha\), and increases in ER\(\beta\). In addition, estradiol replacement returned ER\(\beta\) expression to preovx levels in both DS and DR, but had no effect on ER\(\alpha\). In contrast, in internal mammary arteries from humans, ER\(\beta\) mRNA expression was 10-fold higher than ER\(\alpha\) or GPR30, and 17\(\beta\)-estradiol downregulated ER\(\alpha\), ER\(\beta\), and GPR30.\textsuperscript{10}

Estradiol is usually thought to be renoprotective. For example, in cultured mesangial cells, 17\(\beta\)-estradiol inhibits apoptosis and transforming growth factor (TGF)-\(\beta\) activity and expression.\textsuperscript{11} In addition, estradiol is antiinflammatory.\textsuperscript{12–14} However, with low nitric oxide (NO) and high angiotensin (Ang) II, estradiol exacerbated renal injury via upregulation of renal AT\(1\) receptor expression.\textsuperscript{15} In addition, in women, oral contraceptives increase in BP and albumin excretion,\textsuperscript{16} and occasionally result in hypertension\textsuperscript{17} and biopsy-proven renal damage in the absence of primary renal disease.\textsuperscript{18}

The roles of the ER subtypes in renal function or injury have been studied mainly by using ER\(\alpha\) and ER\(\beta\) knockout (KO) mice. ER\(\alpha\) is mainly responsible for gene regulation because 10 000 genes were upregulated in kidneys of wild-type (WT) and \(\beta\)ERKO mice with estradiol.\textsuperscript{19} \(\beta\)ERKO mice become hypertensive with aging, and males have higher BP,\textsuperscript{20} but both male and female are resistant to age-related glomerular sclerosis, suggesting that renal injury/disease does mediate the hypertension. \(\alpha\)ERKO males are also resistant to age-related glomerular sclerosis, but females exhibit both albuminuria and glomerular sclerosis. Ovx prevents glomerulosclerosis in female \(\alpha\)ERKO, not attributable to the reduction in estradiol or progesterone but to reduction in testosterone.\textsuperscript{21} ER\(\beta\) does provide protection in ischemia/reperfusion injury of hearts (eg, smaller infarct size) in \(\alpha\)ERKO females, but not males.\textsuperscript{22} However, streptozotocin-diabetic \(\alpha\)ERKO females developed higher TGF\(\beta\) expression than WT,\textsuperscript{23} suggesting that ER\(\beta\) may play a role in diabetic nephropathy. ER\(\alpha\) and ER\(\beta\) are present in endothelial cells and vascular smooth muscle cells, but the expression is gender- and vascular bed-dependent.\textsuperscript{6,7} Similarly, the effect of estradiol on vasodilation is dependent on the vascular bed studied. For example, estradiol-mediated vasodilation attributable to nitric oxide (NO), via endothelial NO synthase (eNOS), was absent in femoral and carotid arteries from both \(\alpha\)ERKO and \(\beta\)ERKO mice.\textsuperscript{24} This is consistent with previous studies showing that basal eNOS activity was reduced in \(\alpha\)ERKO...
mice.\textsuperscript{25} In contrast, Al Zubair and colleagues reported that ER\textbeta \textsuperscript{b} agonist caused greater relaxation of female rat mesenteric arteries than did ER\alpha \textsuperscript{a} agonist.\textsuperscript{26} However, both agonists had similar vasodilator effects in males.

**Progestosterone and the Kidney**

Progestosterone receptors have been found mainly in distal tubule cells, although they are present in cortex and medulla of males and females.\textsuperscript{27} The role that progestosterone plays in physiology and pathology of the kidney is not clear. Progesterone has a high affinity for the mineralocorticoid receptor (MR) and can act as an MR antagonist. In rats with DOCA-salt hypertension, mediated by the MR, progestosterone alone, the combination of progesterone and estrogen, but not estrogen alone, attenuated renal damage and preproendothelin mRNA expression,\textsuperscript{28} suggesting that treatment with progesterone may have acted as an MR antagonist in this study. However, progesterone can be degraded in the kidney to metabolites that have lower affinity for the MR.\textsuperscript{29} In contrast, in ovx rats with 5/6 renal ablation, estradiol protected against proteinuria and glomerulosclerosis, but rats treated with estradiol+progesterone exhibited the same renal damage as vehicle-treated rats.\textsuperscript{30} In this case then progesterone promoted renal injury. In another study, early ovx caused a reduction in renal functional reserve and fractional proximal tubular fluid output in middle-aged rats (13 months) that was reversed with estradiol alone or estradiol and progesterone.\textsuperscript{31} Progesterone may also increase sodium reabsorption independent of its effect on the MR, because progesterone alone moderately increased ENaC activity in cortical collecting duct cells, but progesterone and low-dose estradiol increased ENaC activity by 2-fold, whereas high-dose estradiol almost completely inhibited ENaC activity.\textsuperscript{32} Testosterone has also been shown to increase ENaC expression in the kidney,\textsuperscript{33} and progesterone can transactivate the androgen receptor.\textsuperscript{34} Furthermore, progesterone can be metabolized to both testosterone and dihydrotestosterone in the kidney.\textsuperscript{35} Therefore, some of the effects of progesterone on the kidney may be attributable to its androgenic effects.

