

Relationship Between Diurnal Blood Pressure, Renal Hemodynamic Function, and the Renin-Angiotensin System in Type 1 Diabetes

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In patients with diabetes, altered diurnal blood pressure (BP) regulation (high night-to-day [N/D] ratio, or “nondipping”) is associated with increases in albumin excretion and a decline in the glomerular filtration rate (GFR) by an unknown mechanism. Because it is known that renin angiotensin system (RAS) activation and defective glucose control contribute to adverse renal outcomes, we examined renal responses to high glucose and to manipulation of the RAS in adolescents (mean age 14 ± 2 years) with uncomplicated type 1 diabetes, segregated into two groups on the basis of the presence or absence of normal N/D BP ratio. In the first experiment, renal hemodynamic comparisons were made during euglycemia (4–6 mmol/l) and hyperglycemia (9–11 mmol/l), maintained by modified clamp techniques. The induction of hyperglycemia resulted in a significant increase in GFR and filtration fraction (FF) in the high N/D ratio group. In the second experiment, we examined the renal response to graded angiotensin II (Ang II) infusion while subjects were euglycemic and salt replete. High N/D ratio was associated with an enhanced FF response to Ang II. In the third experiment, the N/D ratio and GFR were assessed after 3 weeks of ACE inhibition. This maneuver corrected the high N/D ratio, but it had no effect on glomerular hyperfiltration. These results suggest that RAS activation does not explain the hyperfiltration state, nor can it explain the poor outcomes, at least in this population. However, the observed deleterious hemodynamic responses to high glucose and Ang II and the insensitivity to ACE inhibition may, taken together, provide an explanation for the adverse renal outcomes in patients with type 1 diabetes and high N/D ratio. *Diabetes* 52:1806–1811, 2003

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ABPM, ambulatory blood pressure monitoring; Ang II, angiotensin II; BP, blood pressure; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; HCT, hematocrit; MAP, mean arterial pressure; N/D ratio, night-to-day ratio; PAH, para-aminohippurate; RAS, renin-angiotensin system; RBF, renal blood flow; RVR, renal vascular resistance.

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Ambulatory blood pressure monitoring (ABPM) has been used in the investigation and management of blood pressure (BP) problems for many years. Studies have shown that there is a normal diurnal pattern of BP variation that is characterized by an approximate 10% decrease in BP values during the night (1). Altered diurnal BP regulation, with a tendency for elevated nighttime BP (high night-to-day [N/D] ratio), is referred to as the “nondipping” pattern of BP, and it may be a risk factor for poor clinical outcome, possibly predicting the progression of renal disease (2), increased target organ damage (3–8), and increased cardiovascular morbidity (9–11). Moreover, high N/D ratios are found more commonly in patients with diabetes (12), African Americans (13), and patients with renal disease (14).

In type 1 diabetic patients, a high N/D ratio is associated with several undesirable outcomes, including early morphologic changes in the glomerulus (15), increased albumin excretion (16–19), and an increased rate of decline of the glomerular filtration rate (GFR) in patients with overt diabetic nephropathy (20). Although the mechanism(s) responsible for these associations have not been fully elucidated, subjects with uncomplicated type 1 diabetes and high N/D ratio exhibit higher values for GFR in comparison to subjects with a normal N/D ratio (21), and renal hyperfiltration is a risk factor for diabetic nephropathy (22–25). In one study (26) high N/D ratio was corrected by ACE inhibition, suggesting a role for renin angiotensin system (RAS) activation in this phenomenon. Because of the central role played by the RAS in the pathophysiology of diabetic nephropathy (27–29), we hypothesized that RAS activation may explain both the nondipping phenotype and the poor outcomes. Therefore, we explored RAS function by examining, in three separate but related studies, the renal response to hyperglycemia (known to cause RAS activation) (30–33), the renal response to graded angiotensin II (Ang II) infusion, and the renal response to ACE inhibition in adolescent and young adult subjects with uncomplicated diabetes, segregated into two groups on the basis of the presence or absence of normal N/D ratios.

