

Gender and the Renal Nitric Oxide Synthase System in Healthy Humans

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Background and Objectives: It is widely known that men with kidney disease progress to ESRD at a much greater rate than do women. The mechanism for these gender differences is not clear, but reduced availability of nitric oxide is thought to contribute to the age-related decline in renal plasma flow observed in both healthy men and women. Animal models suggest that the renal vasculature of men may be significantly more dependent on nitric oxide than that of women.

Design, Setting, Participants, & Measurements: Renal plasma flow response to the nonspecific nitric oxide synthase inhibitor nitro-L-arginine methyl ester (L-NAME) was measured by para-aminohippurate clearance technique in 21 healthy, normotensive (8 male, 13 female) individuals in balance on a high-salt diet.

Results: There were striking differences between the genders in the renal hemodynamic response to L-NAME according to age, a difference that remained even after adjustment for other significant covariates. In men, the fall in renal plasma flow induced by L-NAME increased remarkably with increasing age. In women, there was no influence of age on the renovascular response to L-NAME. Neither age nor gender predicted the mean arterial pressure response to L-NAME.

Conclusions: The renal vasculature of men becomes more dependent on nitric oxide with age compared with that of women, suggesting that any renal disease that interferes with nitric oxide production may, over time, cause existent kidney damage to progress more quickly in men relative to women.

Clin J Am Soc Nephrol 2: 916-931, 2007. doi: 10.2215/CJN.00110107

It is widely known that men with kidney disease progress to ESRD at a much greater rate than do women (1–4). Even in healthy men, GFR begins to decline in the fourth decade (5), but this is delayed and attenuated in women (5,6), with similar results observed in renal plasma flow (RPF) (5).

The mechanism for these gender differences is not clear, but reduced availability of nitric oxide (NO), suggested by elevated plasma levels of the endogenous NO synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA) is thought to contribute to the age-related decline in RPF (7). Animal models suggest that the renal vasculature of men may be significantly more dependent on NO than that of women (8–11). There are also reports that in concert with the development of kidney damage, the total NO production falls significantly in the old male rat (12–15). Although no studies published to date have examined the effects of gender on renal NO availability in humans, there is evidence suggesting differences in whole-body NO biosynthesis between healthy premenopausal women and men (16).

These observations raised two questions: Does the dependence of the renal hemodynamics on NO change with age in healthy humans? Are there differences between the genders in terms of renal hemodynamic dependence on NO? We hypoth-

esized that the RPF of men would exhibit greater dependence on NO compared with women and that these differences would increase with age. We therefore examined renal hemodynamic function at baseline and in response to the nonspecific NOS inhibitor nitro-L-arginine methyl ester (L-NAME) in 21 healthy individuals.

Materials and Methods

Participants

Twenty-one healthy, normotensive individuals, ranging in age from 18 to 66 yr, were enrolled in the study. Participants completed an initial medical history and underwent a physical examination, electrocardiogram, and laboratory screening. All participants gave written informed consent. The study protocol was approved by the Brigham and Women's Hospital institutional review board and conducted in accordance with institutional guidelines.

Protocol

Participants were instructed to consume >200 mmol/d sodium and to avoid high-nitrate foods for 4 d before the study. A 24-h urine collection was used to measure sodium, creatinine, and protein excretion. Participants were studied in the supine position after an 8-h fast. At 8 a.m., an intravenous catheter was placed in each arm (one for infusion and one for blood sampling). Fasting plasma glucose concentration was measured at the start of the study. BP was recorded every 15 min by an automatic recording device (Dinamap; Critikon, Tampa, FL). Each participant was given loading doses of 8 mg/kg para-aminohippurate (PAH), followed by constant infusions of PAH at 12 mg/min for 90 min to establish baseline renal hemodynamic measurements, followed by 10 μ g/kg per min L-NAME administered intravenously

Received January 4, 2007. Accepted June 14, 2007.

Published online ahead of print. Publication date available at www.cjasn.org.

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for a total of 10 min. To determine the peak effect of NOS inhibition, PAH clearance and plasma renin activity (PRA) were measured at baseline and at 60, 90, 135, and 180 min after administration of L-NAME. Spontaneously voided urine samples were collected before the study and at 180 min after L-NAME for measurement of urinary nitrates and nitrites.

