Glomerular Function in Spontaneously Diabetic Rats (43329)

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Abstract. **This study was undertaken to determine whether hyperfiltration exists at the single nephron level and whether albumin excretion is increased early in the course of diabetes in Biobreeding rats. Diabetic rats were studied at 8–12 weeks after the onset of diabetes. Control animals were age-matched, diabetes-resistant rats. Urinary and tubular fluid albumin concentrations were measured by polyacrylamide gel electrophoresis. Clearance and micropuncture techniques were used to determine whole kidney and single nephron glomerular filtration rate, renal blood flow, and glomerular capillary pressure. The urinary albumin excretion rate (1.3 ± 0.1 mg/24 hr) and the tubular fluid albumin concentration (4.7 ± 0.7 mg/dl) in the diabetic group were significantly elevated when compared with urinary albumin excretion (0.9 ± 0.1 mg/24 hr) and tubular fluid albumin concentration (2.5 ± 0.5 mg/dl) in the control group. There were no significant differences in glomerular hemodynamics (whole kidney or single nephron glomerular filtration rate or glomerular capillary pressure) between diabetic and control rats. The kidney weight and kidney weight to body weight ratio were significantly higher in diabetic rats when compared with control rats. Early diabetes in Biobreeding rats is characterized by mild albuminuria and increased kidney size, but not glomerular hyperfiltration.**

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Hyperfiltration (glomerular filtration rate > 150 ml/min × 1.73 m^2) (1), kidney enlargement (2–4), and microalbuminuria (20–200 μg/min) (5) may be early indicators of renal disease in patients with insulin-dependent diabetes. Two models of diabetes in rats have been studied to investigate in more detail the renal hemodynamic abnormalities and the morphological changes that are characteristic of human diabetes. The most extensively studied model is one in which diabetes is induced by streptozotocin. Chemically induced diabetes is uniformly associated with increased kidney size, as indicated by an increase in kidney weight and an increase in the kidney weight to body weight ratio (6–14). The enlargement of the kidney precedes any changes in kidney function (15). Regardless of the degree of hyperglycemia, hyperfiltration is not observed when the glomerular filtration rate is expressed in terms of kidney weight (7, 8, 10–14). However, the single nephron glomerular filtration rate (per gram of kidney weight) was moderately elevated in three studies (7, 10, 11). An increase in glomerular capillary pressure was described by Hostetter *et al.* (7), but could not be detected by other studies (11, 12).

The other model is genetic, which is similar to human insulin-dependent diabetes mellitus, and develops diabetes spontaneously (Biobreeding rat) (16–18). In diabetes in the Biobreeding rat, increased kidney size has also been described (19, 20), but again the evidence for hyperfiltration is not convincing. Cohen *et al.* (19) found no evidence of an increase in glomerular filtration rate. An increase in filtration rate was described by Brown *et al.* (20), but only if the data were expressed on the basis of body weight. An increase in albumin excretion in early diabetes has not been clearly demonstrated (19, 20). Due to the absence of studies of single nephron function in the Biobreeding diabetic rat, the present study was undertaken to determine whether hyperfiltration exists at the single nephron level and whether albumin excretion is increased early in the

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course of insulin-dependent spontaneous diabetes mellitus in Biobreeding rats.

Materials and Methods

Male diabetic (n = 15) and control (diabetes-resistant, n = 18) Biobreeding rats were obtained from the University of Massachusetts Medical School (NIH contract colony), Worcester, MA, at 12–15 weeks of age. They were housed in individual cages and were maintained on a 24% protein pellet diet (Teklad, Madison, WI). Diabetic rats received a daily subcutaneous injection of protamine zinc insulin (0.5–0.8 units/100 g body wt in the late morning: Eli Lilly) in the tissues overlying the pectoralis muscles. The insulin dose was adjusted so that diabetic rats routinely exhibited glycosuria (4+ by Testape; Eli Lilly) with no evidence of ketoacidosis (Ketodiastix; Ames). The body weights of diabetic animals were monitored on a daily basis. A weight loss of 10 g or more was attributed to dehydration and the animal received lactated Ringer solution (9 ml of lactated Ringer and 1 ml of 8.4% sodium bicarbonate) subcutaneously. Kidney function was measured in diabetic and control rats at 20–23 weeks of age. The duration of diabetes was 8–12 weeks.

