Sexual Dimorphism of the Aging Kidney: Role of Nitric Oxide Deficiency

GFR falls with aging in humans and rats due to renal vasoconstriction and structural damage. The rate of deterioration is influenced by race/genetic background, environment, and sex, with females protected. Part of the female advantage relates to protective effects of estrogens. There is little information on impact of aging on the distribution/cardiovascular actions of the estrogen receptor subtypes. In rats, androgens may contribute to injury, but in men, high testosterone levels predict cardiovascular health. In women, the association is controversial. Nitric oxide deficiency contributes to the hypertension and renal dysfunction of aging, which may be delayed in the female.

Many studies report an age-dependent fall in the glomerular filtration rate (GFR) during aging in man (105) and rats (6). How much this reflects "normal aging" and how much is due to unbridled cardiovascular and renal disease is unclear. A fall in GFR is not inevitable in man since ~30% of "normal" subjects in longitudinal studies show preserved renal function over many years (52), and GFR can remain stable even in the very old (27). This suggests that co-morbidities such as hypertension, atherosclerosis, glucose intolerance/diabetes, obesity, dyslipidemias, and chronic kidney disease (CKD) contribute importantly to the rate of age-dependent kidney deterioration (8, 37, 45, 111).

In addition, genetic background is a major determinant. African Americans show a more rapid rate of "kidney aging" compared with Caucasians (110). However, it is difficult to dissociate socioeconomic disparities from race/genetics in humans, since diet and environment also have a major impact. The rate of decline in GFR with age varies between laboratory rat strains housed under similar environmental conditions, reinforcing a role for genetic background, where-as high caloric and/or protein intake also exacerbates the rate of age-dependent decline in GFR (6).

Although variable, falls in GFR with advancing age are due to both functional and structural changes. The functional decline is secondary to falls in renal plasma flow (RPF) due to renal vasoconstriction and occurs even in normotensive humans and rats and is exacerbated in hypertensives subjects (3, 28, 51, 90). In the normotensive Munich Wistar (MW) rat, GFR falls slowly due to parallel increases in afferent and efferent renal arteriolar resistances, which maintain normal glomerular blood pressure. Thus, in MWs, the structural damage precedes increased glomerular blood pressure (3), although even in this strain glomerular blood pressure eventually increases (1). Male rats of the MW/WT strain exhibit accelerated kidney disease with aging, yet glomerular blood pressure remains normal (84). Therefore, age-dependent falls in GFR and glomerular injury can occur in the absence of glomerular hypertension. Nevertheless, the sclerosis-prone Sprague-Dawley (SD) rat shows an early rise in glomerular blood pressure as well as age-dependent systemic hypertension (38, 83), which exacerbates the rate of injury development.

Structural changes in aging man include thickening of the glomerular basement membrane (GBM), expansion of the glomerular mesangium, and extracellular matrix leading to glomerular sclerosis. Additional changes include glomerular ischemia and arteriolar, atherosclerosis, and tubulointerstitial injury, all leading to loss of functioning nephrons (45, 51, 59, 70). In aging humans, this loss of function and structural injury occurs so slowly that it usually has no obvious impact. However, the loss of "renal reserve" renders the aged kidney susceptible to failure when other insults are superimposed. In some rat strains, CKD is a major cause of death, presumably reflecting co-morbidities such as hypertension that result from genetic background (90). There are also genetic quantitative trait loci that enhance susceptibility to renal failure by various mechanisms (101).

Thus the severity and rate of development of age-dependent CKD is highly variable and determined by a complex interplay between resistance/susceptibility genes and the diet/environment. There is also a marked sex difference in rate of deterioration of the aging kidney, with females being protected. The remainder of this review will consider some aspects of this sexual dimorphism.

Sexual Dimorphism of the Kidney

Cross-sectional clinical studies show that age-related falls in GFR are delayed in women, and when they do occur they proceed relatively slowly (7, 105). In the rat, female sex almost always confers protection from age-dependent renal dysfunction (6), although this is more evident in the male due to the presence of potent anti-inflammatory steroids.

