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Changes in renal hemodynamics and structure in the aging kidney; sexual dimorphism and the nitric oxide system

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Abstract

With advancing age the kidney shows both functional declines (falls in GFR) and development of structural damage. In most individuals this occurs slowly and does not lead to severe renal impairment unless additional insults are superimposed. There is a pronounced sexual dimorphism with females protected, due both to beneficial effects of the estrogens and damaging effects of androgens, some of which act directly on the glomerular mesangial cell to regulate growth and extracellular matrix production. Nitric oxide is a major factor in regulation of vascular tone and growth and becomes deficient with advancing age, as endothelial dysfunction develops. Although the abundance of the substrate, L-arginine, is well maintained during aging, there are increases in the concentration of circulating endogenous nitric oxide synthase (nNOS) inhibitors, which will contribute, to the endothelial dysfunction. There is a clear sexual dimorphism in the NO system, with pre-menopausal females producing more NO than men. Within the kidney, declines in the abundance and activity of the neuronal form of the nitric oxide synthase (nNOS) correlate with development of disease. In the male rat where injury and dysfunction occurs, nNOS abundance declines markedly, whereas in the protected female, renal nNOS abundance is maintained. Taken together, it is likely that age-dependent declines in NO generation contribute to age-dependent kidney damage.

Keywords

Renal hemodynamics; Aging kidney; Nitric oxide system

1. Introduction

Many clinical studies show that an age-dependent fall occurs in the glomerular filtration rate (GFR) during normal aging in man (Wesson, 1969; Fig. 1) and rats (Baylis and Corman, 1998). However, this fall in GFR is not inevitable since ~30% of subjects in longitudinal studies show preserved renal function over many years (Lindeman et al., 1985). Similarly, the rate of decline in GFR varies between rat strains, with some showing preserved function throughout life, e.g. WAG/Rij. Others exhibit a very rapid rate of age-dependent injury, e.g. Sprague–Dawley, while some shown a graduate decline, e.g. Munich Wistar (Baylis and Corman, 1998). Nevertheless, GFR will fall to some extent with advancing age in most humans and rats due to both functional and structural changes. The functional decline is secondary to falls in renal plasma flow (RPF) due to renal vasoconstriction and this occurs even in normotensive individuals, and is exacerbated in hypertensives (Fig. 2) (Levi and Rowe, 1992; Schmieder et al., 1994; Fliser et al., 1997). Structural changes 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, tubulointerstitial

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injury, arteriolar hyalinization and atherosclerosis, all leading to loss of functioning nephrons (Levi and Rowe, 1992; McLachlan et al., 1987; Neugarten et al., 1999a,b; Kasiske, 1987). Of note, in most individuals this age-dependent loss of function is sufficiently slow that it has no obvious impact in the absence of underlying disease. However, the loss of 'renal reserve' renders the aged kidney susceptible to failure when other insults are superimposed.

2. Sexual dimorphism

There is a marked sexual dimorphism in the aging kidney, with females being protected. Clinical studies show that age-related falls in GFR are delayed in women and when they do occur, proceed relatively slowly (Fig. 1; Wesson, 1969). In the rat, irrespective of strain, female gender confers marked protection from age-dependent declines in GFR, relative to males (Baylis and Corman, 1998), although GFR is lower in the young adult female vs. the male, due to a higher renal vascular resistance (Munger and Baylis, 1988; Baylis, 1994). In terms of structural damage, the findings in humans are equivocal, with McLachlan et al. (1977) reporting a greater vulnerability of men to glomerular sclerosis, while Neugarten et al. (1999a,b) report no sex difference. In rats, however, there is a clear sex difference with females of most strains exhibiting substantial protection (Baylis and Corman, 1998; Baylis, 1994; Erdely et al., 2003).

