The Effect of Diabetes and Sex on Nitric Oxide–Mediated Cardiovascular Dynamics

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Diabetes is associated with impaired cardiovascular responses that are especially prominent in females. Since nitric oxide (NO)–mediated effects on cardiovascular dynamics are altered in diabetes, we evaluated the effect of L-NAME, a nitric oxide synthase (NOS) antagonist, on mean arterial pressure (MAP), heart rate (HR), and selective vascular flows in both male and female normal and diabetic rats as an index of NO activity. Rats were made diabetic using streptozotocin and maintained for 5–6 weeks. Following anesthesia with urethane/α-chloralose, the femoral artery and vein were cannulated for recording and sampling, and flow probes were placed on the iliac, renal, and superior mesenteric arteries. A bolus infusion of L-NAME (10mg/kg) resulted in a rapid +52% and +68% increase in MAP in normal female and male rats, respectively. However, diabetic females' and males' responses were significantly lower (44% and 45%, respectively) when compared with their normal counterparts. The decreased HR in response to the peak pressor effect of L-NAME was more prominent in normal females compared with normal males (–14% vs 2%). The results in diabetic females and males were equivalent (–6% vs –9%, respectively). L-NAME decreased the conductance (flow/MAP) an average of 65% in all three vascular beds in normal female rats. In diabetic females, the iliac and superior mesenteric responses to L-NAME were less, and the renal conductance was contrastingly increased 23%. The response to L-NAME was comparable (–62%) in the renal and superior mesenteric and less (–40%) in the iliacs of normal versus diabetic males. We concluded that diabetes is associated with a decreased pressor response to NOS inhibition. And the impaired constriction response of the renal vessels noted in female diabetic rats may provide a basis for the increased renal pathology observed in diabetic humans.

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Vascular disease is the major complication of diabetes mellitus (1). It can result in modifications in peripheral blood flow at the micro- and macrovasculature levels. However, the exact mechanisms behind these complications remain unclear.

Nitric oxide (NO) is a potent vasodilator, and its production by the endothelium plays an important role in the maintenance of blood pressure and the control of vascular resting tone of different vascular beds (2, 3). The synthesis of NO from L-arginine can be antagonized by a nitric oxide synthase (NOS) antagonist such as N^G-mono-methyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME). These inhibitors have been used to characterize the functional role of NO in the regulation of blood pressure, control of peripheral vascular tone, and regulation of sympathetic nerve discharge (4, 5).

Systemic infusion of L-NAME or L-NMMA results in a dose-dependent pressor effect associated with regional vascular constriction. This pressor effect is consistently associated with bradycardia. These observations reiterate the involvement of NO in the control of regional vascular resistance and blood pressure (6–8).

Reports in the literature indicate that there is a decrease in NO formation in diabetic vessels leading to an impairment or dysfunction of the vascular endothelium (2, 6, 9, 10). In addition to the decreased NO production associated with diabetes, endothelial NO-mediated vasodilation may also be impaired in diabetes (6). It has been suggested that this endothelial dysfunction or reduced response to endothelial NO in diabetes contributes to the development of diabetic vascular diseases (2, 6). Additionally, diabetes-impaired dilatory responses also seem to be influenced by sex (11). We have demonstrated that the vasodilatory response of diabetic female rats to an NO donor is significantly attenuated in the iliac and superior mesenteric vasculature (11). This is consistent with previously observed sex differences (12, 13). Also, more women develop diabetes complications when compared with men and are at a greater disadvantage than men once they develop diabetes (14–17).

Consequently, we hypothesized that in addition to endothelial dysfunction, female rats have more impaired cardiovascular responses when compared with males following

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Table I. Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP), and Heart Rate (HR) in L-NAME–Treated Normal and Diabetic Rats

Group	Body weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal female	254 ± 5 (9)	90 ± 6 (9)	79 ± 4 (9)	469 ± 23 (9)
Diabetic female	213 ± 11[a] (8)	382 ± 48[a] (8)	72 ± 4 (8)	341 ± 9[a] (8)
Normal male	286 ± 3[b] (8)	78 ± 9 (8)	72 ± 2 (8)	419 ± 27 (8)
Diabetic male	230 ± 13[a] (6)	412 ± 32[a] (6)	77 ± 6 (6)	393 ± 20 (6)

Note. The values represent the mean ± SEM. Number in parenthesis = *n*.
[a] $P < 0.05$ vs normals.
[b] $P < 0.05$ vs. normal female, ANOVA.

the induction of diabetes. To examine this hypothesis, we evaluated and compared the effects of the L-NAME-mediated inhibition of NO production on mean arterial pressure, heart rate, and regional blood flow (iliac, renal, and superior mesenteric) in female and male normal and diabetic rats.

