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Transient Glomerular Hyperfiltration in the Streptozotocin-Diabetic Wistar Furth Rat

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ABSTRACT

The glomerular hemodynamic response to streptozotocin (STZ)-induced experimental diabetes differs depending on metabolic control and rat strain used. The present study characterize the glomerular filtration rate (GFR) and other renal parameters, weekly up to eight weeks of diabetes in STZ-diabetic Wistar Furth rats. The STZ-treated rats became diabetic within 24 h after treatment and retained a blood glucose concentration of 20–25 mmol/l throughout the experimental period. The GFR was transiently increased during the first 3–5 weeks after induction of diabetes, but thereafter did not differ from control animals. The renal weight increased by ~50% during the first week after induction of diabetes, thereafter no further increase in weight occurred. The urinary flow rate and urinary osmolar excretion were ~10 times higher in diabetic animals when compared to non-diabetic animals. Although they remained markedly higher than in non-diabetic animals, both the urinary flow rate and the urinary osmolar excretion peaked after 3 weeks of diabetes and thereafter tended to decrease. The urinary sodium and potassium excretions did not differ between non-diabetic and diabetic animals. We conclude that the transient increase in the GFR seen in the human disease, occurs in Wistar Furth rats, which is in contrast to a majority of other rat strains, where the GFR is persistently increased.

INTRODUCTION

Increased glomerular filtration rate (GFR) is a characteristic finding in patients with newly diagnosed IDDM (15). This glomerular hyperfiltration may persist for many years (24), but among the 30–40% diabetics who develop nephropathy (1), GFR begins to decline within a few years after the debut of diabetes (23). Since 1981, several papers have described glomerular hemodynamic changes in streptozotocin-induced diabetic rats (6, 8–10, 13, 14, 26, 27). In a majority of these studies Sprague-Dawley rats or Munich-Wistar rats have been used and a persistent glomerular hyperfiltration has been observed up to 1 year after diabetes was induced (for a review see (18)). However, the glomerular hemodynamic response to experimental diabetes differs greatly depending on metabolic control and the particular rat strain used. Severe hyperglycemia (>30 mmol/l) in Munich-Wistar rats and Sprague-Dawley rats has been shown to be associated with glomerular hypofiltration and

ketoacidosis, whereas moderate hyperglycemia (20 mmol/l) has been observed to increase GFR (8, 13). Some animal strains display increased glomerular capillary pressure after STZ-treatment (Munich-Wistar, Wistar-Kyoto), whereas others do not (Sprague-Dawley, Wistar) (18).

In preliminary experiments using STZ-diabetic Wistar Furth rats, we have noted a markedly decreased tissue oxygen tension throughout the renal parenchyma (20). In both animals studied four or eight weeks after induction of diabetes, the GFR did not differ from controls despite a moderate hyperglycemia (~20 mmol/l) and absence of ketoacidosis. Therefore, the aim of the present study was to characterize the GFR during the course of diabetes in this rat strain.

METHODS

Inbred male Wistar Furth rats, ~10 weeks old, were purchased from B&K, Universal (Sollentuna, Sweden). The animals (n=57) had free access to tap water and standard rat chow (R3, Ewos, Södertälje, Sweden) throughout the study. All experiments were approved by the local animal ethics committee.

Diabetes mellitus was induced by an intravenous injection of STZ in the tail vein (45 mg/kg; dissolved in 0.2 ml saline, Sigma-Aldrich, St Louis, MO, USA). Animals were considered diabetic if blood glucose concentrations rose ≥15 mmol/l within 24 hours after STZ-injection and remained above this level. Blood glucose concentrations were determined with test reagent strips (Medisense, Baxter Travenol, Deerfield, IL, USA) from blood samples obtained from the cut tip of the tail in all animals.

After one to eight weeks of diabetes, the animals were allocated to measurements of GFR. Initial attempts to keep diabetic animals for longer periods failed due to a decline in their physical condition. Normoglycemic animals of corresponding ages were also allocated to GFR measurements to exclude drift in the control values. Control and diabetic animals were anesthetized with an intraperitoneal injection of thiobutabarbital (Inactin, Research Biochemicals International, Natick, MA; 120 mg/kg body weight non-diabetic, 80 mg/kg body weight diabetic animals), placed on an operating table maintained at 37°C and tracheostomized. Polyethylene catheters were inserted into both femoral arteries and the left femoral vein. The right arterial catheter was used to monitor blood pressure (Statham P23dB, Statham Laboratories, Los Angeles, CA), the left arterial catheter was used for blood sampling, whereas the catheter in the vein was used for infusion of saline containing ^{3}H-inulin (American Radiolabeled Company, St Louis, MO, USA). The urinary bladder was catheterized to allow urinary drainage. The left kidney was exposed by a left subcostal flank incision and immobilized in a plastic cup attached to the operating table. The kidney was embedded in cotton wool soaked in saline and its surface was covered with mineral oil (Apoteket, Umeå, Sweden). The left ureter was catherized for urine sampling.

