

Sex Differences in Renal Responses to Hyperglycemia, L-Arginine, and L-NMMA in Humans With Uncomplicated Type 1 Diabetes

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OBJECTIVE—Women exhibit exaggerated renal hemodynamic responses to hyperglycemia, which may promote kidney disease progression. Our aim was to determine if increased nitric oxide generation by L-arginine infusion would reverse this deleterious response to clamped hyperglycemia in women with type 1 diabetes mellitus.

RESEARCH DESIGN AND METHODS—Renal function, blood pressure, and plasma cyclic guanosine monophosphate (cGMP) were measured in 20 men and 15 women with type 1 diabetes mellitus during clamped euglycemia and clamped hyperglycemia. Renal function, blood pressure, and plasma cGMP responses to graded infusions of intravenous L-arginine and N^G-monomethyl-L-arginine (L-NMMA) were measured during clamped hyperglycemia.

RESULTS—Subjects were young, normotensive, normoalbuminuric men and women who adhered to a high-sodium, moderate-protein diet. Plasma cGMP levels during euglycemia were generally lower in men compared with women, and systolic blood pressure (SBP) was higher in men. In response to hyperglycemia, cGMP levels did not change in men but did decline in women ($\Delta -1.10 \pm 0.20$ vs. $\Delta +0.05 \pm 0.20$ pmol/L, between-group effect of hyperglycemia on cGMP; $P = 0.012$). Hyperglycemia also was associated with an increase in SBP, glomerular filtration rate (GFR) (124 ± 6 to 143 ± 7 mL/min/1.73 m²; $P = 0.003$) and filtration fraction (FF) in women, but these parameters did not change in men. In response to L-arginine during hyperglycemia, the increase in cGMP was exaggerated in women versus men and GFR and FF decreased in women only, back toward baseline values observed during clamped euglycemia. L-NMMA infusion did not exaggerate changes in hemodynamic function in response to hyperglycemia.

CONCLUSIONS—L-Arginine reversed the renal hemodynamic effects of hyperglycemia in women, suggesting that nitric oxide is an important regulator of sex-dependent vascular responses to hyperglycemia in humans.

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Human studies have demonstrated a deleterious impact of diabetes mellitus (DM) on endothelial function in women compared with men (1). At the renal microvascular level, we have demonstrated that clamped hyperglycemia is associated with renal hyperfiltration responses in women but not in men (2). Similarly, in type 2 DM, hyperglycemia increases endothelial dysfunction in

women, a finding that was absent in men (3). It is not known, however, if this represents a failure to augment maximally stimulated nitric oxide (NO) bioactivity or an absolute reduction in NO generation by the DM metabolic milieu in women compared with men. An exaggerated effect of DM on endothelial dysfunction in women may, in part, be responsible for the “sex-equalizing” effect

that DM has on cardiovascular mortality and renal disease progression (3,4).

From the renal perspective, we have shown that women with uncomplicated type 1 DM exhibit an augmented renal pressor response to hyperglycemia compared with men, with decreases in renal blood flow (RBF) and effective renal plasma flow (ERPF) and an increase in filtration fraction (FF), suggesting increased efferent renal arteriolar constriction (2). However, the effect of augmenting NO generation with L-arginine, which is the physiological precursor of NO, and the role of NO bioactivity in the pathogenesis of sex-related hemodynamic differences in type 1 DM patients, are unknown.

Accordingly, our aim was to determine if L-arginine, which is the substrate for NO synthase, would reverse sex-dependent renal hemodynamic and blood pressure differences in the response to clamped hyperglycemia in patients with uncomplicated type 1 DM. We hypothesized that baseline plasma cyclic guanosine monophosphate (cGMP) would be elevated in women compared with men and that previously documented hyperglycemia-mediated hemodynamic effects in women would be related to greater cGMP suppression. Second, we hypothesized that NO synthase activation with L-arginine during hyperglycemia would correct the hemodynamic effects of hyperglycemia. Finally, we hypothesized that N^G-monomethyl-L-arginine (L-NMMA) infusion during hyperglycemia would exaggerate the effects of clamped hyperglycemia in women because of further suppression of NO bioactivity.