Analytical Methods

Renal clearance was assessed with PAH (Clinalfa, Läufelfingen, Switzerland) as described previously (17). Urinary albumin concentration was measured by immunonephelometry (Behring, Somerville, NJ). Serum PAH was measured by autoanalyzer. PRA was assayed by RIA (17). Renal vascular resistance (RVR) was calculated using the equation $RVR = [(mean\ arterial\ BP - 12) \times 723/RPF]$. Urinary nitrate and nitrite levels were measured by a modified method of the Griess reaction that converts all nitrate to nitrite using the bacterial enzyme nitrate reductase (18). The colorimetric reaction among nitrite, sulfanilamide, and N-(1-naphthyl) ethylenediamine produces a pink/magenta azo product with a maximum absorbance at 543 nm. Absorbance was read using a Multiskan Ascent microplate photometer (Labsystems, Franklin, MA). Serum and urine creatinine levels were measured by alkaline picrate reactions with a DAX96 (Bayer, Newbury, Berkshire) machine.

Statistical Analyses

The primary analysis tested associations between age and renal hemodynamics according to gender. Study participant baseline and response to L-NAME measures were compared using Wilcoxon sign-rank and Wilcoxon rank sum tests. χ^2 tests were used to compare frequencies. Associations were analyzed by univariate regression analysis (Pearson). In addition, multivariate linear regression analysis was applied to evaluate the relative contributions of covariates to the change in renal hemodynamics in response to L-NAME. The following variables were included: Age, gender, body mass index (BMI), smoking status, mean arterial pressure (MAP), fasting plasma glucose, and baseline RPF. A stepwise multivariate regression selection was used. Statistical analyses were performed using Stata (version 8.2; Stata Corp., College Station, TX) with two-tailed significance levels of 0.05.

Results

Baseline Characteristics

Participant characteristics are presented in Table 1. All participants had normal renal function and BP. There were no differences in baseline RPF ($P = 0.9$) or RVR ($P = 0.9$), MAP ($P = 0.5$), or plasma PRA ($P = 0.7$) between the groups. Women tended to be older and have a greater BMI than men, but this difference did not achieve statistical significance ($P = 0.1$ for age, $P = 0.6$ for BMI).

Renal Hemodynamic Responses to L-NAME

Both men and women had the anticipated decrease in RPF in response to L-NAME ($P = 0.03$ for men, $P = 0.002$ for women), but their responses were not statistically different ($P = 0.5$; Table 2). On univariate analysis, the RPF response to L-NAME did not change with age ($P = 0.09$). However, when the RPF responses to L-NAME were analyzed by gender, there were striking differences between the genders in the renal hemodynamic response to L-NAME according to age ($P < 0.0001$; Figure 1), a difference that remained even after adjustment for all other covariates ($P = 0.001$). In men, the fall in RPF induced by L-NAME showed a striking correlation with increasing age ($r = -0.9$, $P = 0.005$). In women, there was no influence of age on the renovascular response to L-NAME ($r = -0.04$, $P = 0.9$).

The time to maximal renal vasoconstriction in response to L-NAME was not different between the genders ($P = 0.07$). However, after adjustment for age, significant gender-related differences were observed ($P = 0.02$), with the time course to reach the RPF nadir after administration of L-NAME (*i.e.*, maximal effect) decreasing with increasing age in male participants, whereas the time course seemed to increase with increasing age in the female participants (Figure 2).

MAP increased in response to L-NAME in both groups ($P = 0.03$ for men, $P = 0.007$ for women), although the increase in MAP was similar between the genders ($P = 0.7$). Neither age

Table 1. Baseline characteristics

Characteristic	Men ($n = 8$)	Women ($n = 13$)
Age (yr)	36 \pm 4	47 \pm 5
White (%)	89	77
BMI (kg/m^2)	27 \pm 2	29 \pm 1
MAP (mmHg) ^b	88 \pm 3	85 \pm 2
HRT or OC use (women only; %)	N/A	0
Smoker (n [%])	0 (0)	1 (8)
Fasting glucose (mg/dl)	89 \pm 3	87 \pm 4
PRA (ng AngI/ml per h)	0.32 \pm 0.10	0.25 \pm 0.05
Urine Na (mmol/24 h)	348 \pm 30	294 \pm 21
Urinary nitrate/nitrite (μM /mmol creatinine)	56 \pm 10	56 \pm 14
RPF (ml/min per 1.73 m^2) ^b	593 \pm 22	584 \pm 31
RVR (mmHg/ml per min/1.73 m^2)	94 \pm 6	94 \pm 6

^aData are means \pm SE, unless otherwise indicated. AngI, angiotensin I; BMI, body mass index; HRT, hormone replacement therapy; MAP, mean arterial pressure; OC, oral contraceptive; PRA, plasma renin activity; RPF, renal plasma flow; RVR, renal vascular resistance.

