Impact of Renin Angiotensin System Modulation on the Hyperfiltration State in Type 1 Diabetes

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The initial stages of diabetic nephropathy are characterized by glomerular hyperfiltration and hypertension, processes that have been linked to initiation and progression of renal disease. Renin angiotensin system (RAS) blockade is commonly used to modify the hyperfiltration state and delay progression of renal disease. Despite this therapy, many patients progress to ESRD, suggesting heterogeneity in the response to RAS modulation. The role of the RAS in the hyperfiltration state in adolescents with uncomplicated type 1 diabetes was examined, segregated on the basis of the presence of hyperfiltration. Baseline renal hemodynamic function was characterized in 22 patients. Eleven patients exhibited glomerular hyperfiltration (GFR > 135 ml/min), and in the remaining 11 patients, the GFR was <130 ml/min. Renal hemodynamic function was assessed in response to a graded angiotensin II (AngII) infusion during euglycemic conditions and again after 21 d of angiotensin-converting enzyme (ACE) inhibition with enalapril. AngII infusion under euglycemic conditions resulted in a significant decline in GFR and renal plasma flow in the hyperfiltration group but not in the normofiltration group. After ACE inhibition, GFR fell but did not normalize in the hyperfiltration group; the normofiltration group showed no change. These data show significant differences in renal hemodynamic function between hyperfiltering and normofiltering adolescents with type 1 diabetes at baseline, after AngII infusion and ACE inhibition. The response to ACE inhibition and AngII in hyperfiltering patients suggests that vasodilation may complement RAS activation in causing the hyperfiltration state. The interaction between glomerular vasoconstrictors and vasodilators requires examination in future studies.


Diabetic nephropathy is the single most important cause of renal failure in North America, accounting for >40% of prevalent cases (1). The initial stages of renal disease are characterized by renal hemodynamic alterations that result in increased intraglomerular pressure and glomerular hyperfiltration (2–4). This in turn has been associated with cytokine- and growth factor–mediated cell and connective tissue proliferation and fibrosis, mostly on the basis of TGF-β upregulation (5). Several studies (1,6) have linked hyperfiltration to the initiation and progression of diabetic nephropathy. Although some earlier experiments suggested renin angiotensin system (RAS) blunting as the cause of the hyperfiltration state, partly on the basis of decreases in circulating RAS components (7), more recent studies in humans (8) and animals (7) have confirmed that the intrarenal RAS is activated, especially in response to hyperglycemia (6). RAS blockade is currently a well-established therapy in type 1 diabetes (9–13). Although early RAS blockade decreases the relative risk for reaching clinical end points such as time to doubling of serum creatinine, dialysis, or death (9–11); delays the progression from microalbuminuria to macroalbuminuria (9–12); and ameliorates pathologic changes such as glomerular basement membrane thickening (13), it does not completely arrest the progression of diabetic nephropathy in all patients (9–13).

In this study, we describe the renal hemodynamic responses to graded angiotensin II (AngII) infusion and to angiotensin-converting enzyme (ACE) inhibition in a group of normotensive, normoalbuminuric adolescents who had type 1 diabetes and were segregated into groups on the basis of the presence or absence of hyperfiltration. We hypothized that the hemodynamic response to RAS modulation (incremental AngII infusion and ACE inhibition) would not be uniform in these two groups of patients. Our aim was to examine the role of RAS function in the hyperfiltration state and to determine the utility of RAS blockade as a modifier of hyperfiltration.

Materials and Methods

Patients

Participants who fulfilled the following inclusion criteria were asked to participate: Duration of type 1 diabetes >5 yr, Tanner stages 2 to 5 puberty, normoalbuminuria (albumin excretion rate [AER] <20 μg/min on two of three overnight urine collections obtained during the month before study), normal clinic BP, no microvascular disease, and absence of chronic illness other than treated hypothyroidism or mild asthma. The Research Ethics Board at the Hospital for Sick Children (Toronto, Ontario, Canada) approved the protocol. All patients and/or
their parents gave informed consent. Twenty-two adolescents with type 1 diabetes were eligible to participate.