It is reasonable to attribute the sexual dimorphism in kidney aging, with females protected, to the beneficial vascular actions of the estrogens. Indeed, the rate of progression of renal disease in pre-menopausal women is slower than in men and this protection is lost with the onset of menopause, and can be restored with estradiol replacement (Neugarten et al., 2000a; Andreoli, 2000). Female C57Bl6 mice become susceptible to age related glomerulosclerosis after menopause (Zheng et al., 2003). One protective action of estrogens is the potent and direct antigrowth effects on the glomerular mesangial cell and also inhibition of mesangial extracellular matrix accumulation; key events in the development of glomerular sclerosis (Kwan et al., 1996; Dubey and Jackson, 2001; Xiao et al., 2001; Dubey et al., 2002; Guccione et al., 2002). At least some of these actions seem to be independent of estrogen receptors and are mediated by the methoxyestradiol metabolites of estradiol (Xiao et al., 2001; Dubey et al., 2002) although selective estrogen receptor modulators have also been reported to suppress mesangial cell collagen synthesis (Neugarten et al., 2000b). Evidence also suggests that estradiol inhibits TGF- β 1 collagen production (Silbiger et al., 1998) and suppresses type I collagen synthesis via upregulation of the MAP kinase cascade, leading to stimulation of AP-1 activity (Neugarten et al., 1999a,b), although Krepinsky et al. (2002) have reported estradiol-mediated inhibition of MAP kinase activation by mechanical strain.

In addition to direct protective actions on the mesangial cells, estradiol also promotes a number of potent antigrowth signals that act on the mesangium (Dubey and Jackson, 2001), many of which are endothelial in origin, including nitric oxide (NO) (see below).

Apart from the clear benefit of the presence of estrogens, there are some situations in which the androgens provide a risk factor. In the aging Munich Wistar rat, the intact male exhibits a slowly evolving fall in GFR due to mild renal vasoconstriction and glomerular sclerosis (Baylis, 1994). The intact female is protected from these changes, but of note, ovariectomy in the young adult has no impact on the female. In contrast, castration of the young male is protective (Fig. 3) suggesting a primary damaging influence of the androgens. The mechanism of the androgen-induced susceptibility to age-dependent glomerular damage is not known, but does not appear to be hemodynamic. Although the young adult female kidney is vasoconstricted, compared to the male (Munger and Baylis, 1988), the relative tone in afferent and efferent arterioles is similar between the sexes, hence glomerular blood pressure (a primary risk factor for glomerular injury) is not elevated in the male with advancing age (Baylis, 1994). Hypertrophy of the glomerulus provides another potential hemodynamic injury mechanism but although

glomerular volume is greater in the intact male vs. intact female, glomerular volumes are similar between intact and castrated males, suggesting that glomerular hypertrophy is not the primary reason for the susceptibility to injury of the intact male (Baylis, 1994).

There are also non-hemodynamic mechanisms of glomerular injury, many of which target the glomerular mesangial cell and mesangial extracellular matrix (ECM) production. Mesangial extracellular matrix levels are controlled by rate of synthesis and by rate of degradation, by metalloproteases. As shown in Fig. 4, glomerular metalloprotease activity increases with age in intact females and correlates inversely with glomerular injury in all four groups at 18–20 months of age (Reckelhoff and Baylis, 1993). Perhaps the age-dependent increase in glomerular metalloprotease activity seen in intact and ovariectomized females and castrated male rats, is ‘protective’, to compensate for age-dependent increases in mesangial ECM synthesis? This compensation does not occur in the intact male, leaving the kidney vulnerable to ECM accumulation and leading to glomerular sclerosis. There is also evidence that the androgens may stimulate mesangial ECM production, since they cause excessive mesangial expansion and higher levels of glomerular procollagen mRNA levels after subtotal nephrectomy (Lombet et al., 1989).

Therefore, the sex difference in rate of development of age-dependent kidney damage is associated with both the protective estrogens and the damaging androgens. There is less information about the impact of progesterone, which has been reported to enhance some and oppose other estrogen-mediated effects (Orshal and Khalil, 2004). However, multiple pregnancies in women and rats with normal renal function have no adverse impact on aging changes in kidney function and structure (Baylis, 2003), suggesting no harmful effect on the kidney of periodic elevations in progesterone levels.

