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 N⁶-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 (Δ−1.10 ± 0.20 vs. Δ+0.05 ± 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.

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 N⁶-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,
Baseline demographic parameters

| Parameter          | Men (n = 20) | Women (n = 15) |
|--------------------|--------------|----------------|
| Age (years)        | 22 ± 1       | 23 ± 1         |
| Diabetes duration (years) | 17 ± 2    | 18 ± 1         |
| BMI (kg/m²)        | 24 ± 1       | 25 ± 1         |

Baseline biochemistry

| Parameter          | Men (n = 20) | Women (n = 15) |
|--------------------|--------------|----------------|
| HbA1c (%)          | 8.8 ± 0.3    | 8.7 ± 0.5      |
| Hba1c (mmol/mol)   | 74 ± 4       | 72 ± 6         |
| Estrogen (pmol/L in women) | NA       | 179 ± 28      |
| Sodium excretion (mmol/24 h) | 220 ± 14 | 210 ± 13      |
| 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) | 6 ± 1     | 6 ± 2          |

Baseline hemodynamic function

| Parameter          | Men (n = 20) | Women (n = 15) |
|--------------------|--------------|----------------|
| Venous blood glucose (mmol/L) | 4.7 ± 0.1   | 4.9 ± 0.2      |
| Hyperglycemia      | 11.4 ± 0.2   | 11.6 ± 0.2     |

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 acid (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, Laufelfingen, 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, blood pressure, 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 re-
action, 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.
HbA1c was measured by high-performance
liquid chromatography. Plasma insulin levels were measured using
standard techniques (18).

Statistical analysis
Descriptive statistics were used to com-
pare baseline clinical and demographic
characteristics. Our previous data have shown
that the SD of the ΔGFR in re-
sponse 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 to hyper-
glycemia, for a two-sided test with P =
0.05, and with Zα = 1.96, the sample size
should equal 14 in each group. Between-
group comparisons in baseline para-
eters in women versus men were made
using parametric methods (two independ-
ent sample t tests). Within-subject re-
sponses to hyperglycemia, L-arginine,
and L-NMMA were determined by re-
peated 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 characteris-
tics of the cohort. Subjects were young,
normotensive, normoalbuminuric, type 1
DM patients who adhered to the con-
trolled sodium and protein diet. Age, di-
abetes duration, BMI, and HbA1c and
plasma insulin levels during clamped
euglycemia and hyperglycemia were simi-
lar in the two groups (Table 1). In addi-
tion, mean venous blood glucose was
similar in men and women at the be-
ginning 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 hy-
perfiltration (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 hyper-
glycemia on GFR and FF also were signif-
ificant (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,
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 ex-
hibited significant declines in ERPF and
RBF and increases in FF and RVR, and
between-group effects on these parame-
ters were not significant. GFR did not
change in either group and the hyperfil-
tration (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 ≤ 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 ef-
fect of female sex that is observed in non-
DM renal disease is reduced or lost in the
presence of DM (4,19–24). The physio-
logic basis for this lack of sex protection
in DM is unknown. Previous work has
suggested that induction of clamped hy-
perglycemia is preferentially associated
with systemic hypertensive and renal hy-
perfiltration 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 syn-
thesis would reverse the exaggerated pres-
sor response to clamped hyperglycemia in
women with type 1 DM. Our major obser-
vations included that expected increases
in GFR and FF in response to clamped
hyperglycemia were associated with exag-
gerated declines in cGMP in women.
L-NMMA infusion did not significantly
augment the hypertensive and renal ef-
fects of clamped hyperglycemia in women
compared with men. Also, in women, ad-
ministration of L-arginine reduced GFR
and FF but not SBP, back toward values
observed during clamped euglycemic con-
ditions. 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 m²)   | 736 ± 28 | 742 ± 30          | 786 ± 33†         |
| GFR (mL/min/1.73 m²)    | 132 ± 6  | 139 ± 5           | 136 ± 4           |
| FF                      | 0.18 ± 0.01* | 0.19 ± 0.01       | 0.17 ± 0.01       |
| RBF (mL/min/1.73 m²)    | 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 m²) | 663 ± 38 | 703 ± 40          | 728 ± 39          |
| Glomerular filtration rate (mL/min/1.73 m²) | 143 ± 7§  | 134 ± 5‡          | 130 ± 5‡          |
| FF                      | 0.22 ± 0.01§ | 0.19 ± 0.01‡      | 0.18 ± 0.01†      |
| RBF (mL/min/1.73 m²)    | 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 ΔGFR and low-dose and high-dose L-arginine; P = 0.024 and P = 0.004 for ΔFF in women with low-dose and high-dose L-arginine. For the effect of L-arginine in men: P = 0.02 for the ΔERPF at 250 mg/kg; P = 0.037 and P = 0.015 for ΔRBF with the low-dose and high-dose L-arginine, respectively, and P = 0.008 for ΔRVR. ‡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 ΔSBP, P = 0.003 for ΔGFR and ΔFF. ¶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 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.
Type 1 diabetes, nitric oxide, and sex

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 (Δ − 168 ± 22 in men vs. Δ − 187 ± 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 GFR 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 α-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 (25,33,36). 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.

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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 |
|------------------|----------|----------------|-------------------|
| Heart rate       | 62 ± 1   | 60 ± 1         | 58 ± 1            |
| SBP (mmHg)       | 116 ± 2  | 120 ± 3*       | 127 ± 3*          |
| Diastolic blood pressure (mmHg) | 63 ± 2 | 67 ± 2 | 72 ± 2 |
| ERPF (mL/min/1.73 m²) | 704 ± 28 | 651 ± 19† | 561 ± 21† |
| GFR (mL/min/1.73 m²) | 134 ± 5 | 132 ± 5 | 129 ± 5 |
| FF               | 0.19 ± 0.01 | 0.20 ± 0.01‡ | 0.23 ± 0.01‡ |
| RBF (mL/min/1.73 m²) | 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 men (n = 20)

| 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 | 72 ± 2 |
| ERPF (mL/min/1.73 m²)      | 721 ± 43 | 642 ± 40† | 544 ± 33† |
| GFR (mL/min/1.73 m²)       | 150 ± 9* | 146 ± 6 | 150 ± 12 |
| FF                         | 0.21 ± 0.01* | 0.23 ± 0.01‡ | 0.27 ± 0.01‡ |
| RBF (mL/min/1.73 m²)       | 1,123 ± 87 | 974 ± 64‡ | 861 ± 21§ |
| RVR (mmHg/L/min)           | 0.081 ± 0.004 | 0.088 ± 0.005† | 0.111 ± 0.007† |

Type 1 DM women (n = 15)

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 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, tubuloglu- merular 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 vasoconstric- tive 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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