Urinary Protein Excretion. Overnight (18 hr) urine collections were performed 2–3 days prior to micropuncture studies. Rats were housed in metabolism cages with free access to water and a liquid diet (Bioserv F1417; Frenchtown, NJ), similar in composition to the pellet chow. Tail blood samples were obtained at the end of the urine collection for the measurement of protein, cholesterol, glucose, and sodium.

Micropuncture Studies. All animals were glycosuric, with no ketones in the urine on the day of the experiment. Overall kidney function and proximal tubule micropuncture experimentation followed standard usage of the laboratory (21, 22). Food was not withheld from the animals before the experiment. Rats were anesthetized with Inactin (Promonta; 80–100 mg/100 g body wt, ip) and placed on a heated table to maintain rectal temperature at 37–38°C. After the animals were anesthetized, a midline neck incision was made and the trachea was isolated and cannulated. Catheters were inserted into the left jugular vein for infusions during surgery and experimentation, the left internal carotid artery for blood pressure monitoring and blood sampling, and the bladder for urine collections. A left subcostal abdominal incision was made to expose the kidney, which was placed and immobilized in a lucite cup and bathed with warm mineral oil. An intravenous infusion of isotonic saline solution with synthetic inulin (polyfructosan, 4 g/dl) was given at a rate of 0.06 ml/min in diabetic and control rats to maintain stable volume status and for the measurement of glomerular filtration rate. In selected diabetic rats, 1 mCi/ml of [*methoxy*-^{3}H]inulin (ICN Radiochemicals; sp act 42 mCi/g) was also present in the intravenous infusion. Prior to the start of clearance measurements, a small diameter flow transducer (model EP 102; 2 mm) connected to a square-wave electromagnetic flowmeter with attached recorder (model 501; Carolina Medical Electronics, Inc.) was placed around the left renal artery for measurement of renal blood flow. After 45–60 min of equilibration, two clearance periods of approximately 30 min in duration were carried out in each experiment. Blood pressure was monitored continuously from the left internal carotid artery by using a Gould P32 ID transducer (Gould Inc., Oxnard, CA) and monitor (model SP 1405).

Samples of tubule fluid were collected quantitatively from random sites in proximal tubules for determination of single nephron glomerular filtration rate. Collections were taken over a period of 3–7 min after an oil block was established via a 10–12-μm tip pipette filled with Sudan black stained castor oil. The sample volume (20–40 nl) was determined from its measured length in a constant bore capillary tube. After analysis of plasma and tubular fluid inulin, single nephron glomerular filtration rate (collected vol/min × [tubule fluid/plasma]$_{IN}$) was calculated. The proximal tubule albumin concentration was also determined from random site collections of free flow samples. For this collection, the pipette had a longer tip (2 mm), for direct filling of capillary tubes (0.5-μl microcaps) for protein analysis.

In a separate group of animals, afferent glomerular capillary hydraulic pressure was determined indirectly from the sum of stop flow pressure and plasma colloid osmotic pressure (COP). The colloid osmotic pressure of the plasma was determined by the following equation:

$$COP = 1.886C + 0.206C^2 + 0.005C^3$$

in which C is the plasma protein concentration (23). After early segments of proximal tubules were identified by injection of small amounts of 0.9% saline stained with FD & C green, free flow pressure and stop flow pressure in the same tubule were measured with a 3–5-μm micropipette connected to a servo-null micropressure system (World Precision Instruments, North Haven, CT). Stop flow pressures were determined by blocking the proximal tubule with bone wax (24) or Sudan black stained castor oil contained in a 12–14-μm tip micropipette. Stop flow pressures were similar with the two blocking agents. Glomerular transcapillary hydraulic pressure difference was calculated as the difference between glomerular capillary pressure and free flow pressure.

