Kidney International, Vol. 39 (1991), pp. 1176–1183

Disassociation between glomerular hyperfiltration and extracellular volume in diabetic rats

BRYAN J. TUCKER, ROSE C. COLLINS, MICHAEL G. ZIEGLER, and ROLAND C. BLANTZ

Department of Medicine, University of California, San Diego School of Medicine, La Jolla, and Divisions of Nephrology-Hypertension, Veterans Affairs Medical Center, San Diego, California, USA

Disassociation between glomerular hyperfiltration and extracellular volume in diabetic rats. The relationship of the development of glomerular hyperfiltration in diabetes to changes in extracellular fluid volume has not been previously examined. To accomplish this task, male Wistar rats were chronically cannulated in the bladder, femoral artery and vein. Control measurements of glomerular filtration rate (GFR), renal plasma flow (RPF), extracellular fluid volume (ECF), and urinary sodium excretion were performed on two separate days prior to infusion of streptozotocin (65 mg/kg body wt i.v.). After infusion of streptozotocin, the IDDM rats were separated into two groups: untreated IDDM group of rats and IDDM rats treated with insulin at doses sufficient to normalize blood glucose (Ultralente, 2 to 8 IU/day). A third group of normal non-diabetic rats served as time controls. Measurements of renal function occurred at 1, 4, 7, 11, and 15 days after infusion of streptozotocin. Blood glucose in the non-diabetic measurement period averaged 137 ± 30 mg/dl and increased from 412 ± 55 after 24 hours in the untreated diabetic rats to 533 ± 33 mg/dl after 15 days of IDDM. The time controls and the insulin-treated diabetic rats did not differ in blood glucose values at the time measurements were performed. Glomerular filtration rate increased from 1.0 ± 0.1 to 1.7 ± 0.1 ml/min/100 g body wt by day 15 in the untreated diabetic rats with significant increases in GFR within 24 hours. GFR of both time controls and the insulin-treated IDDM rats did not significantly vary during the time of the study. The increase in GFR in the untreated IDDM group was associated with a concomitant increase in RPF. However, ECF decreased in both the insulin treated and untreated groups by one day after streptozotocin infusion and was less than control throughout the 15 day IDDM measurement period. Therefore, the data indicate that the development of hyperfiltration in IDDM is not caused by ECF expansion and cannot be temporally linked to changes in ECF.

Glomerular hyperfiltration is a common observation in early insulin-dependent diabetes mellitus [1–4]. This observation occurs prior to any discernible morphologic injury (other than hypertrophy) to either the systemic microvasculature or the kidneys [5, 6]. Glomerular hyperfiltration is thought to contribute to the development of glomerular capillary hypertension and increased glomerular basement membrane thickening, which in turn is considered to be the precursor to increased urinary protein excretion and glomerular microangiopathies [5, 7]. Since this early elevation in glomerular filtration rate occurs prior to structural changes within the kidney, functional alterations were proposed, and hyperfiltration is best linked to increases in renal plasma flow. However, the cause for increased renal plasma flow is less well defined. A current hypothesis is that fluid volume expansion (either extracellular fluid volume or plasma volume) is a likely candidate inducing hyperfiltration in insulin dependent diabetes (IDDM). The theory that extracellular volume expansion is, in part, responsible for the glomerular filtration is based upon studies which examined extracellular fluid volume (ECF) or plasma volume (PV) in humans [8, 9] or animal models [3, 10] with established IDDM and exhibiting hyperfiltration. If ECF expansion is the cause of glomerular hyperfiltration in early IDDM, then control of extracellular fluid volume could lessen the progression of diabetes-related renal disease and the decline in renal function. Bank et al placed early IDDM rats on salt restricted diets and demonstrated a reduction in glomerular filtration rate compared to rats on normal salt intake, and raised the possibility of this change to reduction of ECF (approximately 10% of ECF) [11]. However, other studies have indicated no change in PV or increased ECF in IDDM [12, 13]. The actual link between expanded ECF and the development of glomerular hyperfiltration in early IDDM has yet to be established.

The purpose of the present study is to determine if ECF expansion can be linked to increased glomerular filtration rate during the initiation and development of IDDM. To accomplish this task, glomerular filtration rate, renal plasma flow, and extracellular fluid volume were measured simultaneously in awake, chronically catheterized rats during normal conditions prior to infusion of streptozotocin and followed for 19 days after development of insulin dependent diabetes. If there were an association between ECF expansion and development of glomerular hyperfiltration, then following the two parameters on a time line basis should provide evidence of functional linkage. Both untreated and insulin treated (sufficient to normalize blood glucose concentrations) IDDM rats were utilized in the present study to provide two models for the examination of the relationship between ECF and GFR.

Methods

All experiments were performed on Wistar rats (Harlan Sprague-Dawley, Indianapolis, Indiana, USA) which were housed at the San Diego Veterans Affairs Medical Center. The weight range of the rats was 280 to 360 g at the time of the studies.

