Induction of hyperglycemia in adult intrauterine growth-restricted rats: effects on renal function

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Lim K, Lombardo P, Schneider-Kolsky M, Hilliard L, Denton KM, Black MJ. Induction of hyperglycemia in adult intrauterine growth-restricted rats: effects on renal function. Am J Physiol Renal Physiol 301: F288–F294, 2011. First published April 20, 2011; doi:10.1152/ajprenal.00564.2010.—Intrauterine growth restriction (IUGR) leads to a reduction in nephron endowment at birth and is linked to renal dysfunction in adulthood. The aim of the present study was to determine whether kidneys of IUGR rat offspring are more vulnerable to a secondary insult of hyperglycemia. IUGR was induced in Wistar-Kyoto rats by maternal protein restriction. At 24 wk of age, diabetes was induced in male IUGR and non-IUGR offspring by streptozotocin injection; insulin was injected daily to maintain blood glucose levels at either a mild (7–10 mmol/l; n = 8/group) or a moderate (10–15 mmol/l; n = 8/group) level. At 32 wk of age, renal function was assessed using ultrasound and [3H]inulin and [14C]paraaminohippurate clearance techniques. Conscious mean arterial blood pressure and heart rate were unchanged in IUGR offspring. Relative kidney length was increased significantly in IUGR offspring, and renal function was altered significantly; of importance, there was a significant increase in filtration fraction, indicative of glomerular hyperfiltration. Induction of hyperglycemia led to marked impairment of renal function. However, the response to hyperglycemia was not different between IUGR and non-IUGR offspring. Maintaining blood glucose levels at a mild hyperglycemic level led to marked improvement in all measures of renal function in IUGR and non-IUGR offspring. In conclusion, while IUGR offspring showed evidence of hyperfiltration, the response to hyperglycemia was similar in IUGR and non-IUGR kidneys in adulthood. Importantly, maintaining blood glucose levels at a mild hyperglycemic level markedly attenuated the renal dysfunction associated with diabetes, even in IUGR offspring.

METHODS

Animals and Diet Treatment

Ten-week-old female and male Wistar Kyoto breeder rats were obtained from the Australian Resource Centre (Perth, Australia). The female rats were divided into two groups and fed either a normal protein diet (NPD) that contained 20% casein, or a low-protein diet (LPD) that contained 8.7% casein, for 2 wk before mating (to familiarize the dams to the diets), during pregnancy, and 2 wk after birth (since nephrogenesis in the rat continues in the first 2 wk postnatally). Normal rat chow and water were administered ad libitum after weaning until 32 wk of age. The semipurified diets were commercially available from Glen Forrest Stockfeeders, Western Australia. The nutrient content of the NPD and LPD semipurified diets was equivalent, except for starch, which was varied to ensure that the diets were close to isocaloric (Table 1) (24). The breeder rats (16 dams) were housed individually and maintained at an ambient temperature of 21°C with a 12:12-h day-night cycle. To prevent stress to the dams, the pups were not handled until 3 days after birth. At postnatal day 3, all litters were reduced to eight pups per dam. Litter size ranged from 8 to 12 pups/litter, and there was no difference in litter size between groups. There were eight dams in each of the NPD

THE PREVALENCE OF TYPE 1 AND type 2 diabetes is currently increasing in developed countries, and it is the leading cause of end-stage renal disease (3, 50). Importantly, the natural progression of renal injury can be prevented or delayed in diabetics by tight glycemic control (28, 38, 42).

Over the past two decades, epidemiological studies have clearly shown an association between low birth weight (birth wt <2.5 kg) and risk of adult renal disease (21, 36, 40, 44, 45). The incidence of low birth weight is increasing in developed countries, largely because of an increasing incidence of placental insufficiency (31, 32) and preterm birth (14a, 14b, 2), and remains highly prevalent in underdeveloped countries largely as a result of maternal malnutrition (9, 13, 15) and infection (1, 4, 22). Importantly, the association between low birth weight and increased risk of adult disease may, in part, be linked to the observed reduction in nephron endowment in individuals that were intrauterine growth restricted (IUGR). Because nephrogenesis is complete in humans by ~36 wk of gestation (19) (and around postnatal day 8 in rats; see Refs. 23 and 41) with no new nephrons formed after this time, a congenital nephron deficit resulting from IUGR is likely to render the kidney vulnerable to secondary postnatal insults, and a number of experimental studies support this concept (27, 33, 52).

