Impaired Saline-Stimulated Diuresis and Natriuresis in the Conscious Hypertensive Glucose-Intolerant Rat

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Background: A major complication of type 2 diabetes is renal dysfunction, a condition that worsens with coexistence of systemic hypertension. However, less is known regarding the renal impact of the prediabetic condition characterized by glucose intolerance. Therefore, we tested the hypothesis that chronic glucose intolerance leads to abnormal renal function, a condition also exacerbated by concurrent systemic hypertension.

Methods: A rat model of concomitant NaCl-sensitive hypertension and glucose intolerance was used to study renal function. Glucose intolerance was produced by administering streptozotocin to neonatal Wistar-Kyoto rats, and systemic hypertension was induced by combining dietary NaCl excess with unilateral nephrectomy. Hemodynamic and renal excretory responses were determined in conscious animals before and after administration of a 30-min isotonic saline challenge (5% of body weight, intravenously).

Results: Nine-month-old glucose-intolerant rats fed a normal NaCl diet did not manifest impairment in saline-stimulated diuresis and natriuresis. By contrast, 3-month-old glucose-intolerant rats fed a high NaCl diet displayed an exaggerated diuretic and natriuretic response to a saline challenge. Although saline-stimulated diuresis and natriuresis were enhanced in the hypertensive rats, they were reduced in the hypertensive glucose-intolerant animals. The reduction in renal excretory function of the latter group was caused by alterations in both glomerular function and tubular reabsorption of fluid and sodium.

Conclusions: The coexistence of systemic hypertension and glucose intolerance result in impaired renal excretory function. This defect could be important, as hypertension can develop before the onset of overt type 2 diabetes. Am J Hypertens 2002;15:58–65 © 2002 American Journal of Hypertension, Ltd.

Key Words: Glucose intolerance, hypertension, salt, renal function.

Glucose intolerance and insulin resistance are prominent features of the prediabetic state that precedes the onset of overt type 2 diabetes.1,2 Another condition that can develop before the onset of type 2 diabetes is hypertension, which is characterized by an abnormality of sodium homeostasis.3–5 Several lines of evidence suggest that coexistence of systemic hypertension and glucose intolerance could increase the risk of significant renal dysfunction relative to either disease alone. First, glucose intolerance and insulin resistance could alter renal sodium handling. Not only is renal glucose transport coupled with sodium flux but, also, insulin exerts an antinatriuretic effect.5,8,9 The latter effects are of importance because the kidney is the final arbiter of body fluid and sodium homeostasis. Second, hypertension is associated with altered renal sodium homeostatic mechanisms.10,11 Third, hypertension potentiates the adverse effects of diabetes and may also potentiate the adverse effects of the prediabetic state of glucose intolerance.3,7,12

To examine the effect of glucose intolerance, both alone and in combination with systemic hypertension, a neonatal streptozotocin-treated rat model was used. It is noteworthy that injection of streptozotocin into an adult rat produces an insulinopenic condition that resembles type 1 diabetes,13 whereas injection of streptozotocin into the neonate rat results in impaired insulin secretion and the progressive development of insulin resistance.14–17 These age-dependent differences in the response to streptozotocin are at-
tributed, in part, to partial regeneration of pancreatic \( \beta \)-cells by the neonate but not the adult rat.\textsuperscript{15} A prominent feature of the neonatal streptozotocin-treated Wistar-Kyoto (WKY) rat is glucose intolerance,\textsuperscript{15,18,19} a condition that precedes the onset of overt type 2 diabetes.\textsuperscript{2} However, the neonatal streptozotocin-treated WKY rat lacks the component of spontaneous hypertension.\textsuperscript{18} Therefore, the effect of prolonged disease duration (ie, 9 months) on renal function in the absence of hypertension can be evaluated. Nonetheless, because the combination of dietary NaCl excess and unilateral nephrectomy elevates blood pressure (BP),\textsuperscript{19,20} the impact of combined systemic hypertension and glucose intolerance can also be examined. A major advantage of this hypertensive glucose-intolerant rat model is the NaCl sensitivity of the hypertension, a condition analogous to the hypertension seen in type 2 diabetes.\textsuperscript{21} Therefore, the neonatal streptozotocin-treated WKY rat provides the flexibility of examining renal function in a model of combined glucose intolerance and NaCl-sensitive hypertension.