^bMean of readings at $t = -10$ min, -5 min, and 0.

Table 2. Response to L-NAME^a

Parameter	Men	Women
MAP (mmHg) ^b	95 ± 3 ^d	93 ± 2 ^d
ΔMAP (mmHg)	7 ± 2	8 ± 2
PRA (ng AngI/ml per h) ^c	0.31 ± 0.10	0.29 ± 0.05
ΔPRA (ng AngI/ml per h)	-0.04 ± 0.05	0.03 ± 0.03
RPF (ml/min per 1.73 m ²) ^c	554 ± 29 ^d	546 ± 29 ^d
ΔRPF (ml/min per 1.73 m ²)	-55 ± 19	-66 ± 6
RVR (mmHg/ml per min/1.73 m ²)	116 ± 12 ^d	120 ± 11 ^d
ΔRVR(mmHg/ml per min/1.73 m ²)	22 ± 7	27 ± 7
Time to nadir response after L-NAME (min)	111 ± 15	143 ± 9
Urinary nitrate/nitrite (μM/mmol creatinine)	58 ± 11	63 ± 13
ΔUrinary nitrate/nitrite (μM/mmol creatinine)	0.4 ± 22	7 ± 13

^aData are means ± SE unless otherwise indicated. L-NAME, nitro-L-arginine methyl ester.

^bMean of two readings at peak response to L-NAME.

^cMean of two readings at nadir of response to L-NAME.

^d $P < 0.05$ versus baseline.

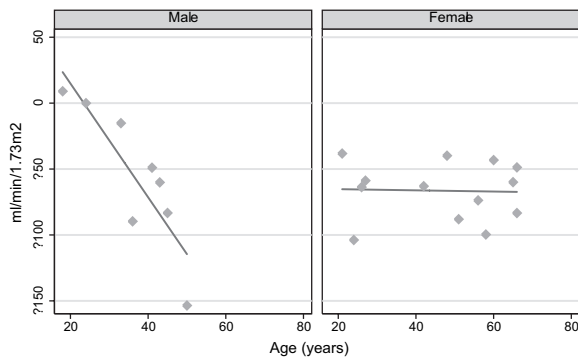


Figure 1. Renal plasma flow (RPF) response to nitro-L-arginine methyl ester (L-NAME) versus age, according to gender. Men: $r = -0.90$, $P = 0.005$; women: $r = -0.04$, $P = 0.9$.

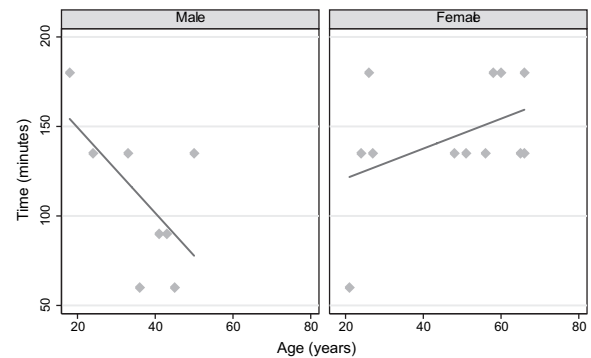


Figure 2. Time to RPF nadir after administration of L-NAME. Men: $r = -0.61$, $P = 0.1$; women: $r = 0.44$, $P = 0.1$.

nor gender influenced the MAP response to L-NAME ($P = 0.8$ for age, $P = 0.6$ for gender). There was no correlation between the change in MAP and the change in RPF in response to NOS inhibition ($P = 0.2$). Whereas RVR increased significantly in both men and women, there was no significant gender difference ($P = 0.6$), even after adjustment for age.

The change in urinary nitrates and nitrites in response to L-NAME did not differ between the groups ($P = 0.9$) and was not associated with either age ($P = 0.9$) or gender ($P = 0.9$). Plasma PRA did not change in either group in response to L-NAME ($P = 0.5$ versus baseline for men, $P = 0.3$ versus baseline for women, $P = 0.3$ for men versus women).