This sex-related difference is not linked to a direct sex steroid effect, in this example, thyroxine or estrogen (1). The female advantage in reducing the risk of effective renal plasma flow (ERPF) and increased renal resistances, which is prominent in men (17), should be noted. Cross-sectional studies show that women vs. men with hypertension (65) and diabetes (66) have higher ERPF (factors 2-3) and vessels (factors 3-4) than men (17). However, as expected, a small group of women is hypertensive and shows a marked deficiency in ERPF (factors 3-4) and vessels (factors 3-4) compared with normotensive women (65, 66).

In the aging kidney, renal arteriolar resistances, which maintain normal renal hemodynamics, elevate in hypertensive mice (36) and rats (6). This rate of deterioration is secondary to Seeder et al., 1994 (53, 117) and Barlow et al., 1995 (65, 66); aged females show a decrease in resistances, reinforcing a role for genetic background, where-as high caloric and/or protein intake also exacerbates the rate of age-dependent decline in GFR (6).

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age-dependent declines in GFR, irrespective of strain (6), although GFR is lower in the young adult female vs. the male due to a higher renal vascular resistance (4, 65).

The preservation of GFR in the female has been linked to a delayed fall in renal plasma flow. For example, in the meta-analysis by Wesson (165), the effective renal plasma flow (ERPF) begins to fall later in women than in men. This difference is particularly prominent in a recent clinical study on potential renal transplant donors (7) with ERPF falling by more than 10% per decade in normal men between ages 20 and 50 and by ~1% per decade in women over the same ages. By age 76, however, the values of ERPF (factored for surface area) were similar in a small group of normotensive men and women (46). The falls in ERPF are due to renal vasoconstriction. It should be noted that care must be used when ERPF is calculated from PAH clearance since renal extraction of PAH is reportedly lower in women than in men (17). However, we observed a lower RPF in the women vs. men using insulin clearance factored by extraction (65) and glomerular plasma flow calculated from single nephron filtration fraction and SNGFR (3).

In the aging male MW rat, both afferent and efferent renal arteriolar resistances increase in parallel, whereas these resistances tend to decline with age in the initial vasoconstricted female kidney (and in this strain, in both sexes, the glomerular blood pressure remains constant with age). There are no other studies on sexual dimorphism in glomerular blood pressure in aging. As a surrogate measure, the filtration fraction tends to increase slightly from ~16 to 22% in normal men (perhaps suggesting a mild increase in glomerular blood pressure), while remaining stable at ~20% in women between ages 20 and 60 (7).

In terms of structural damage, the findings in humans are equivocal, with McLachlan et al. (59) reporting a greater vulnerability of men to glomerular sclerosis, whereas Neugarten et al. (70) reported no sex difference. In rats, however, there is a clear sex difference with females of most strains exhibiting substantial protection (3, 6, 24) (FIGURE 1).

Role and Actions of Estrogen

It is likely that the sexual dimorphism in kidney aging, with females protected, is associated with the beneficial vascular actions of the estrogens. Indeed, the rate of progression of renal disease in women is slower than in men (68). Female C57Bl6 mice become susceptible to age-related glomerulosclerosis after menopause (115), and estrogen supplementation reverses glomerular sclerosis in male TGF-β overexpressing mice (39) and female sclerosis-prone ROS-1/N mice (23). The vascular actions of estrogen are complicated, however, as indicated by the recent unfavorable clinical trials on hormone replacement therapy (HRT) in postmenopausal women (40, 87). The type of HRT employed is critical since, although native 17β-estradiol is likely to be beneficial, the complex conjugated equine estrogens (containing many estrogens, progestins, androgens, and ‘other substances’), used in WHI and HERS, have less predictable actions (20). Age of initiation of HRT is also critical, and older women showed less benefit and/or increased cardiovascular risk compared with women in whom HRT was initiated at or close to menopause (20). This could reflect impact of aging and/or loss of endogenous estrogen on estrogen receptor (ER) distribution and hence estrogen responsiveness (86).