**Methods**

**Effects of Prolonged Glucose Intolerance on Kidney Function**

Wistar-Kyoto rats obtained from Harlan Laboratories (Indianapolis, IN) were bred at the Medical College of Georgia animal facility. To produce the model of glucose intolerance, 2-day-old male neonatal rats (\( n = 6 \)) were injected intraperitoneally with 90 mg/kg of streptozotocin.\textsuperscript{14,19} Control littermates (\( n = 6 \)) received an injection of citrate buffer (0.1 mol/L, pH 4.5). The animals were weaned at 21 days of age and maintained at constant humidity (60% ± 5%), temperature (24°C ± 1°C), and light cycle (0600 to 1800 h). Unless otherwise specified, the animals had free access to food and water.

At about 6 weeks of age, each rat underwent a glucose tolerance test; the test was repeated at about 9 months of age, just before initiating the renal function studies. After an overnight fast, blood glucose concentrations were determined before (ie, fasting) and after administration of a glucose challenge (2 g/kg intraperitoneally).\textsuperscript{18,19}

To prepare for the renal function studies in conscious animals, each rat underwent instrumentation, under ether anesthesia, with femoral vessel cannuli and a stainless steel bladder catheter.\textsuperscript{18–20,22} Two days after surgery, each rat was placed in an environmental conditioning unit (Braintree, MA); all rats were conditioned to the environmental parameters. A 30-min volume expansion (5% of the body weight) was initiated with isotonic saline containing \( ^3 \textrm{H} \)-inulin.\textsuperscript{18,20} Urine samples were collected at 15, 30, 60, and 90 min after initiation of the saline volume load. At the midpoint of each urinary collection period, 0.2 mL of arterial blood sample was collected for determination of plasma radioactivity and electrolyte concentration; the blood sample was replaced with an equal volume of isotonic saline.

\( \text{Na}^+ \) and \( \text{K}^+ \) were measured by flame photometry and used to calculate the sodium and potassium excretion rates.\textsuperscript{18,20} The GFR and fractional excretion of fluid (FEH2O) and sodium (FENa+) were calculated by using standard clearance formulas.\textsuperscript{20}

**Effects of Combined Glucose Intolerance and Hypertension on Kidney Function**

The glucose-intolerant rat was produced as described above. At 4 weeks of age, the animals were anesthetized with ether and underwent either a right nephrectomy (UNX) or sham operation.\textsuperscript{18–20,23} After a 2-week period to allow for compensatory renal hypertrophy, each rat underwent a glucose tolerance test as described above; the test was repeated at 12 weeks of age, just before beginning the renal function studies. Thereafter (ie, at 6 weeks of age), the animals were switched to a high (8%) NaCl diet for the remaining 6 weeks of the study. Renal function studies were conducted when the animals reached 3 months of age. As shown previously, dietary NaCl excess caused an increase in systolic blood pressure (SBP) in the UNX but not in the sham-operated rats.\textsuperscript{20} The protocol outlined above produced four experimental groups with the following designations: 8% sham control (normotensive control, \( n = 4 \)), 8% sham glucose-intolerant (normotensive glucose-intolerant, \( n = 4 \)), 8% UNX control (hypertensive control, \( n = 7 \)), and 8% UNX glucose-intolerant (hypertensive glucose-intolerant, \( n = 6 \)). Before performing the renal function studies, the animals were housed individually in metabolic cages. After 3 days of acclimation, two consecutive 24-h urine samples were collected. Daily protein excretion was determined and expressed as the average of two 24-h samples.\textsuperscript{21}

The protocol for renal function studies, the analysis of urine and plasma samples, and the calculation of data were carried out as outlined above. At the conclusion of the studies the animals were euthanized with an injection of pentobarbital (50 mg/kg intravenously) and the kidney weights (KW) were determined for each animal.

