Renal Enlargement Precedes Renal Hyperfiltration in Early Experimental Diabetes in Rats

MARTIN BAK,*† KLAUS THOMSEN,† THORA CHRISTIANSEN,‡ and ALLAN FLYVBJERG*
*Medical Research Laboratories, Institute of Experimental Clinical Research, †Institute for Basic Psychiatric Research, and ‡Magnetic Resonance Research Centre, Aarhus University Hospital, Denmark.

Abstract. The order of appearance between renal hypertrophy and hyperfunction in early experimental diabetes is still disputed. The reason for previous discrepant results is believed to be methodologic problems, as most previous studies of renal function have been performed in anesthetized animals. In the present study in nondiabetic and streptozotocin-diabetic animals, renal volume was measured by a noninvasive magnetic resonance imaging technique, while renal function parameters were measured in conscious, chronically catheterized animals. To avoid artifacts caused by the procedures associated with induction of streptozotocin-diabetes (fasting, brief anesthesia, and transient hypoglycemia) on renal growth and function, diabetic animals were injected with insulin to obtain euglycemia for 4 d before study start. At day 0, insulin administration was withdrawn and all animals developed hyperglycemia within 12 h. Renal volume and kidney function were measured on days 0, 1, 2, 3, 5, and 7. Renal enlargement was detectable at day 1 (20%) and reached an increase of 40% at day 7. No changes were seen in effective renal plasma flow or effective renal vascular resistance within the first 7 d after development of hyperglycemia. GFR tended to rise on day 5 and was increased by 16% at day 7. The absolute proximal reabsorption showed a pronounced rise (30%) at day 7, whereas no change was seen in the proximal tubular fluid output. It is concluded that renal enlargement precedes renal hyperfunction in the early phase after onset of experimental diabetes.

The diabetic kidney hypertrophy-hyperfunction syndrome is a well established phenomenon that may contribute to later development of diabetic kidney disease. Renal enlargement occurs very shortly after induction of hyperglycemia, and it has been shown that the protein content rises in parallel to the kidney weight. Similarly, an increased protein/DNA ratio has been measured after a few days, indicating hypertrophy of the cells (1,2).

Several experimental studies have reported increased kidney function in animals with moderate hyperglycemia (3,4). Although the existence of a tight relationship between increased renal mass and hyperfunction is well described in the well established diabetic state, the order of appearance of changes between renal volume and function in the initial dynamic phase after onset of diabetes is still controversial (5,6).

The above-mentioned controversy may be founded in artifacts associated with induction of diabetes and the fact that renal function parameters have usually been measured in anesthetized animals (7). Due to anesthesia and other types of stress, the GFR may be influenced in diabetic rats relative to control rats (8).

The aim of this study was to examine the time relationship between changes in renal volume and function in the early phase of experimental diabetes under circumstances in which the possible drawbacks of previous protocols were avoided. Our results show that increase in kidney size precedes the increase of GFR by several days.

Materials and Methods

Animals

Specific pathogen-free adult female Sprague Dawley rats (Møllegaards Avlslab., Eiby, Denmark) weighing 200 to 260 g were used. Rats were housed one per cage in a room with a 12:12 h artificial light cycle, temperature 21 ± 2°C, and humidity 55 ± 2%. The rats were fed a standard diet (R3; Lactamin, Stockholm, Sweden) containing 21% protein, 200 mmol/kg Na+, and 200 mmol/kg K+ for at least 2 wk before initiation of the experiment. Three days before the experiment, the diet was changed to a diet containing lithium chloride, 10 to 12 mmol/kg dry weight, to obtain measurable plasma lithium concentrations without influencing renal function (9). Rats had free access to food and tap water throughout the experiment.

One week before the experiment, the animals were anesthetized with halothane/N2O. Using aseptic surgical techniques, sterile Tygon™ catheters (Norton Performance Plastics, Arkon, OH) were advanced into the abdominal aorta and the inferior vena cava via the femoral vessels. A sterile chronic suprapubic catheter was implanted into the bladder. All catheters were produced and fixed, with minor modifications, as described previously (7). After instrumentation, the rats were infused with saline subcutaneously (5 ml) and given a long-acting analgesic, Buprenorphinum (Temgesic™; Reckitt & Colman, Hull, United Kingdom), subcutaneously and housed individually. After a recovery period of 5 to 6 d, the rats were acclimatized to restriction by daily training sessions in restraining cages. The duration of each daily session was gradually increased from 1 to 3 h a day.
Induction of Diabetes

