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Impact of gender on the renal response to angiotensin II

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Background. It is clear that women with renal disease progress to end stage at a slower rate than do men. We hypothesized that this protection may result from gender-mediated differences in responses to angiotensin II (Ang II), which has known hemodynamic effects that are thought to promote renal disease progression. We examined sex differences in renin-angiotensin system (RAS) function by measuring renal hemodynamic function and circulating plasma components of the RAS at baseline and in response to graded infusions of Ang II.

Methods. We studied two groups of normal healthy subjects, 24 men and 24 women, mean age 28 ± 1 years, ingesting a controlled sodium and protein diet. We examined baseline concentrations of angiotensin converting enzyme, plasma renin activity, Ang II, and aldosterone. Inulin and paraaminohippurate clearance techniques were used to estimate effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) at baseline and in response to graded Ang II infusion (0.5, 1.5, and 2.5 ng/kg/min).

Results. Mean baseline values for mean arterial pressure and aldosterone were lower in women, whereas values for plasma Ang II, GFR, ERPF, and filtration fraction (FF) did not differ. In response to Ang II, both groups exhibited a similar increase in mean arterial pressure and a decline in ERPF. GFR was maintained during Ang II infusion only in men, resulting in an augmentation of FF. In women, GFR declined in parallel with ERPF, and the FF response was significantly blunted. 17β-Estradiol plasma concentrations influenced the ERPF response to Ang II infusion, with higher levels predicting a blunting of the decrease. The GFR response was not affected.

Conclusions. The renal microcirculation in sodium-replete women may respond differently to Ang II than that of men, with the female sex predicting a lesser augmentation of FF and possibly a blunted increase in intraglomerular pressure. The mechanism remains obscure, but these contrasting responses may help to explain gender-mediated differences in renal disease progression.

The impact of gender on the function of the renin-angiotensin system (RAS) is unclear. It is well known that estrogen plays a role in the regulation of some components of the RAS. For example, a promoter region in the angiotensinogen gene is responsive to estrogen [1], and administration of exogenous estrogen raises plasma, hepatic, and renal concentrations of angiotensinogen, suggesting that angiotensin II (Ang II), the effector substance of the RAS, might be elevated in females. However, in nonpregnant ewes, there is a gradual, progressive rise in plasma renin activity (PRA) and circulating Ang II after ovariectomy [2, 3]. Most studies in humans examining the relationship between gender, estrogen status, and the RAS have relied on the measurement of baseline plasma concentrations of components of system. Results have been conflicting, with groups suggesting that the system is activated [4, 5] or blunted [6]. Few studies exist that use dynamic maneuvers to examine differences in RAS function between men and women. Those that have done so have provided some intriguing information. Ishibashi et al have demonstrated a significant blunting of the response of PRA to sodium restriction in older hypertensive women compared with men [7], and Magness et al have shown that estrogen can interfere with the vascular response to Ang II, at least in pregnant women [8].

A complete understanding of RAS function in normal premenopausal women would be of interest. It is clear that women progress to end-stage renal failure after a renal insult at a slower rate than do men [9–12], and Ang II has both renal hemodynamic and mitogenic effects that have been implicated in renal disease progression [13]. Therefore, we undertook a systematic analysis of the baseline components of the RAS and the renal and peripheral responsiveness to Ang II in 48 normal healthy men and premenopausal women, all ingesting a controlled sodium and protein diet, to determine whether a discernible gender difference exists. Our rationale reflected the possibility that the pattern of renal responsiveness to Ang II might represent a physiological process that could help to clarify gender-mediated differences in disease progression.

METHODS

Subjects

Fifty normal healthy men and women were recruited to participate in the study. Their mean age was 28 ± 1 years (range 20 to 35 years). Each subject underwent a detailed history and physical examination by a qualified internist. All were white, normotensive, nonobese, and

[1] **See Editorial, p. 336.**

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nonsmokers. No subject was ingesting any regular medications; specifically, none of the women were users of oral contraceptive medications. The study was performed with the approval of the University of Toronto Human Subjects Review Committee and with the informed written consent of each subject.

All subjects were counseled to adhere to a diet that maintained their normal caloric intake, their sodium intake to more than 200 mmol/day, and their protein intake to 1 to 1.5 g/kg/day for seven days prior to the study. A 24-hour urine sample was obtained one day prior to the study to determine urine sodium and urea excretion. Data from subjects were not analyzed if urine sodium excretion was less than 180 mmol/day, resulting in the exclusion of two subjects, one man and one woman. Therefore, data from 48 subjects were analyzed. Protein intake was estimated from the urine urea concentration and was corrected for body weight. On the day of the testing, the volunteer subjects reported to the Renal Physiology Laboratory of the Toronto Hospital. All studies were conducted at 0830 hours, after an overnight fast, with the subjects lying supine in a warm, quiet room.