There are two major ER subtypes, α and β, and the data on renal and vascular distribution of the subtypes is sparse and controversial. Rogers and colleagues report that ERα predominates in female SD rat kidney cortex, whereas ERβ predominates in the male (86). In contrast, Lu et al. (56) report the opposite with sham nephrectomized male SDs showing greater ERα abundance vs. females. The renal ERs distribution is inversely related to 17β-estradiol level in normal male and female rats (86) and in the Dahl R rat where renal cortical ERα falls with ovariectomy (26), although ovariectomy increases ERβ in both Dahl S and R. However, the impact of 17β-estradiol level on the ERs is location specific, and ER expression is under complex control (61). Aging has variable impact on ER distribution, with slight declines in both subtypes in the vasculature of old female SHR (107), whereas ERα mRNA and protein increases in the kidney of the old female mouse, with decreases in the male (93). Of course, posttranslational changes can also modify receptor activity independent of mRNA and protein abundance, but there is little information on impact of aging. The relative role of these ER subtypes in renal and cardiovascular pathology is unclear, although ERβ activation may be injurious in some situations. Studies by Lane and colleagues using ER knockout mice (ERKO) suggest that ERs may be injurious by mediating the compensatory hypertrophy after uninephrectomy and with diabetes since the αERKO is protected from glomerular enlargement and matrix accumulation (55, 99). Maric and colleagues reported increased renal ERα in Type 1 diabetic female rats together with a fall in plasma estradiol (104). In contrast, ERα is required for vascular repair from atherosclerosis in mice of both sexes (61), and ERs depletion in Dahl S rats on high salt is associated with worsening hypertension and renal damage (26). ERα increases in old female (protected) mouse kidneys (83), and mesangial cells from GS prone mice express decreased ERα and ERβ (77). In addition, αERKO female (but not male) mice develop albuminuria and glomerular damage with age (22). In experimentally induced CKD (5/6 nephrectomy), greater falls occur in ERα in males (susceptible to injury) than females, although the final

Kidney

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levels of ERs post NX were not much different between the sexes (56), so the significance of ERs abundance in this model is unclear. In some settings, ERs may be protective since the EREKO develops age-dependent hypertension (116) and EREKO females develop greater cardiac injury following ischemia/reperfusion (31).

The ERs are nuclear receptors, form homo- and heterodimers, and can activate transcription by interaction with estrogen response elements in target genes and also by direct interactions with transcription factors (61). Of note, after uterus and pituitary, the kidney has the greatest number of estrogen-regulated genes. The ERs seems to be primarily responsible since estrogen-dependent renal gene regulation is intact in ovarioectomized (EREKO mice given 17β-estradiol but suppressed in the EREKO) (41). There are also nongenomic, rapid actions of estrogen on membrane-bound ERs (both α and β) (61), although their role in kidney has not yet been defined. Some of the actions of estrogen are independent of estrogen receptors and are mediated by the methoxyestradiol metabolites of estradiol (19, 108). The actions of estrogen are therefore complex and sometimes unpredictable. Renoprotective actions of estrogens (both ER dependent and independent) include suppression of glomerular mesangial cell growth and inhibition of mesangial extracellular matrix accumulation; key events in the development of glomerular sclerosis (19, 21, 34, 49, 67, 71, 95, 108, 115). Loss of these protective actions after menopause contribute to the decline in renal function and development of structural damage in aging women. Although usually beneficial, estrogen is not universally good for the kidney. There are ER-independent actions of estrogen on kidney that increase the pressure and production of renal injury (FIGURE 2), suggesting a primary damaging influence of the male gonadal hormone milieu. There is no sex difference in glomerular blood pressure in this strain with aging, and although glomerular volume is greater in the intact male vs. intact female, glomerular volumes are similar between intact (vulnerable) and castrated (protected) males. This suggests that androgens do not promote kidney damage via hemodynamic mechanisms (3).