3. Nitric oxide

Nitric oxide (NO) is made in many locations in the body and has powerful hemodynamic effects, to lower vascular tone. NO is also recognized as an anti-growth agent that can inhibit mesangial cell growth and extracellular matrix production. Therefore, if chronic NO deficiency develops, this could lead to a state of vasoconstriction with increased blood pressure, as well as unrestrained mesangial growth and/or matrix over-production. There is substantial evidence that chronic NO deficiency occurs in various types of chronic renal disease (CRD) and may contribute to progressive loss of function and evolving structural damage (Baylis, 2001). There is also significant evidence to suggest that NO deficiency develops during normal aging. Endothelial NO-dependent vasodilation is impaired with advancing age in normal man and rats (Lyons et al., 1997; Cernadas et al., 1998; Bode-Boger et al., 2003). Total body NO production can be estimated from the urinary excretion of NO_x ($\text{NO}_x = \text{NO}_2 + \text{NO}_3$; the stable oxidation products of NO), providing that the NO_x intake is controlled. There is clear evidence that $\text{U}_{\text{NO}_x} \text{V}$ falls in the aging (male) rat (Reckelhoff et al., 1994; Sonaka et al., 1990; Hill et al., 1997; Erdely et al., 2003), denoting a reduction in total NO production. In man, we were unable to document a fall in $\text{U}_{\text{NO}_x} \text{V}$ with age (Schmidt et al., 2001) although Lyons et al. (1997) did report reductions in $\text{U}_{\text{NO}_x} \text{V}$ in healthy old age. There is clearly a reduction in endothelial NO activity with age, leading to endothelial dysfunction. In fact, oral arginine supplementation can reverse the endothelial dysfunction in the aged, suggesting that a defect in NO production does occur even in healthy aging subjects (Bode-Boger et al., 2003).

There is also a reported sexual dimorphism in the NO system, with pre-menopausal women making more total NO than males (Forte et al., 1998) and a greater abundance of the constitutive NO synthases (NOS) in young adult female vs. male rat kidney (Reckelhoff et al., 1998; Neugarten et al., 1997). In addition, estrogen exerts marked stimulatory actions on endothelial NO at both genomic and non-genomic levels, and in addition to antioxidant actions of estrogens

may prolong the active life of NO (Orshal and Khalil, 2004). Estrogen also stimulates nNOS at the gene level and may inhibit iNOS expression although this is controversial (Ceccatelli et al., 1996; Fadel et al., 2003; Hayashi et al., 1997; Mershon et al., 2002; Noris et al., 2000; Orshal and Khalil, 2004; You et al., 2003). The situation with androgens is also variable with testosterone-induced inhibition of NO-dependent vasodilation being reported in some parts of the circulation and stimulation in others (Orshal and Khalil, 2004). Regional variations in the effect of testosterone on the nNOS abundance and activity has also been reported (Gonzalez-Hernandez and Gonzalez, 2000). In contrast to the inhibitory actions of testosterone in some vascular beds, the 'female' androgen DHEA is stimulatory on NO (Orshal and Khalil, 2004).

Another complicating factor is the complex relationship between NO and ANG II, given the known regulation of the renin-angiotensin system by the sex hormones. In general angiotensin II stimulates NO release and influences NOS enzyme expression and in the kidney, the NOS proteins often colocalize with components of the renin-angiotensin system (Millatt et al., 1999). The activity of the renin-angiotensin system is highly regulated by the sex hormones, with testosterone exerting stimulatory effects on plasma renin activity and angiotensinogen (Reckelhoff, 2001; Ellison et al., 1989). Further, increased activity of ANG II stimulates superoxide production via NADPH oxidase, an effect that is more prominent in the male vs. female SHR due to the stimulatory actions of testosterone (Reckelhoff, 2001). In contrast, estrogen inhibits ANGII actions including the induction of eNOS and stimulation of NADPH oxidase (Gragasin et al., 2003). Thus, in general the female hormones that stimulate NO production tend to exert inhibitory actions on ANGII production, while the reverse is true for testosterone.

Furthermore, male rats are more susceptible to low dose, chronic NOS inhibition-induced kidney damage, due both to renovascular protective effects of the estrogens and deleterious renovascular actions of androgens (Verhagen et al., 2000). We therefore conducted a series of studies to investigate the NO system in the aging rat, with particular regard to sexual dimorphism.