In this study, we address the hypothesis that a congenital nephron deficit, as a result of IUGR, renders the kidney vulnerable to a secondary insult of hyperglycemia leading to exacerbated renal dysfunction in adulthood. We propose that these changes occur independent of changes in arterial pressure and can be attenuated by lowering blood glucose to a mild hyperglycemic level. Hence, our aim was to examine the effect of induction of hyperglycemia on renal function in IUGR adult rat offspring when blood glucose levels were maintained at mild or moderate levels. To induce IUGR, we used a model of maternal protein restriction, during pregnancy and lactation, where the growth trajectory of the offspring remains lower after birth (no catch up in growth) (52) and the offspring do not develop hypertension (52). In this model, we have previously shown that there is about a 25% reduction in nephron endowment in the IUGR offspring (51, 52).

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and LPD groups. When the pups were weaned, one male pup per dam was allocated to each of the control, mild hyperglycemic, and moderate hyperglycemic groups. Hence, there was always only one pup per litter in each of the experimental groups. At 24 wk of age, rats assigned to the hyperglycemic groups were injected with a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) to induce diabetes/hyperglycemia. Before injection of STZ, there was no difference in the blood glucose levels between the LPD and NPD offspring (4.03 ± 0.07 and 4.16 ± 0.04 mmol/l, respectively). Control rats (n = 8) were injected with saline. Three days after STZ injection, blood glucose levels were maintained at either a mild blood glucose level in the rats assigned to the mild hyperglycemic group (7–10 mmol/l; n = 8) or at a moderate blood glucose level in those rats assigned to the moderate hyperglycemic group (10–15 mmol/l; n = 8). To do this, blood glucose levels were measured daily (via a drop of blood from the tail artery), and insulin was injected (2–4 IU) subcutaneously to maintain blood glucose levels within the nominated range. The animal experiments were approved by the Monash University Biomedical Ethics Committee, and treatment and care of the animals conformed to the Australian Code of Practice for the care and use of animals for scientific purposes.

### Ultrasound Analyses of Renal Function

Renal ultrasound was performed at 31 wk of age using a Philips HDI5000 SonoCT system (Universal Diagnostic Solutions). Rats (n = 6 or 7 rats/group) were anesthetized with isoflurane, and the hair over the back of the rat was shaved. Warmed coupling gel was applied to the rats’ skin to ensure good contact with the L 12–5-MHz transducer. The kidneys were located using B-mode imaging, and the maximum long axis was measured. Each renal artery was located using color Doppler initially, and then peak renal artery blood flow velocity was measured with spectral Pulsed Doppler using the smallest sample gate size (1 mm). Each variable was measured in five to six consecutive traces, and the mean peak amplitude was used in the analysis. The ultrasound setting selected was pediatric-neorenal (18, 34).

### Measurement of Heart Rate and Mean Arterial Pressure

At 32 wk of age, rats (n = 8/group) were anaesthetized, and a catheter was inserted in the tail artery. Following an hour of recovery, data collection began. Mean arterial pressure and heart rate were measured for 30 min in conscious rats (52).