Study protocol

An 18-gauge peripheral venous cannula was inserted into an antecubital vein for infusions of inulin, paraaminohippurate (PAH), and Ang II, and another cannula was placed in the opposite arm for blood sampling. Each subject voided and then drank sufficient water in the first 45 minutes to induce a water diuresis. Approximately 200 ml were ingested in each hour of the protocol to maintain an adequate urine output for collection of spontaneously voided samples. Hemodynamic parameters [mean arterial pressure (MAP), heart rate] were measured throughout the study by an automated sphygmomanometer (Dinamapp) and were recorded once in each half hour of the protocol. Renal hemodynamics were measured using inulin and PAH clearance techniques. After collecting blood for inulin blank, hematocrit (Hct), Ang II, angiotensin converting enzyme (ACE) level, and, in the women, 17β-estradiol, and urine for inulin blank, a priming infusion containing 25% inulin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, inulin and PAH were infused continuously at a rate calculated to maintain their respective plasma concentrations constant at 20 and 1.5 mg/dl. After a 90-minute equilibration period, three timed urine collections of a 20-minute duration each were then obtained, and blood was collected for Hct, aldosterone, and PRA.

A solution of Ang II (2.5 mg/vial) was prepared by dissolving the diluent in normal saline to produce a concentration of 0.5 mg/ml. Two hundred and fifty ml normal saline were then added to 0.2 ml Ang II to produce a concentration of 400 ng/ml. Ang II was infused at three doses, 0.5, 1.5, and 2.5 ng/kg/min, each dose for 30 minutes. Subjects remained supine except to void. Blood was collected once during each Ang II infusion period for Hct, aldosterone, PRA, inulin, and PAH, and urine was collected for sodium, inulin, and PAH. MAP was also measured at the midpoint of each infusion. A further collection of both blood and urine was obtained at the end of the Ang II infusion, after a 30-minute recovery period.

Sample collection and analytical methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3,000 r.p.m. for 10 minutes at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Inulin concentrations in plasma and urine were measured by a modified method of Walser, Davidson and Orloff [14], and PAH concentration by a spectrophotometric method according to Brun [15]. The mean of the final two clearance periods represents glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), expressed per 1.73 m^2. Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1-Hct). Renal vascular resistance (RVR) was derived by dividing MAP by the RBF. Serum sodium concentration was measured by an ion-selective electrode method, and urine sodium by a flame photometry method.

Serum angiotensin converting enzyme (ACE) activity was measured by a spectrophotometric method using an ACE kinetic test kit (Buhlmann Laboratories AG, Schonembuch, Switzerland). Briefly, serum samples and calibrators (20 μl) were allowed to react with a synthetic peptide (N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine) (200 μl) in a 96-well microplate. The enzyme kinetics at 37°C were followed by the decrease in absorbance at 340 nm for 15 minutes with a Bio-Tek microplate reader (model # Ceres UV 900 Hdi; Bio-Tek, Burlington, VT, USA).

Angiotensin II was measured by radioimmunoassay. Blood was collected into prechilled tubes containing ethylenediaminetetraacetic acid and angiotensinase inhibitor (0.1 ml Bestatin Solution; Buhlmann Laboratories, Schonembuch, Switzerland). After centrifugation, plasma samples were stored at −70°C until analysis. On the day of analysis, plasma samples were extracted on phenylsilylsilica columns. A competitive radioimmunoassay kit supplied by Buhlmann Laboratories AG (Schonembuch, Switzerland) was used to measure the extracted Ang II. The detection limit of the assay was approximately 2.0 pg/ml, with a precision of 8.3% intra-assay variation and 9.0% interassay variation.

Plasma renin activity was determined by the quantitation of angiotensin I generation by radioimmunoassay using the New England Nuclear Kit. Aldosterone was

Table 1. Baseline characteristics

Parameter	Men ($N = 24$)	Women ($N = 24$)	P value
Age *years*	28 ± 1	27 ± 1	NS
BMI *kg/m²*	25 ± 1	23 ± 1	0.004
MAP *mm Hg*	91 ± 2	82 ± 2	0.0008
$U_{Na}V$ *mmol/day*	220 ± 17	208 ± 14	NS
Protein intake *g/kg/day*	1.15 ± 0.06	1.12 ± 0.04	NS
Hct	0.430 ± 0.005	0.379 ± 0.005	0.0001
ACE *U/liter*	44 ± 2	48 ± 2	NS
Ang II *pg/ml*	8 ± 2	9 ± 3	NS
Aldosterone *pmol/liter*	231 ± 18	188 ± 23	0.05
PRA *ng Ang I/liter/min*	0.17 ± 0.02	0.22 ± 0.07	NS

Abbreviations are: BMI, body mass index; MAP, mean arterial pressure; $U_{Na}V$, 24-hour urine sodium excretion; protein intake, calculated from 24-hour urine urea excretion; Hct, hematocrit; ACE, angiotensin converting enzyme; Ang II, angiotensin II; PRA, plasma renin activity.

measured by radioimmunoassay, using the Coat-A-Count system. Plasma 17β-estradiol was determined by radioimmunoassay using the DPC kit (Drug Products Corporation, Los Angeles, CA, USA).