**Testosterone and the Kidney**

The kidney can synthesize testosterone and dihydrotestosterone because it contains the cytochrome P450 enzymes that are necessary.\textsuperscript{35} Similar to the estradiol receptors, the complete nephron localization for the androgen receptor (AR) has not been elucidated. Mesangial cells, glomeruli, proximal tubules, and cortical collecting ducts contain AR.\textsuperscript{21,36} Treatment of normotensive rats with dihydrotestosterone increases proximal sodium reabsorption, via an Ang II–mediated mechanism, and BP,\textsuperscript{37} but neither GFR nor AT\textsubscript{1} receptor binding was affected. This is in contrast to acute infusion of testosterone that increases GFR and renal plasma flow and reduces renal vascular resistance (Reckelhoff, unpublished results, 1998). Therefore, androgens have both chronic and acute effects on the kidney.

Male rats experience a more rapid reduction in GFR and more renal injury with age than females.\textsuperscript{37–40} However, the reduction in GFR cannot be fully accounted for by the level of glomerular injury found in the kidneys, suggesting that aging is a state of renal vasoconstriction. For example, in male normotensive rats, aged 20 to 22 months, GFR was reduced by 50% compared to young males and yet only 20% of their glomeruli exhibited sclerosis.\textsuperscript{38} In aging male SHR, GFR was reduced by 30% and renal vascular resistance was increased 30%, whereas less than 5% of glomeruli had sclerosis.\textsuperscript{39} Castration of aging SHR and Munich Wistar rats prevents reductions in GFR and glomerular injury.\textsuperscript{37,39} In addition, castration prevents the increase in renal vascular resistance. Castration of male rats with renal-wrap hypertension also attenuated the glomerular injury and proteinuria.\textsuperscript{41} When castrated rats or ovx females with renal-wrap hypertension were treated with dihydrotestosterone, glomerular injury was exacerbated.

Testosterone can mediate renal injury in females. Testosterone supplements increased expression of AR and TGF-\Beta in glomeruli of ovx B6 mice,\textsuperscript{21} and AR antagonism protects against renal injury in female TGR(mREN2)27.\textsuperscript{42} The concept that androgens may also be harmful to kidneys of females is important because postmenopausal women may produce more androgens as they age.\textsuperscript{35} Plasma testosterone levels are decreased in most men with aging and other chronic diseases, such as CKD.\textsuperscript{43} Thus many investigators believe that renal disease is independent of androgens. However, because the kidney is capable of synthesizing androgens,\textsuperscript{35} it is not clear whether reductions in plasma testosterone reflect similar changes in renal testosterone.

**Diabetes, Hyperlipidemia, and Sex Steroids**

Before puberty, renal complications of diabetes (DM) are rare, but the incidence of renal disease in DM sharply increases after puberty.\textsuperscript{44} In a large clinical trial of 27 805 individuals with type 1 DM, male sex was an independent risk factor associated with the development of CKD.\textsuperscript{45} Other studies have also shown that the “female protective factor” is lost in the presence of diabetes.\textsuperscript{46} Perhaps because of a decrease in plasma estradiol as found in female rats with streptozocin-induced type 1 DM,\textsuperscript{47} 17\Beta-Estradiol replacement reversed renal fibrosis.\textsuperscript{48} Similarly, in hypertensive type II DM, postmenopausal women, 17\Beta-estradiol replacement was also shown to be protective against renal disease.\textsuperscript{49} Metabolic syndrome, including insulin resistance, type II DM, and dyslipidemia, constitutes a major risk factor for developing CKD. In a recent study, a positive correlation between the number of components of metabolic syndrome and the incidence of CKD was found.\textsuperscript{50} Of interest, no gender difference in the incidence of CKD was reported, with women having the same incidence as men.\textsuperscript{50} In Zucker (fa/fa) rats, a model of type 2DM and hyperlipidemia, there are gender differences in vascular reactivity, where the males exhibit an impaired response to vasoconstrictors and to endothelial-mediated vasodilation.\textsuperscript{51} Males exhibited a paradoxical decrease in vascular resistance in response to thromboxane agonist and blunting of the response to Ang II. In addition, Zucker males exhibited a blunted vasodilatory response to acetylcholine and a blunted vasoconstrictor response to nitro-L-arginine methyl ester (L-NAME), inhibitor of NOS. Castration improved vascular responses.\textsuperscript{52} However,
in aging Zucker rats, ESRD was found to be the major cause of mortality, and a gender difference was not observed (males: 91.1%, females: 93.3%). Although it is possible that a reduction in estradiol could have played a role in lack of protection in females, administration of estradiol to ovx Zucker rats impaired renal function further and led to profound glomerulosclerosis. These data suggest that in presence of metabolic syndrome, type II DM, or dyslipidemia, the effects of sex steroids on renal disease are unpredictable.