The animals were allowed a 60 minute equilibration period followed by 30–60 min to estimate GFR by determining the clearance of inulin. ^{3}H-inulin (dissolved in saline) was initially given as a bolus dose of 5 μCi and then infused (5 ml × kg^{-1} × h

Table 1. Body weight, mean arterial blood pressure and blood glucose concentration in non-diabetic and 1- to 8-week-STZ-treated Wistar Furth rats.

	Mean arterial blood pressure (mmHg)	Blood glucose (mM)	Body weight (g)
Control (n=7)	125±1	6.0±0.2	318±9
Diabetes 1 week (n=5)	114±2	21.8±1.2[a]	313±7
2 weeks (n=5)	112±4	24.8±0.6[ab]	290±7[a]
3 weeks (n=5)	114±1	25.3±0.5[ab]	290±7[a]
4 weeks (n=6)	117±2	24.5±1.2[ab]	272±6[ab]
5 weeks (n=6)	114±7	25.7±0.7[ab]	269±6[ab]
6 weeks (n=6)	117±3	26.8±1.0[ab]	246±9[abcde]
7 weeks (n=7)	115±2	25.4±1.1[ab]	251±5[abcd]
8 weeks (n=10)	113±3	25.3±0.5[ab]	247±8[abcdef]

All values are means±SEM. **a** denotes P<0.05 vs control group, **b** denotes P<0.05 vs. 1 week diabetic group, **c** denotes P<0.05 vs. 2 week diabetic group, **d** denotes P<0.05 vs. 3 week diabetic group, **e** denotes P<0.05 vs. 4 week diabetic group and **f** denotes P<0.05 vs. 5 week diabetic group. Comparison made with ANOVA and, when appropriate, followed by Fishers PLSD test.

non-diabetic; 10 ml × kg × h diabetic animals) via the femoral vein catheter. Urine and blood samples were taken for subsequent analyses. The blood sample was collected at the midpoint of the urine collection period. The radioactivity of ^{3}H-inulin in the plasma (10 µl) and urine (1 µl) samples was measured by liquid scintillation. The GFR was then calculated as clearance of ^{3}H-inulin using the formula GFR= U × V/P, where U denotes the concentration of inulin in the urine sample, V denotes the urine flow rate (ml/min), and P denotes the concentration of inulin in the plasma sample. The urine volumes were measured gravimetrically, the osmolality by use of a freezing point technique (Model 3MO, Advanced Instruments, MO, USA) and the urinary sodium and potassium concentrations by use of a flame photometer (IL543, Instrumentation Lab, Milan, Italy). At the end of each experiment, the kidneys were removed, blotted and weighed.

Statistical analysis. All values are given as means±SEM. Multiple comparisons between data were performed using analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test ((4), Statview, Abacus Concepts, Berkeley, CA, USA). For all comparisons, P<0.05 was considered statistically significant.

RESULTS

All normoglycemic animals weighed 277–348 g (Table 1). Animals rendered diabetic by injection of STZ weighed approximately 320 g (range 286–330 g), but gradually declined in weight during the course of diabetes. The non-diabetic animals had a blood glucose concentration of 4–6 mmol/l (Table 1). Administration of STZ made the animals hyperglycemic (blood glucose concentration >15mmol/l) within 24 h. All STZ-treated animals remained diabetic when measurements of renal function were performed 1–8 weeks later. The mean arterial blood pressure was similar in all rats (Table 1).