Statistical analysis

Data were analyzed by gender and are presented as mean ± SEM. Between group baseline differences were determined using nonparametric methods (Wilcoxin Rank Sums). Within subject and between group examination of the responses to Ang II was by repeated measures analysis of variance and Bonferroni correction. Correlation coefficients were used to discern any relationships between 17β-estradiol plasma concentrations and the renal and humoral responses to Ang II infusion. Women were then divided into quartiles on the basis of 17β-estradiol plasma concentrations, and the quartiles with the highest (more than 300 pmol/liter) and the lowest (less than 100 pmol/liter) levels were compared again using Wilcoxin Rank Sums and repeated measures analysis of variance. All statistical analyses were performed using the statistical package SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

The baseline characteristics of the two groups are shown in Table 1. There were no significant differences in age, 24-hour urine sodium excretion ($U_{Na}V$), and serum ACE concentrations. Although there was a significant difference in urea excretion (425 ± 18 mmol/day in men, 309 ± 18 in women), calculated protein intake corrected per kg of body weight did not differ significantly. There were significant differences in body mass index, Hct, and MAP. Ang II levels were equivalent in the men compared with the women. PRA was numerically, but not significantly, increased in women. Aldosterone levels were significantly lower in women compared with men.

Renal and peripheral responses to angiotensin II

As can be seen from Table 2, at baseline, women exhibited a strikingly lower arterial pressure than did men, but no significant differences were detectable in ERPF, RBF, or FF. GFR was numerically but not significantly increased in women compared with men. In

Table 2. Responses to angiotensin II by gender

Parameter	Baseline	0.5 ng	1.5 ng	2.5 ng	Recovery
Responses to Ang II in men					
MAP *mm Hg*	92 ± 1	93 ± 1	96 ± 2[a]	99 ± 2[a]	90 ± 2
GFR *ml/min/1.73 m²*	100 ± 3	98 ± 4	100 ± 5	103 ± 4	103 ± 2
ERPF *ml/min/1.73 m²*	588 ± 21	460 ± 23[a]	413 ± 16[a]	389 ± 20[a]	590 ± 38
RBF *ml/min/1.73 m²*	1020 ± 30	790 ± 32[a]	711 ± 29[a]	670 ± 28[a]	1007 ± 74
FF	0.17 ± 0.01	0.22 ± 0.01[a]	0.25 ± 0.01[a]	0.27 ± 0.01[a]	0.18 ± 0.01
RVR *mm Hg/liter/min*	92 ± 4	126 ± 5[a]	140 ± 8[a]	156 ± 5[a]	99 ± 6
$U_{Na}V$ *μmol/min*	391 ± 24	214 ± 16[a]	156 ± 12[a]	128 ± 10[a]	259 ± 26
Aldosterone *pmol/liter*	231 ± 18	334 ± 20[a]	442 ± 24[a]	518 ± 39[a]	357 ± 34
PRA *ng Ang I/liter/min*	0.17 ± 0.02	0.17 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Responses to Ang II in women					
MAP *mm Hg*	82 ± 2[b]	83 ± 2	89 ± 2[a]	90 ± 2[a]	80 ± 2
GFR *ml/min/1.73 m²*	107 ± 5[b]	95 ± 5[ac]	93 ± 5[ac]	95 ± 5	104 ± 5
ERPF *ml/min/1.73 m²*	586 ± 31	451 ± 25[a]	416 ± 32[a]	390 ± 26[a]	584 ± 36
RBF *ml/min/1.73 m²*	945 ± 50	713 ± 39[a]	656 ± 51[a]	609 ± 43[a]	910 ± 57
FF	0.19 ± 0.01	0.21 ± 0.01[c]	0.22 ± 0.01[ac]	0.24 ± 0.01[a]	0.18 ± 0.01
RVR *mm Hg/liter/min*	92 ± 7	124 ± 9[a]	154 ± 14[a]	163 ± 13	94 ± 6
$U_{Na}V$ *μmol/min*	335 ± 22	184 ± 15[a]	143 ± 12[a]	111 ± 10[a]	210 ± 16
Aldosterone *pmol/liter*	188 ± 23[b]	306 ± 36[a]	423 ± 44[a]	552 ± 54[a]	359 ± 36
PRA *ng Ang I/liter/min*	0.22 ± 0.07	0.21 ± 0.08	0.24 ± 0.1	0.18 ± 0.08	0.24 ± 0.08

Abbreviations are: GFR, glomerular filtration rate (corrected per 1.73 m²); ERPF, renal plasma flow (corrected per 1.73 m²); FF, filtration fraction; RVR, renal vascular resistance; $U_{Na}V$, urinary sodium excretion; PRA, plasma renin activity.