There are many non-hemodynamic mechanisms of glomerular injury, some of which target the glomerular mesangium. The androgens stimulate mesangial extracellular matrix (ECM) production and cause excessive mesangial expansion and higher levels of glomerular procollagen mRNA levels after subtotal nephrectomy (54). Also, glomerular metalloprotease activity increases with age in intact females but not in males, and castration of the male restores glomerular metalloprotease activity as well as protecting against glomerular injury (86). Elliot et al. reported that, although ovariec-
tomy promotes the uERKO mouse from glomerular sclerosis, testosterone supplementation promotes glomerular sclerosis (22). Studies by Beckhoff and colleagues in the SHR show that androgen receptor antagonism lowers BP in the male, whereas testosterone administration to the female raises BP (79).

In contrast, in men, higher androgens correlate with lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular effects of estradiol, higher and 17β-estradiol but lower BP, arterial wall thickness, and CAD (53). Low androgen levels are associated with increased CVD risk (44). Testosterone administration to the female raises BP (79). Androgens and anticardiovascular
Androgens and estrogens interact with many systems that impact on cardiovascular and renal function and structure. This article concludes with some considerations of interactions with nitric oxide (NO) that have major influences on cardiovascular health.
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High sodium intake. The fall in UNOxV correlates with developing endothelial dysfunction (58), and although there is no clinical data available on sex differences in UNOxV with aging, the appearance of endothelial dysfunction is delayed in aging women vs. men (15). There is also sexual dimorphism in the NO system in young adults, with premenopausal women making more total NO than men (29) and a greater abundance of the constitutive NO syntheses (NOS) in young adult female vs. male rat kidney (69, 81).

The sex differences in NO are due, in part, to estrogens, which have multiple potent actions to stimulate NO production from both eNOS and the neuronal NOS isoform that is predominant in the kidney epithelium. Estrogen stimulates NO directly at both genomic and nongenomic levels, indirectly by reduction of essential co-factors, or high oxidant state can also cause decreased NO production (4, 5).

Substrate Availability

Circulating l-arginine levels are well maintained in healthy aged humans (46) and in the male rat (63), although after a 12-h fast plasma l-arginine concentration falls in the aging male SD rat (82), suggesting that food deprivation stress may induce substrate deficiency (4, 5). Increases in NOS inhibitors, such as asymmetric dimethylarginine (ADMA), have been reported in the aging male rat (57, 109). ADMA levels also rise in humans in healthy old age, in both men and women, correlating with declines in renal plasma flow (46). Although there is no sex difference in plasma ADMA at 69 and 25 yr of age (46), the increase is delayed in women (91). There are probably multiple mechanisms of increased plasma ADMA with age. Renal clearance falls with age (see above), but this is a minor route of ADMA removal in humans (~15%), whereas almost no intact ADMA is excreted in the urine in the rat (72, 73). The predominant method ofADMA removal is via dimethylaminohydrolase (DMAH) which has the highest activity in the liver. ADMA accumulates as a delayed rise in renal cortical and brain cerebellar levels of ADMA at 69 and 25 yr of age (46), the increase is significant in men and may contribute to the development of endothelial dysfunction, seen in humans and rats (10, 16, 58). Oral arginine supplementation reverses endothelial dysfunction in healthy aged humans (10), suggesting substrate deficiency and/or an increased competitive inhibitor with aging. In addition, decreased abundance or activity of the NOS enzymes, due to reduction in protein content, reduced availability of essential co-factors, or high oxidant state can also cause decreased NO production (4, 5).