Received for publication February 26, 1990
and in revised form January 23, 1991
Accepted for publication January 24, 1991

Chronic cannulation procedure

Rats received at least six hours of training by sitting quietly in a restraining cage at intervals of not more than three hours at any one time in preparation for the conscious studies. After the training was completed the animals were anesthetized with a short duration anesthetic [initial i.p. injection with 65 mg/kg body wt methohexital sodium (Brevital) and i.v. doses of 5 to 10 mg/kg body wt of Brevital thereafter as required]. Sterile Tygon catheters were placed in the left femoral artery and vein. The collateral vascular supply to the leg is sufficient to permit maintenance of adequate vascular perfusion. The vascular catheters were threaded under the skin, exteriorized at the back of the neck, primed with a solution of 25% mannitol and 1,000 U/ml heparin (1:1) and plugged with pins. A 14 gauge steel cannula, enclosed in Silastic tubing, was implanted in the bladder and plugged with a stainless steel pin also covered in silastic so that the animal was able to void normally through the urethra, thus reducing the likelihood of bladder infection. This is a modification of the preparation described in detail by Gellai and Valtin [14] and has been utilized in other studies from our laboratory [15]. The animals were allowed to recover for five to seven days after the cannulation procedure prior to the initial experiments.

Specific experimental protocols

Untreated diabetics ($N = 6$, group 1). After recovery from the cannulation procedure, the rats were placed in a restraining cage and the first of two control measurement periods was performed prior to the infusion of streptozotocin and onset of insulin dependent diabetes. Two to three days following the first control measurement a second control measurement period was performed. Immediately after the second control measurement period, streptozotocin (65 mg/kg body wt, Sigma Chemical Co., St. Louis, Missouri, USA) was infused (approximately 1 min to perform infusion) through the femoral vein catheter while the rat was awake and the animal returned to its cage. Measurements were repeated at 1, 4, 7, 11, 15, and 19 days after induction of diabetes. Day 19 data was eliminated from the study due to deteriorating health of some rats with developing ketoacidosis, clogging of the vascular catheters, incomplete urine collections and agitation of the animal in the cage resulting in significantly compromised renal function measurements in the restraining cage during the study, as well as incomplete data collection at this time point. Also, if blood sugar did not increase to levels >300 mg/dl within four days after streptozotocin and it exhibited significant glycosuria, the animal was discarded.

Treated diabetic rats ($N = 6$, group 2). The protocol for the treated group of rats is essentially the same as the untreated diabetic except that the rats were treated during their diabetic phase of the study with a long acting insulin (Ultralente, NOVO Pharmaceuticals) given in the late afternoon of each day to correspond to the nighttime feeding habits of the animals. Blood glucose concentration was monitored daily via the femoral artery catheter and the following dose metered accordingly (2 to 8 U/day s.c.) to maintain a blood glucose concentration at near normal values. All animals in this group exhibited an occasional higher than normal blood glucose during the treatment phase and was used as an indication of successful establishment of diabetes.

Normal non-diabetic rats ($N = 4$, group 3). The protocol and measurement period sequence was the same as in the group 1 and 2 rats except that after the second measurement period, vehicle without streptozotocin was infused and no insulin was given to these animals. This group was utilized to provide a control for the effects of the multiple measurement procedures over time and to determine if the protocol exerted any impact on renal function or ECF.

Measurement protocol in the conscious rat

The rats were placed in a restraining cage five to seven days after recovery from surgery and two control, pre-diabetic measurement periods were performed to assess renal function and ECF in the conscious non-surgically stressed condition in each of the three groups: untreated diabetic, treated diabetic, and normal time controls. The bladder pin was removed to allow collection of urine. The arterial line was connected to a pressure transducer for measurement of mean arterial pressure (MAP) and was also utilized for blood sampling. A constant intravenous infusion of ^{3}H inulin (100 μCi/dl) and para-aminohippuric acid (PAH) (1 to 2 g/dl) was given at a rate of 5 $\mu l \cdot min^{-1} \cdot 100$ g body wt^{-1} through the venous line [15]. Differences in urinary output in groups 2 and 3 and the primary infusion were matched with an additional infusion of isotonic saline solution to maintain constant volume. Inulin distribution space was measured as an index of extracellular fluid volume during a 60-minute equilibration period [15]. In some rats the equilibration period was extended to 80 minutes to assure that complete equilibration was achieved. This was verified by comparing the plasma ^{3}H inulin concentration in the first blood sample following the equilibration period to the following collections. Less than 5% increase in plasma ^{3}H inulin concentration in the second blood sample obtained 20 minutes following the first post-equilibration blood sample was utilized as the criteria for establishment of equilibrium. This measurement was determined by monitoring both input and urinary output of ^{3}H inulin. At the end of the equilibration period the total amount of inulin in the rat was determined and assumed to be homogeneously distributed in extracellular space. A blood sample was obtained and the concentration of inulin in plasma water was measured. The total amount of inulin in the rat divided by the plasma water concentration on inulin yielded an index of extracellular volume. This procedure has been utilized previously by this laboratory [15]. A 100 μl blood sample was obtained to determine plasma catecholamine levels at the end of the equilibration period. Also, at the end of the equilibration period, three to four 20-minute urine collections were obtained and the volumes measured. Arterial blood collections (~140 μl of whole blood) were performed bracketing each urinary collection period and analyzed for hematocrit, blood glucose concentration, and inulin, PAH, and plasma protein concentrations. Urine was analyzed for ^{3}H inulin and PAH concentration. Studies that have been performed in glycosuric humans have demonstrated that errors in measurement of effective renal plasma flow can occur due to binding of glucose to PAH [16] and can be eliminated with addition of NaOH during the collection of urine [16]. We added 10 μl of 2 N NaOH in the urine collection vials in some of the initial studies, but found

this to have minimal impact on the measurements of PAH clearance in the rat and discontinued the practice. Na^+ and K^+ concentrations were also measured in both serum and urine. At the end of the experiments, the erythrocytes were reconstituted with sterile saline and reinfused into the rat. These experiments were repeated after infusion of streptozotocin or vehicle as indicated in the specific experimental protocols until day 19 at which time the final collections were obtained and the animal euthanized for examination of the catheters for signs of infection.