Discussion

Our study examined the renal hemodynamic response to acute NOS blockade by L-NAME infusion in healthy, normotensive men and women. The RPF of men became significantly more dependent on NO with age. In stark contrast, the influence of NOS inhibition on the renal hemodynamics of women did not vary with age. The difference in the age-dependent renovascular constrictor response to L-NAME between the genders was

not reflected in the MAP response to the NOS inhibitor, which did not vary with age and was not different between the groups, thus indicating only a difference in intrarenal, rather than systemic, NOS activity between men and women.

Many factors link the NOS system and the development of altered vascular function and hemodynamics. High glucose augments NO production in the renal cortex (19), and early nephropathy in diabetes is associated with increased intrarenal NO production (20); however, all of our participants were free of diabetes and had fasting glucose levels in the euglycemic range. Kielstein *et al.* (7) reported increased plasma values of the endogenous NOS inhibitor ADMA in elderly hypertensive individuals compared with age-matched normotensive control subjects, but all of our participants were normotensive. Expression of most of the genes that are known to belong to the NOS system have been detected in human adipose tissue, and the genes encoding endothelial NOS (eNOS), inducible NOS, and cGMP-dependent protein kinase-1 are expressed at higher levels in obese women (21). However, despite that the women in our study had a higher BMI (although not statistically significant), the renal hemodynamics of men still had greater dependence on NO with age.

There is increasing animal and human evidence that progression of chronic kidney disease is associated with a deficiency of NO (22), and experimentally induced NO deficiency produces renal disease (23). Rising plasma levels of ADMA have been associated both with decreasing RPF levels and with increasing age in healthy individuals (7) as well as worsening renal function in humans (24–26). Gender-dependent differences in ADMA plasma concentration were previously shown in healthy volunteers in different age groups. Men show a linear increase in ADMA levels with age; in contrast, women who were younger than 50 yr had significantly lower ADMA plasma levels than male individuals of any age, although ADMA plasma concentrations increased in a curvilinear manner after 50 yr of age (27), which may reflect hormonal status. Thus, it is probable that circulating ADMA levels were higher in the male compared with the female participants in our study.

It is likely that general peripheral NO production (by eNOS) is regulated differently than renal NO production (by eNOS and neuronal NOS), as suggested by the divergence between MAP and RPF responses in this study. Circulating ADMA is more likely to influence general total peripheral resistance rather than RPF and RVR. However, because renal ADMA content is high and renal breakdown of ADMA by dimethylarginine dimethylaminohydrolase is also significant (28), gender differences in the kidney could have an impact on both circulating and intrarenal ADMA levels. Although no studies published to date have examined the effects of gender on renal NO availability in humans, there is evidence suggesting differences in whole-body NO biosynthesis between healthy premenopausal women and men (16).

Animal models suggest differences in dependence of the renal vasculature on NO, depending on gender. Verhagen *et al.* (29) demonstrated that mild NOS inhibition resulted in significantly greater increases in proteinuria in male rats compared with female rats. The kidneys of male Han:SPRD rats, a model of polycystic kidney disease, are susceptible to the effects of L-NAME, although the kidneys of their female counterparts are not (11). Chronic L-NAME increased RVR by 130% in male rats but by only 60% in female rats and decreased RPF by 40% in male rats but had no effect on female rats (8). Erdely *et al.* (15) reported that elderly male Sprague-Dawley rats, a strain in which the male develops significant renal pathology, have reduced renal NOS activity and NOS protein abundance compared with both age-matched female rats and young male rats, suggesting that renal NO deficiency in the aging male rat may contribute to age-dependent kidney damage. In a rat model of renal wrap hypertension, Ji *et al.* (30) demonstrated more severe renal injury in male compared with female rats and attributed this sexual dimorphism to differences in renal endothelial and neuronal NO production.

The mechanism explaining the differences between the genders in terms of progression of renal pathology remains elusive. The endothelial NOS gene G894→T polymorphism is a determinant of both baseline renal hemodynamic function and the hemodynamic response to angiotensin II (AngII) in men but not in women (31). The renal perfusion pressure is greater in male rats in response to AngII than in female rats, and this difference

remains between the genders after blockade of NOS by L-NAME (10), suggesting that although the renal vasculature of male rats is more sensitive to the vasoconstrictor effects of AngII, it is also more dependent on the vasodilator effects of NO compared with female rats. It therefore is possible that because male kidneys are more dependent on NO, removal of this vasodilator uncovers the presence of more vasoconstrictors, such as AngII and endothelin-1, in men compared with women, which would accelerate progression of renal disease.