**Hypertension and Sex Steroids**

Hypertension is more prevalent and more severe in men than women. However, the sex difference in BP is lost after menopause. Sex differences in renal injury in humans follow the BP; ie, hypertensive men have a higher incidence of ESRD than women. The mechanisms are not clear, but changes in sex steroid–mediated differences in glomerular capillary pressure \( (P_{gc}) \) may play a role. Glomerular hypertension is a key factor in the pathogenesis of progressive glomerulosclerosis and renal failure, independent of the initial insult. For example, in response to Ang II infusion, men and women exhibited similar increases in BP and decline in effective renal plasma flow \( (ERPF) \), whereas GFR was maintained only in men, resulting in an increase in filtration fraction \( (FF) \). In women, GFR declined in parallel with ERPF, and thus there was little increase in FF, which is an indication of \( P_{gc} \). Thus \( P_{gc} \) in men likely increased more in response to Ang II than in women, which if sustained over time, would lead to greater glomerular injury in men.

Hypertension in animal models is associated with the renal injury, although the extent depends on the age, the model, and the experimental design. In the SHR, BP is higher in males, but renal injury only occurs with aging, but \( P_{gc} \) is elevated in males by age 9 months, before any renal damage. Although BP increases in female SHR after cessation of estrous cycling to levels not different or even higher than males, females exhibit little glomerulosclerosis compared to males. Whether \( P_{gc} \) is increased in aged female SHR has not been determined.

Whether and how sex steroids could affect \( P_{gc} \) is not clear, however. Testosterone has been shown to have modulating effects on calcium channels. For example, chronic testosterone causes an increase in T type calcium currents. Alternatively, L-type calcium currents are blocked by testosterone. Feng and colleagues reported that both L- and T-type calcium channels are present in afferent and efferent arterioles. It is possible that androgens could modulate these calcium currents leading to preglomerular vasodilation leading to an increase in \( P_{gc} \). Alternatively, estradiol could have the opposite effect on calcium channels and thus protect glomeruli from increased transmission of the systemic BP, protecting against increased \( P_{gc} \).

**Sex Steroids and Renin-Angiotensin System**

Inhibition of the renin-angiotensin system delays the progression of diabetic and nondiabetic renal diseases. Testosterone increases proximal sodium reabsorption via Ang II–mediated mechanisms and increases intrarenal angiotensinogen expression. If renin does not work at its Vmax, as in humans and rats, an increase in renin substrate would cause an increase in Ang II production. Testosterone could promote renal injury by a hemodynamic mechanism in which it stimulates Ang II production, leading to increases in sodium reabsorption in the proximal tubule with a concomitant reduction of sodium reaching the macula densa, resulting in a decrease in glomerular afferent resistance (compared to females) allowing greater transmission of systemic BP to the glomerular capillary.

Estrogen may protect against renal injury via its effects on components of the renin-angiotensin system. Estrogen reduces the number of \( \text{AT}_1 \) receptors in many tissues, including the kidney, and attenuates tissue responsiveness to Ang II. Estrogen increases angiotensinogen levels, mainly in the liver, but appears to decrease plasma renin activity. However, the lack of protection from primary cardiovascular events with estrogen replacement found in the Women’s Health Initiative (WHI) study suggests that changes in the estrogen responsiveness occur after menopause. Whether the changes in estrogen responsiveness with age are attributable to changes in expression/intracellular signaling/transcription activation of the ERs or other mechanisms remain to be determined.