Analytical Methods. Plasma and urine polyfructosan were analyzed using the anthrone method (25); tubule fluid inulin was analyzed by a micromodification of the same method (26). Corrections were made

for glucose in plasma and urine (27). The validity of these corrections was confirmed by comparing the clearance of polyfructosan and [*methoxy*-^{3}H]inulin in four diabetic rats. The ratio of cold versus radioactive inulin values was 1.0 ± 0.1 (n = 4) for whole kidney and 0.90 ± 0.05 for single nephron glomerular filtration rate. A microcontinuous gradient gel electrophoresis procedure was used for the separation and quantitation of proteins, including albumin, in plasma (diluted 1/51) and urine (21). Plasma sodium concentrations were measured by flame photometry (FLM3 Flame Photometer; Radiometer, Copenhagen). The plasma glucose concentration was determined by the hexose kinase method (Boehringer-Mannheim). Cholesterol concentration in the plasma was determined by using the Cholesterol HP Kit from Boehringer-Mannheim. The water content of the kidney was determined from the difference between wet and dry weights. The kidney was dried to constant weight in an oven at 90°C for 48 hr.

The kidneys were fixed in buffered formalin and embedded in paraffin. Sagittal sections of the kidneys were stained with hematoxylin and eosin, and by the periodic acid-Schiff reaction for histologic examination by light microscopy (28). Two of the investigators (B. N. and L. G. F.) evaluated the sections blindly.

Statistical Analysis. Student's t test was used for statistical comparison. A P-value less than 0.05 was considered significant. All values are given as mean ± SE.

Results

Plasma Composition. Diabetic rats were severely hyperglycemic and their urine flow rates were twice that of control rats (Table I). There were no significant differences between diabetic and control groups in sodium, total protein, or albumin concentrations. However, cholesterol concentrations in the diabetic rats were lower than in the control group.

Body and Kidney Weight. Kidney wet weights were increased in diabetic rats even though the body weights of diabetic rats were significantly less than those of control rats (Table II). Kidney dry weights in the diabetic group were also increased, but they did not reach statistical significance ($P < 0.1$). A similar magnitude increase in mean kidney wet weight and dry weight in the diabetic group was observed (88% and 89%, respectively). The water content of kidney tissue, expressed as the percentage of kidney wet weight, was not different between the groups.

Protein Excretion. Albumin excretion was elevated in diabetic rats whether it was expressed per rat or per gram of kidney weight (Table III). Consistent with the significant increased urinary albumin excretion rate, the proximal tubule fluid albumin concentration was nearly 2-fold higher in the diabetic animals. The urinary protein excretion was not elevated.

Renal Function. The whole animal glomerular filtration rate, renal blood flow rate, and renal plasma flow rate were the same in diabetic and control rats when the data were expressed as absolute values per animal (ml/min) or per gram of kidney weight (ml/min × g kidney wt) (Table IV). Mean hematocrit and arterial pressure during the acute experiments were similar in both diabetic and control groups. The plasma hematocrit at the end of the micropuncture experiment was identical to the presurgical concentration in both groups and indicates that the extracellular volume status was not altered during the experimental procedure. The plasma glucose concentration in control and diabetic rats was similar to the presurgical concentration (Table I). During the micropuncture experiment, there was no change in the plasma glucose concentration in either group.

The single nephron glomerular filtration rate was also similar between the diabetic and control animals (Table V). The glomerular capillary pressure and net filtration pressure were not increased in the diabetic group. Proximal tubule fluid reabsorption, indicated by the ratio of tubule fluid to plasma inulin concentration, was identical in both groups.