Materials and Methods

Design. Normal and diabetic female and male (n = 15, 14, 13, and 10, respectively), Wistar rats (BW: 250–275 g) were used in our experimental procedures. Animals were kept in a controlled environment with a 12:12-hr light:dark cycle and a 23°C room temperature with free access to water and food.

Streptozotocin Injections and Blood Glucose Determination. Diabetes was induced in normal rats by a single intravenous tail vein injection of streptozotocin (STZ) (50 mg/kg dissolved in sodium citrate, 0.1 m*M*, pH 4.5). Five days later the induction of diabetes was confirmed by measurements of blood glucose collected from the orbital sinus using a glucose analyzer (Yellow Springs Instrument Co., Yellow Spring, OH). The diabetic state was verified by a nonfasting blood glucose >300 mg/dl. Animals were used 4–6 weeks post-STZ injection without insulin supplements.

Surgical Procedures and Experimental Measurements. On the day of the study and following a 24-hr fast, normal or diabetic rats were anesthetized with urethane (0.5 mg/kg) and α-chloralose (70 mg/kg) and placed on a heating pad to maintain their body temperature. A tracheotomy was performed to diminish respiratory obstructions, and catheters with heparinized saline were placed into the femoral artery and veins. The venous catheter was used for blood sample collection and infusions. The femoral artery cannula was used for cardiovascular recording.

Pulsed-Doppler blood flow transducers (Crystal Biotech Co., Hopkinton, MA) were placed around the iliac, renal, and superior mesenteric arteries. The arterial catheter was connected to a pressure transducer (Argon Co., Athens, TX), and the flow probes connected to a pulsed-Doppler flowmeter (Baylor Electronics: Instrumentation Development Laboratories, Houston, TX). The Biowindows Software Program and a micro-5000 signal processing system (Modular Instruments, Malvern, PA) were used to monitor cardiovascular responses. The Biowindows Program was used to record all cardiovascular parameters: mean arterial pressure (MAP), heart rate (HR), and blood flow (Hz Ds units).

Experimental Protocol. Normal and diabetic female and male rats were given a single bolus injection of saline or N-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg). This dose was demonstrated to be maximally stimulatory in previous studies (7, 8, 18). Recording for the evaluation of the response was begun 5 min after establishing a stable baseline. Mean arterial pressure (MAP), heart rate

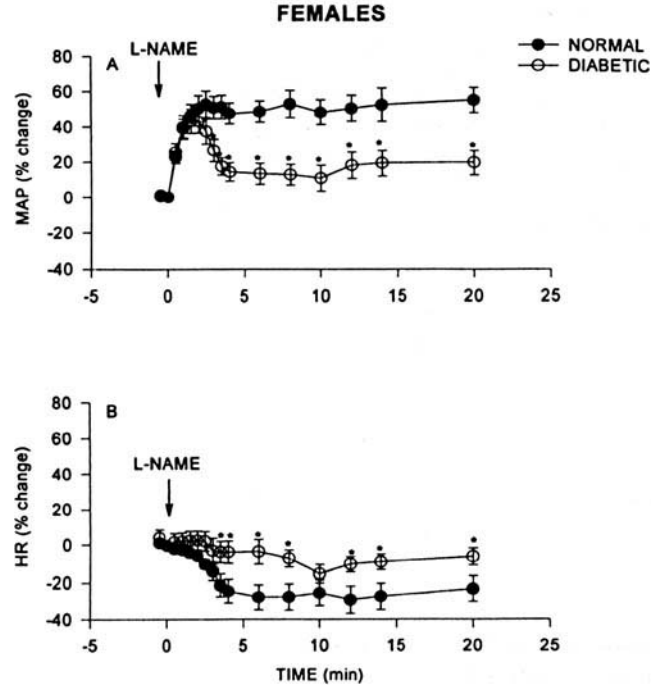

Figure 1. The effect of L-NAME (10 mg/kg) on (A) mean arterial pressure (MAP) and (B) heart rate (HR) in normal (n = 9) and diabetic (n = 8) female rats. *$P < 0.05$ vs normal female rats. Two-way ANOVA group effect normal versus diabetic female MAP, $P = 0.0001$ and normal versus diabetic female HR, $P = 0.0001$.