### Determination of Effective Renal Plasma Flow, Glomerular Filtration Rate, and Urinary Sodium and Protein Concentrations

The rats were reanaesthetized using inactin (150 mg/kg body wt), [3H]insulin (20 μCi/ml), and [14C]para-aminohippurate (PAH) (0.1 μCi/ml) and were infused via a jugular vein catheter at 3 ml/h. A catheter was inserted in the urinary bladder to allow urine collection and used for clearance calculations (16, 17). After a 1-h equilibration period following [3H]insulin and [14C]PAH infusion, urine was collected for 30 min. A 1-ml arterial blood sample was taken at the midpoint of the urine collection and used for clearance and hematocrit measurements, respectively. On completion of the renal function studies, while the rats were still anaesthetized, the kidneys were perfusion fixed using 4% paraformaldehyde in 0.1 mol/l phosphate buffer at a perfusion pressure of 120 mmHg (39). Urinary sodium concentration was measured using a RapidChem 744 Electrolyte Analyzer (Bayer Australia Limited). Urinary protein concentrations were determined by the Bradford method (Thermo Scientific, Rockford, IL).

### Histological Analysis of Glomerulosclerosis

Whole kidneys were embedded in paraffin, sectioned at 4 μm, and stained with periodic acid-Schiff reagent to highlight the extracellular matrix and counterstained with hematoxylin to identify the cells. In a representative intact section, taken through the hilus, evidence of glomerulosclerosis was assessed by systematically sampling glomeruli throughout the kidney sections (46).

### Statistical Analysis

All data are expressed as means ± SE. Statistical analyses were undertaken using GraphPad Prism (version 5.00; GraphPad Software, San Diego, CA). Body weights at 3 days of age were analyzed using an unpaired t-test. At 32 wk of age, data were analyzed using a two-way ANOVA with the following factors: maternal diet (P0; LPD or NPD), hyperglycemic treatment (Pr; control, mild, or moderate), and their interaction (P0 × Pr). A Tukey’s post hoc test was also applied to data to examine whether there were significant differences between groups. Statistical significance was accepted at the level of P < 0.05.

### RESULTS

#### Body Weight and Kidney Weight

Body weight was significantly lower (24% reduction, P < 0.001) at 3 days of age in the LPD offspring compared with NPD offspring (4.4 ± 0.1 and 5.8 ± 0.2 g, respectively). At 32 wk of age, the body weights of the LPD offspring remained significantly lower (P0 < 0.0001) compared with the NPD offspring. When hyperglycemia was induced, there was a significant decrease in body weight (P1 < 0.0001) in both the NPD and LPD hyperglycemic groups (Table 2). There was a significant reduction (P2 < 0.0001) in kidney weights in the LPD offspring compared with NPD controls (Table 2). However, when adjusted to body weight, there was no significant difference in relative kidney weight between the NPD and LPD offspring. Induction of hyperglycemia had no significant effect on kidney weights or kidney weight-to-body weight ratio in all groups (Table 2).

#### Mean Arterial Pressure and Heart Rate

At 32 wk of age, there was no significant difference in conscious mean arterial pressure or heart rate between LPD and NPD offspring. Increasing levels of hyperglycemia did not affect mean arterial pressure or heart rate in LPD or NPD offspring (Table 2).

#### Renal Ultrasound Assessments

Kidney length. Maximum kidney length was not significantly different between LPD and NPD offspring at 32 wk of age.
age, and induction of hyperglycemia did not affect kidney length. However, when adjusted to body weight, there was a significant increase ($P_D = 0.007$) in relative kidney length in the LPD offspring compared with NPD controls, and induction of hyperglycemia also led to a significant increase ($P_T = 0.004$) in relative kidney length, although the response was not different between groups (Table 2).

Peak systolic renal blood flow velocity. There was a significant reduction in relative peak systolic renal blood flow velocity in LPD offspring ($P_D = 0.008$) and as a result of hyperglycemia ($P_T = 0.0006$). The response to hyperglycemia was not different between LPD and NPD offspring. Post hoc analyses showed that moderate hyperglycemia led to a significant reduction in peak renal blood flow velocity per kidney weight in the LPD offspring, and this was restored back to control values when blood glucose levels were reduced in the mild hyperglycemic group (Fig. 1).