RESEARCH DESIGN AND METHODS

Twenty men and 15 women with type 1 DM participated in this study (Table 1). Inclusion criteria were: duration of type 1 DM ≥ 5 years; age 18 years or older; blood pressure $< 140/90$ mmHg; normoalbuminuria on 24-h urine collection; and no history of renal disease or macrovascular disease or regular medications other than insulin,

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Table 1—Baseline characteristics and biochemistry

Parameter	Men (n = 20)	Women (n = 15)
Baseline demographic parameters		
Age (years)	22 ± 1	23 ± 1
Diabetes duration (years)	17 ± 2	18 ± 1
BMI (kg/m ²)	24 ± 1	25 ± 1
Baseline biochemistry		
HbA _{1c} (%)	8.8 ± 0.3	8.7 ± 0.5
HbA _{1c} (mmol/mol)	74 ± 4	72 ± 6
Estrogen (pmol/L in women)	NA	179 ± 28
Sodium excretion (mmol/24 h)	220 ± 14	210 ± 15
Protein intake (g/kg/day)	1.02 ± 0.05	0.92 ± 0.06
Plasma cGMP (pmol/L)	4.20 ± 0.42	5.71 ± 0.67
Plasma insulin (pmol/L)		
Euglycemia	48 ± 13	33 ± 7
Hyperglycemia	46 ± 11	47 ± 23
Venous blood glucose (mmol/L)		
Euglycemia	4.7 ± 0.1	4.9 ± 0.2
Hyperglycemia	11.4 ± 0.2	11.6 ± 0.2
Baseline hemodynamic function		
Heart rate	67 ± 2	73 ± 3
SBP (mmHg)	116 ± 2*	109 ± 2
Diastolic blood pressure (mmHg)	65 ± 1	63 ± 2
ERPF (mL/min/1.73 m ²)	722 ± 43	700 ± 36
GFR (mL/min/1.73 m ²)	128 ± 9	124 ± 6
FF	0.19 ± 0.02	0.18 ± 0.01
RBF (mL/min/1.73 m ²)	1,160 ± 75	1,070 ± 66
RVR (mmHg/L/min)	0.075 ± 0.003	0.078 ± 0.005

Data are mean ± SD. A 24-h urine collection was used to evaluate dietary adherence through the determination of urinary sodium and urea excretion. Protein intake was calculated from the urea excretion using the formula protein = [(urine urea excretion × 0.18) + 14] / weight. RBF was derived using ERPF / (1 - hematocrit), and RVR was derived by dividing the mean arterial pressure by the RBF. NA, not available. *P = 0.004 for blood pressure in men in women at baseline.

including oral contraceptives. Female subjects were studied during the follicular phase of the menstrual cycle, determined by cycle day and measurement of 17β-estradiol levels. The Research Ethics Board at the University Health Network approved the protocol and all subjects gave informed consent.

Assessment of renal parameters

To maintain suppression of endogenous renin-angiotensin system (RAS) activity, subjects adhered to a high-sodium (>140 mmol/day) and moderate-protein (<1.5 g/kg per day) diet during the 7-day period before each experiment, as described previously (5). On 2 consecutive days, brachial artery blood pressure (Critikon, Tampa, FL) and renal hemodynamic parameters were obtained after a 6-h modified clamp during clamped euglycemia (day 1, 4–6 mmol/L) and hyperglycemia (day 2, 9–11 mmol/L) (5). On the euglycemic day, renal hemodynamic function [glomerular filtration rate (GFR)

and ERPF] were estimated using inulin and *p*-aminohippuric (PAH) clearance techniques. In brief, a 16-gauge peripheral venous cannula was inserted into the left antecubital vein for infusion of glucose and insulin, and a second cannula was inserted for blood sampling more distally. Blood glucose was measured every 5–10 min and the insulin infusion was adjusted to maintain euglycemia. After the desired level of ambient glycemia was maintained for 6 h, a third intravenous line was inserted into the right arm and was connected to a syringe infusion pump for administration of inulin and PAH. Plasma cGMP also was measured as a marker of NO production (5). After collecting blood for inulin and PAH 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-min

equilibration period, blood was collected for inulin, PAH, and hematocrit. Blood was further collected every 30 min for 60 min for inulin and PAH, and GFR and ERPF were estimated by steady-state infusion of inulin and PAH, respectively (5).