[a] $P < 0.05$ vs. baseline value

[b] $P < 0.05$ vs. baseline value in men

[c] $P < 0.05$ vs. response to Ang II in men

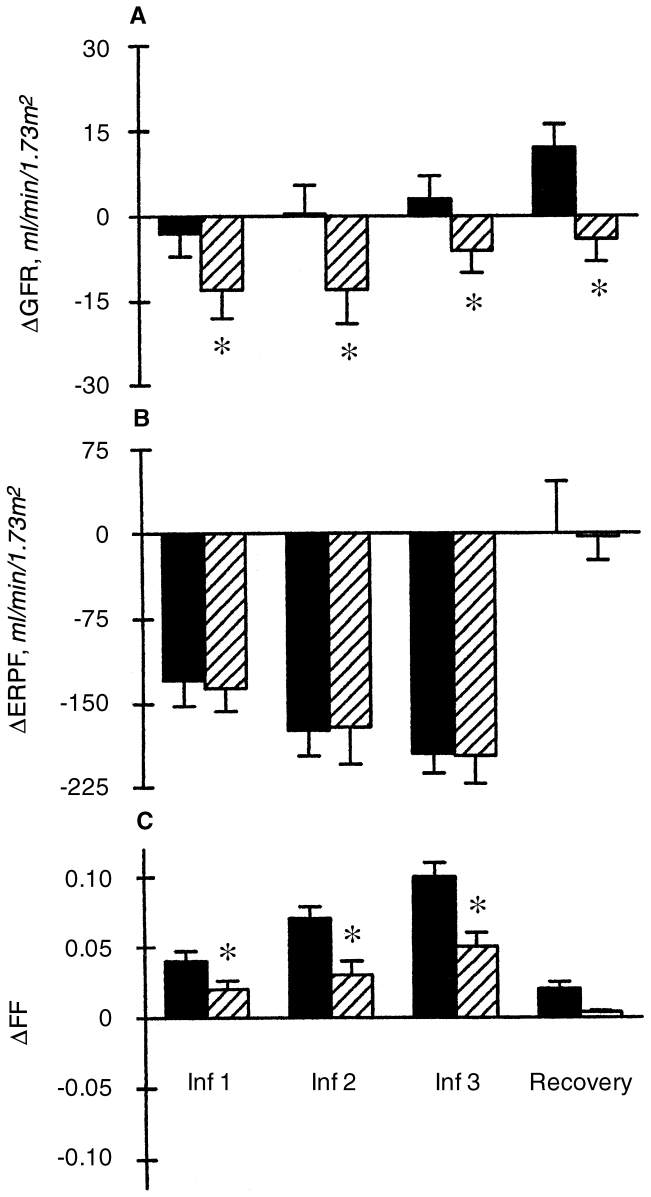

Fig. 1. Change in the glomerular filtration rate (*A*; ΔGFR), renal plasma flow (*B*; ΔERPF) and filtration fraction (*C*; ΔFF) in response to angiotensin (Ang) II infusion at 0.5 ng/kg/min (Inf 1), 1.5 ng/kg/min (Inf 2), 2.5 ng/kg/min (Inf 3), and at recovery in men (■) and women (▨). *$P < 0.05$ vs. response in men.

response to Ang II infusion, both groups exhibited equivalent small magnitude increases in arterial pressure that were statistically significant when compared with baseline. As can be seen from Figure 1, there was a significant decline in ERPF, which was equivalent in both groups. The response of GFR differed between groups, with men responding to Ang II infusion by maintaining GFR and women experiencing a significant decline in GFR that paralleled the fall in ERPF and that was especially evident at the smallest infusion rate of Ang II. The GFR did not return to baseline until the recovery period. The