Renal Function

Renal vascular resistance. Renal vascular resistance (uncorrected for kidney wt) was significantly greater in the LPD control group compared with the NPD control group ($P < 0.02$; $10.3 \pm 0.6$ vs. $8.1 \pm 0.6$ mmHg·min$^{-1}$·l$^{-1}$, respectively), although this difference was no longer evident when renal vascular resistance was corrected for kidney weight. Renal vascular resistance (corrected for kidney wt) was greater in the hyperglycemic groups ($P_T = 0.009$), but the response was not different between the LPD and NPD offspring ($P_{D \times T} = 0.9$). The effect of hyperglycemia on renal vascular resistance per kidney weight was attenuated with improved glycemic control (in the mild hyperglycemic groups) (Fig. 2A).

Renal plasma flow. Absolute renal plasma flow (ml/min) was lower in control LPD offspring compared with control NPD offspring ($P < 0.004$; $6.0 \pm 0.3$ vs. $7.8 \pm 0.4$ ml/min, respectively). When adjusted to kidney weight, there was no significant difference in renal plasma flow per kidney weight between NPD and LPD offspring. However, hyperglycemia led to a marked decrease in relative renal plasma flow per kidney weight ($P_T = 0.0016$). By maintaining blood glucose levels at or below 10 mmol/l in the mild hyperglycemic NPD and LPD offspring, relative renal plasma flow was not significantly different (Tukey’s post hoc analyses) compared with their respective controls (Fig. 2B).

Glomerular filtration rate. Total glomerular filtration rate (GFR) was significantly lower in control LPD compared with NPD offspring ($P < 0.001$; $1.4 \pm 0.2$ vs. $1.7 \pm 0.03$ ml/min, respectively). When adjusted for kidney weight, there was no significant difference in GFR per kidney weight between LPD and NPD offspring ($P_D = 0.8$). Hyperglycemia led to a marked decline ($P_T = 0.002$) in GFR per kidney weight, but, when blood glucose levels were reduced in the mild hyperglycemic offspring, GFR per kidney weight was not different from their respective controls (Fig. 2C).

Urine flow rate, sodium excretion rate, and urinary protein excretion. Urine flow rate was significantly lower in LPD offspring compared with NPD offspring ($P < 0.001$; $0.012 \pm 0.002$ vs. $0.007 \pm 0.001$ ml/min in NPD and LPD controls, respectively) but was not different when adjusted to kidney weight. Induction of hyperglycemia led to a significant increase in urine flow rate/kidney weight ($P_T = 0.0002$), but the response was not different between the LPD and NPD offspring. By lowering blood glucose levels (in the mild hyperglycemic groups), urine flow rate and relative urine flow rate were restored to the level of the control group. Urinary sodium excretion was not significantly different between any of the groups ($P_D = 0.4$, $P_T = 0.14$, and $P_{D \times T} = 0.16$; data not shown). Urinary protein excretion

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**Table 2.** Body weight, mean arterial pressure, heart rate, kidney weight, and relative kidney length at 32 wk of age in control NPD and LPD offspring and those exposed to mild or moderate hyperglycemia from 24 to 32 wk of age.

<table>
<thead>
<tr>
<th></th>
<th>NPD</th>
<th>LPD</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Mild</td>
<td>Mod</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>455 ± 8</td>
<td>429 ± 6</td>
<td>405 ± 3</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>102 ± 2</td>
<td>101 ± 1</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>300 ± 1</td>
<td>301 ± 1</td>
<td>301 ± 1</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>1.80 ± 0.07</td>
<td>1.74 ± 0.09</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>Relative kidney wt, mg/g</td>
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<td>4.07 ± 0.23</td>
<td>4.29 ± 0.18</td>
</tr>
<tr>
<td>Relative kidney length, mm</td>
<td>0.045 ± 0.001</td>
<td>0.048 ± 0.002</td>
<td>0.050 ± 0.002</td>
</tr>
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</table>

Values are means ± SE. Mild, mild hyperglycemic group; Mod, moderate hyperglycemic diet; Cont, control diet. Data were analyzed by a 2-way ANOVA with maternal diet (D: NPD or LPD), treatment (T; mild or moderate), and maternal diet × treatment (D×T) interaction as factors.
tended to be greater in the LPD offspring ($P_D = 0.056$), and, although hyperglycemia was associated with an increase in urinary protein excretion ($P_T = 0.12$) (Fig. 2, D and E).