The rats were randomly allocated into diabetic (D) and control (C) groups. Control rats (C) were not treated with streptozotocin (STZ) or insulin. In the diabetic groups (D), diabetes was induced by intravenous injection of STZ (40 mg/kg body wt) in acetic 154 mmol/L NaCl (pH 4.5) after 12 h of food deprivation. Eighteen hours after STZ administration, and daily thereafter, the animals were weighed, urinalyses were performed for glucose and ketones using Neostix 4T™ (Amers Limited, Stoke Poges, Slough, United Kingdom), and tail vein blood glucose was determined by Haemoglucomat 1-44T™ and Refloxit II™ reflectance meter (Boehringer Mannheim, Mannheim, Germany). Insulin treatment with a very long-acting, heat-treated Ultralente Insulin™ (Novo Nordisk A/S, Bagsvaerd, Denmark) was initiated 18 h after administration of STZ after having checked that all animals had blood glucose levels above 15 mmol/L. Insulin was given in an initial dose of 4 to 8 U, followed by 1 to 3 U daily for 4 d to attain euglycemia (10). Day 0 was defined as the day of insulin withdrawal. This preexperimental insulin treatment was carried out to attain recovery after fasting, brief anesthiesia, transient STZ-induced hypoglycemia, and acute STZ toxicity on renal function parameters in the initial phase.

Experimental Protocols

The rats were randomized to renal clearance or magnetic resonance imaging (MRI) measurement. MRI scanning could not be performed in the rats used for clearance experiments due to the implanted steel bladder catheter.

Renal Clearance Protocol

The experiments were carried out between 8 a.m. and 1 p.m. The rats were transferred to a restraining cage and connected to infusion pumps via the vein catheter and to a BP transducer via the arterial catheter. Urine was collected in three periods of 20 min preceded by an equilibration period of 105 min. Throughout the experiment, a half isotone saline (77 mM NaCl) was infused at a rate of 70 μl/min to maintain a minimum urine flow necessary for accuracy of the bladder emptying. 14C-tetraethylamonium bromide (0.83 μCi/ml; New England Nuclear, Boston, MA), together with 3H-inulin (2.5 μCi/ml; Amersham, Rainhain, United Kingd) and LiCl (13 mmol/L), were infused together with the saline as markers of effective renal plasma flow (ERPF), GFR, and tubular fluid delivery from proximal tubules (Vprox), respectively. A bolus of markers four times the continuous infusion velocity was given in the first 15 min. Blood samples (200 μl) were drawn from the arterial catheter after 105 and 165 min. Blood substitution with donor blood was given after each blood sample. Mean arterial BP was recorded continuously using a Unisense transducer (Baxter, Irvine, CA) connected to a preamplifier and PC registration. Clearance experiments were carried out in diabetic rats on day 0 (n = 5), days 1, 2, 3, and 5 (n = 6), and day 7 (n = 8), and in the control group at day 0 (n = 5), day 5 (n = 4), and day 7 (n = 8).

MRI Protocol

Kidney volume was estimated by a validated MRI technique in a separate group of animals (11). Images were obtained using a Sisco 300/183 Horizontal Bore Scanner (Sisco, Sunnyvale, CA) operating at 7 tesla. Fifteen minutes after barbital injection, the animals were injected intravenously with 200 μl of Gadolinium (Gd(DTPA); Schering, Germany), and T1-weighted Spin Echo pictures were obtained within the next 10 min, with echo time = 10 msec and recovery time = 500 msec. Inplant resolution was 0.3 mm × 0.3 mm and slice thickness was 1 mm. The slice gab varied between 0.1 and 0.7 mm covering the kidney volume within 16 slices.

Analysis

Urine volume was determined by gravimetric means. Li+ concentration was determined in plasma and urine by flame emission spectrophotometry and atomic absorption spectrophotometry, respectively. 14C-tetraethylamonium (TEA) and 3H-inulin in plasma and urine were determined by dual label liquid scintillation counting (Wallac™ model 1409; Helsinki, Finland). Sample (15 μl) and 285 μl of water were mixed with 2.5 ml of scintillation liquid (Ultima Gold™; Packard Instruments, Meriden, CT). Correction of dpm was performed by automatic efficiency control.

Calculations

Renal clearances (C) were calculated by the standard formula:

\[ C = \frac{U \times V}{P} \]  

where U is urine concentration, V is urine flow rate, and P is plasma concentration.

In previous studies, the renal extraction fraction of TEA has been shown to approximate 90%, and the validity of TEA as an estimate of ERPF has been documented (12,13). With the concentration of TEA used in this study, TEA is without effects on efferent renal sympathetic nerve activity in rats (14). By use of CTEA, Cin, and CLI, the following parameters were calculated:

\[ \text{ERPF} = \frac{C_{\text{TEA}}}{C_{\text{IN}}} \]  
\[ \text{GFR} = \frac{C_{\text{IN}}}{C_{\text{LI}}} \]  
\[ V_{\text{prox}} = \frac{C_{\text{IN}}}{C_{\text{LI}}} \]  
\[ \text{APR} = \frac{C_{\text{IN}} - C_{\text{LI}}}{\text{MAP} - 5} \times (1 - \text{Hct})/C_{\text{TEA}}, \text{where Hct is the hematocrit.} \]

It is assumed that the renal venous pressure was 5 mmHg throughout the experiment.