RESEARCH DESIGN AND METHODS

Subjects. ABPM was conducted with a SpaceLabs 90207 ABP-monitor (SpaceLabs, Madison, WI), as previously described (17), in consecutive patients attending the Hospital for Sick Children diabetic clinic. N/D ratios were derived from a mean of the day and night systolic and diastolic readings,

and they were considered high if the ratios were >1 SD above the mean for height- and sex-adjusted adolescent reference values. Each subject was studied on six separate occasions, and only those with consistent evidence of N/D ratio status were segregated into the two groups. High N/D ratio was defined as a systolic N/D ratio of >0.92 and diastolic ND ratio of >0.86 . A total of 20 adolescent and young adult male and female subjects with type 1 diabetes (10 with high N/D ratio and 10 with normal N/D ratio) were recruited to participate in the study. Their mean age was 14 ± 2 years. Each subject underwent a detailed history and physical and laboratory examination. All were insulin dependent and were studied within 15 years of diagnosis. They were otherwise healthy nonsmokers who were normotensive and on no medications except for insulin. No women were users of oral contraceptive medications. All women were studied in the follicular phase of the menstrual cycle. No subject had evidence of retinopathy, microalbuminuria (as measured in a 24-h sample by radioimmunoassay), a decrease in creatinine clearance, or an orthostatic decline in BP, as determined by a qualified pediatrician. The study was performed with the approval of the Hospital for Sick Children and the University of Toronto human subjects review committees. Written informed consent was obtained from each subject and their parents.

All subjects were counseled to adhere to a diet that maintained normal caloric intake, sodium intake of 150–200 mmol/day, and protein intake up to $1.5\text{--}2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 7 days before each study day. Compliance was assessed by a 24-h urine collection obtained 1 day before each study for measurement of sodium and urea excretion. Subjects were included if the excretion of sodium and urea were 2–3 and 3–6 mmol/kg, respectively, in 24 h. Protein intake was calculated from the urea excretion using standard equations. No subjects were excluded on this basis. All subjects refrained from caffeine for 48 h before each study. Subjects were admitted to the Clinical Investigation Unit of the Toronto Hospital for Sick Children the evening before each study day. All studies were conducted at 0830 h after an overnight fast, with the subjects lying supine in a warm quiet room.

Study protocol

Study 1. Each subject was studied twice, once after 12 h of euglycemia and once after 12 h of hyperglycemia without glucosuria. On the evening before each study, an 18-gauge peripheral venous cannula was inserted into an antecubital vein for infusion of insulin, and a second 19-gauge sampling line was inserted in the contralateral arm for blood sampling. Insulin was infused at an average rate of 0.9 ± 0.01 units/h during the night and continued throughout the study. During the prestudy period, blood glucose levels were measured every hour, and euglycemia (blood glucose 4.0–6.0 mmol/l) and hyperglycemia (9–11 mmol/l, a plasma level chosen to avoid glucosuria and activation of the RAS due to volume contraction secondary to osmotic diuresis) were maintained by varying the insulin infusion rate (30,31,34). Subjects then presented to the Physiology Laboratory at 0800 h the next morning with the appropriate blood glucose level. A third venous catheter was inserted for infusion of inulin and para-aminohippurate (PAH). Plasma glucose levels were measured every 30 min by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA), and minor adjustments were made in the insulin infusion rate to maintain glucose in the desired range. Mean arterial pressure (MAP) and heart rate were measured every 5 min throughout the study by an automated sphygmomanometer (Dinamapp), and the mean result for each period was recorded once during each half hour of the protocol.

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. Subjects remained supine at all times. After a 90-min equilibration period and during each half hour for 90 min, blood was collected for inulin, PAH, and hematocrit (HCT), and GFR and effective renal plasma flow (ERPF) were estimated by steady-state infusion of inulin and PAH according to the calculation method described by Schnurr et al. (35). GFR and ERPF represent the mean of the final two clearance periods.

Study 2. On a separate occasion, the renal hemodynamic response to graded Ang II was assessed during euglycemia. The same subjects underwent identical prestudy preparation for 7 days before the experiment. They were admitted to the Clinical Investigation Unit, and a similar protocol was followed to ensure blood glucose levels were maintained at 4–6 mmol/l. For this portion of the study, insulin was infused at an average rate of 0.9 ± 0.02 units/h. Renal hemodynamic function was assessed using the previously described inulin and PAH clearance techniques.