The use of animals in this study conformed to institutional guidelines on care and use of laboratory animals.
The fasting blood glucose levels were modestly higher in the 9-month-old rats that had been treated with streptozotocin at 2 days of age (121 ± 7 for streptozotocin group v 93 ± 4 mg/dL for control; P < .05). Furthermore, the streptozotocin-treated animals were clearly glucose-intolerant. After glucose challenge, the blood glucose content of the streptozotocin-treated rats reached a higher peak value and remained elevated for a more prolonged period of time than did the glucose levels in the control rats (353 ± 19 v 260 ± 31 [30 min], 297 ± 17 v 237 ± 18 [1 h], and 256 ± 26 v 159 ± 18 mg/dL [2 h]; P < .05).

The baseline rates of fluid and sodium excretion tended to be higher in the glucose-intolerant rats than in the controls (18.9 ± 2.0 v 12.8 ± 1.7 μL/min; sodium, 0.8 ± 0.3 v 0.4 ± 0.1 μEq/min). When either group of animals was subjected to a saline load, the rates of fluid and sodium excretion increased, with the largest increase occurring 30 min after initiation of the saline load (time course data not shown). The percentage of the fluid load that was excreted within 90 min of the challenge was similar in the control and the glucose-intolerant groups (39 ± 3 v 49 ± 8, respectively). However, the percentage of the sodium load that was excreted during this period was slightly higher in the glucose-intolerant rats than in the controls (52 ± 5 v 37 ± 4, respectively; P < .05). The baseline GFR was similar in the two groups (1.1 ± 0.1 v 1.1 ± 0.2 mL/min/g KW, respectively). After a saline load, GFR rose to a similar degree in the two groups. There was also an increase in the fractional excretion of fluid and sodium, with the cumulative (0 to 90 min) fractional excretion of sodium being greater in the glucose-intolerant group than in the control group (10.5% ± 0.7% v 7.9% ± 0.6%, respectively; P < .05). The baseline potassium excretion rate (2.7 ± 0.3 v 2.6 ± 0.3 μEq/min) as well as the amount of potassium excreted within 90 min of initiating the saline load (438 ± 30 v 455 ± 19 μEq) were similar in the glucose-intolerant and the control groups.

### Table 1. Features of the 3-month-old experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>Body Weight (g)</th>
<th>Kidney Weight (g)</th>
<th>Kidney Weight/Body Weight (mg/g)</th>
<th>Protein Excretion (mg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control (normotensive)</td>
<td>4</td>
<td>263 ± 4</td>
<td>2.4 ± 0.1</td>
<td>9.0 ± 0.3</td>
<td>71 ± 7</td>
</tr>
<tr>
<td>Sham glucose-intolerant (normotensive)</td>
<td>4</td>
<td>254 ± 10</td>
<td>2.0 ± 0.1*</td>
<td>7.8 ± 0.2*</td>
<td>74 ± 12</td>
</tr>
<tr>
<td>UNX control (hypertensive)</td>
<td>7</td>
<td>248 ± 4</td>
<td>1.6 ± 0.0†</td>
<td>6.5 ± 0.2†</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>UNX glucose-intolerant (hypertensive)</td>
<td>6</td>
<td>266 ± 7</td>
<td>1.6 ± 0.1†</td>
<td>6.2 ± 0.1†</td>
<td>83 ± 12</td>
</tr>
</tbody>
</table>

UNX = unilaterally nephrectomized.

* P < .05 compared with sham control group.
† P < .05 compared with their respective sham counterparts.

### Statistical Analysis

All data were analyzed by the analysis of variance (significance criteria of P < .05) with Duncan’s test used for comparison of the mean values. All data are reported as means ± SEM.

### Results

#### Effect of Prolonged Glucose Intolerance on Kidney Function

The BW of the 9-month-old glucose-intolerant rats was lower than that of the age-matched control group (409 ± 5 v 440 ± 9 g; P < .05). However, KW (3.06 ± 0.06 v 2.81 ± 0.12 g) and the KW to BW ratio of the control and glucose-intolerant rats were similar (7.0 ± 0.1 v 6.9 ± 0.3). No significant differences were noted between the control and glucose-intolerant groups relative to either the mean arterial pressure (129 ± 3 v 137 ± 3 mm Hg) or the heart rate (319 ± 9 v 314 ± 13 beats/min). In agreement with other studies, the mean arterial pressure and heart rate were not significantly affected by the administration of an acute isonicotic volume load (data not shown).