On the hyperglycemic day, after baseline blood pressure, renal and plasma cGMP measurements were obtained, L-arginine (Clinalpha, Laüfelfingen, Switzerland) was administered at incremental low and moderate doses (100 mg/kg over 30 min and then 250 mg/kg over 30 min) to probe renal hemodynamic effects without confounding systemic hypotensive effects associated with higher doses (6–12). Renal function and circulating cGMP measurements were assessed at the end of each L-arginine infusion period, as described in previous experiments (6).

To further assess the interaction between ambient glycemia and NO synthase inhibition, participants subsequently underwent a graded intravenous infusion of L-NMMA at 1 and 3 mg/kg during clamped hyperglycemia, as previously described (13). Renal function, blood pressure, and circulating cGMP measurements were assessed at the end of each L-NMMA infusion period. The L-NMMA infusion was administered ~1–2 weeks after the L-arginine phase of the experiment. All experiments were performed in the same warm (25°C), temperature-controlled room and in a dark, quiet environment after 10 min of rest in the supine position.

Sample collection and analytical methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was separated, placed on ice, and then stored at –70°C before the assay. Inulin and PAH were measured in serum by colorimetric assays using anthrone and *N*-(1-naphthyl)ethylenediamine, respectively (14–16). The mean of two baseline clearance periods represent GFR and ERPF, expressed per 1.73 m². The RBF was derived using ERPF / (1 - hematocrit), and renal vascular resistance (RVR) was derived by dividing the mean arterial pressure by the RBF. All renal hemodynamic measurements were adjusted for body surface area (14,16).

To assess NO formation (17), cGMP levels were measured at baseline and 30 min after the 100 mg/kg and 250 mg/kg L-arginine doses and at each phase of the L-NMMA infusion. The cGMP assay is

based on the competition between cGMP in the standards or samples and a cGMP–acetylcholinesterase conjugate (cGMP tracer) for a limited number of cGMP-specific rabbit antibody binding sites. The rabbit antibody–cGMP complex (either free or tracer) binds to the mouse monoclonal antibody IgG that is coated onto the well. The plate is washed to remove the unbound reagent and then Ellman reagent (acetylthiocholine and 5,5'-dithiobis-2-nitrobenzoic acid, a substrate to acetylcholinesterase) is added to the well. The product of this enzymatic reaction, 5-thio-2-nitrobenzoic acid, has a distinct yellow color and absorbs strongly at 412 nm. The intensity of the color is proportional to the amount of cGMP tracer bound to the well, which is inversely proportional to the amount of free cGMP present in the standards or sample (13).

Urinary albumin excretion rate was determined from an overnight urine collection using immunoturbidimetry. HbA_{1c} was measured by high-performance liquid chromatography. Plasma insulin levels were measured using standard techniques (18).

Statistical analysis

Descriptive statistics were used to compare baseline clinical and demographic characteristics. Our previous data have shown that the SD of the Δ GFR in response to hyperglycemia is ~ 3 mL/min/1.73 m² (2). To have an 80% power to detect a 10 mL/min/1.73 m² between-group difference in the GFR response hyperglycemia, for a two-sided test with $P = 0.05$ and with $Z_{\alpha} = 1.96$, the sample size should equal 14 in each group. Between-group comparisons in baseline parameters in women versus men were made using parametric methods (two independent sample t tests). Within-subject responses to hyperglycemia, L-arginine, and L-NMMA were determined by repeated measures ANOVA, and two-sided $P < 0.05$ was considered to be significant. All statistical analyses were performed using SPSS 19.0.