Table 3. Baseline characteristics in groups segregated by estrogen levels

Parameter	High estrogen ($N = 8$)	Low estrogen ($N = 9$)	P value
Age *years*	28 ± 1	27 ± 1	NS
17β-estradiol *pmol/liter*	380 ± 26	71 ± 10	0.0001
BMI *kg/m²*	22 ± 1	23 ± 1	NS
$U_{Na}V$ *mmol/day*	206 ± 30	210 ± 24	NS
Protein intake *g/kg/day*	1.15 ± 0.07	1.15 ± 0.06	NS
Hct	0.384 ± 0.01	0.379 ± 0.007	NS
ACE *U/liter*	50 ± 4	48 ± 3	NS
Ang II *pg/ml*	12 ± 4	9 ± 2	NS
Aldosterone *pmol/liter*	252 ± 37	154 ± 20	0.04
PRA *ng Ang I/liter/min*	0.36 ± 0.13	0.12 ± 0.02	0.04
MAP *mm Hg*	84 ± 2	79 ± 2	0.05
GFR *ml/min/1.73 m²*	109 ± 10	109 ± 6	NS
ERPF *ml/min/1.73 m²*	546 ± 28	630 ± 49	0.05
RBF *ml/min/1.73 m²*	886 ± 43	1018 ± 80	0.04
FF	0.20 ± 0.02	0.17 ± 0.009	0.05
RVR *mm Hg/liter/min*	96 ± 6	83 ± 4	0.04
$U_{Na}V$ *μmol/min*	343 ± 43	346 ± 31	NS

Abbreviations are: BMI, body mass index; $U_{Na}V$, 24-hour urine sodium excretion; Hct, hematocrit; ACE, angiotensin converting enzyme; Ang II, angiotensin II; PRA, plasma renin activity; MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; $U_{Na}V$, urinary sodium excretion.

FF increased strikingly in men and showed a blunted increase in women. There were no differences in the response of $U_{Na}V$ between the two groups.

Impact of plasma 17β-estradiol concentrations

Eight women exhibited 17β-estradiol levels greater than 300 pmol/liter, and nine had plasma levels less than 100 pmol/liter. The baseline characteristics of these groups can be found in Table 3. Estrogen status appeared to influence aldosterone and PRA, MAP, ERPF, and FF. We were unable to detect any significant difference between the two groups in the renal hemodynamic response to Ang II infusion, although there may have been a trend toward augmented declines in ERPF in the low estrogen state ($P = 0.08$; Fig. 2).

Using correlation coefficients, however, the ERPF response to Ang II was significantly correlated with plasma 17β-estradiol concentrations. As plasma levels increased, the response to Ang II was blunted. No relationship was detectable between plasma levels and the GFR response. There was a significant relationship between the aldosterone response to Ang II infusion and plasma 17β-estradiol concentrations, with increased plasma levels blunting the response (Fig. 3).

DISCUSSION

In this study, we examined the baseline components of the RAS and renal and peripheral responses to Ang II in young healthy men and women. The rationale for this study was twofold. First, significant gender differ-