Statistical Analyses

All values are presented as mean ± SEM. Overall statistical analysis comparisons were performed by one-way ANOVA (between groups and within groups) or two-way ANOVA for two-way classified data (group and time). Individual comparisons within (day 7 versus day 0) or between (control versus diabetes on day 7) groups were performed by subsequent use of t test for unpaired data. Differences were considered statistically significant at P < 0.05.

Results

Changes in Body Weight and Blood Glucose in Diabetic Animals after Withdrawal of Insulin Treatment

The body weight of the diabetic rats showed a decrease during the first days after development of hyperglycemia and then became stable (Table 1). The control rats showed no change in body weight within the 7 d (data not shown). The
blood glucose concentration was in the normal range at day 0, and rose within 24 h to values above 15 mmol/L in both groups of STZ-treated rats intended for either MRI or renal clearance measurements (Table 1). In the days preceding day 0, the blood glucose concentrations were in the normal range in the diabetic rats (5.3 ± 1.3, 8.5 ± 1.5, and 5.6 ± 1.7 mM at days 3, 2, and 1 before day 0, respectively). In the control rats, the blood glucose concentrations were in the normal range at the days of measurement (data not shown). None of the diabetic animals developed ketonuria after insulin withdrawal. There were no significant changes in mean arterial BP (approximately 110 mmHg) (data not shown) in any of the renal clearance animals. The plasma lithium concentration in the rats used for renal clearance was within the appropriate range in both groups, although it was slightly lower in the diabetic than in the control group (0.14 ± 0.006 mM versus 0.19 ± 0.01 mM).

**Table 1. Body weight and blood glucose after induction of diabetes in the two groups of rats examined by MRI scanning or by clearance measurements**

<table>
<thead>
<tr>
<th>Day</th>
<th>Body Weight (g)</th>
<th>Blood Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRI</td>
<td>Clearance</td>
</tr>
<tr>
<td>0</td>
<td>249 ± 5</td>
<td>262 ± 4</td>
</tr>
<tr>
<td>1</td>
<td>242 ± 2</td>
<td>252 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>231 ± 5</td>
<td>242 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>227 ± 2</td>
<td>237 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>229 ± 3</td>
<td>236 ± 3</td>
</tr>
<tr>
<td>7</td>
<td>226 ± 4</td>
<td>239 ± 7</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM. MRI, magnetic resonance imaging.*

When studying the time pattern at which renal enlargement and renal hyperfiltration occur in the initial phase after induction of experimental diabetes, there are some technical problems to overcome. After administration of STZ, a state of hypoglycemia develops caused by release of insulin from the destroyed β cells of Langerhans. To avoid effects of transient hypoglycemia in the present study, we administered insulin for 4 d after STZ until the experiment was initiated by insulin withdrawal. Another difficulty associated with studying early renal changes in diabetes is the fact that GFR is influenced by anesthesia and other types of stress. This problem was overcome in the present study by use of conscious rats fully recovered from the operation and trained to participate in the experiment. Furthermore, there are technical problems with maintaining fluid balance during the clearance measurements, as diabetic rats show increased urine flow rates and unreplaced fluid loss may result in a decreased GFR, which could mask the effects of diabetes on kidney function. In the present study, this was overcome by administration of half isotonic saline at a rate slightly higher than that necessary to maintain fluid balance in the diabetic rats. Surprisingly, the urine flow rate showed a tendency to be lower in the diabetic rats than in the control rats in the present study. Because the hematocrit values tended to be lower in the diabetic group compared with the control group, the lower urine flow rates did not seem to be a consequence of volume depletion. Another possibility is that the diabetic rats might be more stressed than the control rats. Previous investigations in rats with early STZ-induced diabetes have reported an increased urinary excretion of catecholamines (15,16), and therefore a certain degree of stress in the diabetic group cannot be excluded. However, if this were the case, an increased level of catecholamines would be an integrated part of the disease and therefore not invalidate the results. Finally, body weight of hyperglycemic diabetic rats decreases in contrast to that of normoglycemic control rats. If GFR is divided by body weight as practiced in some studies, the increase of GFR in diabetic animals is overestimated (17,18). Accordingly, functional renal data in the present study are given as absolute values to avoid this artifact. MRI scanning was used to estimate changes of the kidney volume in the same animal over time. This method allows reliable noninvasive estimate of kidney volume in both diabetic and nondiabetic rats. The...
volumes measured by MRI are a good estimate of the kidney volume obtained in perfusion-fixed kidneys (11).