**Sex Steroids and Endothelin**

There is a paucity of studies regarding the interaction among sex hormones, the endothelin system, and renal disease. In female-to-male transsexuals, testosterone treatment increases plasma endothelin levels. DOCA-salt rats, renal injury associated with ovx in females is ameliorated by endothelin \( (\text{ET}_1) \) receptor antagonism. After warm ischemia, the early recovery of renal blood flow is delayed in male rats compared to females, due to a greater increase in renal vascular resistance in males, likely mediated in part by increased endothelin, because preproendothelin mRNA was elevated in kidneys of males but not females. In uninephrectomized spontaneously hypertensive-stroke prone rats (SHRsp), ovx females exhibited significantly greater glomerular damage and greater endothelin expression than estradiol-treated animals.

Ang II has been shown to increase expression of preproendothelin in the kidney. Because androgens increase angiotensinogen and plasma renin activity, it is possible that androgens could increase Ang II leading to increased endothelin and subsequent renal injury. Alternatively, because estradiol can downregulate \( \text{AT}_1 \) receptor expression, estrogen should prevent the increase in endothelin and protect against renal injury. This hypothesis remains to be tested.

**Sex Steroids and Oxidative Stress**

Growing evidence links oxidative stress and renal disease, including drug-induced nephrotoxicity, IgA nephropathy, ischemia-reperfusion injury, and diabetic nephropathy. Men with these diseases exhibit more renal injury than women. Estradiol is an antioxidant, and ovx increases renal NADPH oxidase activity and glomerulosclerosis index in female renal-wrap hypertensive rats; estradiol replacement abrogated these changes. Ovx also worsens adriamycin-induced nephropathy and augments oxidative stress, which
Table. How Sex Steroids May Modulate Renal Injury

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Sex Steroid Effects</th>
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</thead>
<tbody>
<tr>
<td>Renal Ang II</td>
<td>Estradiol</td>
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<tr>
<td>AT1 receptors</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Endothelin</td>
<td>Testosterone/DHT</td>
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<tr>
<td>Oxidative stress</td>
<td>antioxidant</td>
</tr>
<tr>
<td>Vascular effect</td>
<td>antioxidant</td>
</tr>
<tr>
<td>Sodium reabsorption</td>
<td>vasoconstrictor</td>
</tr>
</tbody>
</table>

Ang II indicates angiotensin II; AT1, angiotensin type 1; dec, decrease; inc, increase.

was prevented by estradiol. Androgens are thought to increase oxidative stress. In humans, men have higher levels of indicators of oxidative stress than age-matched, premenopausal women. However, postmenopausal women have higher levels of oxidative stress than do their premenopausal counterparts. Few studies have examined sex differences in renal injury and the role of oxidative stress. BP in male SHR is reduced with antioxidants, such as tempol or apocynin, but not in females. Sullivan and colleagues reported that the activities of superoxide dismutase and catalase were augmented in kidneys from male SHR compared to females.

Renal antioxidant enzyme expression is higher in male SHR than in females. However, unlike in male WKY rats, male SHR fail to upregulate renal expression of antioxidant enzymes when oxidative stress is increased with molsidomine. SHR fail to upregulate renal expression of antioxidant enzymes when oxidative stress is increased with molsidomine. Whether oxidative stress is higher in male or female SHR is dependent on the tissue and on the age of the animals. In kidneys, oxidative stress, measured as increases in F2-isoprostane or lucigenin chemiluminescence, is slightly lower or similar in females and males. However, we have not examined whether renal damage in aging male SHR is ameliorated with chronic antioxidants, although BP is reduced, and therefore renal injury is also likely lessened. Whether in humans antioxidant therapy can attenuate or prevent CKD is unclear at the present.

Perspectives

The role that sex steroids play in CKD and in the progression to ESRD in humans is not clear. Sex steroids may not be causative of CKD in humans but are likely to be permissive of or protective against progression to ESRD. The mechanisms by which sex steroids could modulate renal disease are many, but in the Table, some of the possible mechanisms and how the sex steroids could affect these mechanisms are shown. Estradiol is mainly antioxidant, vasodilatory, and Ang II action-“inhibitory,” although not overtly so. The effect of estradiol on renal endothelin is not clear. Progesterone has been less well studied, but it is likely to be vasoconstricting chronically, antioxidant, antinatriuretic. The effect of progesterone on Ang II, AT1 receptor, and endothelin is not clear. Androgens, testosterone, and dihydrotestosterone are chronically vasoconstricting, prooxidant, antinatriuretic, and Ang II and endothelin-action stimulatory. Discovery of the mechanisms by which sex steroids impact CKD in humans will allow improvement of treatment paradigms that could be made specific if the patient is male or female. The role played by sex steroids in renal disease remains an exciting area for research in humans or animals.

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None.

References


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