RESULTS

Baseline characteristics

Table 1 describes the clinical characteristics of the cohort. Subjects were young, normotensive, normoalbuminuric, type 1 DM patients who adhered to the controlled sodium and protein diet. Age, diabetes duration, BMI, and HbA_{1c} and

plasma insulin levels during clamped euglycemia and hyperglycemia were similar in the two groups (Table 1). In addition, mean venous blood glucose was similar in men and women at the beginning of the vascular studies during clamped euglycemia and hyperglycemia (Table 1), indicating that the desired level of ambient glycemia was achieved.

Plasma cGMP levels during clamped euglycemia were generally lower in men compared with women (Table 1; $P = 0.056$), and systolic blood pressure (SBP) was higher in men. Baseline renal hemodynamic function parameters were similar in the two groups (Table 1) and similar proportions of male and female participants exhibited baseline renal hyperfiltration (GFR > 135 mL/min/1.73 m², 35% of men vs. 33% of women).

Response to clamped hyperglycemia in men and women

In response to clamped hyperglycemia, cGMP levels did not change in men but did decline in women, and the between-group effect of hyperglycemia on cGMP was significant (Fig. 1). As expected from our previous work, induction of clamped hyperglycemia also was associated with significant increases in SBP, GFR, and FF in women but not in men (Tables 1 and 2). Between-group effects of hyperglycemia on GFR and FF also were significant (Tables 1 and 2).

Response to L-arginine during clamped hyperglycemia

In response to L-arginine infusion during hyperglycemia, the increase in cGMP was exaggerated in women versus men at the 250 mg/kg dose (Fig. 2). For renal hemodynamic parameters, in response to L-arginine during clamped hyperglycemia, GFR and FF decreased in women only,

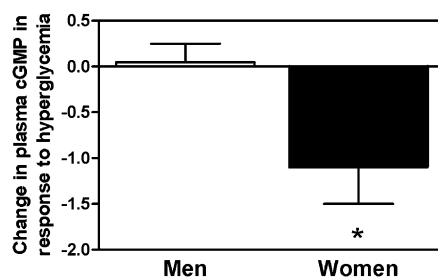


Figure 1—The effect of clamped hyperglycemia on cGMP in men and women with uncomplicated type 1 DM (mean \pm SD). * $P = 0.012$ for the effect of hyperglycemia on plasma cGMP in men compared with women.

back toward baseline values observed during clamped euglycemia, although GFR remained numerically higher at the end of the hyperglycemic L-arginine infusion compared with the euglycemic baseline ($P =$ not significant). As expected, SBP did not change in either group in response to L-arginine during clamped hyperglycemia.

Response to L-NMMA during clamped hyperglycemia

Effects on SBP, GFR, and FF at baseline on the hyperglycemic L-NMMA day were similar to effects at baseline during the hyperglycemic L-arginine day (Table 3). In response to L-NMMA, both groups exhibited significant declines in ERPF and RBF and increases in FF and RVR, and between-group effects on these parameters were not significant. GFR did not change in either group and the hyperfiltration (GFR) response to hyperglycemia was not affected by L-NMMA in women. At the end of the L-NMMA infusion, FF was higher in women compared with men ($P = 0.01$). cGMP declined in both groups after L-NMMA ($P \leq 0.004$) and nadir cGMP levels were similar in men versus women (3.67 ± 0.41 pmol/L vs. 3.89 ± 0.51 pmol/L; $P = 0.74$ for between-group effect).