Fig. 5 shows a simplified version of the nitric oxide (NO) synthesis pathway. It is evident that net NO deficiency could occur by a number of mechanisms. For example, reductions in substrate availability (L-arginine) either ingested or from the endogenous source (kidney), could lead to NO deficiency. Increases in endogenous NOS inhibitors such as methylated arginine analogs, including asymmetric dimethyl arginine (ADMA), will reduce NOS activity. A decreased abundance or activity of the NOS enzymes, due either to reduction in protein content, reduced availability of essential co-factors or high oxidant state would also lead to NO deficiency. Any of these events either alone or acting together, would reduce the total amount of NO generated, which would be measurable as a reduced production rate of NO_x . Other, more down stream events could reduce NO-dependent activity after NO formation, for example by increased NO inactivation with superoxide anion, by decreasing the access of NO to its target tissue, (as occurs with a deposition of advanced glycosylated end products) and/or alteration in the NO receptors and their transduction mechanisms. There is evidence both of increased oxidant stress and formation of advanced glycosylated end products, in aging; however, our studies have focused on the first steps in the pathway which control the total amount of NO generated.

As shown in Fig. 6, while U_{NO_x} V falls in the aged male (Sprague-Dawley) rat, the total NO production is preserved in the aging female. As also shown, the kidney develops severe structural damage with age in the Sprague-Dawley male while the female is protected (Erdely et al., 2003). Falls in U_{NO_x} V have also been related to age-dependent kidney damage in the male Fischer 344. When maintained on a normal protein diet U_{NO_x} V fell and kidney damage

developed but dietary protein restriction was associated with preserved U_{NO_x} V and structure (Sonaka et al., 1990), suggesting that there may be a cause and effect relationship.

We first investigated the substrate (arginine) availability in aging rats, since declines in arginine availability had been reported previously in the aging male Sprague–Dawley (Reckelhoff et al., 1994). Endogenous arginine synthesis involves a series of enzymatic reactions in the small intestine leading to formation of citrulline, which is then transported in the blood to the kidney for enzymatic conversion to L-arginine. We found that expression of the genes involved in renal arginine synthesis and small intestinal citrulline synthesis were either unchanged or upregulated with age in the male Sprague–Dawley rat. In addition, the plasma, renal cortical and brain cerebellar levels of L-arginine were unchanged by age, which taken together, indicates that substrate availability is preserved in the aged male rat. Furthermore, there was no sex difference in arginine biosynthetic capacity at any age (Mistry et al., 2002). There is also evidence that circulating L-arginine levels are well maintained in normal aging man (Kielstein et al., 2003).

Even if the substrate levels are maintained, increases in the competitive inhibitors, such as asymmetric dimethyl arginine (ADMA), will inhibit NOS. There are two studies in which increased plasma ADMA levels have been reported in the aging male rat (Xiong et al., 2001; Lu et al., 2002). Further, recent observations have indicated that plasma ADMA levels rise in man in healthy old age, correlating with declines in renal plasma flow (Kielstein et al., 2003) and that the endothelial dysfunction seen in normal aging can be improved with L-arginine supplementation (Bode-Boger et al., 2003). There is currently no information on any sex differences in circulating ADMA or any other endogenous NOS inhibitors.

In addition to declines in systemic NO production, there is now evidence that renal NOS generation falls as age-dependent kidney damage develops. We recently reported a fall in the abundance of the neuronal NOS (nNOS) in the aging male Sprague–Dawley, correlating with decreased NOS activity (Erdely et al., 2003). The fall in renal cortical nNOS begins in middle age when injury is moderate and declines further as the kidney damage increases in severity, in the old rat (Figs. 6 and 7). We have also observed that renal cortical nNOS abundance falls in other models of CRD (Baylis, 2001). There is also a late fall in the renal cortical abundance of the endothelial (e)NOS in the old male rat kidney (Fig. 7) but this is preceded by the kidney damage and is likely to be a consequence, rather than a cause of the injury process. Of note, the female rat, which is protected from the age-dependent kidney damage, also shows preserved nNOS (and eNOS) protein levels (Fig. 7). We find no difference in NOS protein abundance in renal cortex between young male and female rats (Erdely et al., 2003) as also reported by Neugarten et al. (1997), although increased medullary NOS and NOS in whole kidney homogenates have been observed in female vs. male SD (Neugarten et al., 1997; Reckelhoff et al., 1998). We have also observed that in the 5/6 renal mass reduction model, the Wistar Furth rat which develops no kidney damage, also shows a preserved renal nNOS abundance (Baylis, 2001). This reinforces the suggestion that declines in renal nNOS abundance and activity may play a causal role in the progression of various forms of CRD, including age-dependent damage.