Measurement of plasma volume

In a separate group of rats ($N = 20$), chronic cannulation was performed of the femoral artery and vein but not the bladder catheter. The animals were allowed to recover for five to seven days prior to any experiments or induction of insulin dependent diabetes with streptozotocin (65 mg/kg body wt i.v., Sigma Chemical Co.).

The rats were divided at random into four groups ($N = 5$ in each group) and measurements of plasma volume were performed in the following conditions: 1) Control, untreated with streptozotocin; 2) 24 hours after induction of streptozotocin diabetes; 3) seven days after the induction of streptozotocin diabetes; and 4) 15 days after the induction of streptozotocin diabetes. None of the rats in streptozotocin-induced diabetes groups received any exogenous insulin or were treated for hyperglycemia in any fashion in an effort to parallel the protocol of the untreated diabetic group in which ECF was obtained. However, these rats were only utilized once for plasma volume measurements since gamma emitting radioisotopes were used and the animals could not be returned to the animal facility.

After the rat was resting quietly in the restraining cage, blood was withdrawn from the femoral artery catheter for measurement of hematocrit, plasma protein concentration and blood glucose concentration (<100 μl). Following the initial blood withdrawal, 1 ml of blood was removed and centrifuged. When the centrifugation was complete, the plasma was reinfused into the animal and the remaining red blood cells were labeled with Cr^{51} as described by Sterling [17] and Sterling and Gray [18] for measurement of plasma volume as previously performed in this laboratory [19]. Albumin labeled by I^{125} (ICN Radiochemicals, Irvine, California, USA) was also used as a plasma marker. The radiolabeled red blood cells and the I^{125} albumin were then combined in isotonic saline and after aliquots were obtained to determine total radioactivity, the remaining volume was measured and infused into the rat. Within five minutes of the infusion, a 200 μl sample of whole blood was removed and placed in a tube for counting on a gamma counter. Blood was also withdrawn at 15, 30, and 60 minutes after the infusion of the radiolabel. The zero time intercept for the I^{125} albumin and Cr^{51} labeled erythrocytes was utilized as measures of plasma volume. If there was not good agreement with the two methods, then the study was discarded.

Analytic methods

Total filtration rates were calculated as previously described [15, 20]. Renal plasma flow was determined from the clearance of PAH. A PAH extraction ratio of 0.85 was utilized; this has been previously shown to an average extraction ratio for the rat [14, 21]. Systemic protein concentration was determined by analysis of femoral artery plasma. Both refractometric and the method of Lowry et al [22] was utilized. Plasma and urinary Na^+ and K^+ concentrations were determined by flame photometry (Instrumentation Laboratory, Lexington, Massachusetts, USA).

Norepinephrine, epinephrine, and dopamine concentrations in systemic plasma in all three groups were determined by a highly sensitive radioenzymatic assay for catecholamines previously described [23] and utilized by this laboratory [15].

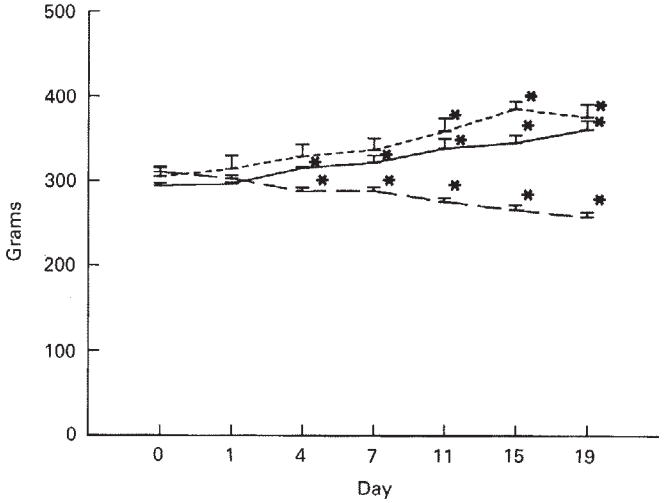

Fig. 1. *Effect of untreated (IDDM, long dashed line) and insulin treated diabetes (TR IDDM, short dashed line) on body weight.* IDDM rats lost weight significantly during the time course of the study ($P < 0.05$). TR IDDM rats' body weight increased at the same rate as the time controls (solid line). Since there were marked differences in body weight by day 15 in untreated versus treated diabetic rats, renal function was normalized to 100 g body weight in the following figures. $*P < 0.05$ compared to respective pre-diabetic control measurements (day 0 values).

Statistical analysis

Significance of the data between control and experimental conditions was determined by analysis of variance and paired Student's *t*-test where appropriate in the conscious rat studies [24]. Time series analysis was performed by a modification of Tukey analysis [24]. Comparisons between groups were analyzed by unpaired *t*-test to determine significant differences. The animals were not compared to the time control group but rather each individual rat's control values prior to administration of streptozotocin. All data values are given as the means ± SE.