**Filtration fraction.** Filtration fraction was significantly greater in LPD offspring compared with NPD offspring ($P_D = 0.02$); this was associated with renal plasma flow being $\sim 24\%$ and GFR being $\sim 18\%$ lower in LPD compared with NPD.
offspring. Induction of hyperglycemia had no effect on filtration fraction in all groups ($P_T = 0.7$) (Fig. 2F).

**Histological Analysis of Glomerulosclerosis**

There was no evidence of glomerulosclerosis in the kidneys of any of the experimental groups. The glomerulosclerosis index score was zero in all groups.

**DISCUSSION**

The findings of the present study clearly demonstrate marked impairment of renal function in response to hyperglycemia in all groups, and this was independent of a prenatal LPD-induced nephron deficit. Importantly, the response to hyperglycemia was not influenced by nephron endowment. Animals with a congenital nephron deficit were no different from controls in their ability to recover from the adverse effects of moderate hyperglycemia.

**IUGR and Kidney Growth**

In this study, there was no significant difference in relative kidney weight; however, there was a significant increase in relative kidney length in the IUGR offspring, as measured in vivo using ultrasound. It is likely that this renal hypertrophy is a compensatory response in an attempt to maintain renal function when there is a marked reduction in nephron endowment. In this model of maternal protein restriction, there is an approximate 25% reduction in nephron number (52).

**IUGR is Linked to Adult Renal Disease**

In recent years, epidemiological evidence clearly demonstrates an association between low birth weight and adult renal disease (21, 36, 40, 44, 45). In a recent meta-analysis by White et al. (45), it was shown that individuals with low birth weight (birth wt <2.5 kg) have a 70% greater risk of developing chronic kidney disease in later life. In support of these epidemiological studies, evidence of altered renal function is a consistent finding in experimental models of IUGR (48, 49, 52). In the present study, there were marked differences in renal hemodynamics and function in LPD offspring compared with NPD offspring. On a whole kidney basis, renal vascular resistance was greater and renal plasma flow, GFR, and urine flow rate were reduced in LPD offspring. Some of these differences could be accounted for by the reduction in body size and thus kidney size in the IUGR offspring such that there was no difference in GFR when adjusted for kidney weight. However, although renal plasma flow was ~24% lower in the LPD offspring, GFR was ~18% lower, resulting in the filtration fraction being significantly greater in LPD offspring, indicative of glomerular hyperfiltration. The greater filtration fraction was associated with a modest proteinuria and evidence of altered renal growth (greater relative renal length) in the LPD offspring. Hyperfiltration, by definition, must be the result of an increase in glomerular filtration pressure and/or an increase in the glomerular capillary permeability coefficient. Previously, in glial-derived neurotrophic factor homozygous mice, we demonstrated that a deficit in glomerular capillary surface area, associated with a congenital nephron deficit, can be corrected for in adulthood by an increase in the total length of glomerular capillaries, without an increase in arterial pressure (39). Thus we suggest that hyperfiltration in the LPD offspring is associated with an increase in glomerular capillary filtration surface area and/or permeability; further studies are required to address this hypothesis. The alterations we observed in renal function in the LPD offspring are at this stage modest; however, it is likely that these would be accentuated with age.

Our findings of no difference in the levels of glomerulosclerosis, renal plasma flow relative to kidney weight, or GFR relative to kidney weight in LPD offspring support our previous findings in this LPD model at 24 wk of age (52). To the contrary, other laboratories have reported a significant reduction in GFR per gram of kidney tissue in adult LPD offspring (47), which may relate to an elevation in blood pressure and/or catch-up growth in the offspring in these previous studies (8, 47). In our LPD model, which is similar to many other laboratories (12, 20, 30, 43), the offspring do not develop hypertension, that is, the LPD offspring remain normotensive in adulthood (51, 52).