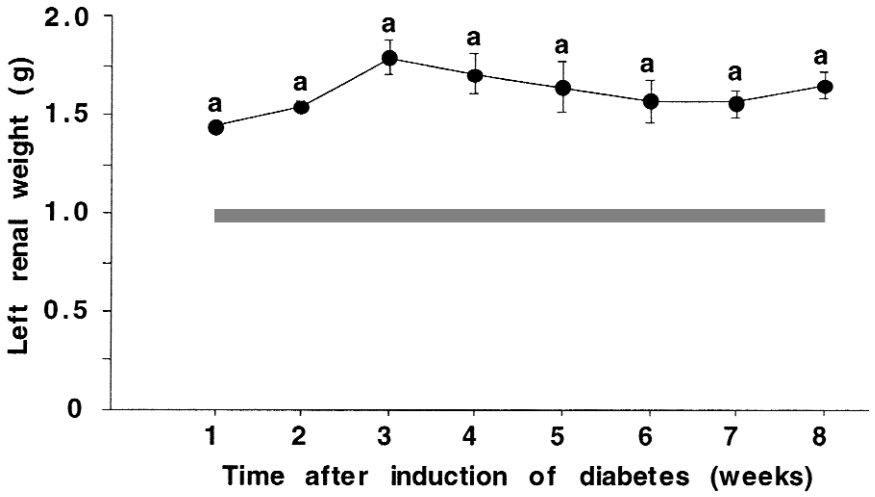

Fig. 1. The left kidney weight in non-diabetic (grey bar) and 1- to 8-week-STZ-diabetic (filled circles) Wistar Furth rats. All values are given as means±SEM for 5-10 animals. **a** denotes P<0.05 when compared to non-diabetic animals.

The left kidney weight increased by ~50% during the first week after induction of diabetes, thereafter no further increase in weight was obtained (Figure 1). The GFR increased transiently during the first 3–5 weeks after induction of diabetes, but thereafter did not differ from control animals (Figure 2). In control animals, neither renal weight nor GFR correlated to body weight (r=0.62, P=0.21 and r=0.28, P=0.56, respectively). The urinary flow rate was ~10 times higher in diabetic animals when compared to non-diabetic animals (Table 2). Eight weeks after induction of diabetes, urinary flow rate had decreased when compared to 3-week-diabetic animals. Urinary osmolar excretion was also ~10 times greater in diabetic animals, but was somewhat

Table 2. Urinary flow rate and urinary excretion of sodium, potassium osmolytes in non-diabetic and 1- to 8-weeks-STZ-treated Wistar Furth rats.

	Urinary flow rate (µl/min× kidney)	Sodium excretion (µmol/min× kidnet)	Potassium excretion (µmol/min× kidney)	Osm excretion (µOsm/min× kidney)
Control (n=7)	1.07±0.09	0.024±0.003	0.25±0.06	1.92±0.30
Diabetes				
1 week (n=5)	21.98±3.35[a]	0.110±0.082	0.63±0.14	19.19±2.66[a]
2 weeks (n=5)	29.48±3.65[a]	0.071±0.023	0.63±0.18	23.39±3.30[a]
3 weeks (n=5)	29.78±3.14[a]	0.070±0.017	0.70±0.08	24.56±1.25[a]
4 weeks (n=6)	23.53±5.17[a]	0.115±0.047	0.49±0.12	20.52–4.18[a]
5 weeks (n=6)	25.29±3.17[a]	0.131±0.024	0.85±0.14	22.46±2.11[a]
6 weeks (n=6)	20.19±3.95[a]	0.195±0.090	0.70±0.18	17.26±2.96[a]
7 weeks (n=7)	20.47±4.92[a]	0.178±0.102	0.58±0.15	16.20±2.94[ab]
8 weeks (n=10)	19.81±2.72[ab]	0.094±0.027	0.64±0.13	16.83±2.56[ab]

All values are means±SEM. **a** denotes P<0.05 vs control group and **b** denotes P<0.05 vs. 3 weeks diabetic group. Comparison made with ANOVA and, when appropriate, followed by Fishers PLSD test.