CONCLUSIONS—The protective effect of female sex that is observed in non-DM renal disease is reduced or lost in the presence of DM (4,19–24). The physiologic basis for this lack of sex protection in DM is unknown. Previous work has suggested that induction of clamped hyperglycemia is preferentially associated with systemic hypertensive and renal hyperfiltration effects in women compared with men (2). The goal of this study was to determine if the administration of an L-arginine infusion to augment NO synthesis would reverse the exaggerated pressor response to clamped hyperglycemia in women with type 1 DM. Our major observations included that expected increases in GFR and FF in response to clamped hyperglycemia were associated with exaggerated declines in cGMP in women. L-NMMA infusion did not significantly augment the hypertensive and renal effects of clamped hyperglycemia in women compared with men. Also, in women, administration of L-arginine reduced GFR and FF but not SBP, back toward values observed during clamped euglycemic conditions. There was no impact on these measures in men.

Table 2—Hemodynamic responses to a graded infusion of L-arginine during clamped hyperglycemia in men and women with type 1 diabetes

Type of response	Baseline	L-Arginine 100 mg	L-Arginine 250 mg
Type 1 DM men (n = 20)			
Heart rate (beats per min)	68 ± 3	65 ± 3	71 ± 3
SBP (mmHg)	116 ± 2*	117 ± 2‡	118 ± 2‡
Diastolic blood pressure (mmHg)	64 ± 2	63 ± 2	65 ± 2
ERPF (mL/min/1.73 m ²)	736 ± 28	742 ± 30	786 ± 33†
GFR (mL/min/1.73 m ²)	132 ± 6	139 ± 5	136 ± 4
FF	0.18 ± 0.01*	0.19 ± 0.01	0.17 ± 0.01
RBF (mL/min/1.73 m ²)	1,120 ± 50*	1,316 ± 114†	1,273 ± 62†
RVR (mmHg/L/min)	0.077 ± 0.003*	0.069 ± 0.004	0.067 ± 0.003†
Type 1 DM women (n = 15)			
Heart rate (beats per min)	66 ± 2	67 ± 3	68 ± 3
SBP (mmHg)	113 ± 3§	114 ± 2	114 ± 3
Diastolic blood pressure (mmHg)	61 ± 2	62 ± 1	62 ± 1
Effective renal plasma flow (mL/min/1.73 m ²)	663 ± 38	703 ± 40	728 ± 39
Glomerular filtration rate (mL/min/1.73 m ²)	143 ± 7§¶	134 ± 5†	130 ± 5†
FF	0.22 ± 0.01§¶	0.19 ± 0.01†	0.18 ± 0.01†
RBF (mL/min/1.73 m ²)	1,035 ± 10	1,081 ± 62	1,119 ± 81
RVR (mmHg/L/min)	0.085 ± 0.007	0.077 ± 0.004	0.074 ± 0.004

Data are mean ± SD. *For between-group differences at baseline hyperglycemic hemodynamic parameters: $P = 0.004$ for SBP; $P = 0.009$ for FF; $P = 0.018$ for RBF; and $P = 0.004$ for RVR. †For within-group changes in response to L-arginine in women: $P = 0.002$ for Δ GFR and low-dose and high-dose L-arginine; $P = 0.024$ and $P = 0.004$ for Δ FF in women with low-dose and high-dose L-arginine. For the effect of L-arginine in men: $P = 0.02$ for the Δ ERPF at 250 mg/kg; $P = 0.037$ and $P = 0.015$ for Δ RBF with the low-dose and high-dose L-arginine, respectively; and $P = 0.008$ for Δ RVR. ‡For between-group differences in mean blood pressure values during the L-arginine infusion: $P = 0.014$ at 100 mg/kg L-arginine and $P = 0.015$ at 250 mg/kg L-arginine. §For within-group effects of clamped hyperglycemia: $P = 0.012$ for Δ SBP; $P = 0.003$ for Δ GFR and Δ FF. ¶For between-group differences in the effects of clamped hyperglycemia: $P = 0.009$ for GFR and FF.