Table II. Mean Arterial Pressure (MAP), Heart Rate (HR), Basal, Absolute, Percentage Change Plateau, and Peak Responses in L-NAME (10 mg/kg) in Normal and Diabetic Rats

Group	Basal MAP (mm Hg)	Peak MAP (mm Hg)	MAP Plateau (mm Hg)	MAP Plateau (% change)	Basal HR (beats/min)	Post L-NAME HR (beats/min)
Normal female	79 ± 4 (9)	120 ± 3 (9)	117 ± 1 (9)	49 ± 1 (9)	469 ± 23 (9)	405 ± 18 (9)
Diabetic female	72 ± 4 (8)	104 ± 3[a] (8)	81 ± 1[a] (8)	15 ± 2[a] (8)	341 ± 9[a] (8)	321 ± 20[a] (8)
Normal male	72 ± 2 (8)	121 ± 4 (8)	113 ± 1[b] (8)	58 ± 2[b] (8)	419 ± 27 (8)	429 ± 24 (8)
Diabetic male	77 ± 6 (6)	112 ± 2[a] (6)	99 ± 1[a,c] (6)	30 ± 1[a,c] (6)	393 ± 20 (6)	357 ± 19[a] (6)

Note. The values represent the mean ± SEM. Number in parenthesis = *n*.
[a] $P < 0.05$ vs normal counterpart.
[b] $P < 0.05$ vs normal female.
[c] $P < 0.0001$ vs diabetic female, ANOVA.

(HR), and blood flow (iliac, renal, and superior mesenteric) were monitored continuously.

Blood samples, 0.2 ml with saline replacement, were collected prior to the study. The blood sample was centrifuged and used for glucose analysis using a glucose analyzer.

Calculations and Data Analysis. The data presented are averages of 30-sec intervals for the reported periods following control saline or L-NAME treatment. All data are expressed as means ± SEM. Iliac, renal, and mesenteric vascular conductances were calculated by dividing mean iliac, renal, and mesenteric Pulsed-Doppler blood flow (BF) by MAP. Baroreflex sensitivity index was calculated as HR divided by MAP (HR/MAP). The MAP, HR, and conductance values of figures were expressed as percentage change from basal. One-way ANOVAs were used to determine the differences between groups. Differences observed were further evaluated using Newman-Keuls *posthoc* analysis. A two-way ANOVA with repeated measures was used to determine differences in response over time and were further evaluated using *posthoc* analysis. Significance was set at $P < 0.05$.