In conclusion, while age-dependent injury and declines in function are not inevitable, they will occur to some extent in most individuals. There is a pronounced sexual dimorphism with females protected due both to the protective estrogens and the lack of damaging androgens. There is also clear evidence of decreased total NO production, NOS activity and abundance in the aging male rat kidney (Erdely et al., 2003). While substrate (arginine) production is preserved, there are increases in the circulating NOS inhibitor, ADMA, in the aging male rat that would promote generalized declines in NO production. In the kidney, both major constitutive isoforms (eNOS and nNOS) are reduced, with declines in nNOS occurring early.

In contrast, there is no systemic or renal NOS deficiency in the aging female rat. We suggest that the loss of the renal nNOS may play a role in the progression of the age-dependent kidney damage and that this is related to CRD rather than aging 'per se' (Baylis, 2001). Furthermore, the more robust renal NO system in the female may in part explain the slower rate of progression in women with non-diabetic renal disease (Seliger et al., 2001).

4. Uncited reference

Elliot et al. (2003).

References

- Andreoli SP. Hormone replacement therapy in postmenopausal women with end-stage renal disease. *Kidney Int* 2000;57:341–342. [PubMed: 10620218]
- Baylis C. Age-dependent glomerular damage in the rat: dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. *J Clin Invest* 1994;94:1823–1829. [PubMed: 7962527]
- Baylis C. Impact of pregnancy on underlying renal disease. *Adv Ren Replace Ther* 2003;10:31–39. [PubMed: 12616461]
- Baylis C. Nitric oxide deficiency; both consequence and cause of chronic renal disease (CRD). *Hypertens Nephrol* 2001;5:193–201.
- Baylis C, Corman B. The aging kidney: insights from experimental studies [invited editorial]. *J Am Soc Nephrol* 1998;9:699–709. [PubMed: 9555673]
- Bode-Boger SM, Muke J, Surdacki A, Brabant G, Boger RH, Frolich JC. Oral L-arginine improves endothelial function in healthy individuals older than 70 years. *Vasc Med* 2003;8:77–81. [PubMed: 14518608]
- Ceccatelli S, Grandison L, Scott RE, Pfaff DW, Kow LM. Estradiol regulation of nitric oxide synthase mRNAs in rat hypothalamus. *Neuroendocrinology* 1996;64:357–363. [PubMed: 8930936]
- Cernadas MR, Sanchez de Miguel L, Garcia-Duran M, Gonzalez-Fernandez F, Millas I, Monton M, Rodrigo J, Rico L, Fernandez P, de Frutos T, Rodruigez-Feo JA, Guerra J, Carmelo C, Casado S, Lopez-Farre A, et al. Expression of constitutive and inducible NOS in the vascular wall of young and aging rats. *Circ Res* 1998;83:279–286. [PubMed: 9710120]
- Dubey RK, Jackson EK. Estrogen-induced cardiorenal protection: potential cellular, biochemical and molecular mechanisms. *Am J Physiol Ren Physiol* 2001;280:F365–F388.
- Dubey RK, Gillespie DG, Keller PJ, Imthurn B, Zacharia LC, Jackson EK. Role of methoxyestradiols in the growth inhibitory effects of estradiol on human glomerular mesangial cells. *Hypertension* 2002;39:418–424. [PubMed: 11882583]
- Elliot SJ, Karl M, Berho M, Potier M, Zheng F, Leclercq B, Striker GE, Striker LJ. Estrogen deficiency accelerates progression of glomerulosclerosis in susceptible mice. *Am J Pathol* 2003;162:1441–1448. [PubMed: 12707027]
- Ellison KE, Ingelfinger JR, Pivor M, Dzau VJ. Androgen regulation of rat renal angiotensinogen messenger RNA expression. *J Clin Invest* 1989;83:1941–1945. [PubMed: 2723066]
- Erdely A, Greenfeld Z, Wagner L, Baylis C. Sexual dimorphism in the aging kidney; inverse relationship between injury and nitric oxide system. *Kidney Int* 2003;63:1021–1026. [PubMed: 12631083]
- Fadel PJ, Zhao W, Thomas GD. Impaired vasomodulation is associated with reduced neuronal nitric oxide synthase in skeletal muscle of ovariectomized rats. *J Physiol* 2003;549:243–253. [PubMed: 12665606]
- Fliser D, Franek E, Joest M, Block S, Mutschler E, Ritz E. Renal function in the elderly: impact of hypertension and cardiac function. *Kidney Int* 1997;51:1196–1204. [PubMed: 9083286]
- Forte P, Kneale BJ, Milne E, Chowienzyk PJ, Johnston A, Benjamin N, Ritter JM. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension* 1998;32:730–734. [PubMed: 9774371]