Light microscopic studies of kidneys from diabetic and control rats showed no abnormalities in glomeruli, tubules, or vessels.

Discussion

We found mild albuminuria without an increase in total urinary protein excretion in early diabetes (8–12 weeks duration) of Biobreeding rats with severe hyperglycemia (plasma glucose concentration > 425 mg/dl). In a previous study, Cohen *et al.* (29) noted a fractional increase of albumin in urinary protein in diabetic Biobreeding rats at 4 months of age. These observations correspond with previous observations in

Table I. Plasma Composition and Urine Flow Rate from Overnight Collection

Group (n)	Glucose (mg/dl)	Sodium (mEq/liter)	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Urine flow rate (μl/min)
Control (14)	159 ± 7	140 ± 1	92 ± 5	6.8 ± 0.2	3.0 ± 0.2	7.1 ± 0.3
Diabetic (12)	501 ± 75[a]	138 ± 2	74 ± 1[a]	7.1 ± 0.3	3.0 ± 0.2	16.4 ± 1.7[a]

[a] Significantly different from control at $P < 0.05$.

Table II. Body and Kidney Weight

Group (n)	Body wt (g)	Kidney wet wt (g)	Kidney dry wt (g)	Water (%)	Kidney wt/body wt
Control (9)	463 ± 12	2.61 ± 0.06	0.59 ± 0.03	77.3 ± 0.6	0.0057 ± 0.0001
Diabetic (11)	361 ± 9[a]	2.96 ± 0.12[a]	0.66 ± 0.03	78.2 ± 0.5	0.0082 ± 0.0003[a]

[a] Significantly different from control at $P < 0.05$.

Table III. Urinary and Tubule Fluid Protein

Group	Urinary protein excretion (mg/24 hr)	Urinary protein excretion (mg/24 hr × g kidney wt)	Urinary albumin excretion (mg/24 hr)	Urinary albumin excretion (mg/24 hr × g kidney wt)	Tubular fluid albumin (mg/dl)
Control	18.4 ± 1.0 (14)[a]	7.8 ± 0.3 (14)	0.9 ± 0.1 (14)	0.3 ± 0.05 (14)	2.5 ± 0.5 (19)
Diabetic	21.8 ± 1.9 (12)	7.5 ± 0.5 (12)	1.3 ± 0.1[b] (12)	0.4 ± 0.03[b] (12)	4.7 ± 0.7[b] (12)

[a] Numbers in parentheses are the number of observations (tubular fluid) or number of animals (urinary protein and albumin excretion).
[b] Significantly different from control at $P < 0.05$.

Table IV. Whole Kidney Hemodynamics

	Control	Diabetic
Glomerular filtration rate (ml/min)	2.74 ± 0.05 (9)[a]	3.19 ± 0.34 (12)
(ml/min × g kidney wt)	1.00 ± 0.09 (9)	1.13 ± 0.12 (12)
Renal blood flow (ml/min)	14.2 ± 0.90 (6)	13.0 ± 1.60 (4)
(ml/min × g kidney wt)	5.45 ± 0.37 (6)	4.28 ± 0.68 (4)
Renal plasma flow (ml/min)	6.94 ± 0.57 (6)	5.89 ± 0.70 (4)
(ml/min × g kidney wt)	2.68 ± 0.22 (6)	1.93 ± 0.29 (4)
Mean arterial pressure (mm Hg)	130 ± 4 (9)	120 ± 3 (12)
Hematocrit (%)	51 ± 2 (9)	51 ± 1 (12)
Urine flow rate (μl/min × g kidney wt)	10.4 ± 1.3 (9)	29.3 ± 4.2[b] (12)
Plasma glucose concentration[c] (mg/dl)	192 ± 20 (9)	502 ± 76[b] (12)

[a] Numbers in parenthesis are the number of animals.
[b] Significantly different from control at $P < 0.05$.
[c] Plasma glucose concentration is the mean of three samples taken during the micropuncture experiment.