Circulating NOS Inhibitors

Increases in NOS inhibitors, such as asymmetric dimethylarginine (ADMA), have been reported in the aging male rat (57, 109). Plasma ADMA levels also rise in humans in healthy old age, in both men and women, correlating with declines in renal plasma flow (46). Although there is no sex difference in plasma ADMA at 69 and 25 yr of age (46), the increase is delayed in women (91). There are probably multiple mechanisms of increased plasma ADMA with age. Renal clearance falls with age (see above), but this is a minor route of ADMA removal in humans (~15%), whereas almost no intact ADMA is excreted in the urine in the rat (72, 73). The predominant method of

NO intake is controlled. Total NO production (from UNOxV) falls in the aging male SD rat as age-dependent CKD develops, whereas, in the female, UNOxV remains unchanged with age, and kidney damage is delayed (FIGURE 2) (24, 38, 82, 97). The UNOxV also correlates inversely with the kidney damage in the aging male Fischer 344; when maintained on a normal protein diet, UNOxV falls and kidney damage develops, but dietary protein restriction is associated with preserved UNOxV and structure (97). In humans on normal sodium intake, UNOxV falls with age (58, 89), although this age difference is lost on either low or
ADMA removal is by metabolism by dimethylarginine dimethylamino-hydrolase (DDAH), and the kidney has the highest levels of DDAH of any organ. It is possible that DDAH activity falls with age, contributing to ADMA accumulation. This would certainly explain the delayed rise in women since estrogen stimulates DDAH activity (39). Synthesis of ADMA might also increase with aging, since protein methyltransferase 1 (PRMT1), which methylates arginine, is activated by oxidative stress (11).

**NOS Protein Activity/Abundance**

The abundance of endothelial eNOS declines with age and oxidative stress increases, which will both impair endothelial NO production and cause endothelial dysfunction (112). In addition to declines in systemic endothelial NO production, renal NOS generation falls as age-dependent kidney damage develops. As shown in FIGURE 3, decreased renal cortical abundance of the neuronal NOS (nNOS) occurs in the aging male SD (24). Renal eNOS abundance also falls late, but the fall in renal cortical nNOS begins in middle age, precedes injury, and declines further as the kidney damage increases in severity, in the old rat. Renal cortical nNOS abundance also falls in other models of CKD (69, 81). However, we find no difference in either eNOS or nNOS protein abundance in renal cortex between young male and female rats (24) as also reported for nNOS by Neugarten et al. (69), although increased medullary eNOS and eNOS in whole kidney homogenates have been observed in female vs. male SD (69, 81).

In summary, the total NO production falls with age in the male rat and in humans (no sex difference data available). Given the stimulatory actions of the estrogens on NO production, at least regional NO production is likely to be greater in premenopausal women than in men and decline after menopause. The impact of androgens on NO production is variable and unclear at present but may be net protective in men. The age-dependent fall in total NO production is not due to substrate limitation but increases in circulating antagonists likely contribute. The abundance of the renal nNOS also likely contributes to the age-dependent CKD, contributing to the greater susceptibility of the male kidney to age-dependent damage.

In conclusion, although age-dependent kidney damage promotes general cardiovascular disease and likely hastens progression of kidney damage. The loss of the renal nNOS may also play a role in the progression of the age-dependent kidney damage. Furthermore, the more robust NO systems in the female may in part explain the slower rate of progression from a vulnerable to an active state can also be driven by low-dose estrogen (4a). The role of estrogen in renal and renal endothelial dysfunction is complex, and competitive inhibitor studies are needed to determine if estrogen maintains renal NOS activity in male rats. Although substrate deficiency (2) has been proposed as a cause of the NO deficiency in aging, we find no difference in either eNOS or nNOS protein abundance in renal cortex between young male and female rats. *Significant difference between young and other ages.*

**FIGURE 1.** The relative abundance of the endothelial NOS protein and the neuronal NOS protein in homogenates of renal cortex from young, middle-aged, and old male (M) and female (F) Sprague-Dawley rats. **Significant difference (P < 0.05)** between male and female. **Significant difference between young and other ages.** Figure is drawn from data described in Ref. 24 and reproduced from Ref. 4a with permission from Elsevier.
progression in women with non-diabetic renal disease [92]. The possibility that some of the cardiovascular actions of androgens are mediated via estrogen is intriguing and warrants further study.■

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