**Protocol and Evaluations**

Patients adhered to a sodium-replete (150 to 200 mmol/d) and moderate-protein (1 to 1.5 g/kg per d) diet during the 7-d period before each experiment. Diets were evaluated twice during the month leading up to the study and also on days 5 and 6. For ascertainment of compliance, patients underwent an overnight urine collection to determine the urinary sodium and urea excretion on days 5 and 6. Protein intake was calculated from the urea excretion using standard methods (14). Patients were studied when the excretion of sodium and urea was 2 to 3 and 3 to 6 mmol/kg, respectively, in 24 h. All patients met these study criteria. Patients also had ambulatory BP monitoring performed on three occasions leading up to the experiment. We measured average 24-h systolic BP (SBP) and diastolic BP (DBP) as well as night-day (N/D) ratio. Although other valid methods exist for defining N/D ratios (15), nocturnal dipping was defined according to our previously published definition in a similar adolescent patient population (14), in which N/D ratios were derived from a mean of the day and night systolic and diastolic values (14). Ratios were considered high when they were >1 SD above the mean for height- and gender-adjusted adolescent reference values (14). High N/D systolic ratio therefore was defined as >0.92, and high diastolic ratio was defined as >0.86. Nighttime was defined as 11:00 p.m. to 7:00 a.m.

Euglycemic conditions (blood glucose 4 to 6 mmol/L) were maintained for 10 to 12 h preceding and during all investigations. In both phases of the experiment, blood glucose was maintained by a modified glucose clamp technique as described previously (14). Female patients were studied during the early follicular phase of the menstrual cycle, determined by counting days of the menstrual cycle and measuring 17β-estradiol levels. None of the female patients was a user of oral contraceptive medications.

Experiments were carried out on two separate days. Patients were admitted to the Clinical Investigation Unit at the Hospital for Sick Children the evening before each day of the study, as described previously (14). A 16-gauge peripheral venous cannula was inserted into a vein in one arm for infusion of glucose and insulin, and a second blood sampling line was inserted into a vein in the contralateral arm. Blood glucose was measured every hour, and the insulin infusion was adjusted to maintain euglycemia. Studies were conducted the following morning after an overnight fast with patients lying in the supine position in a warm, quiet room. A third intravenous line was inserted into the arm contralateral to the insulin infusion and was connected to a syringe infusion pump for infusions of insulin and paraaminopropiophenone (PAH) and AngII. Mean arterial pressure (MAP) and heart rate were measured before each blood sample throughout the study by an automated sphygmomanometer (Dinamapp, Criticon, Tampa, FL). After blood was collected for insulin and PAH blank, a priming infusion that contained 25% insulin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, insulin and PAH were infused continuously at a rate calculated to maintain their respective plasma concentrations constant at 20 and 1.5 mg/dL. Patients remained supine at all times. After a 90-min equilibration period, blood was collected for insulin, PAH, and hematocrit (Hct). Blood was collected further every 30 min for 60 min, and GFR and effective renal plasma flow (ERPF) were estimated by steady-state infusion of insulin and PAH according to the calculation method described by Schnurr et al. (16).

A solution of AngII (Clinalfa, Laülfingen, Switzerland; 51.2 μg/vial) was prepared by dissolving the diluent in normal saline to produce a concentration of 400 ng/mL. AngII was infused at two doses, 1 and 3 ng/kg per min, each dose for 30 min. Patients remained supine at all times. Blood was collected once during each AngII infusion period for Hct, insulin, and PAH. MAP was measured at the midpoint of each infusion. An additional collection of blood was obtained at the end of the AngII infusion, after a 30-min recovery period.

Patients then were initiated on enalapril (0.1 mg/kg per d for 1 wk and then 0.1 mg/kg twice daily for 2 wk). After a total of 21 d, patients were admitted to the Clinical Investigation Unit on day 2, were again rendered euglycemic as outlined above, and again underwent renal hemodynamic testing using insulin and PAH clearance.

**Sample Collection and Analytical Methods**

Blood samples that were collected for inulin and PAH determinations were centrifuged immediately at 3000 rpm for 10 min at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Insulin and PAH were measured in serum by colorimetric assays using anthrone and N-(1-naphthyl) ethylenediamine, respectively. The mean of the final two baseline clearance periods represent GFR and ERPF, expressed per 1.73 m². Filtration fraction (FF) was determined as the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1 − Hct). Renal vascular resistance (RVR) was derived by dividing the MAP by the RBF. All renal hemodynamic measurements were adjusted for body surface area.

Urinary AER was determined from three timed overnight urine collections. Urinary albumin concentration was determined by immunoturbidimetry (17). Glycosylated hemoglobin (HbA₁c) was measured by HPLC.