Body weight was similar among the four 3-month-old groups (Table 1). As expected, total KW was significantly higher in the two sham-operated, two-kidney groups than in the rats with only one kidney. However, total KW was less in the sham-operated glucose-intolerant rats than in the sham-operated controls. No differences were noted between the KWs of the UNX glucose-intolerant and UNX control rats (Table 1).

The fasting blood glucose level was similar among the four groups (Fig. 1A). After glucose challenge, the blood glucose concentration of the two glucose-intolerant groups remained significantly higher than that of the two control groups (Fig. 1A). Neither uninephrectomy nor the high NaCl diet affected blood glucose levels.

Systolic blood pressure fell within the normal range in both the sham-operated glucose-intolerant and the sham-operated control groups. Indeed, the lack of an effect of dietary NaCl excess on the BP of the normotensive sham-operated, two-kidney WKY rat has been consis-
tently demonstrated.\textsuperscript{22,24} On the other hand, unilateral nephrectomy was associated with a significant increase (approximately 25 mm Hg; \( P < .05 \)) in the SBP of both the glucose-intolerant and control rats (Fig. 1B).

The baseline excretion rates of fluid (22.5 ± 2.1 \( \mu \text{L/min} \)) and sodium (5.6 ± 0.5 \( \mu \text{Eq/min} \)) were lower in the normotensive control than in the normotensive glucose-intolerant rats. When the data were normalized relative to KW, one important difference was uncovered: namely, the baseline fluid and sodium excretion rates became greater in the hypertensive control rat than in the normotensive control rat (Table 2). None-

| Table 2. Urinary fluid, sodium, and potassium excretion of 3-month-old experimental groups |
|---------------------------------|-------------|----------------|----------------|----------------|
| Group                           | Baseline Fluid (\( \mu \text{L/min/g KW} \)) | Baseline \( \text{Na}^+ \) (\( \mu \text{Eq/min/g KW} \)) | Baseline \( \text{K}^+ \) (\( \mu \text{Eq/min/g KW} \)) | Total \( \text{K}^+ \) (\( \mu \text{Eq/90 min} \)) |
| Sham control (normotensive)     | 9.6 ± 1.0  | 2.2 ± 0.3       | 0.6 ± 0.1       | 220 ± 27       |
| Sham glucose-intolerant (normotensive) | 22.0 ± 5.5* | 4.3 ± 0.7*     | 0.4 ± 0.1      | 182 ± 14       |
| UNX control (hypertensive)      | 23.1 ± 5.6* | 5.1 ± 0.6*     | 0.9 ± 0.1      | 125 ± 7‡       |
| UNX glucose-intolerant (hypertensive) | 20.7 ± 5.5* | 4.3 ± 1.1*     | 1.1 ± 0.3†      | 180 ± 18       |

KW = kidney weight; other abbreviation as in Table 1.

\* \( P < .05 \) compared with sham control group.

\dagger \( P < .05 \) compared with sham glucose-intolerant group.

‡ \( P < .05 \) compared with other groups.
theless, no differences in the baseline fluid and sodium excretion rates of the hypertensive glucose-intolerant and the normotensive glucose-intolerant rats were noted on normalization of the data (Table 2).

The 3-month-old control rats excreted about 50% and 80% of the administered fluid and sodium loads within 90 min of the saline volume challenge (Fig. 2A and B). A larger percentage of the administered loads is excreted by the 3-month-old normotensive glucose-intolerant sham-operated rats than by the normotensive control sham-operated rats (Fig. 2A and B). Interestingly, the hypertensive control (UNX) and the normotensive control sham-operated rats excrete similar absolute percentages of the administered loads (Fig. 2A and B). However, when the data were normalized relative to KW (Fig. 2C and D), the hypertensive control (UNX) rats were found to excrete significantly more fluid and sodium than the normotensive control sham-operated group (Fig. 2C and D). In marked contrast to the control rats, unilateral nephrectomy in the glucose-intolerant rats fed a high NaCl diet was associated with a decline in saline-stimulated diuresis and natriuresis, an effect that was evident in both the normalized (Fig. 2C and D) and absolute excretion data (Fig. 2A and B).