**Renal Function When IUGR is Combined With Hyperglycemia**

It has long been recognized that diabetes leads to renal disease, and, at the present time, diabetic nephropathy (associated with types 1 and 2 diabetes) is the leading cause of end-stage renal disease (3, 5, 11, 14, 35). In the present study, the renal response to an elevation in blood glucose, contrary to our hypothesis, was not different in the LPD and NPD kidneys. However, our findings suggest that, over a prolonged period of time, the renal dysfunction associated with hyperglycemia might have become aggravated in IUGR offspring. Indeed, there have been a number of experimental studies that have demonstrated vulnerability of IUGR kidneys to secondary renal insults (10, 20, 29, 52). Central to the pathological processes within the kidney associated with diabetes are high blood sugar levels (37) and the subsequent formation of advanced glycation end products (AGEs; formed by the glycosylation of proteins) (26). Of particular relevance, in previous studies, we have shown that IUGR rat offspring are vulnerable to infusion of AGEs in the absence of high blood glucose levels. We showed marked accumulation of AGEs within the kidneys of LPD offspring with a congenital nephron deficit and a marked upregulation of key molecules associated with renal disease (52). Hence, it is likely that AGE formation, which is well known to be high in STZ-diabetic rats, may be the mediator of the renal dysfunction observed in the present study.

**Maintaining Blood Glucose Levels at a Mild Hyperglycemic Level Attenuates the Renal Dysfunction in Diabetic Offspring**

It is popular opinion that the reduced nephron endowment associated with IUGR is a major contributor to the elevated risk of renal disease in subjects that were born of low birth weight (21, 25, 27). Indeed, it is the loss of a critical reserve of glomeruli through disease that ultimately leads to end-stage renal disease; therefore, it is likely that disease progression will be accelerated when nephron endowment is already reduced at disease onset (29). Hence, the question arises that is of utmost clinical importance; can the renal disease in diabetic IUGR offspring be prevented and/or attenuated given that they have...
a reduced nephron endowment? Encouragingly, the findings of the present study demonstrate that this is achievable by maintaining blood glucose at a mild hyperglycemic level. We showed that, if the blood glucose levels in hyperglycemia rats were maintained at or below 10 mmol/l by daily injection of insulin, then the deleterious effects on renal function could be markedly attenuated and, importantly, GFR per gram of kidney tissue was restored to normal, even in the presence of a congenital nephron deficit. Hence, the findings reinforce the importance of tight glycemic control in the prevention of diabetic renal disease.

Renal Doppler Ultrasound is a Good Noninvasive Measure of In Vivo Renal Blood Flow

Importantly, in the current study, we have shown that Doppler ultrasound measurements of relative peak renal blood flow velocity are in accordance with renal plasma blood flow measurements as determined with PAH clearance techniques; certainly, a 25% difference in renal blood flow is readily detected. Hence, validation of the measurements of renal blood flow velocity measurements with “gold standard” PAH clearance techniques provides the opportunity now to assess in vivo renal blood flow over time within the same animal in longitudinal renal programming studies.

In conclusion, we have shown that IUGR, because of maternal protein restriction, does not result in an increase in arterial pressure in adulthood, yet there is evidence of renal hyperfiltration. Thus we suggest that physiological adaptations in renal growth can adequately compensate for a congenital nephron deficit to normalize whole kidney renal function in the absence of an increase in arterial pressure. However, contrary to our hypothesis, renal function in adult IUGR offspring was not more adversely affected to superimposed hyperglycemia. Induction of hyperglycemia led to a marked decline in renal function (increased renal vascular resistance and reduced GFR) in all groups of offspring. Maintaining blood glucose levels at a mild hyperglycemic level markedly attenuated the adverse effects of hyperglycemia, and, importantly, IUGR offspring were equally able to recover to near-normal function when glycemic control was improved. These findings thus highlight the importance of maintaining tight glycemic control in diabetic subjects to prevent or retard the progression of renal disease.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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RENAI AL FUNCTION IN ADULT HYPERGLYCEMIC IUGR RATS