**Statistical Analyses**

The data were analyzed on the basis of filtration status. Results are presented as mean ± SEM. Between-group comparisons of all parameters at baseline were made using parametric methods (unpaired t test). Within-patient and between-group differences in the response to AngII and ACE inhibition were determined by repeated measures ANOVA with Bonferroni correction in the case of AngII and paired t test in the case of ACE inhibition. All statistical analyses were performed using the statistical package SAS (SAS Institute Inc., Cary, NC).

**Results**

**Baseline Characteristics**

Eleven patients fulfilled the criteria for normofiltration (GFR ≤ 130 ml/min per 1.73 m²), and 11 patients fulfilled the criteria for hyperfiltration (GFR ≥ 135 ml/min per 1.73 m²). At baseline, the two groups were similar in age, gender distribution, body mass index, HbA₁c, and diabetes duration (Table 1). There were no differences in SBP, DBP, or MAP between the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperfiltration Group (n = 11)</th>
<th>Normofiltration Group (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>5/6</td>
<td>6/5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>15.0 ± 2.0</td>
<td>15.4 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 2.0</td>
<td>22.2 ± 1.9</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>8.45 ± 1.0</td>
<td>8.50 ± 1.2</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>11.7 ± 3.1</td>
<td>11.5 ± 3.1</td>
</tr>
</tbody>
</table>

*Data are mean ± SEM. BMI, body mass index; HbA₁c, glycosylated hemoglobin
two groups. The proportion of patients with a nocturnal dipping BP pattern was not significantly different between the two groups, although there were a greater number of nondippers in the hyperfiltration group (Table 2). Ambulatory BP monitoring showed a significantly lower average 24-h SBP in the hyperfiltration group compared with the normofiltration group (Table 2). The hyperfiltration group exhibited a mean GFR of 177.7 ± 54.1 ml/min per 1.73 m² compared with a GFR of 111.3 ± 14.6 ml/min per 1.73 m² in the normofiltration group (P = 0.0008). FF also was augmented significantly in the hyperfiltration group (0.24 ± 0.03 versus 0.17 ± 0.03 in the normofiltration group; P = 0.009). No significant differences were noted in RBF or ERPF. Mean values for RVR were numerically lower in the group; early follicular phase of the menstrual cycle.

**Response to AngII**

Graded AngII infusion (Table 3) increased the MAP in both of the groups to the same degree, but the renal hemodynamic response differed between the two groups. The hyperfiltration group experienced a significant reduction in GFR from 177.7 ± 54.1 to 159.4 ± 57.8 ml/min per 1.73 m² (P = 0.04), whereas there was no significant change in GFR in the normofiltration group. The change in GFR was significantly different between groups (P = 0.007; Table 3, Figure 1). Whereas both groups exhibited significant decrements in ERPF and RBF and increments in FF and RVR, the magnitude of the fall in ERPF (P = 0.001 versus response of normofiltration group) and RBF (P = 0.04 versus response of normofiltration group) was greater in the hyperfiltration group.

**Table 2. Baseline systemic and renal hemodynamic function by GFR**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperfiltration Group</th>
<th>Normofiltration Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>108.9 ± 11.8</td>
<td>113.0 ± 14.2</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>59.8 ± 6.2</td>
<td>59.8 ± 10.0</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>77.2 ± 7.8</td>
<td>78.0 ± 7.2</td>
</tr>
<tr>
<td>24-h SBP</td>
<td>112.8 ± 8.6b</td>
<td>120.8 ± 14.4</td>
</tr>
<tr>
<td>24-h DBP</td>
<td>66.5 ± 10.3</td>
<td>69.6 ± 12.7</td>
</tr>
<tr>
<td>Nocturnal dippers</td>
<td>4/11</td>
<td>7/11</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>177.7 ± 54.1b</td>
<td>111.27 ± 14.6</td>
</tr>
<tr>
<td>ERPF (ml/min per 1.73 m²)</td>
<td>771.8 ± 218.7</td>
<td>658.9 ± 110.0</td>
</tr>
<tr>
<td>FF</td>
<td>0.24 ± 0.08b</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>RBF (ml/min per 1.73 m²)</td>
<td>1269.2 ± 391.6</td>
<td>1083.1 ± 184.7</td>
</tr>
<tr>
<td>RVR (mmHg/L per min)</td>
<td>65.6 ± 16.7</td>
<td>73.5 ± 10.9</td>
</tr>
</tbody>
</table>

aData are mean ± SEM. DBP, diastolic BP; ERPF, effective renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; SBP, systolic BP. bP < 0.05 for hyperfiltering group compared to normofiltering group.