Table V. Superficial Single Nephron Function

	Control	Diabetic
Single nephron glomerular filtration rate (nl/min)	40.1 ± 2.7 (29)[a]	38.0 ± 2.3 (31)
(nl/min × g kidney wt)	32.3 ± 2.5 (29)	27.8 ± 1.7 (31)
Free flow pressure (mm Hg)	12.3 ± 0.3 (16)	12.3 ± 0.2 (23)
Stop flow pressure (mm Hg)	28.3 ± 1.1 (8)	26.5 ± 0.5 (15)
Arterial plasma colloid osmotic pressure (mm Hg)	13.7 ± 2.0 (5)	14.8 ± 0.5 (8)
Afferent glomerular capillary hydraulic pressure (mm Hg)	42.1 ± 1.0 (8)	41.2 ± 0.5 (15)
Glomerular transcapillary hydraulic pressure difference (mm Hg)	29.5 ± 1.1 (8)	28.9 ± 0.6 (15)
Tubular fluid/plasma inulin concentration	1.81 ± 0.09 (29)	1.81 ± 0.13 (35)

[a] Numbers in parenthesis are the numbers of observations.

human studies that microalbuminuria may occur in the absence of abnormal proteinuria in early diabetes (5). Increases in albumin excretion have also been found in streptozotocin-induced diabetic rats (9, 11, 30–32). The mild albuminuria of diabetes in the Biobreeding rat was accompanied by an increase in proximal tubular fluid albumin concentration in superficial nephrons and may represent a change in glomerular macromolecule permeability.

In agreement with other investigators using the Biobreeding model of diabetes (19, 33), we found no elevation in the whole kidney glomerular filtration rate or renal blood flow, when expressed per gram of kidney wet weight, in rats with severe hyperglycemia. We further observed that single nephron glomerular filtration rate or glomerular capillary pressure was not increased in diabetic Biobreeding rats with severe hyperglycemia. Our findings are different from those observed in streptozotocin-treated diabetic rats. A decrease in the whole kidney and single nephron glomerular filtration rate, with no change in glomerular capillary pressure, was observed in streptozotocin-treated rats with severe hyperglycemia (7). The decrease in the single nephron glomerular filtration rate was attributed to increased

renal vasoconstriction resulting in a decrease in glomerular plasma flow rate. The cause of the renal vasoconstriction was not a decrease in blood volume (7). In streptozotocin-treated rats with mild hyperglycemia (i.e., plasma glucose < 425 mg/dl), an increase in single nephron glomerular filtration rate and glomerular capillary pressure has been reported (7). Whether an increase in these parameters occurs in Biobreeding diabetic rats with mild hyperglycemia remains to be investigated.

Previous studies in diabetic Biobreeding rats (19, 20) have also reported an increase in kidney size. Our findings indicate that an increment in kidney dry weight and not water content was responsible for the increase in kidney size. A similar observation has been reported in streptozotocin-treated diabetic rats (6). Using morphometric techniques, previous studies found that the increase in kidney size in the diabetic Biobreeding rats was accompanied by thickening of the glomerular basement membrane, with no increase in the fractional mesangial area (19, 20). This finding is different from the streptozotocin-induced model of diabetes, in which an increase in the glomerular basement membrane thickness (34) and fractional mesangial area (35) was observed. An increase in the proximal tubule size has also been reported in streptozotocin-treated rats (36). Whether proximal tubule enlargement occurs in diabetic Biobreeding rats is unknown. Similar to the streptozotocin-induced model of diabetes (37, 38), the increase in kidney size in the Biobreeding rat does not appear to be correlated with glomerular hemodynamic changes. Factors that promote kidney growth, such as insulin-like growth factor, may be the mechanism whereby increased kidney size occurs in diabetes (38, 39).

In summary, diabetic Biobreeding rats with severe hyperglycemia exhibit mild albuminuria and increased kidney size, with no change in glomerular hemodynamics.

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