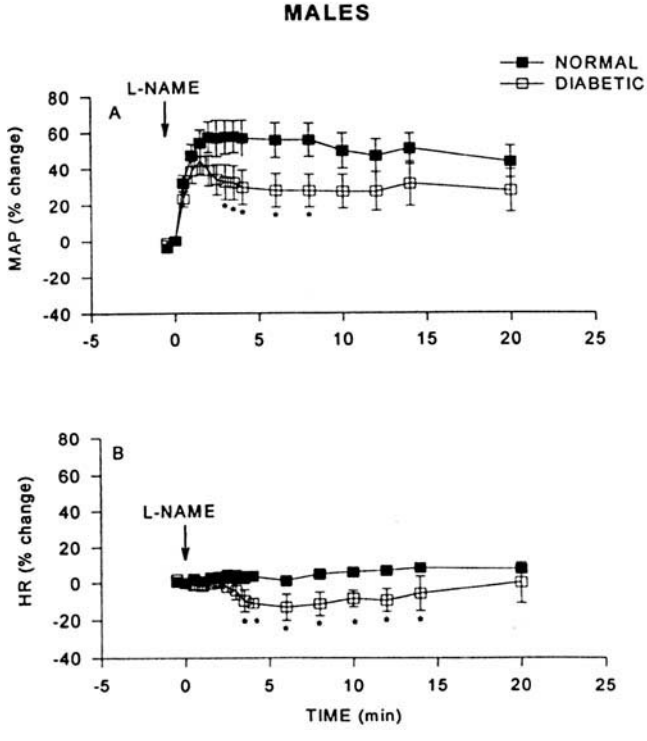

Figure 2. The effect of L-NAME (10 mg/kg) on (A) mean arterial pressure (MAP) and (B) heart rate (HR) in normal ($n = 8$) and diabetic ($n = 6$) male rats. *$P < 0.05$ vs normal male rats. Two-way ANOVA group effect normal versus diabetic male MAP, $P = 0.0001$; normal versus diabetic male HR, $P = 0.0001$.

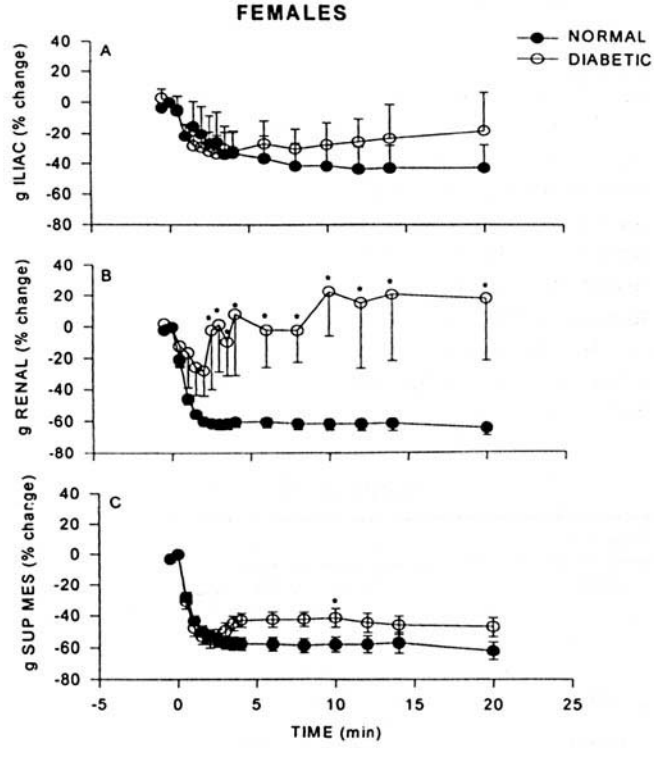

Figure 3. The effect of L-NAME (10 mg/kg) on (A) iliac, (B) renal, and (C) superior mesenteric arteries conductance (g) in normal ($n = 9$) and diabetic female (8) rats. *$P < 0.05$ vs normal female. Two-way ANOVA group effect normal versus diabetic female (g) Renal, $P = 0.0001$ and normal versus diabetic female (g) Sup Mes, $P < 0.005$.