A solution of Ang II (51.2 $\mu\text{g}/\text{vial}$; Clinalfa, Läufelfingen, Switzerland) was prepared by dissolving the diluent in normal saline to produce a concentration of 100 $\mu\text{g}/\text{ml}$. Then, 22 ml of normal saline was added to 0.22 ml of Ang II solution to produce a concentration of 400 ng/ml. Ang II was infused at two doses, 1 and 3 $\text{ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, for 30 min each. Subjects remained supine at

TABLE 1

Clinical characteristics of groups segregated by N/D ratio

Parameter	Normal N/D ratio	High N/D ratio
Age (years)	15 ± 2	15 ± 2
Diabetes duration (years)	10 ± 2	11 ± 3
HbA _{1c} (%)	9 ± 1	$8 \pm 1^*$
BMI (kg/m^2)	25 ± 3	23 ± 2
Daytime MAP (mmHg)	85 ± 4	$80 \pm 7^*$
Urine Na ⁺ (mmol/day)	167 ± 14	148 ± 9
Protein intake ($\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$)	1.2 ± 0.06	1.2 ± 0.04
Creatinine clearance (ml/s)	2 ± 0.4	2 ± 0.5

Data are means \pm SE. Urine Na⁺, 24-h urine sodium excretion. * $P < 0.05$ vs. normal N/D ratio group.

all times. Blood was collected once during each Ang II infusion period for HCT, inulin, and PAH. MAP was measured at the midpoint of each infusion. A further collection of blood was obtained at the end of the Ang II infusion, after a 30-min recovery period.

Study 3. On a separate occasion, after initiation of oral enalapril (0.1 mg/kg daily \times 1 week and then 0.1 mg/kg b.i.d. \times 2 weeks), ABPM was again conducted on two occasions. The subjects were admitted to the Clinical Investigation Unit, were rendered euglycemic as outlined above, and again underwent renal hemodynamic testing using inulin and PAH clearance.

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. The mean of the final two clearance periods represent GFR and 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 $1 - \text{HCT}$. Renal vascular resistance (RVR) was derived by dividing MAP by the RBF.

Statistical analysis. Subjects were segregated into subgroups on the basis of the presence or absence of normal N/D ratio. Data are presented as means \pm SE. Within-subject and between-group comparison of all parameters at baseline were made using nonparametric methods (Wilcoxon's rank-sum test). Within-subject and between-group differences in the response to high glucose, graded Ang II infusion, and ACE inhibition were determined by repeated-measures ANOVA and Bonferroni correction. All statistical analyses were performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Baseline parameters. Baseline clinical parameters are shown in Table 1. Mean values for age, duration of diabetes, urine sodium excretion, protein intake, and BMI were similar in the two groups. Mean values for HbA_{1c} and MAP were significantly lower in the high N/D ratio group compared with the normal N/D ratio group.

Response to high glucose. Renal and systemic hemodynamic results at baseline and in response to high glucose are shown in Table 2 and Fig. 1. The high N/D ratio group exhibited glomerular hyperfiltration that was further augmented by glucose. In contrast, GFR values did not change in the normal N/D ratio group during the hyperglycemic clamp. The normal N/D ratio group exhibited a small but significant increase in RVR, whereas RVR did not change in the high N/D ratio group during the hyperglycemic clamp.

Response to Ang II. Graded infusion of Ang II led to predictable declines in RBF and increases in FF and RVR in both groups of diabetic subjects (Table 3). However, Ang II led to a decline in GFR in the normal N/D ratio group, whereas GFR was maintained in the high N/D ratio group, resulting in a greater increase in FF.

Response to ACE inhibition. Administration of enalapril for 3 weeks resulted in a restoration of normal N/D ratio.