Due to the above-mentioned problems, it is not surprising that conflicting results have been published. The findings in the present report that renal growth is the primary event after rise of blood glucose in diabetic rats is in agreement with one previous study (6) in which renal growth occurred after 4 d, whereas hyperfiltration was not observed until after 10 d. However, because that study was carried out in acutely operated rats it cannot be excluded that this may have influenced GFR in the same way as described in nondiabetic animals (7). In another study, which was carried out in anesthetized diabetic rats, the kidney size and GFR increased in parallel with significant changes emerging at day 3 after injection of STZ (5). In one study, renal volume and GFR were measured simultaneously, but GFR was estimated on the basis of creatinine clearance, which is not a generally accepted measure of GFR (19). Altogether, no study has indicated that hyperfiltration occurs before the renal growth, and the increase of GFR therefore seems to occur either at the same time or subsequent to the renal growth. Accordingly, hyperfiltration is hardly responsible for the initial kidney growth in diabetes, whereas it is possible that initial tubular and glomerular growth is involved in the increase of GFR.

The mechanism responsible for the renal growth is unknown, but several growth factors have been suggested as mediators of kidney growth during experimental diabetes in rodents, particularly growth hormone and insulin-like growth factor I (20,21).

Other conditions characterized by increased GFR and kidney weight are pregnancy (22), unilateral nephrectomy (23), and high protein intake (24). In these conditions, an increase of GFR due to increase of ERPF is the primary event that over a period of days is followed by kidney growth. This growth is probably due to adaptive changes in tubular function, which prevents the urinary loss of water and electrolytes (25). In general, the absolute proximal reabsorption rises in parallel with the increase in GFR, and the fractional reabsorption in the proximal tubules remains unaltered or is slightly lowered (26). This is seen during pregnancy (27), unilateral nephrectomy (28), and protein loading (29). The renal changes in diabetes are different from other conditions with hyperfiltration (unilateral nephrectomy, pregnancy, and high protein intake) in that the absolute proximal reabsorption increases numerically equal to or more than GFR as observed in the present and previous studies (30), resulting in an increase of the fractional proximal tubular fluid reabsorption. This is the outcome to be expected if an increase in proximal tubular reabsorption were the primary functional event after induction of diabetes. A primary increase in proximal tubular reabsorption will tend to lower the hydrostatic pressure in the proximal tubule and thereby stimulate the inflow (GFR) and inhibit the proximal tubular fluid outflow (V_{prox}), thus preventing the increase of V_{prox} normally observed when GFR increases. The outcome therefore supports the notion outlined above that increased proximal tubular fluid reabsorption is a primary event in diabetes that contributes to the rise in GFR.

Figure 1. Changes in kidney volume, GFR, absolute proximal tubular fluid reabsorption (APR), and proximal tubular fluid output (V_{prox}) in diabetic and control animals from day 0 to day 7 after withdrawal of insulin and development of hyperglycemia in the diabetic group. The dotted lines indicate the levels before insulin withdrawal in the diabetic groups. Data are given as means ± SEM. The control group is depicted in gray bars and the diabetic group in black bars. n = 4 to 9.

*P < 0.05, control versus diabetic; †P < 0.05, days 1, 2, 3, 5, and 7 versus day 0 for diabetic rats.
In conclusion, renal enlargement after induction of diabetes in rats seems to precede increases in kidney function. The change in kidney function, at least in part, may be a consequence of enhanced proximal tubular fluid reabsorption rate.

Acknowledgments
This study was supported by the Danish Medical Research Council (Grant 9700592), the Research Foundation of Aarhus University, the Danish Kidney Foundation, the Ruth König Petersen Foundation, the Novo Foundation, the Aage Louis-Hansen Memorial Foundation, the Nordic Insulin Foundation, the Eva and Henry Frænkels Memorial Foundation, the Danish Health Research Council (Grant 9600822), and The Institute of Experimental Clinical Research, Aarhus University Hospital. We are grateful to Professor Hans Ørskov for critically reading the manuscript and to Mrs. Else Tusch, Jette Birk, Karen Mathiassen, Kirsten Nyborg, and Nina Rosenqvist for excellent technical assistance.

References
(G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. 


24. Kaysen GA, Rosenthal C, Hutchison FN: GFR increases before renal mass or ODC activity increase in rats fed high protein diets. 


27. Atherton JC, Bielinska A, Davison JM, Haddon I, Kay C, Samuels R: Sodium and water reabsorption in the proximal and distal nephron in conscious pregnant rats and third trimester women. 


29. Christensen S, Marcussen N, Petersen JS, Shalmi M: Effects of uninephrectomy and high protein feeding on lithium-induced chronic renal failure in rats. 

30. Vallon V, Blantz RC, Thomson S: Homeostatic efficiency of tubuloglomerular feedback is reduced in established diabetes mellitus in rats. 