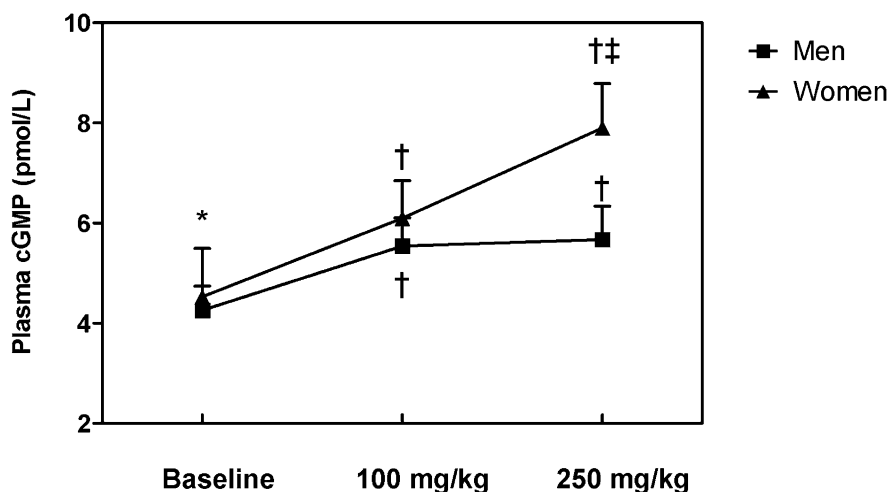


Figure 2—The effect of L-arginine on plasma cGMP during clamped hyperglycemia in men and women with uncomplicated type 1 DM (mean ± SD). * $P = 0.001$ for the cGMP level during clamped hyperglycemia compared with clamped euglycemia in women. †Within-group changes in cGMP compared with the baseline hyperglycemic value in women. $P = 0.001$ for 100 mg/kg dose L-arginine and $P = 0.0001$ at 250 mg/kg dose L-arginine. For men, $P = 0.0001$ and $P = 0.0001$ for cGMP responses at low and high doses, respectively. ‡ $P = 0.012$ for the response to L-arginine in women compared with men.

Experimental evidence has shown that estrogen alters endothelial reactivity by modulating NO production, leading to systemic vascular and renal protection in females without DM (25–27). Although chronic estrogen exposure upregulates NO, thereby providing vascular protection in females without DM, this interaction may be more complex in the context of DM (26,28–30).

The goal of the current study was to determine if NO synthase activation with L-arginine would reverse sex-dependent renal hemodynamic and blood pressure differences in the response to clamped hyperglycemia in type 1 DM patients. We observed that expected increases in GFR and FF in response to clamped hyperglycemia were associated with exaggerated declines in cGMP in women. This finding suggests that exaggerated hyperglycemia-induced renal effects observed in DM women are partly related to a decline in NO bioactivity (3). Consistent with our findings, it was recently reported that estrogen increases NO production by neuronal NO synthase under normal glucose conditions in animals with streptozotocin-induced DM, but that under high-glucose conditions this effect is attenuated (31). In humans with type 2 DM, NO synthase blockade with L-NMMA decreases ERPF to a greater extent in women compared with men, also suggesting high baseline renal NO bioactivity (25).

In a cohort of hyperfiltering (GFR ≥ 135 mL/min/1.73 m²) patients with type 1 DM, we recently demonstrated that NO inhibition with L-NMMA during clamped euglycemia leads to modest declines in GFR and more important suppression of ERPF and cGMP, consistent with dominant preglomerular vasoconstrictive effects observed in animals (13,32). In contrast, our first major observation in the current study was that clamped hyperglycemia alone led to significant increases in FF and GFR and a decline in cGMP with minimal ERPF effects in women, suggesting a dominant postglomerular decline in NO bioavailability. L-NMMA during clamped hyperglycemia in the current study did not exaggerate between-group changes in hemodynamic function or plasma cGMP. Furthermore, consistent with our previous work performed during clamped euglycemia (13), predominant declines in ERPF and RBF compared with effects on GFR suggest that L-NMMA infusion during clamped hyperglycemia also exerts a dominant preglomerular effect.