- Gonzalez-Hernandez T, Gonzalez MC. Gender differences and the effect of different endocrine situations on the NOS expression pattern in the anterior pituitary gland. *J Histochem Cytochem* 2000;48:1639–1648. [PubMed: 11101632]
- Gragasin FS, Xu Y, Arenas IA, Kainth N, Davidge ST. Estrogen reduces angiotensin II-induced nitric oxide synthase and NAD(P)H oxidase expression in endothelial cells. *Arterioscler Thromb Vasc Biol* 2003;23:38–44. [PubMed: 12524222]
- Guccione M, Silbiger S, Lei J, Neugarten J. Estradiol upregulates mesangial cell MMP-2 activity via the transcription factor AP-2. *Am J Physiol Ren Physiol* 2002;282:F164–F169.
- Hayashi T, Yamada K, Esaki T, Mutoh E, Iguchi A. Effect of estrogen on isoforms of nitric oxide synthase: possible mechanism of anti-atherosclerotic effect of estrogen. *Gerontology* 1997;43 (Suppl 1):24–34. [PubMed: 9187936]
- Hill C, Lateef AM, Engels K, Samsell L, Baylis C. Basal and stimulated nitric oxide in control of kidney function in the aging rat. *Am J Physiol* 1997;272:R1747–R1753. [PubMed: 9227586]
- Kasike BL. Relationship between vascular disease and age-associated changes in the human kidney. *Kidney Int* 1987;31:1153–1159. [PubMed: 3599655]
- Kielstein JT, Bode-Boger SM, Frolich JC, Ritz E, Haller H, Fliser D. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 2003;107:1891–1895. [PubMed: 12681993]
- Krepinsky J, Ingram AJ, James L, Ly H, Thai K, Cattran DC, Miller JA, Scholey JW. 17beta-Estradiol modulates mechanical strain-induced MAPK activation in mesangial cells. *J Biol Chem* 2002;277:9387–9394. [PubMed: 11777903]
- Kwan G, Neugarten J, Sherman M, Ding Q, Fotadar U, Lei J, Silbiger S. Effects of sex hormones on mesangial cell proliferation and collagen synthesis. *Kidney Int* 1996;50:1173–1179. [PubMed: 8887275]
- Levi, M.; Rowe, JW. Renal function and dysfunction in aging. In: Seldin, DW.; Giebisch, G., editors. *The Kidney: Physiology and Pathophysiology*, 101. Vol. Chapter 101. Raven Press; New York: 1992. p. 3433-3456.
- Lombet JR, Adler SG, Anderson PS, Nast CC, Olsen DR, Glasscock RJ. Sex vulnerability in the subtotal nephrectomy model of glomerulosclerosis in the rat. *J Lab Clin Med* 1989;114:66–74. [PubMed: 2738450]
- Lu R, Hu CP, Wu XP, Liao EY, Li YJ. Effect of age on bone mineral density and the serum concentration of endogenous nitric oxide synthase inhibitors in rats. *Comp Med* 2002;52:224–228. [PubMed: 12102566]
- Lyons D, Roy S, Patel M, Benjamin N, Swift CG. Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. *Clin Sci* 1997;93:519–525. [PubMed: 9497788]
- McLachlan MS, Guthrie JC, Anderson CK, Fulker MJ. Vascular and glomerular changes in the ageing kidney. *J Pathol* 1977;121:65–78. [PubMed: 874633]
- Mershon JL, Baker RS, Clark KE. Estrogen increases iNOS expression in the ovine coronary artery. *Am J Physiol Heart Circ Physiol* 2002;283:H1169–H1180. [PubMed: 12181148]
- Millatt LJ, Abdel-Rahman EM, Siragy HM. Angiotensin II and nitric oxide: a question of balance. *Regul Pept* 1999;81:1–10. [PubMed: 10395403]
- Mistry S, Greenfield Z, Morris S, Baylis C. The ‘intestinal–renal’ arginine biosynthetic axis in the aging rat. *Mech Age Dev* 2002;123:1159–1165.
- Munger K, Baylis C. Sex differences in renal hemodynamics in rats. *Am J Physiol* 1988;254:F223–F231. [PubMed: 3344806]
- Neugarten J, Ding Q, Friedman A, Le J, Silbiger S. Sex hormones and renal nitric oxide synthases. *J Am Soc Nephrol* 1997;8:1240–1246. [PubMed: 9259350]
- Neugarten J, Gallo G, Silbiger S, Kasike B. Glomerulo-sclerosis in aging humans is not influenced by gender. *Am J Kidney Dis* 1999a;34:884–888. [PubMed: 10561145]
- Neugarten J, Medve I, Lei J, Silbiger SR. Estradiol suppresses mesangial cell type I collagen synthesis via activation of the MAP kinase cascade. *Am J Physiol* 1999b;277:F875–F881. [PubMed: 10600934]
- Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. *J Am Soc Nephrol* 2000a;11:319–329. [PubMed: 10665939]