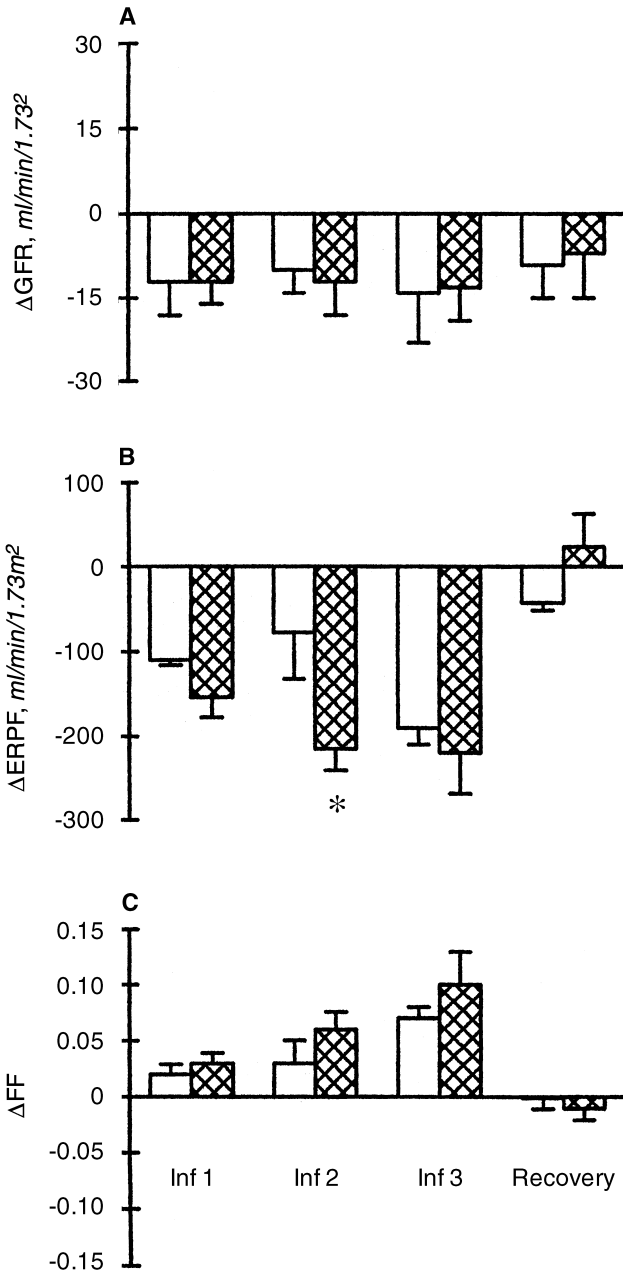

Fig. 2. Change in the glomerular filtration rate (*A*; ΔGFR), renal plasma flow (*B*; ΔERPF) and filtration fraction (*C*; ΔFF) in response to Ang II infusion at 0.5 ng/kg/min (Inf 1), 1.5 ng/kg/min (Inf 2), 2.5 ng/kg/min (Inf 3), and at recovery in women with plasma 17β-estradiol concentrations >300 pmol/liter (□) and women with plasma 17β-estradiol concentrations <100 pmol/liter (■). *$P < 0.05$ vs. response in women with high plasma 17β-estradiol concentrations.

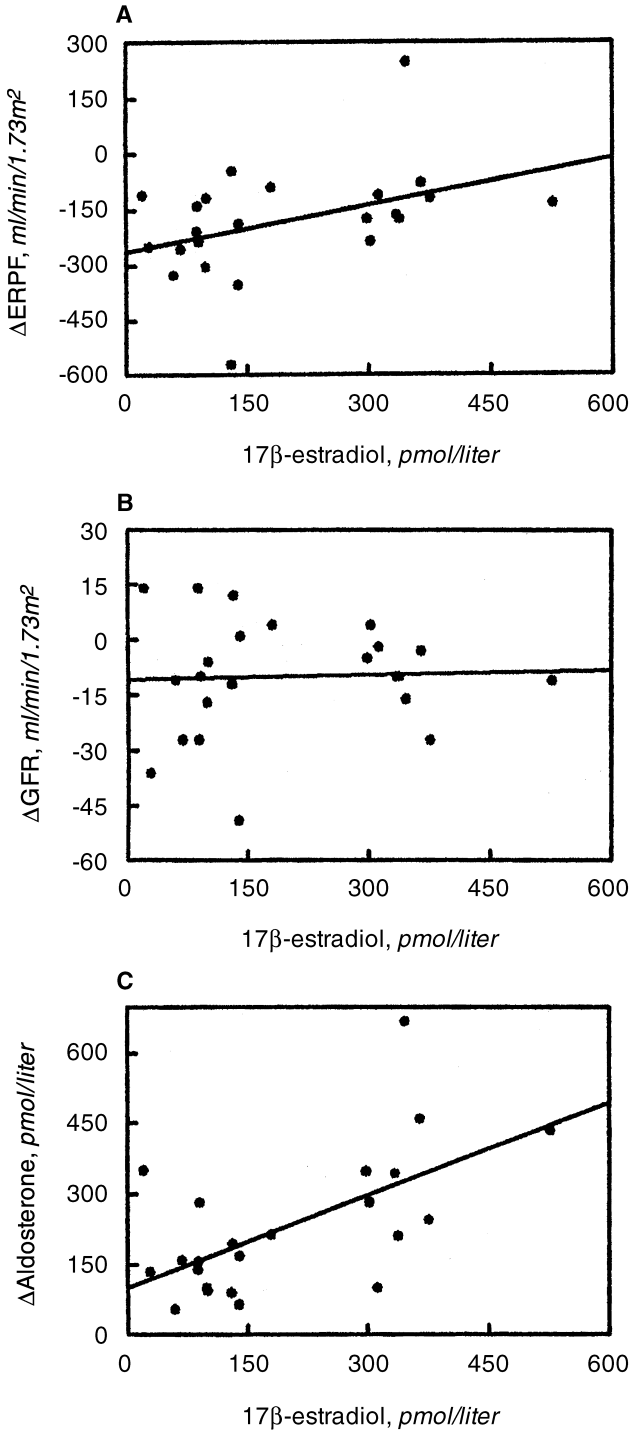

Fig. 3. Relationship between 17β-estradiol plasma concentrations and change in renal plasma flow (*A*; ΔERPF; $P = 0.03$, $r = 0.39$), glomerular filtration rate (*B*; ΔGFR; $P = 0.77$, $r = 0.05$), and aldosterone (*C*; ΔAldosterone; $P = 0.008$, $r = 0.49$), in response to angiotensin (Ang) II infusion (1.5 ng/kg/min).

ences exist in the progression of renal disease between men and women, and the RAS has been implicated in disease progression [9–12]. Second, some confusion exists in the literature regarding the activity of the RAS in women. We hypothesized that if gender-mediated differences in RAS function exist, at least some could be discerned by examining baseline components of the RAS and the hemodynamic response to Ang II.