Table 3—Hemodynamic responses to a graded infusion of L-NMMA during clamped hyperglycemia in men and women with type 1 diabetes

Type of response	Baseline	L-NMMA 1 mg/kg	L-Arginine 3 mg/kg
Type 1 DM men (n = 20)			
Heart rate	62 ± 1	60 ± 1	58 ± 1
SBP (mmHg)	116 ± 2	120 ± 3*	127 ± 3*
Diastolic blood pressure (mmHg)	63 ± 2	67 ± 2	75 ± 2
ERPF (mL/min/1.73 m ²)	704 ± 28	651 ± 19†	561 ± 21†
GFR (mL/min/1.73 m ²)	134 ± 5	132 ± 5	129 ± 5
FF	0.19 ± 0.01	0.20 ± 0.01‡	0.23 ± 0.01‡
RBF (mL/min/1.73 m ²)	1,157 ± 57	1,029 ± 43§	1,007 ± 64§
RVR (mmHg/L/min)	0.075 ± 0.004	0.085 ± 0.005	0.106 ± 0.007
Type 1 DM women (n = 15)			
Heart rate (beats per min)	68 ± 2	67 ± 3	67 ± 2
SBP (mmHg)	113 ± 3	110 ± 4	120 ± 4
Diastolic blood pressure (mmHg)	64 ± 1	63 ± 2	77 ± 2
ERPF (mL/min/1.73 m ²)	721 ± 43	642 ± 40†	544 ± 33†
GFR (mL/min/1.73 m ²)	150 ± 9*	146 ± 6	150 ± 12
FF	0.21 ± 0.01*	0.23 ± 0.01‡	0.27 ± 0.01‡¶
RBF (mL/min/1.73 m ²)	1,123 ± 87	974 ± 64§	861 ± 21§
RVR (mmHg/L/min)	0.081 ± 0.004	0.088 ± 0.005	0.111 ± 0.007

Data are mean ± SD. *P = 0.01 for between-group differences in GFR and FF at baseline during the hyperglycemic L-NMMA infusion; P < 0.0001 for SBP level in men vs. women at 1 mg/kg and 3 mg/kg L-NMMA. †In men: P = 0.015 and P < 0.0001 for within-group changes in ERPF at low and high doses of L-NMMA, respectively; for women: P < 0.0001 and P < 0.0001 for within-group changes in ERPF at low and high doses of L-NMMA, respectively. ‡In men: P = 0.031 and P < 0.0001 for within-group changes in FF at low and high doses of L-NMMA, respectively; for women: P < 0.0001 and P < 0.0001 for within-group changes in FF at low and high doses of L-NMMA, respectively. §In men: P = 0.013 and P < 0.0001 for within-group changes in RBF at low and high doses of L-NMMA, respectively; for women: P < 0.0001 and P < 0.0001 for within-group changes in RBF at low and high doses of L-NMMA, respectively. ¶P = 0.01 for the peak FF in women vs. men. ||In men, P = 0.001 and P < 0.0001 for within-group changes in RVR at low and high doses of L-NMMA, respectively; for women: P < 0.0001 and P < 0.0001 for within-group changes in RVR at low and high doses of L-NMMA, respectively.

Our data therefore suggest that women exhibit enhanced NO bioactivity in the postglomerular circulation at baseline during clamped euglycemia; clamped hyperglycemia alone quenches postglomerular NO, leading to an increase in GFR and FF with lesser effects on ERPF, and these effects are reversible with infusion of L-arginine. When L-NMMA was administered during clamped hyperglycemia, our findings suggested that NO bioactivity was already maximally suppressed by hyperglycemia in the postglomerular circulation and the decline in ERPF ($\Delta -168 \pm 22$ in men vs. $\Delta -187 \pm 28$ mL/min/1.73 m² in women) and increase in RVR may have instead reflected preglomerular vasoconstriction. Unfortunately, segmental resistances cannot be assessed in human studies of whole organ function and should be further clarified in animal models.