**Response to RAS Blockade**

Whereas ACE inhibition (Table 4) led to significant decreases in MAP in both groups of patients, the renal hemodynamic response to ACE inhibition differed between the groups. After ACE inhibition, GFR and FF declined in the hyperfiltration group but still remained in the hyperfiltration range. GFR in the normofiltration group was unchanged, as was FF. The GFR and FF responses to ACE inhibition were significantly different between groups (P = 0.004 and P = 0.04, respectively; Table 4, Figure 1). Whereas the normofiltration group exhibited increases in RBF and ERPF in response to ACE inhibition, the hyperfiltration group experienced numerical decreases (P = 0.03 versus response of the normofiltration group). The normofiltration group exhibited a decline in RVR in response to ACE inhibition, whereas the hyperfiltration group experienced no change (P = 0.03 versus response of normofiltration group).

**Discussion**

The aim of this study was to determine the contribution of the RAS to the hyperfiltration state in normoalbuminuric, normotensive adolescents with type 1 diabetes. This was achieved by examining the renal hemodynamic response to AngII infusion and ACE inhibition in a group of patients with uncomplicated type 1 diabetes, with and without hyperfiltration. The major findings in patients who were studied under euglycemic conditions were as follows (1) AngII infusion resulted in a significant decrease in GFR in the hyperfiltration group with maintenance of GFR in the normofiltration group, and (2) RAS blockade with enalapril reduced but did not normalize GFR in the hyperfiltration group.

Diabetic hyperfiltration is thought to be due in part to RAS-mediated vasoconstriction, and experimental studies suggest that this may occur at the efferent arteriole, leading to an increase in intraglomerular pressure (2). In vitro studies have demonstrated that increased intraglomerular pressure, via shear-stress forces on the endothelium, can result in mesangial matrix formation and mesangial cell proliferation by increasing formation of types I and IV collagen, laminin, fibronectin, TGF-β1, and AngII receptor mRNA (18–22). These factors then produce the characteristic mesangial matrix increase and eventually the sclerotic and irreversible glomerulopathy of diabetes (18–22). Several mechanisms, such as oxidative stress and activation of protein kinase Cβ in addition to altered hemodynamic function, are seen as central to the pathogenesis of diabetic nephropathy (23). Human studies in type 1 diabetes have strongly correlated hyperfiltration with an increased risk for diabetic nephropathy in long-term observational studies (24,25).

Our first major finding was that after AngII infusion, the two groups showed different responses in GFR and FF as well as a quantitative difference in the reduction in RBF. The typical renal response to AngII is a decline in ERPF, maintenance of GFR, and an augmentation of FF (26). The normofiltration group responded in a normal manner, with maintenance of GFR despite a fall in ERPF. In contrast to the normofiltration group, the hyperfiltration group exhibited a significant decline in GFR accompanied by a fall in ERPF. Although these whole-
kidney clearance studies do not allow us to discern specific alterations in segmental arteriolar resistances, one possible interpretation of the data is that the hyperfiltration group responded with a relatively greater increase in afferent resistance. Although the major experimental effect of AngII is efferent constriction (27), it also has been shown to have important afferent arteriolar vasconstrictive effects at high concentrations in micropерfused rabbit renal arterioles (28) and in studies that induce RAS blockade with ACE inhibition or angiotensin receptor blockade in rat arterioles (29). In addition, the vasconstrictive effects of AngII may be modulated by vasodilatory mediators. For example, nitric oxide is thought to counteract the constrictive effects of AngII may be modulated by vasodilatory mediators. For example, nitric oxide is thought to counteract the constrictive effects of AngII may be modulated by vasodilatory mediators. For example, nitric oxide is thought to counteract the constrictive effects of AngII may be modulated by vasodilatory mediators. For example, nitric oxide is thought to counteract the constrictive effects of AngII may be modulated by vasodilatory mediators. For example, nitric oxide is thought to counteract the constrictive effects of AngII.
blockade in similar patients with type 1 diabetes may be clarified by the ongoing Renin Angiotensin System Study (42,43).