- Neugarten J, Acharya A, Lei J, Silbiger S. Selective estrogen receptor modulators suppress mesangial cell collagen synthesis. *Am J Physiol Ren Physiol* 2000b;279:F309–F318.
- Noris M, Todeschini M, Zappella S, Bonazzola S, Zoja C, Corna D, Gaspari F, Marchetti G, Aiello S, Remuzzi G. 17beta-estradiol corrects hemostasis in uremic rats by limiting vascular expression of nitric oxide synthases. *Am J Physiol Ren Physiol* 2000;279:F626–F635.
- Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R233–R249. [PubMed: 14707008]
- Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension* 2001;27:1199–1208. [PubMed: 11358929]
- Reckelhoff JF, Baylis C. Glomerular metalloprotease activity is suppressed by androgens in the ageing kidney. *J Am Soc Nephrol* 1993;3:1835–1838. [PubMed: 8329677]
- Reckelhoff JF, Kellum JA, Blanchard EJ, Bacon EE, Wesley EJ, Kruckeberg WC. Changes in nitric oxide precursor, L-arginine and metabolites, nitrate and nitrite, with aging. *Life Sci* 1994;55:1895–1902. [PubMed: 7990649]
- Reckelhoff JF, Hennington BS, Moore AG, Blanchard EJ, Cameron J. Gender differences in the renal nitric oxide (NO) system: dissociation between expression of endothelial NO synthase and renal hemodynamic response to NO synthase inhibition. *Am J Hypertens* 1998;11:97–104. [PubMed: 9504456]
- Schmidt RJ, Beierwaltes WH, Baylis C. Aging and alterations in dietary sodium intake on total nitric oxide production in normal man. *Am J Kidney Dis* 2001;37:900–908. [PubMed: 11325670]
- Schmieder RE, Schachinger H, Messerli FH. Accelerated decline in renal perfusion with aging in essential hypertension. *Hypertension* 1994;23:351–357. [PubMed: 8125562]
- Seliger SL, Davis C, Stehman-Breen C. Gender and the progression of renal disease. *Curr Opin Nephrol Hypertens* 2001;10:219–225. [PubMed: 11224697]
- Silbiger S, Lei J, Ziyadeh FN, Neugarten J. Estradiol reverses TGF-beta1-stimulated type IV collagen gene transcription in murine mesangial cells. *Am J Physiol* 1998;274:F1113–F1118. [PubMed: 9841504]
- Sonaka I, Futami Y, Maki T. L-arginine–nitric oxide pathway and chronic nephropathy in aged rats. *J Gerontol Biol Sci* 1990;49:B157–B161.
- Verhagen AM, Attia DM, Koomans HA, Joles JA. Male gender increases sensitivity to proteinuria induced by mild NOS inhibition in rats: role of sex hormones. *Am J Physiol Ren Physiol* 2000;279:F664–F670.
- Wesson, LG, Jr. *Physiology of the Human Kidney*. Grune and Stratton; New York: 1969. Renal hemodynamics in physiological states; p. 96-108.
- Xiao S, Gillespie DG, Baylis C, Jackson EK, Dubey RK. Effects of estradiol and its metabolites on glomerular endothelial NO synthesis and mesangial cell growth. *Hypertension* 2001;37:645–650. [PubMed: 11230350]
- Xiong Y, Yuan LW, Deng HW, Li YJ, Chen BM. Elevated serum endogenous inhibitor of nitric oxide synthase and endothelial dysfunction in aged rats. *Clin Exp Pharmacol Physiol* 2001;28:842–847. [PubMed: 11553026]
- You HJ, Kim JY, Jeong HG. 17 beta-estradiol increases inducible nitric oxide synthase expression in macrophages. *Biochem Biophys Res Commun* 2003;303:1129–1134. [PubMed: 12684053]
- Zheng F, Plati AR, Potier M, Schulman Y, Berho M, Banerjee A, Leclercq B, Zisman A, Striker LJ, Striker GE. Resistance to glomerulosclerosis in B6 mice disappears after menopause. *Am J Pathol* 2003;162:1339–1348. [PubMed: 12651625]