Our second major observation was that L-arginine reduced GFR and FF in women only, back toward values observed during clamped euglycemia. These changes were associated with an increase in cGMP, suggesting that hyperglycemia-induced suppression of NO synthase bioactivity can be reversed in women by the provision of substrate. The mechanism for the decline in GRF and FF cannot be determined in this human physiology study but, as we have suggested in previous work, the observed changes are consistent with postglomerular vasodilatory effects (2). Alternatively, effects on tubuloglomerular feedback may be involved.

In contrast with renal hemodynamic function data suggesting increased NO bioactivity, NO bioactivity is blunted in systemic vascular smooth muscle cells obtained from female rats with DM. This has been attributed to activation of the

estrogen receptor β -subtype, which inhibits inducible NO synthase (33). The resulting decline in NO bioactivity may, in part, explain the blunted systemic arterial response to NO synthase blockade with L-NAME in female rats with DM compared with male rats with DM (28). Reduced systemic NO bioactivity also may explain the greater suppression of systemic endothelial function in women compared with men with type 1 DM (34). In contrast, plasma cGMP levels were generally higher at baseline during clamped euglycemia in women in our cohort, in conjunction with significantly lower blood pressure, suggesting high-baseline, euglycemic NO bioactivity. These lower baseline blood pressure values in women are consistent with studies of healthy individuals and type 2 DM patients (25,35,36). During clamped hyperglycemia, however, L-arginine increased cGMP but did not influence blood pressure. Our findings therefore show that although baseline euglycemic NO bioactivity was higher in women, a small reduction in NO bioactivity with clamped hyperglycemia was associated with exaggerated hypertensive responses that were not influenced by L-arginine. Whereas other systemic neurohormonal factors such as the RAS may be involved, our results suggest a greater dependence on NO bioactivity for the maintenance of "normal" systemic vascular function in women with uncomplicated type 1 DM.

This study raises some important issues that require further study. First, previous studies have highlighted an important interaction between hyperglycemia, macrovascular disease, and the development of kidney disease. Future work should determine if sex differences in renal risk are related to differences in endothelial function or arterial stiffness. Second, the mechanisms responsible for the interaction between NO and hyperglycemia remain unclear and may relate to either feedback interactions between NO and vasoconstrictor pathways, such as the RAS, or effects on tubular function. Novel agents such as sodium glucose cotransport-2 inhibitors and adenosine antagonists may be used to elucidate the role of NO as a modulator of sex-dependent effects in humans. Finally, in light of the intriguing physiological differences that hyperglycemia induces in men versus women, clinical studies should consider the influence of sex as a risk factor for the development of diabetic nephropathy.

Our study does have limitations. We attempted to minimize the effect of the small sample size in our study by using homogeneous study groups and by using a careful prestudy preparation phase with a focus on known factors that influence neurohormonal activation. In addition, we decreased variability by using a study design that allowed subjects to act as their own controls. Nevertheless, the small sample size may have limited our ability to detect between-group differences in certain parameters such as baseline plasma cGMP levels. Next, because NO synthase isoforms are widely expressed in different anatomical compartments, we were unable to determine the specific origin of cGMP in this intact human study (37). In addition, tubuloglomerular feedback likely is an important factor leading to renal hemodynamic function changes in DM and may have been influenced by L-arginine. Because of the existing complexity of this set of experiments, tubular factors were not assessed and should be examined in future protocols. Finally, effects on segmental resistances by clamped hyperglycemia or L-NMMA may have either withdrawal of NO bioactivity or unopposed action of vasoconstrictive hormones such as angiotensin II. The influence of NO-RAS interactions on vascular function also need to be further elucidated.

In conclusion, our results suggest that NO bioactivity may contribute to the exaggerated renal effects of hyperglycemia in women with type 1 DM. L-arginine reverses the renal hyperfiltration effect of hyperglycemia in women, suggesting that NO is an important contributor to the regulation of normal vascular function in women with type 1 DM.

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D.Z.I.C. researched data and wrote the manuscript. J.W.S. and E.B.S. co-wrote the manuscript. D.Z.I.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for

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