Results

Effect of untreated and insulin treated IDDM on growth, blood pressure, blood glucose, and systemic catecholamines

All three groups of rats exhibited similar initial body weights at the start of the measurement protocols (294 ± 3 g in control, 310 ± 5 g in the untreated diabetic group, and 304 ± 11 g in the insulin treated diabetic rats). As depicted in Figure 1, the growth of the treated diabetics and time controls were not different, with a final weight of 360 ± 10 in time controls and 375 ± 15 g in the insulin-treated diabetic rats. This represents a 22%

Table 1. Effect of diabetes on mean arterial pressure

	Control	Day 1	Day 4	Day 7	Day 11	Day 15
			mm Hg			
Time controls	101 ± 2	98 ± 2	104 ± 3	105 ± 3	115 ± 5[a]	106 ± 3
IDDM	101 ± 2	100 ± 2	100 ± 3	95 ± 1[a]	93 ± 2[a]	97 ± 3
Treated IDDM	107 ± 1	106 ± 1	106 ± 1	107 ± 2	110 ± 3	113 ± 3

[a] $P < 0.05$ compared to respective Control period

weight gain in the time controls and a 23% weight gain in the treated diabetic rats. However, the untreated diabetic group actually lost a significant amount of body weight (from 310 ± 5 to 257 ± 6 g, $P < 0.05$), a 17% decrease in body weight during the time course of the study (Fig. 1).

Mean arterial pressure (MAP) was not significantly different among the three groups and did not change markedly during the time course of the study (Table 1). MAP in the time control group was 101 ± 1 mm Hg at the beginning of the study and fluctuated between 98 ± 2 and 115 ± 5 mm Hg during the 15 daytime matched study period. The untreated IDDM group exhibited a MAP of 101 ± 2 mm Hg prior to the induction of diabetes, and thereafter ranged from 93 ± 2 to 100 ± 3 mm Hg during the course of the 15 day study (Table 1). In the treated IDDM group, MAP was 107 ± 1 in the pre-diabetic measurement periods and ranged from 106 ± 1 to 113 ± 3 mm Hg during the treated IDDM portion of the study (Table 1). Overall, the untreated IDDM group exhibited a lower MAP during the IDDM portion of the study compared to the time controls or the treated IDDM groups. Glomerular hyperfiltration could not be associated with increases in blood pressure in the untreated IDDM group.

Blood glucose concentrations varied significantly between the untreated IDDM rats and the time controls or the treated IDDM rats, as depicted in Figure 2. Daily measurement of blood glucose and injection of insulin to normalize blood glucose was performed in the treated IDDM group with the resulting values at the time of the measurement periods shown in Figure 2. The pre-insulin injection values of blood glucose determined by afternoon measurements in the treated group (2 to 4 p.m.) resulted in slightly different blood glucose values than those measured at the time of the studies, 237 ± 14 mg/dl.

Awake, unstressed, plasma epinephrine, norepinephrine, and dopamine concentrations are depicted in Table 2. Epinephrine levels did not change after induction of IDDM compared to the control pre-diabetic measurement periods in both the untreated and treated IDDM groups. There were no changes in plasma epinephrine levels in the time control group (Table 2). Dopamine concentrations varied considerably in the individual rats, but the values during the time course of the study after induction of IDDM was not different than the respective pre-diabetic control values (Table 2). Also, there were no changes in plasma norepinephrine from pre-diabetic values to values after induction of IDDM in either the untreated or treated IDDM groups. The time controls did not exhibit significant alterations in plasma norepinephrine concentrations during the time course of the study (Table 2). These data indicate that events associated with either treated or untreated diabetes did not alter plasma catecholamines and, as indicated by the time controls, the study protocol and the chronic cannulation procedure do not appear to produce any changes in circulating catecholamine concentrations.

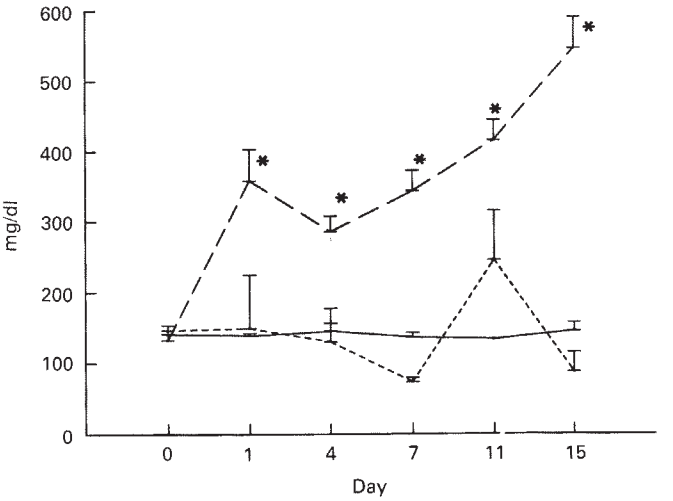

Fig. 2. *Changes in blood glucose during the 15 day study period in IDDM (long dashed line), insulin treated diabetics (TR IDDM, short dashed line), and time controls (solid line).* IDDM rats rapidly increased their blood glucose and developed glycosuria within 24 hours. Blood glucose in TR IDDM rats did not significantly differ from day 0 (pre-streptozotocin infusion control measurements) during the time of the study or compared to the time control group. *$P < 0.05$ compared to respective control measurements (day 0 values).