The key findings in this study are as follows: (*a*) At

baseline, women exhibited significantly lower MAP, but no differences in renal and peripheral hemodynamic function. (*b*) No significant differences existed in baseline components of the RAS except for plasma aldosterone, which was reduced in women. (*c*) Although both groups experienced a similar decline in ERPF in response to Ang II infusion, the GFR response differed, with men exhibiting maintenance and an augmentation in FF and women demonstrating a decline in GFR in parallel with ERPF and a blunted increase in FF. (*d*) Women with plasma 17β-estradiol levels of more than 300 pmol/liter exhibited elevations in concentrations of aldosterone and PRA, decreased ERPF, and increased FF and RVR compared with women with plasma 17β-estradiol levels of less than 100 pmol/liter, suggesting that higher estrogen plasma concentrations may activate the intrarenal RAS. (*e*) There was a significant correlation between plasma 17β-estradiol levels and the ERPF response to Ang II, but no correlation between 17β-estradiol levels and the GFR response.

The baseline renal and peripheral hemodynamic results deserve comment. Few recent studies exist that compare renal hemodynamic function in men and women. In Munich-Wistar rats studied in the conscious, unanesthetized state, Munger and Baylis [16] noted that females exhibit lower whole-kidney GFR and RPF and higher RVR. Micropuncture studies performed under anesthesia revealed that single-nephron GFR and plasma flow were significantly lower, and afferent and efferent resistances were significantly higher in females, but that the transglomerular hydraulic pressure difference was similar in both sexes. When whole-kidney and single-nephron data for male and female rats were expressed per gram kidney weight, no differences remained significant. Ovariectomy elevated GFR and plasma flow to a point intermediate between intact females and males, such that significant differences were not detectable between them. Remuzzi et al used the Munich-Wistar rat model to demonstrate that arterial pressure and whole-kidney and single-nephron filtration rates were reduced in female compared with male rats, differences that persisted after correction of whole-kidney data for kidney weight [17]. Plasma flow differences did not reach statistical significance when normalized for kidney weight. Studies in humans are also conflicting. In 1969, Wesson concluded from a compilation of early studies performed in humans that GFR and RBF were lower in women than in men [18]. In a more recent study by Slack and Wilson, which retrospectively examined inulin and PAH clearance data from 141 healthy renal transplant donors, no gender differences were noted in GFR or RBF [19]. This study did not control for protein or sodium intake prior to the study, and there was a large age range. In this study, our results from this homogeneous age-matched group show that although there is a great deal of variation, the GFR and ERPF in women is at least equal to men after correcting for body surface area. Although plasma estrogen concentrations influenced RBF, RVR and FF, there appeared to be little impact on GFR.

Our results also indicate a gender difference in arterial pressure. Little information is available on arterial pressure in nonhypertensive groups segregated by gender. One such study by Wiinberg et al used 24-hour ambulatory blood pressure to determine sex-stratified normal values and found that systolic blood pressure was significantly higher in men than in women [20]. In this study, even in women with elevated estrogens where arterial pressures tended to be higher, MAP was decreased compared with men.

We also noted that aldosterone plasma concentrations were significantly lower in women when compared with men, even though sodium intake was equivalent. Estrogen status appeared to influence aldosterone, PRA, and possibly Ang II, with high levels augmenting these RAS components. It is known that estrogen stimulates transcription of the angiotensinogen gene in the liver [1]. Although the midcycle increase of 17β-estradiol in healthy women is thought not to be sufficient to increase plasma angiotensinogen [21], we have clearly shown an impact of estrogen levels on components of the RAS.

An important observation from our data was that a clear gender difference exists in the renal hemodynamic response to Ang II. It is known that the highest sensitivity to the vascular effects of Ang II is found in the kidney and that infusion of low doses that do not affect systemic blood pressure can induce a substantial constriction of renal vessels [22], resulting in a decline in RBF. GFR tends to be maintained because of relative changes in afferent and efferent resistance, with constriction proportionally greater in the efferent arteriole, resulting in an increased glomerular pressure [23, 24]. Our findings suggest that female sex predicts a blunted FF response to Ang II, possibly signifying lesser increases in intraglomerular pressure. It is interesting to note that striking and equivalent declines in ERPF and RBF and increases in RVR were noted in both men and women, making it difficult to explain our findings on the basis of gender differences in relative efferent and afferent resistance changes. Although our results might suggest an augmented afferent or blunted efferent arteriolar constrictive response to Ang II in women, the fact that the change in ERPF was equivalent in both groups makes this an unlikely assumption. It is known that Ang II reduces the ultrafiltration coefficient (Kf), another determinant of GFR, probably due to mesangial cell contraction. The possibility exists that the Kf response to Ang II is more pronounced in women, but because Kf cannot be measured in human subjects, we are unable to comment on this aspect of the renal microcirculation. Unfor-