Taken together, the incomplete amelioration of hyperfiltration in response to ACE inhibition and the decline in GFR in response to AngII suggest a significant role for arteriolar vasodilation in the maintenance of hyperfiltration in this group of patients. In support of a role for enhanced vasodilatory activity in the hyperfiltration group was the finding of a lower ambulatory SBP. Although speculative, this suggests that vasodilation also is important in the systemic circulation, as previously reported (44). It is important to note, however, that whatever the mechanisms that are responsible for these effects, ACE inhibitor-mediated reductions in GFR and FF seem to be blunted in these hyperfiltering adolescents with type 1 diabetes.

The divergent renal hemodynamic responses to RAS blockade in the two groups also deserve comment. As can be seen from Table 4, the responses of all parameters except MAP were directly opposite in the hyperfiltration group compared with the normofiltration group. Therefore, hyperfiltering patients with diabetes exhibit significantly different renal hemodynamic function when contrasted with patients with a normal GFR and are not merely the same patients with differences in extracellular fluid volume, protein intake, circulating glucose levels, and Hba1c; these parameters having been controlled in our study. The two groups also exhibited subtle differences in their systemic BP: A greater number of nocturnal nondippers were found in the hyperfiltration group, although this did not reach statistical significance. Interpretation of this finding is limited by the small number of patients but may reflect underlying vascular dysfunction in this group, predisposing them to the earlier development of such complications as microalbuminuria (15,45).

This study has important limitations. The sample size was small, which may have limited our ability to detect significant differences in some parameters, such as baseline RVR and AngII-mediated changes in FF and RVR. We attempted to minimize the effect of the small sample size by using homogeneous study groups and by careful prestudy dietary preparation. Furthermore, the effect of variations in estrogen on renal hemodynamic and RAS function in female patients was controlled by studying only those who were nonusers of oral contraceptive medications and only during the follicular (low estrogen) phase of the menstrual cycle. We also used a study design that allowed each patient to act as his or her own control, thereby decreasing variability. We did not have a hyperfiltration group to use as a control (without RAS modulation), and the possibility exists that the GFR response to RAS modulation represented regression toward the mean. This is unlikely, however, because the pattern of renal hemodynamic function was consistent with changes in renal vasomotor tone with RAS blockade and stimulation. Another potential limitation of this study is the dose and duration that were chosen for the ACE inhibitor therapy, in that a further reduction in hyperfiltration may have been achieved with a longer period or a higher dose of enalapril.

**Conclusion**

Our aim was to establish the relative importance of RAS activation in the maintenance of the hyperfiltration state in patients with type 1 diabetes. Our findings show that glomerular hyperfiltration during euglycemia is a critical determinant of the hemodynamic response to RAS modulation in patients with type 1 diabetes. Furthermore, vasodilatory influences may complement RAS activation in mediating the hyperfiltration state, so RAS blockade may not correct hyperfiltration in all patients with uncomplicated type 1 diabetes.

**Acknowledgments**

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We thank the nurses in the Clinical Investigation Unit, Hospital for Sick Children, for invaluable assistance with the protocol. We also thank Clinalfa for providing AngII and PAH for these experiments.

**References**


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**Table 4. Mean systemic and renal hemodynamic responses to ACEI by GFR**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperfiltration Group</th>
<th>Normofiltration Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Euglycemia</td>
<td>Post-ACEI</td>
</tr>
<tr>
<td>MAP</td>
<td>77.2 ± 7.8</td>
<td>70.6 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GFR</td>
<td>177.7 ± 54.1</td>
<td>142.8 ± 41.0&lt;sup&gt;d,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ERPF</td>
<td>771.8 ± 218.7</td>
<td>699.3 ± 199.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FF</td>
<td>0.24 ± 0.08</td>
<td>0.21 ± 0.05&lt;sup&gt;d,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RBF</td>
<td>1269.2 ± 391.6</td>
<td>1140.2 ± 339.4&lt;sup&gt;d,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RVR</td>
<td>65.6 ± 16.7</td>
<td>68.1 ± 21.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are mean ± SEM. MAP = 0.16; GFR = 0.004; ERPF = 0.03; FF = 0.04; RBF = 0.03; RVR = 0.03. ACEI, angiotensin-converting enzyme inhibitor.

<sup>b</sup>p < 0.05 versus baseline values.

<sup>c</sup>P < 0.05 versus response of normofiltration group.


33. Skorecki KL, Ballermann BJ, Rennke HG, Brenner BM:


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