TABLE 2
Renal response to hyperglycemia in groups segregated by N/D ratio

Parameter	Normal N/D ratio		High N/D ratio	
	Euglycemia	Hyperglycemia	Euglycemia	Hyperglycemia
GFR (ml · min ⁻¹ · 1.73 m ⁻²)	128 ± 46	127 ± 17	142 ± 31	157 ± 28*†
ERPF (ml · min ⁻¹ · 1.73 m ⁻²)	698 ± 124	502 ± 107	756 ± 242	704 ± 158
RBF (ml · min ⁻¹ · 1.73 m ⁻²)	1,113 ± 183	1,006 ± 121	1,250 ± 454	1,166 ± 289
FF	0.18 ± 0.05	0.21 ± 0.03	0.20 ± 0.05	0.23 ± 0.05
RVR (mmHg · l ⁻¹ · min ⁻¹)	77 ± 13	84 ± 18	64 ± 16‡	67 ± 9†
MAP (mmHg)	83 ± 5	83 ± 9	74 ± 9‡	75 ± 9

Data are means ± SE. **P* < 0.05 vs. baseline; †*P* < 0.05 vs. response of normal N/D ratio group; ‡*P* < 0.05 vs. baseline value of normal N/D ratio group.

Before initiation of enalapril treatment, both the systolic and diastolic N/D BP ratios were greater in the high N/D ratio group, as expected based on group selection criteria (systolic N/D ratio 0.96 ± 0.05 vs. 0.87 ± 0.03 and diastolic N/D ratio 0.92 ± 0.1 vs. 0.78 ± 0.07, both *P* < 0.05). After 20 days of enalapril, the N/D BP ratio decreased in only the high N/D ratio group, removing differences between the two groups (systolic N/D ratio 0.92 ± 0.05 vs. 0.90 ± 0.04 and diastolic 0.85 ± 0.09 vs. 0.82 ± 0.07, *P* < 0.05). In the normal N/D ratio group, the GFR decreased from 128 ± 46 to 109 ± 23, ml · min⁻¹ · 1.73 m⁻² (*P* < 0.05) in response to enalapril, whereas in the high N/D ratio group, the GFR remained unchanged (142 ± 31 to 145 ± 39 ml · min⁻¹ · 1.73 m⁻², NS). The FF decreased from 0.18 ± 0.05 to 0.16 ± 0.02 (*P* < 0.05) in the normal N/D ratio group, but it was unchanged in the high N/D ratio group (0.20 ± 0.05 to 0.20 ± 0.05, NS).

DISCUSSION

In diabetic patients, high N/D ratio has been associated with adverse outcomes, such as increased urinary albumin excretion (16–19), autonomic neuropathy (36), an increased rate of decline of renal function in those with diabetic nephropathy (20), intensive insulin treatment (suggesting nighttime hypoglycemia and sympathetic activation) (37), and renal morphologic abnormalities suggestive of early nephropathy (15). In normotensive normoalbuminuric type 1 diabetic patients without any degree of autonomic dysfunction according to traditional cardiovascular tests, a high N/D ratio is associated with glomerular hyperfiltration (21). It is unclear whether a high N/D ratio

is a cause of vascular dysfunction (due to, for example, an increased duration of higher BP) or is a surrogate marker of a phenotype at risk for these disease end points. Because of the central role played by RAS in the initiation and progression of diabetic nephropathy, and because of a previous study linking RAS to abnormal nocturnal BP patterns (26), we examined RAS activity groups with high and normal N/D ratios. Our major findings were that 1) the high N/D ratio group exhibited baseline glomerular hyperfiltration (despite lower MAP and better long-term glucose control) and a deleterious renal hemodynamic response to high glucose, characterized by a further increase in GFR and FF; 2) the high N/D ratio group exhibited an augmented renal hemodynamic response to Ang II, characterized by an increase in FF compared with the normal N/D ratio group, suggesting enhanced intraglomerular pressure; and 3) enalapril treatment ameliorated the high N/D ratio but had no impact on glomerular hyperfiltration, suggesting a renal insensitivity to ACE inhibition.