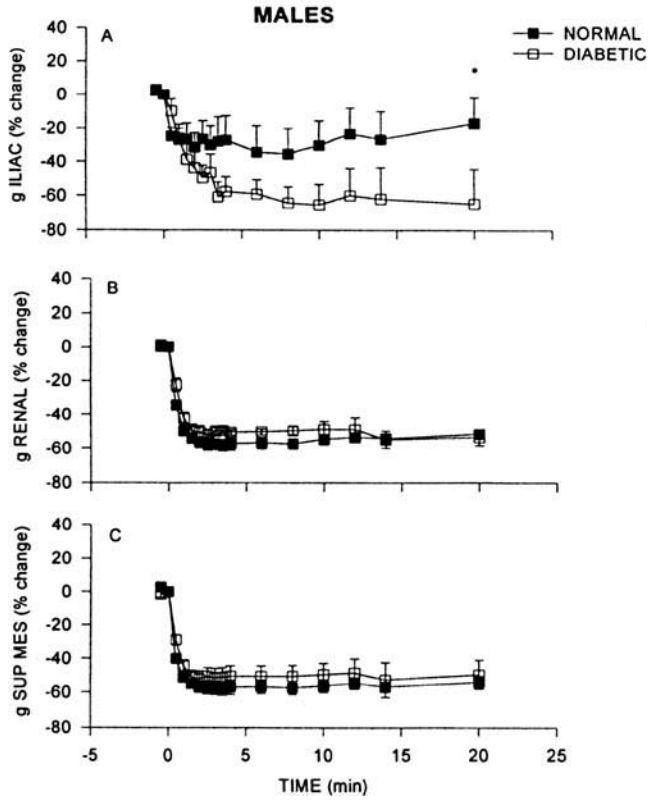

Figure 4. The effect of L-NAME (10 mg/kg) on (A) iliac, (B) renal, and (C) superior mesenteric arteries conductance (g) in normal (n = 8) and diabetic (n = 6) male rats. *P < 0.05 vs normal male rats. Two-way ANOVA group effect normal versus diabetic male (g) Iliac, P = 0.001 and normal versus diabetic male (g) Sup Mes, P = 0.001.

Results

The body weights of female and male diabetic rats were decreased (−16% and −20%, respectively), and the HRs were decreased (27% and −6%, respectively) compared with normals. The blood glucoses were increased ≈370% in the diabetic females and males when compared with normals (Table I). Basal mean arterial pressure was not different between the groups (Table I).

The systemic administration of the NOS inhibitor consisted of a single bolus injection of L-NAME (0.2 ml) *via* the femoral vein. L-NAME increased the MAP in normal and diabetic female rats (Fig. 1A). Diabetic females had a lower peak MAP and plateau pressure in response to L-NAME when compared with normal female rats (−13% and −31%, respectively; Fig. 1A and Table II). The HR in response to the pressor effect of NO antagonist was significantly decreased in the normals compared with diabetics (−14% and −6%, respectively; Fig. 1B and Table II).

L-NAME increased the MAP in normal and diabetic males. Again, the peak pressor and plateau response to NO suppression was less (−7% and −12%, respectively) in diabetic males when compared with normals (Fig. 2A and Table II). The HR in response to NO antagonist-induced pressor response was decreased (−9%) in diabetic males but not in normals (Fig. 2B, Table II).

In female rats, L-NAME decreased the conductance in all three vascular beds in normal animals (Fig. 3A, 3B, & 3C). However, this response was attenuated in the superior mesenteric bed of diabetic animals (Fig. 3C). Contrastingly, L-NAME increased conductance (+23%) in the renal vessels of diabetic females (Fig. 3B).

In male rats, L-NAME decreased the conductance in all three vascular beds in normal and diabetic animals (Fig. 4). However, this decrease was significantly greater in the iliac vascular bed of the diabetic animals (Fig. 4A).

In this study we calculated the HR/MAP ratio as an index to baroreflex sensitivity following L-NAME administration. The increased MAP following L-NAME significantly lowered this index for each of the illustrated time points when compared witih their respective basal periods (Table III). The basal index during the control period was significantly decreased (−24%) in diabetic females when compared with normal females, and tended to remain lower following L-NAME administration (Table III). The basal index of diabetic males was also lower but was not different from normals following L-NAME administration.

Discussion

The present study indicates that the NOS antagonist, L-NAME acts to increase the blood pressure significantly in normal and diabetic female and male rats. These findings are consistent with previous observations of NO's action in the regulation of mean arterial pressure (2, 7, 18). We ob-

Table III. Baroreflex Sensitivity Index (HR/MAP) to L-NAME (10 mg/kg)

Time (min)	Normal female	Diabetic female	Normal male	Diabetic male
−0.50	6.00 ± 0.39 (9)	4.83 ± 0.30 (8)	5.82 ± 0.33 (8)	5.16 ± 0.21 (6)
1.75	3.91 ± 0.21[a] (9)	3.36 ± 0.20[a] (8)	3.72 ± 0.28[a] (8)	3.50 ± 0.13[a] (6)
2.00	3.77 ± 0.19[a] (9)	3.36 ± 0.20[a] (8)	3.70 ± 0.32[a] (8)	3.63 ± 0.11[a] (6)
10.00	2.95 ± 0.23[a] (9)	3.67 ± 0.37[a] (8)	4.01 ± 0.34[a] (8)	3.73 ± 0.41[a] (6)

Note. The values represent the mean ± SEM. Number in parenthesis = n. Baroreflex sensitivity index was calculated as heart rate (beats/min) divided by mean arterial pressure (mm Hg).
[a] P < 0.01 vs time −0.50 min, ANOVA.

served that the pressor response to L-NAME was significantly greater in normals compared with diabetic rats. These results are comparable with the study by Abiru *et al.* (2) and Kiff *et al.* (8) in which the pressor response induced by L-NAME was significantly attenuated in STZ-diabetic rats compared with control. It is suggested that the failure of the NOS inhibitor to have a comparable pressor effect in diabetics may be associated with a decreased NO synthesis or release from endothelial cells in diabetic animals leading to its decreased effect of NO and its contribution to cardiovascular control (6).