Effects of untreated and treated IDDM on renal hemodynamics

Urine flow from day 1 to day 15 is depicted in Figure 3 and is compared to the control period (indicated by day 0). Urine flow increased significantly within 24 hours of IDDM and remained greater than control throughout the study period. Urine flow was also significantly increased in the insulin-treated IDDM rats which maintained relatively good glycemic control at the time of the measurement period. The untreated IDDM group demonstrated marked glycosuria whereas the treated group had no glucose in the urine during the measurement period. The time control group did not exhibit any significant change in urine flow during the course of the study (Fig. 3).

Sodium excretion rate increased significantly after 24 hours in the untreated IDDM group and remained significantly increased during the course of the study. In the insulin-treated IDDM group, urinary sodium excretion was also significantly increased after onset of diabetes compared to the pre-diabetic phase of the study. However, the magnitude of the increase was less than that of the untreated IDDM group (Fig. 4).

The increase in glomerular filtration rate in the untreated

Table 2. Effect of diabetes on plasma catecholamines

	Time controls		IDDM		Treated IDDM	
	Con.	Con.	Con.	Diabetic	Con.	Diabetic
Epinephrine	71 ± 24	83 ± 19	65 ± 6	79 ± 24	118 ± 10	159 ± 30
Norepinephrine	69 ± 4	97 ± 15	196 ± 53	256 ± 39	163 ± 28	145 ± 26
Dopamine	41 ± 9	36 ± 8	195 ± 55	308 ± 82	56 ± 3	71 ± 16

Data are presented as pg/ml. Abbreviation Con. is control group.

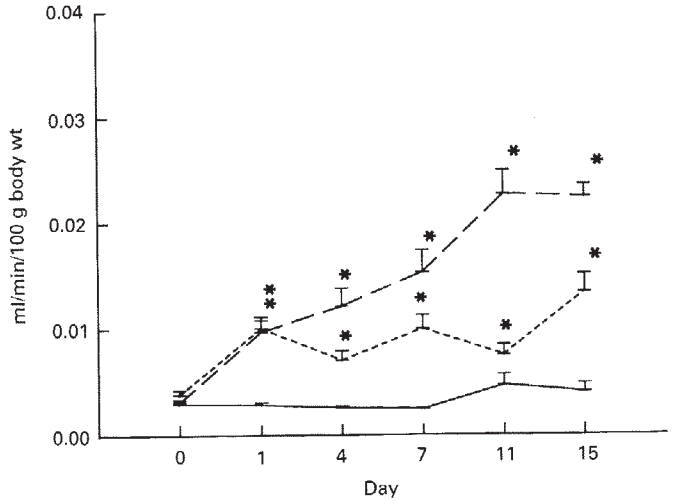

Fig. 3. *Effects of untreated (IDDM, long dashed line) and insulin treated (TR IDDM, short dashed line) on urine flow during the study period.* The solid line represents the time controls which did not significantly alter urine flow during the time course of the study. Urine flow significantly increased in both IDDM and TR IDDM rats compared to control (day 0) and to the time controls (except for TR IDDM to time controls at day 11). However, by day 11, IDDM rats had a significantly greater urine flow compared to TR IDDM rats. *$P < 0.05$ compared to respective day 0 control measurements.

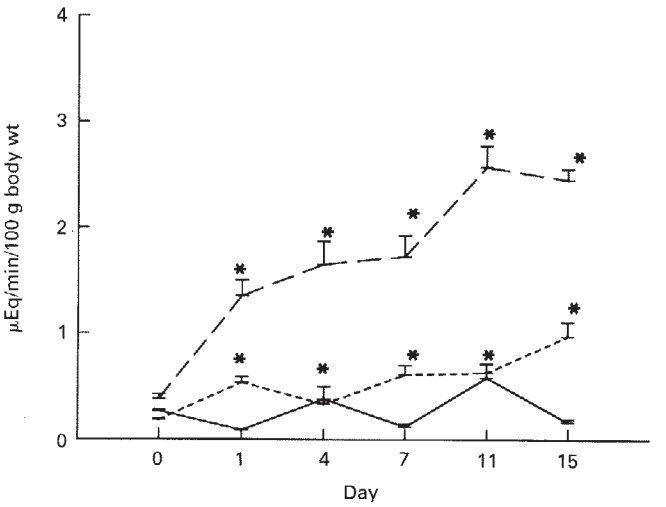

Fig. 4. *Alterations in sodium excretion rate during the first 15 days of untreated (long dashed line) and insulin treated IDDM (short dashed line).* IDDM rats demonstrated a significant increase in $U_{Na}V$ in day 1 and remained elevated during the study period ($P < 0.05$). $U_{Na}V$ in TR IDDM did not have the same magnitude of increase as in the IDDM rats and at days 4 and 11 did not differ from time control values (solid line). *$P < 0.05$ compared to respective day 0 measurements.