Similar to our first major finding, it has been reported that diabetic patients with a high N/D ratio exhibit hyperfiltration (21). Another recent study (15) has reported that a high N/D ratio is related to hyperfiltration, basement membrane thickening, and mesangial matrix expansion in the glomeruli of adolescents with uncomplicated type 1 diabetes. It is possible that the increased burden of nocturnal hypertension is instrumental in these changes, but in our study of adolescents and young adults, the high N/D ratio group exhibited lower MAP over 24 h than the group with normal N/D ratios, and values for nocturnal BP were similar. The decrease in RVR in this group suggests the possibility that, rather than higher pressures impacting negatively on renal function, more pressure may be transmitted to the glomerulus, resulting in increased intraglomerular pressure. In response to hyperglycemia, the high N/D ratio group exhibited a further increase in GFR and FF. We (30,31) and others (32,33) have previously shown that hyperglycemia without glucosuria results in activation of the intrarenal RAS. The mechanism(s) responsible for our observations could have been related to different effects of high glucose on RAS function in the two groups. For example, activation of the intrarenal RAS, leading to efferent arteriolar constriction and increased glomerular capillary pressure, might have been more vigorous in the high N/D ratio group because of selective upregulation of Ang II receptor expression, thus accounting for the rise in FF and maintenance of GFR. However, it is important to note that hyperglycemia did not increase RVR in the high N/D group, suggesting other mechanisms.

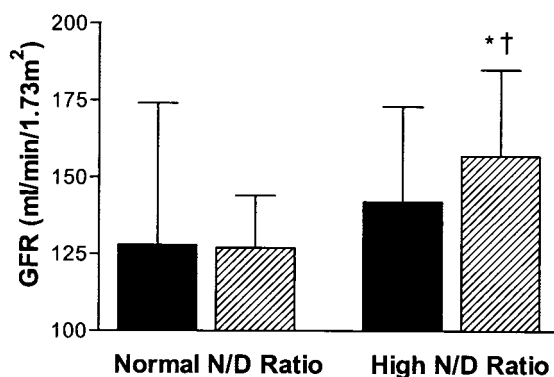


FIG. 1. GFR response during euglycemia (■) and hyperglycemia (▨) in subjects with type 1 diabetes segregated on the basis of N/D BP ratio. **P* < 0.05 compared with GFR during euglycemia; †*P* < 0.05 compared with response of normal N/D ratio group.

TABLE 3
Renal response to AngII in groups segregated by N/D ratio

Parameter	Normal N/D ratio				High N/D ratio			
	Baseline	1 ng · kg ⁻¹ · min ⁻¹	3 ng · kg ⁻¹ · min ⁻¹	Recovery	Baseline	1 ng · kg ⁻¹ · min ⁻¹	3 ng · kg ⁻¹ · min ⁻¹	Recovery
GFR (ml · min ⁻¹ · 1.73 m ⁻²)	128 ± 46	118 ± 41	113 ± 29*	117 ± 35	142 ± 31	142 ± 25	141 ± 24†	137 ± 22
ERPF (ml · min ⁻¹ · 1.73 m ⁻²)	698 ± 124	561 ± 90	480 ± 101*	502 ± 107	756 ± 242	617 ± 175	532 ± 158*	616 ± 190
RBF (ml · min ⁻¹ · 1.73 m ⁻²)	1,250 ± 454	1021 ± 334	881 ± 291*	1,012 ± 337	1,166 ± 289	990 ± 289	829 ± 203*	909 ± 243
FF	0.18 ± 0.05	0.21 ± 0.06	0.24 ± 0.04*	0.24 ± 0.06	0.20 ± 0.05	0.24 ± 0.04	0.28 ± 0.06*	0.23 ± 0.05
RVR (mmHg · l ⁻¹ · min ⁻¹)	77 ± 13	101 ± 19*	134 ± 31*	110 ± 29	64 ± 16‡	89 ± 27	119 ± 34*	86 ± 24
MAP (mmHg)	83 ± 5	90 ± 8	101 ± 10*	85 ± 5	74 ± 9‡	83 ± 9	97 ± 16*	81 ± 11

Data are means ± SE. **P* < 0.05 vs. baseline; †*P* < 0.05 vs. response of normal N/D ratio group; ‡*P* < 0.05 vs. baseline value of normal N/D ratio group.