The administration of an NOS antagonist resulted in regional vasoconstriction in all three vascular beds (iliac, renal, and superior mesenteric) in normal female and male rats. These results are also in agreement with previous reports in normal and diabetic male rats (1–7, 7–21). However, it can be noted that in our study, diabetic male rats exhibit an enhanced constrictor response in the iliac bed in response to NOS inhibition, suggesting that there is a shift toward enhanced constrictor tone versus dilatation in skeletal muscle in diabetic males. On the other hand, the renal bed of diabetic female rats failed to constrict or had an increased conductance following administration of the NOS antagonist.

There is substantial evidence for the contribution of endothelial nitric oxide in the control of regional vascular differential conductances *in vivo* (7, 22). The different responses to L-NAME in the iliac, renal, and superior mesenteric bed could be due to different degrees of regulatory influence of NO versus neural control on other mechanisms in the control of conductances in the different vascular beds. Investigators have demonstrated consistently that the inhibition of NO leads to an enhanced sympathoexcitatory response that results in an exaggerated vasoconstriction (22–26). Therefore, we suggest that this sympathoexcitatory response is exacerbated in diabetic male rats and may account for the increased vasoconstriction in the iliac bed in response to L-NAME.

Diabetes is also characterized by abnormal renal hemodynamics, vasodilation, pronounced glomerular hyperperfusion, and hyperfiltration (18, 20, 27). Reports have demonstrated that such abnormalities are completely abolished by the administration of L-NAME to diabetic rats (18, 20). Our results in normal and diabetic males are consistent with the above findings. On the contrary, our diabetic female rats exhibited increased renal blood flow. It is well documented that estrogen enhances NO production (28–30). Specifically, it has been demonstrated that chronic estrogen administration enhances NO production from endothelium. We believe that the renal vasodilatory response to L-NAME can be attributed to the combination of enhanced tendency for vasodilation in females coupled with the dramatic increase in mean arterial pressure. Consequently, discrepancies in the diabetic female and male rats' renal responses could be a combined dysfunction in synthesis/release of NO and sympathetic discharge (22).

Our observations of the blood flow dynamics in response to NOS inhibition in the superior mesenteric vascular bed of normal female and male rats are consistent with previous studies (31). Since the ability of NOS inhibition by L-NAME to decrease diabetic females' superior mesenteric conductance is compromised when compared with normal females, basal NO production might be more compromised in diabetic female rats (32).

A note for consideration is the observation that L-NAME has been demonstrated to have muscarinic cholinergic receptor antagonist properties (33, 34). However, this has not been supported for M1-, M2-, and M3-receptor–mediated responses in rats or rabbits (35). Nevertheless, we do not believe that this antagonist characteristic plays any significant role in the interpretation of our responses.

The L-NAME pressor responses were associated with bradycardia for all four groups of animals and were consistent with previous studies in female and male dogs, and male rats (7, 21). Our studies also supported the observation that normal females have a more sensitive baroreflex function compared with diabetic females and normal and diabetic males (36, 37).

In summary, bolus administration of L-NAME resulted in a significant pressor effect and bradycardia in all four groups of animals, and the pressor response was significantly attenuated in diabetic animals and to a greater degree in diabetic females. Blockade of NOS by L-NAME decreased conductance in all three vascular beds in female and male normal animals. In diabetic female rats, NOS inhibition resulted in a decrease in conduction of the iliac and superior mesenteric vascular bed but an increase in renal conductance. However, the decreased conductance in response to NOS inhibition was greater in the iliac bed of diabetic males. Consequently, this study suggests that the diabetic rat model exhibits an impaired baroreceptor response in conjunction with an impaired NO production. The altered pressor response and impaired constrictal response in the renal vessels in female diabetic rats may provide an explanation for the clinical observation of increased renal pathology and cardiovascular deterioration observed in humans.

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