IDDM rats was significant after 24 hours of untreated streptozotocin-induced diabetes, and increased progressively during the time course of the study (Fig. 5). Overall, absolute GFR (non-normalized for body wt) increased from 3.2 ± 0.1 ml/min in the control measurements to 4.3 ± 0.1 ml/min during untreated IDDM ($P < 0.05$). In the treated IDDM group, GFR/100 g body weight did not increase during the time course of the study (Fig. 5). Absolute GFR increased compared to the pre-diabetic control measurements from 3.0 ± 0.1 to 3.7 ± 0.1 ml/min ($P < 0.05$) but this increase was consistent with growth and significantly less than the increase observed in the untreated IDDM group ($P < 0.05$). There was no significant change in GFR/100 g body weight in the time controls and only modest increases in absolute GFR during the time course of the study.

Renal plasma flow normalized to body weight demonstrated a marked and consistent increase after streptozotocin induced diabetes in the untreated IDDM group (Fig. 6). This increase in RPF/100 g body weight did not occur with either the treated IDDM group or the time controls (Fig. 6). In the untreated IDDM group, absolute RPF during the overall period increased from 11.8 ± 0.3 in the pre-diabetic control measurements to 13.1 ± 0.4 ml/min ($P < 0.05$). In the treated IDDM group, RPF increased from 10.4 ± 0.3 to 12.6 ± 0.3 ml/min, but this increment was consistent with growth during the study period. The time controls demonstrated an initial RPF of 10.7 ± 0.3 ml/min and did not significantly change during the remaining period of the study (11.4 ± 0.4 ml/min).

Effect of untreated and insulin treated diabetes on extracellular fluid volume

Extracellular fluid volume (ECF, % of body wt) was not significantly altered during the course of the study in the time control group (indicated by the line in Fig. 7). However, after streptozotocin induced diabetes in both the untreated and treated groups, ECF decreased significantly after 24 hours and remained at levels less than the pre-diabetic control measurements during the entire 15 day course of the study (Fig. 7). Overall, the decrease in ECF was greater in the untreated than the treated diabetic group ($P < 0.05$).

These data indicate that the early glomerular hyperfiltration observed in untreated insulin-dependent diabetes is associated with increases in renal plasma flow but cannot be correlated to increased extracellular fluid volume.

Effect of untreated insulin dependent diabetes on plasma volume

Plasma volume (PV) was 2.60 ± 0.11% of body wt (11.6 ± 0.8 ml) in the control rats, 2.53 ± 0.14% of body wt (13.1 ± 1.1 ml)

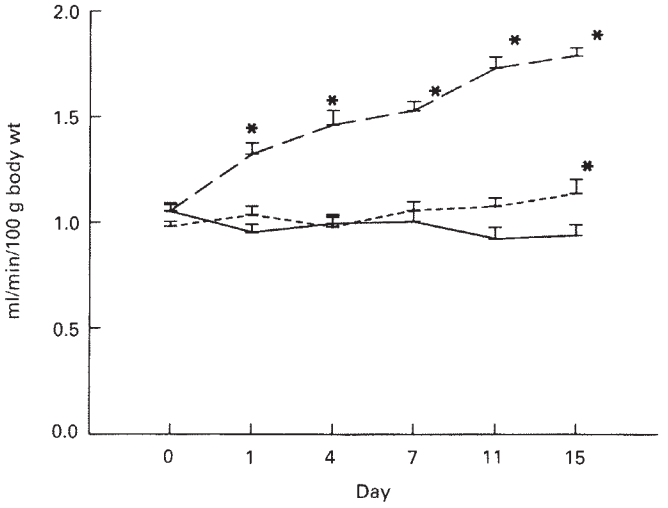

Fig. 5. *Changes in glomerular filtration rate (GFR) during the first 15 days of untreated (long dashed line) and insulin treated (short dashed line) IDDM.* IDDM rats exhibited an increased GFR after 24 hours and continued to further increase during the 15 day study period. GFR in the TR IDDM rats did not significantly change during the 15 day period and were not different from the time controls (solid line) except at the day 15 time point. *$P < 0.05$ compared to respective day 0 measurements.

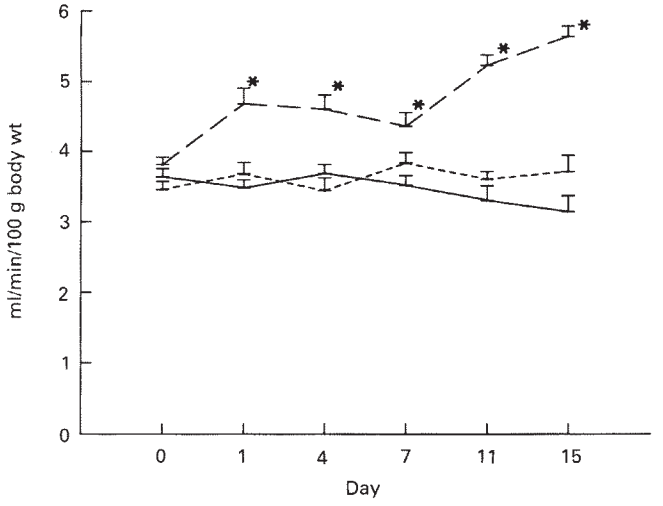

Fig. 6. *Changes in renal plasma flow (RPF) during the first 15 days of untreated (long dashed line) and insulin treated (short dashed line) IDDM.* RPF in diabetic rats increased significantly after 24 hours post-streptozotocin infusion and remained elevated for the duration of the study ($P < 0.05$) as well as significantly differing from the time control group. RPF in TR IDDM was not significantly changed from control. The solid line represents the time controls and did not significantly change during the time course of the study. *$P < 0.05$ compared to respective day 0 measurements.