tunately, the results from these experiments in intact kidney function cannot offer any further information that would clarify the gender differences in the response of the renal microcirculation to Ang II. It is important to note, however, whichever mechanism is operative in women, the Ang II-mediated augmentation of FF, and possibly intraglomerular pressure, appears to be blunted. There is compelling evidence from studies using pharmacological blockers of the RAS that the Ang II-mediated increase in intraglomerular pressure is one of the major mechanisms operative in the progression of renal disease [25–28] and that reductions in glomerular pressure can ameliorate glomerular injury. Therefore, this phenomenon may explain in part some of the protection from renal disease progression noted in women. The mitogenic effect of Ang II has also been implicated in disease progression [13]. In mesangial cells, Ang II increases ^{3}H-thymidine incorporation, induces hypertrophy, and stimulates collagen and actin synthesis [29]. Rosenberg and Hostetter studied *in vivo* intrarenal infusion of Ang II into rat kidney and noted an increased expression of early growth response genes [30]. Any sex-related differences in the growth-promoting properties of Ang II cannot be discerned from our present data, but it is tempting to speculate that gender differences may also exist in this aspect of the Ang II response.

Other investigators have noted a gender difference in the response of ERPF to Ang II infusion. In a recent study by Hopkins et al [31], gender was a significant covariate in predicting the renal plasma flow change to Ang II infusion, with women exhibiting a significantly greater decline in ERPF. We could discern no gender difference in this parameter in our subjects, although the mean age and prestudy preparation were similar. Hopkins et al did not stratify subjects by phase of the menstrual cycle, and infusion rates of Ang II differed from this study, possibly explaining the variations in results. Our data demonstrate that estrogen status influences baseline renal and peripheral vascular function, with increasing levels tending to elevate both MAP and FF. In addition, there was a significant influence of estrogen plasma concentrations on the ERPF response to Ang II, with increasing levels tending to blunt the response. Both of these observations are consistent with an estrogen-mediated activation of the intrarenal RAS, in that higher intrarenal Ang II levels may be expected to cause a refractoriness to exogenous Ang II.

Findings from our study provide few clues to the mechanisms underlying the gender differences in the GFR response to Ang II. Estrogen status appeared to have little impact, with both groups of women exhibiting a decline in GFR in parallel with ERPF. Evidence exists that suggests that estrogen may directly influence Ang II receptor number [32], but its impact on renal tissue is controversial, with some authors suggesting a modulating effect [33] and others not [32]. The possibility exists that our findings may be unrelated to the RAS, but may be indicative of gender differences in counter-regulatory responses such as renal nitric oxide release. Locally produced nitric oxide has been shown to act directly to blunt the renal vasoconstrictor actions of Ang II by providing an opposing vasodilatory stimulus [34]. Renal medullary endothelial nitric oxide synthase has been shown to be significantly augmented by estrogen replacement in oophorectomized female rats [35]. Exhaled nitric oxide has been shown to be elevated in women during the luteal phase of the menstrual cycle [36], although this is controversial [37]. Although activity of this counter-regulatory system was not evaluated in this study, the fact that estrogen concentrations were not predictive of the GFR response to Ang II makes it is less likely that estrogen-mediated renal nitric oxide release was responsible for our GFR findings. The mechanism, therefore, must remain speculative until further study.

In summary, this series of experiments suggests that gender predicts the renal hemodynamic response to Ang II in sodium-replete human subjects. In men, GFR was maintained and FF was augmented, whereas in women, GFR declined in parallel with ERPF, and the rise in FF was blunted. Whereas estrogen status appeared to influence both baseline components of the RAS and renal and peripheral hemodynamics, the GFR response to Ang II could not be explained by this mechanism. Although a complete understanding of sex differences in Ang II-mediated vasoconstriction of the renal microcirculation cannot be discerned from this study of whole-kidney function, we have provided a possible hypothesis to explain the impact of gender on the progression of renal disease. Further studies are required both to evaluate other aspects of RAS function in women and to determine the mechanisms underlying our findings.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin-converting enzyme; Ang II, angiotensin II; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; HCT, hematocrit; Kf, ultrafiltration coefficient; MAP, mean arterial pressure; PAH, paraaminohippurate; PRA, plasmin renin activity; RAS, renin angiotensin system; RBF, renal blood flow; RVR, renal vascular resistance; $U_{Na}V$, urinary sodium excretion.

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