Our second major finding was that the high N/D ratio group demonstrated an augmented FF response to Ang II. This finding further suggests that mechanisms other than RAS activation may be contributing to the baseline findings of hyperfiltration and increased FF, because one might have expected a blunting of the renal hemodynamic response due to Ang II receptor downregulation. Whatever the mechanism, it is possible that this increased sensitivity to Ang II may contribute to the association of high N/D ratios with adverse outcomes. Ang II increases glomerular capillary pressure (which is an important determinant of glomerular injury) and, as reviewed by Wolf and Ziyadeh (38), is also currently regarded as a trophic hormone that stimulates cell growth and proto-oncogene expression in kidney cells and results in the formation and release of a number of cytokine mediators of the sclerosing process. Although the augmented hemodynamic response to Ang II in the high N/D ratio group does not necessarily imply differences in the growth-promoting properties of Ang II in this group, it is tempting to speculate that such an effect may account, at least in part, for the association between high N/D ratios and adverse clinical outcomes.

Our third major finding was the observation that, as previously documented, ACE inhibition resulted in a correction of the abnormal N/D ratio (26), but it did not ameliorate glomerular hyperfiltration. Recent data from Hollenberg et al. (29) demonstrated that 80% of subjects with type 1 diabetes in their study exhibited evidence of intrarenal RAS activation, as suggested by a vigorous renal vasodilator response to ACE inhibition and Ang II receptor blockade. Interestingly, we noted no such renal vasodilator response, and the GFR, ERPF, and FF results were unchanged by treatment with enalapril. This result, in concert with the augmented response to Ang II infusion, casts further doubt on the hypothesis that RAS activation was the underlying etiology for the renal hemodynamic abnormalities in the high N/D ratio group.

It remains possible that differences in the activity of vasodilatory systems such as the nitric oxide pathway or the cyclo-oxygenase system, rather than RAS activation, may have contributed to the hemodynamic responses in the two groups. In this regard, a recent study of streptozotocin-induced diabetic rats with moderate hyperglycemia reported increased cyclooxygenase-2 expression in

the renal cortex, and inhibition of this system normalized hyperfiltration (39). Furthermore, it is possible that, as suggested by Vallon et al. (40), hyperfiltration in the high N/D group was caused by a primary increase in proximal tubular reabsorption stimulated by sodium-glucose cotransport, resulting in the suppression of tubulo-glomerular feedback activity. In this regard, in a study by Vallon et al. (41), the activity of two proximal tubular sodium-glucose cotransporters, sodium-glucose cotransporter-1 and -2, were inhibited by the administration of phlorizin directly into the proximal tubules of streptozotocin-induced diabetic rats, resulting in an increase in the sodium content of the distal tubular fluid and a reduction in single-nephron GFR. It is possible that selective upregulation of either glomerular vasodilatory systems or tubular reabsorptive systems in the high N/D ratio group may have explained our findings. However, this must remain speculative, and further studies will be necessary because our present studies were not designed to test these hypotheses.

Potential confounding variables that could have impacted on renal hemodynamic function include differences in sodium and protein intake between groups (42,43). To ensure against this, each subject was counseled to adhere to a controlled sodium and protein diet, and compliance was verified by 24-h urine collections. Another parameter that can impact on renal function is long-term glucose control. HbA_{1c} was measured in both groups before study, and surprisingly, the results suggested that glucose control was better in the high N/D ratio group. The possibility also exists that nocturnal hypoglycemia and sympathetic activation could cause high N/D ratios (37). Of note, there were no differences in mean plasma norepinephrine levels in the two groups of diabetic subjects, although we recognize that this is an insensitive measure of sympathetic nervous system function. Furthermore, sympathetic activation could not explain the baseline renal hemodynamic findings and the augmented responses to Ang II infusion and to high glucose. We have previously shown differences in RAS activity throughout the menstrual cycle in premenopausal female subjects (44), and sex differences exist in the renal response to Ang II, mediated in part by high estrogen (45). To control for this, all female subjects were studied in the follicular phase of the menstrual cycle.

In summary, we have examined the contribution of the

RAS to the renal hemodynamic abnormalities in subjects with uncomplicated diabetes who exhibit high N/D ratios. Although our results did not suggest a role for RAS activation, we did demonstrate deleterious renal responses to both high glucose and Ang II and renal insensitivity to ACE inhibition, which, taken together, may help explain the association between high N/D ratio and adverse renal outcomes in patients with type 1 diabetes.

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