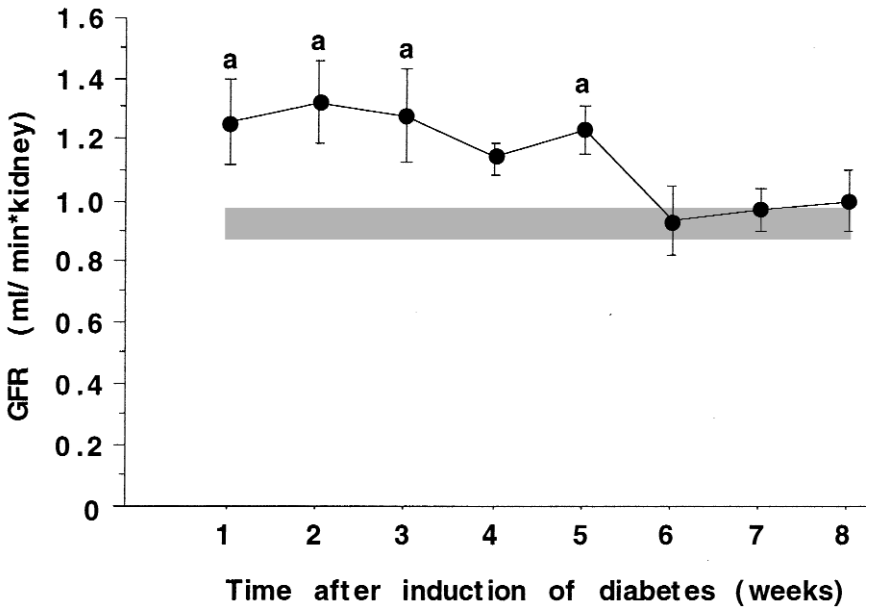

Fig. 2. The glomerular filtration rate (GFR) of the left kidney in non-diabetic (grey bar) and 1- to 8-week-STZ-diabetic (filled circles) Wistar Furth rats. All values are given as means±SEM for 5–10 animals. **a** denotes $P<0.05$ when compared to non-diabetic animals.

lower in the 7- and 8-week-diabetic animals when compared to 3-week-diabetic animals (Table 2). The urinary sodium and potassium excretions did not differ between non-diabetic and diabetic animals (Table 2).

DISCUSSION

Previous studies in STZ-diabetic rats have revealed that the glomerular hemodynamic responses are not only dependent on metabolic control, but also on the rat strain used (18). STZ-treatment (45–55 mg/kg intravenously) of male Wistar Furth rats produces a reproducible diabetes with blood glucose concentrations of 20–25 mmol/l in the absence of ketoacidosis (20). However, the renal changes in this strain during manifest diabetes remain to be determined. Since a pivotal criterion for adequate animal models in pathological research is a close similarity to the human disease, the present study aimed to elucidate the timely course of GFR changes in this strain of rats. Interestingly, a transient increase in the GFR, as in the human disease, was observed in Wistar Furth rats. This is in contrast to most other investigated rat strains where the GFR is persistently increased (cf. (18)). In this context it should be noted that there was no correlation between age and GFR changes in the control animals, as also previously described (3).

The mechanism(s) involved in the glomerular hyperfiltration is heavily debated. Investigators have characterized the functional effects of diabetes on the various seg-

ments of the glomerular microvasculature (for a review see (17)). Many substances have also been invoked as humoral mediators of vasodilation in the diabetic glomerulus (for a review see (21)). Also, a prior increase in proximal reabsorption capacity, with subsequent reduced tubuloglomerular feedback response has been implicated as a cause for the increased GFR (22). A prerequisite for the latter theory is an enlargement of the kidney that fore goes the glomerular hyperfiltration, while the tubuloglomerular feedback can evoke changes in the GFR in the manner of seconds (5). The order of appearance between renal hypertrophy and GFR-increase is disputed, but they both occur at an early stage (16). In the Wistar Furth rat we also observed that the renal weight increased, concomitant with the increase in GFR, as early as 1 week after induction of diabetes. Whereas the GFR increase returned to normal over a period of 3–5 weeks, the increase in renal weight was stable throughout the 8-week experimental study period. Several factors have been forwarded to explain the increase in renal weight, predominantly IGF-1 (2, 7), but also e.g. TGF-b (11), angiotensin II (25) and atrial natriuretic peptide (12).

We have previously noted a progressive increase in urinary albumin excretion after inducing diabetes with STZ in the Wistar-Furth rat (19). This albumin excretion could not be explained by STZ-nephrotoxicity, but by the diabetic state (Palm et al, unpublished observations). Our present finding of initial glomerular hyperfiltration in the Wistar-Furth rat, therefore, seems to be accompanied by disturbed glomerular function.

Consistent with our previous results (20), we found an increase in the urinary excretion of osmolytes in the diabetic Wistar-Furth rats. This increase is likely to mainly represent exaggerated urinary excretion of glucose and proteins (e.g. albumin). The urinary excretion of sodium and potassium also tended to be increased in diabetic animals, but did not reach statistical significance. Although both the urinary flow rate and urinary osmolar excretion remained markedly higher than in non-diabetic animals, both parameters peaked early during the course of diabetes and thereafter tended to decrease. This suggests an adaptive response in the urinary flow rate with secondary changes in the osmolar excretion after several weeks of diabetes. The exact mechanism for this remains to be determined.

In conclusion, the present study investigated the timely course of GFR changes in Wistar Furth rats. In contrast toa majority of other investigated rat strains where the GFR is persistently increased (cf. (18)), a transient increase in the GFR, as in the human disease, was recorded in Wistar Furth rats. We also observed an increase in renal weight within 1 week after inducing diabetes with no further increase during the 8-week experimental study period.

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