Fig. 3A and B illustrates the effect of saline volume loading on both GFR, and Fig. 3C and D the fractional excretion of fluid and sodium. For all four experimental groups, volume loading caused an initial increase, followed by a decrease in GFR. By 15 min, a significant difference in GFR was noted between the two hypertensive and the two normotensive groups (Fig. 3A). When the data were expressed as the percentage of baseline, the saline-stimulated elevation in GFR was attenuated in the hypertensive glucose-intolerant group relative to the other groups (Fig. 3B). Significantly, the fractional excretion of fluid and sodium was higher in the normotensive glucose-intolerant rat (sham-operated glucose-intolerant) than in the normotensive control (sham-operated control) rat. Similarly, the hypertensive control (UNX control) group displayed a marked increase in the fractional excretion of fluid and sodium compared with the normotensive control (sham-operated control) group. Yet, when hypertension and glucose intolerance were combined, the fractional excretion fell relative to either the normotensive glucose-intolerant rats or the hypertensive controls (Fig. 3C and D).

Baseline potassium excretion was similar in the normotensive glucose-intolerant rats and the normotensive controls. Unilateral nephrectomy caused an increase in the baseline rate of potassium excretion in the glucose-intolerant and control groups (Table 2). Potassium excretion increased in all groups after administration of a saline volume load. Nonetheless, the amount of potassium excreted over the 90-min cumulative period was less in the UNX control group than in the other three groups (Table 2). The amount of protein excretion was similar in the four groups (Table 1). In agreement with a previous report,25
daily food and fluid intake were similar in the control and glucose-intolerant rats on either diet (data not shown).

Discussion
Effect of Glucose Intolerance on Kidney Function

The present study shows that the 9-month-old glucose-intolerant rat does not manifest impaired saline volume-induced diuresis and natriuresis, a feature that we also reported for the younger glucose-intolerant rat fed a normal NaCl diet. Indeed, these responses are exaggerated in the 3-month-old glucose-intolerant rat fed a high NaCl diet (Fig. 2). Interestingly, patients with uncomplicated type 2 diabetes do not display a deficit in the excretory responses to saline volume loading. However, 2 to 4 weeks after injection of streptozotocin, the anesthetized type 1 diabetic rat displays a marked reduction in the diuretic and natriuretic responses to saline volume loading. Thus, whereas marked impairment of the volume receptor reflex is a feature of the normotensive type 1 diabetic rat, the normotensive glucose-intolerant rat appears to use this mechanism efficiently.

The effects of diabetes on the volume reflex mechanism have received considerable attention, mainly because it is an important regulator of both blood volume and BP. Through this mechanism, changes in blood volume (eg, through the administration of a saline volume load) activate neuronal and humoral pathways that regulate renal and cardiovascular functions, thereby preserving blood volume homeostasis. The integrated cardiovascular and renal responses to volume expansion result from the coordinated activation of various components of the volume reflex. These include: 1) the afferent limb (volume receptors, electromechanical coupling, and afferent fibers); 2) central sites for neuronal processing of the afferent input; and 3) the efferent limb (release or action of humoral factors and renal sympathetic nerve activity).

The contribution made by individual components of the volume reflex to the exaggerated saline volume-induced renal responses in the glucose-intolerant rat remains largely uninvestigated. Nonetheless, at the level of the kidney, the data provide two lines of evidence supporting the notion that an alteration in tubular reabsorption activity, rather than the filtered load, is the most important factor contributing to the elevation in renal excretory function. First, the fractional excretion of fluid and sodium is significantly greater in young normotensive glucose-intolerant than the normotensive control rats fed the high NaCl diet. Second, the elevation in the glomerular filtration rate in response to saline loading is similar in the normotensive glucose-intolerant and the normotensive control rats.