Manipulation of the sex hormones replicates the effects of gender, suggesting that it is the hormonal milieu, rather than actual structural differences, that accounts for the gender disparity in progression of kidney disease (32). Although estradiol is a potent stimulus for formation of eNOS and subsequent generation of NO (33), controversy exists over whether it is the presence of androgen or the absence of estrogen (29,34–37) that promotes the acceleration of renal injury. The rate of progression of renal disease in premenopausal women is slower than in men, and this protection is lost with the onset of menopause but can be restored with estradiol replacement (38,39). Certainly, exogenous estrogen intake in the form of hormone replacement therapy increases NO production (40); however, although several of our female study participants were of postmenopausal age, none was taking hormone replacement therapy, suggesting factors other than a lack of estrogen account for the age-related increased renal hemodynamic dependence on NO observed in men.

The results of our study must be interpreted within its limitations. It is possible that our study time course was too short; by measuring the RPF up to only 180 min after L-NAME administration, we may not have captured the point of maximal renal vasoconstriction in response to L-NAME, particularly in young men or older women, although the possibility of gender and age differences with respect to the pharmacokinetics of L-NAME is in itself of interest. We only used a single low dose of L-NAME, and it is possible that the lack of response to acute low-dosage NOS inhibition in our young male participants in fact reflected a very large NO production, with the dosage of L-NAME administered being inadequate to evoke any renal hemodynamic effect; however, the limitations imposed by human clinical research precluded the use of a higher dosage. We did not control the amount of L-arginine, which normalizes vascular dysfunction in patients with hypercholesterolemia (41), ingested by our study participants; however, all of our participants were normocholesterolemic, and, as such, this should not have unduly influenced our results. In addition, oral administration of L-arginine for 6 mo to participants with glomerulonephritis did not change proteinuria, GFR, or RPF compared with a control group that received placebo (42). Similarly, we did not control for vitamin supplementation in our participants. There is some evidence that co-infusion of vitamin C with L-arginine results in a significant increase in RPF in individuals with glomerular disease (43) and in smokers compared with nonsmokers (44). However, all our participants were healthy, and the only smoker in our study population was female, which would potentially bias our findings only toward the null hypothesis. We did not control for phase of the men-

strual cycle in our premenopausal participants; however, that the RPF response to L-NAME in the postmenopausal participants did not change with age argues against estrogen being the only factor involved in renal NO production, although clearly estrogen status cannot be discounted. Although there were no apparent differences in urinary excretion of nitrites and nitrates between the genders in response to L-NAME, measurements of acute changes in these compounds should be interpreted cautiously, because they may reflect altered tubular handling of nitrates rather than the acute activity of the systemic and/or renal NO systems (45).

The differences found between the genders in terms of renal hemodynamic dependence on NO are striking. Individuals with renal disease were not studied as part of this investigation. However, our results suggest that if a patient were to develop a renal disease that interferes with NO production, then this may, over time, cause existent kidney damage to progress more quickly in men relative to women. The results of our study may also have implications in terms of therapy of renal disease in men and women. Infusion of L-arginine, a substrate of NOS, in experimental animals increases RPF and GFR and decreases proteinuria (46). In rats after five-sixths nephrectomy, supplementation with tetrahydrobiopterin, a key co-factor of NOS, initiated 24 h after surgery and maintained for 8 wk, preserved systolic BP, reduced proteinuria, and prevented the development of glomerular mesangial expansion (47). Given that renal disease is associated with decreased production of NO, it is tempting to speculate that supplementation with these agents may prove to be beneficial in humans with renal disease, particularly men. Although the biologic effects seen in our physiology study were large, clearly, large-scale prospective studies will need to be performed before recommendations regarding clinical practice can be made.

Acknowledgments

This work was supported by a biomedical fellowship from the Kidney Foundation of Canada (to S.B.A.) and grants from the National Institutes of Health (T32 HL-07609, P01AC00059916, and 1P50ML53000-01 to N.K.H.).

This work was presented in part at the annual meeting of the Canadian Society of Nephrology; Calgary, Alberta, Canada; May 2005.

We are grateful to Charlene Malarick, RN, BSN, Caroline Coletti, BSc, MAT, and Padmavathi Peddakotla, BSc, for expert technical assistance.

Disclosures

None.

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