after 24 hours of untreated streptozotocin-induced diabetes, 2.67 ± 0.16% body wt (11.8 ± 0.5 ml) after seven days, and 2.89 ± 0.13% of body wt (12.1 ± 0.8 ml) after 15 days. There were no significant changes in PV either by absolute volume or by percent of body wt at any time point during insulin dependent diabetes compared to control values. PV was maintained at values not different from control values even though ECF decreased significantly during the same time period in early

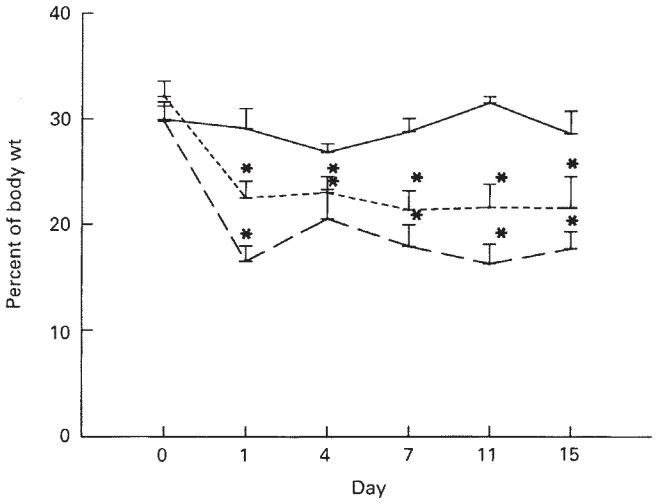

Fig. 7. *Alterations in extracellular fluid volume (ECF) during the first 15 days of untreated (long dashed line) and insulin treated (short dashed line) IDDM.* ECF in IDDM rats significantly decreased in the first 24 hours and remained at levels significantly less than control measurements ($P < 0.05$) and was significantly less than the time controls (excluding day 4). ECF in TR IDDM rats was also significantly decreased compared to control (day 0) values ($P < 0.05$). The solid line represents ECF in the time controls which did not change significantly during the period of the study. *$P < 0.05$ compared to respective day 0 measurements.

insulin dependent diabetes. However, no plasma volume expansion was observed to associate with the development of glomerular hyperfiltration.

Discussion

Increases in glomerular filtration rate or hyperfiltration is a common phenomena associated with insulin dependent diabetes in both human and animal models [1–4]. One school of thought is that hyperfiltration in the clinical condition is associated with eventual proteinuria and increasing glomerulopathy leading to renal failure [5, 6]. When blood glucose control is well maintained, hyperfiltration does not occur as observed in the early stages of IDDM in the present study and in longer term animal models of insulin dependent diabetes as well as in the human [6]. Proper glycemic control has also been associated with either a reduction or a prolongation of the onset of proteinuria [25]. Several investigators have proposed that the hyperfiltration in IDDM contributes, through glomerular capillary hypertension, to the development of diabetic nephropathy [5, 9]. However, the mechanism(s) producing glomerular hyperfiltration in IDDM is less well defined. A popular hypothesis is that increased renal plasma flow resulting in glomerular hyperfiltration is due to expansion of either plasma [3, 10] or extracellular fluid volume [1, 10]. There is some support for the hypothesis that volume expansion provides the mechanism for hyperfiltration. Hostetter, Troy and Brenner [3] found a 38% increase in plasma volume after four to six weeks of poorly controlled insulin-dependent diabetes in rats. Mogensen reported increased ECF in humans with IDDM who also exhibited increased GFR [1]. However, both clinical and animal studies have been published demonstrating that plasma volume and/or ECF was decreased in established insulin-dependent

diabetes, in which hyperfiltration persisted [12, 13]. The intent of the present study was to determine if changes in ECF could be related to changes in glomerular filtration rate from the onset of streptozotocin induced diabetes in the rat. The awake animal preparation was considered appropriate due to previous observations that anesthesia will cause a plasma volume/ECF shift [26]. In addition, monitoring the pertinent values in an individual animal throughout the development of hyperfiltration provided a temporal criteria to link increased GFR to volume expansion.

The findings of the present study indicate that ECF expansion is not a requirement for increased glomerular filtration rate. In fact, glomerular hyperfiltration developed under conditions of volume depletion in the awake animal model of IDDM. These data provide a strong argument for the dissociation of increased GFR and ECF expansion. The increase in GFR was progressive over time, whereas a reduction in ECF occurred within 24 hours of induction of diabetes and remained constant thereafter. Glomerular filtration increased rapidly in the untreated IDDM group with significant increments achieved at 24 hours after induction of insulin dependent diabetes. This elevation in GFR was associated with increased plasma flow in the untreated IDDM group. Since the increase in GFR was almost immediate, this change cannot be readily attributed to either renal hypertrophy or morphologic alterations. Carney, Wong and Dirks have also demonstrated early development of hyperfiltration in the rat [27] but did not attribute the increase to any specific mechanism. These data indicate that volume expansion is an unlikely primary cause for the glomerular hyperfiltration observed in early insulin dependent diabetes, although if present, may further magnify the increases in GFR.