The reason for the glucose intolerance–related alteration in renal tubular reabsorption activity is open to speculation. One possibility is that the exaggerated response to volume overload in glucose-intolerant rats is
related to a differential contribution from the pressure-diuresis-natriuresis mechanism. However, the observation that BP is similar between the two-kidney glucose-intolerant and control rats fed the high NaCl diet is inconsistent with this notion. Another possibility is that enhanced diuretic and natriuretic responses of the 3-month-old glucose-intolerant rat represent resistance to the sodium-retaining effects of insulin. Indeed, insulin resistance is a characteristic feature of the glucose-intolerant rat. However, the sodium-retaining property of insulin is reportedly preserved in type 2 diabetic patients. Nonetheless, the dietary NaCl intake of these patients is unclear. Clearly, further studies examining the effect of glucose intolerance and insulin resistance on renal sodium handling in relation to dietary NaCl manipulation are warranted.

**Effect of Combined Glucose Intolerance and Hypertension on Kidney Function**

Systemic hypertension is a common coexisting disorder in diabetes mellitus. Type 1 diabetic patients usually develop hypertension in association with overt diabetic nephropathy. By contrast, hypertension can develop either before or after the onset of overt type 2 diabetes. The incidence of both hypertension and type 2 diabetes increases with age, accounting for the observation that more than 90% of individuals with a dual diagnosis of diabetes and hypertension suffer from type 2 diabetes. Therefore, systemic hypertension is recognized as a feature of early type 2 diabetes, acting both as a risk factor for nephropathy and a contributor to its progression. Because glucose intolerance precedes the onset of fasting hyperglycemia and overt type 2 diabetes, coexistence of hypertension and glucose intolerance could either accelerate or exacerbate the eventual manifestation of renal dysfunction in this population.

The most important finding of this study is that the combination of hypertension and glucose intolerance causes a reduction in saline volume–induced diuresis and natriuresis. However, glucose intolerance alone does not impair renal function, whereas hypertension alone leads to an exaggerated renal response to saline loading. Although the mechanism underlying the effects of combined hypertension and glucose intolerance are not known, BP was similar in the two hypertensive groups, suggesting that the reduction in renal excretory function by the hypertensive glucose-intolerant group may involve an impairment in the pressure-natriuresis-diuresis relationship.

Several lines of evidence suggest that alterations in both glomerular function and tubular reabsorption activity contribute to the reduction in saline volume-induced diuresis and natriuresis in the hypertensive glucose-intolerant rat. First, the saline-stimulated rise in the GFR is attenuated in the hypertensive glucose-intolerant rat in comparison to the normotensive glucose-intolerant rat (eg, two-kidney glucose-intolerant rat fed the high NaCl diet). By contrast, no difference in GFR was noted between the hypertensive control and normotensive control groups (Fig. 3A and B). Thus, similar to human type 2 diabetes with coexisting systemic hypertension, concomitant systemic hypertension and glucose intolerance in rats is associated with glomerular dysfunction. Second, the hypertensive control rats displayed augmented fractional excretion of fluid and sodium but not an increase in the glomerular filtration rate. This is consistent with other reports indicating that the effect of high BP on diuresis and natriuresis is primarily mediated by a reduction in tubular reabsorption rather than an increase in the filtered load. By contrast, the fractional excretion of fluid and sodium tended to be lower in the hypertensive glucose-intolerant rats than in the normotensive glucose-intolerant rats, an effect that contributes to the reduction in excretion.

In conclusion, the normotensive glucose-intolerant rats fed a high NaCl diet in this study displayed an elevation in saline volume–induced diuresis and natriuresis. Similarly, NaCl-induced hypertensive control rats displayed a marked enhancement in the excretory efficiency of the remaining kidney. By contrast, the NaCl-induced hypertensive glucose-intolerant rats manifested a reduction in the diuretic and natriuretic efficiency of the remaining kidney. Although the deleterious impact of high BP on target organ function in diabetes is well recognized, this study clearly suggests that the systemic hypertension also exerts adverse consequences on kidney function during the “lead-in” phase (that is, impaired glucose tolerance) to overt type 2 diabetes.

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**References**