Fig. 1.

Glomerular filtration rate, GFR (measured by inulin or 24 h creatinine clearances) in cross-sectional studies in normal men and women of different ages (Wesson, 1969). Solid lines represent mean and broken lines represent 1 SD (Reprinted with permission from Wesson, 1969).

Fig. 2.

Effective renal plasma flow, ERPF (measured by PAH clearances) and the calculated renal vascular resistance, RVR, in the following four groups: young healthy normotensive subjects (young NT, $n = 24$; 13 males; mean age 26 ± 3 years); elderly healthy normotensive subjects (elderly NT, $n = 29$; 13 males; 68 ± 7 years); elderly treated and untreated hypertensive patients (elderly HT; $n = 25$; 13 males; 70 ± 6 years); and elderly patients with compensated mild to moderate heart failure (elderly HF; $n = 14$; six males; 69 ± 6 years). Figure reproduced from Fliser et al. (1997) with permission.

Fig. 3.

GFR (from inulin clearances), % glomerular sclerosis and 24 h urine total protein excretion ($U_{\text{protein V}}$) in 18–20 month old intact males and females and castrated males and ovariectomized females of the Munich Wistar rat strain. GFR data is expressed as a % of the young adult (4 month old) value for each group. Figure is drawn from data described in Baylis (1994).

Fig. 4.

Glomerular metalloprotease activity in a homogenate of isolated glomeruli from intact young (4 monthly old) adult male and female, and 18–20 month old intact males and females, castrated (cast) males and ovariectomized (OVX) female Munich Wistar rats. Figure is drawn from data described in Reckelhoff and Baylis (1993).

Fig. 5.
Simplified scheme showing the biosynthetic pathway for nitric oxide (NO) production in vivo.

Fig. 6.

The 24 h urinary excretion of $\text{NO}_2 + \text{NO}_3$ (NO_x), $\text{U}_{\text{NO}_x} \text{ V}$ (upper panel) and the % of damaged glomeruli (i.e. those showing segmental and global sclerosis) in young adult (3–5 m) and old (18–22 m) male (M) and female (F) Sprague–Dawley rats. Figure is drawn from data described in Erdely et al., 2003.

Fig. 7.

The relative abundance of the endothelial NOS (eNOS) protein (upper panel) and the neuronal NOS (nNOS) protein in homogenates of renal cortex from young, middle-aged and old male (M) and female (F) Sprague-Dawley rats. Figure is drawn from data described in Erdely et al., 2003. * denotes a significant difference ($p < 0.05$) between male and female. # denotes a difference between young and other ages.