One potential cause for volume depletion in the untreated IDDM group is the early observation of polyuria and increased sodium excretion. Although fluid and electrolyte intake was not monitored in this study, one can presume that at least sodium balance was in deficit. The loss in fluid from the ECF is approximately 34 ml after the first day based upon the untreated rats' change in ECF (Figs. 1 and 7). However, body weight in the untreated diabetic group only decreased by 8 ± 2 g after 24 hours (Fig. 1). Therefore, extracorporeal losses cannot account for the total decrease in ECF. The most likely reservoir for the majority of fluid exiting the ECF is the intracellular space. In the untreated IDDM group, if most of the shift of fluid was into the intracellular space, this would be indicative of early intracellular volume expansion with the intracellular space slowly returning to original, prediabetic, volume status as the animal lost weight during the time course of the study since ECF was constant during the diabetic phase of this study (Fig. 7).

In the treated IDDM group, which were maintained with relatively tight glycemic control, GFR and RPF normalized to body weight remained constant. However, fractional ECF decreased after 24 hours post-induction of streptozotocin and remained at less than control values throughout the period of the study, again, most likely a shift of fluid to intracellular space. This decrease in ECF may have been maintained by sodium losses due to increased sodium excretion that was not compensated by increased intake, potentially due to episodic hyperglycemia during periods when the renal function and ECF measurements were not performed. It is evident in the present study that insulin treatment did not ameliorate the decrease in ECF, although GFR and RPF were normalized. In either case, untreated or treated IDDM, if the volume status of the diabetic rat is characterized by a decreased ECF, then dietary salt restriction should have a considerable effect on GFR such that even a small volume decrease in ECF of 5 to 10 ml could reduce GFR. This was observed by Bank et al [11] in hyperfiltering diabetic rats in that GFR was normalized with decreased sodium intake.

Plasma volume in the untreated diabetic rats did not increase and cannot be a factor in the development of glomerular hyperfiltration. Despite the significant decrease in ECF in untreated diabetic rats, PV was maintained at values not different from control. This shift in PV/ECF ratio may indicate a change in the hydrostatic or oncotic forces at the peripheral capillary beds modifying the intra- to extravascular fluid distribution.

To summarize, the increase in glomerular filtration rate in early streptozotocin induced diabetes is associated with an increase in renal plasma flow, but there was no correlation of GFR or RPF to changes in extracellular fluid or plasma volume. The present study indicates that volume expansion need not be a factor in the development of glomerular hyperfiltration observed under the conditions of the present study. Factors other than volume expansion should be considered as a mechanism for the development of glomerular hyperfiltration in insulin dependent diabetes. Both untreated and insulin treated IDDM rats exhibited a decreased extracellular fluid volume compared to control values, indicating that insulin treatment sufficient to produce normoglycemia and maintain GFR and RPF at normal values was unable to maintain a normal extracellular to intracellular fluid balance.

Acknowledgments

Portions of this manuscript were presented at the annual meeting of the American Society of Nephrology, 1988 and was abstracted in the January, 1989 issue of *Kidney International*. This work was supported by a grant DK39900 from the National Institutes of Health.

Reprint requests to Bryan J. Tucker, Nephrology Research, V-151, University of California, San Diego, La Jolla, California 92093, USA.

References

1. MOGENSEN CE: Glomerular filtration rate and renal plasma flow in short term and long term juvenile diabetes mellitus. *Scand J Clin Lab Invest* 28:91–100, 1971
2. CHRISTIANSEN JS, GAMMELGAARD J, FRANDSEN M, PARVING H-H: Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin dependent diabetics. *Diabetologia* 20:451–456, 1981
3. HOSTETTER TH, TROY JL, BRENNER BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
4. BANK N, LAHORRA MAG, AYNEDJIAN HS, SCHLONDORFF D: Vasoregulatory hormones and the hyperfiltration of diabetes. *Am J Physiol* 254:F202–F209, 1988
5. ZATZ R, BRENNER BM: Pathogenesis of diabetic microangiopathy. *Am J Med* 80:443–453, 1986
6. MOGENSEN CE: Definition of diabetic renal disease in insulin-dependent diabetes mellitus based on renal function tests, in *The Kidney and Hypertension in Diabetes Mellitus*, edited by MOGENSEN CE, Boston, Martinus Nijhoff Publishing, 1988, p. 7
7. DITZEL J, BORCHNER-MORTENSEN J, RODBRO P: Elevated glomerular filtration rate in early diabetes may not be explained by increased sodium reabsorption secondary to an impairment in renal

tubular handling of phosphate. *Horm Metab Res* (Suppl) 11:87–89, 1981
8. FAUCHALD P, NORSETH J, JERVELL J: Transcapillary colloid osmotic gradient, plasma volume and interstitial fluid volume in long-term Type 1 (insulin-dependent) diabetes. *Diabetologia* 28: 269–273, 1985
9. HOSTETTER TH, RENNKE HG, BRENNER BM: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med* 72:375–380, 1982
10. ILSTRUP KM, KEANE WF, MICHELS LD: Intravascular and extracellular volumes in the diabetic rat. *Life Sci* 29:717–724, 1981
11. BANK N, LAHORRA MAG, AYNEDJIAN HS, WILKES BM: Sodium restriction corrects hyperfiltration of diabetes. *Am J Physiol* 254: F668–F676, 1988
12. OLSHAN AR, O'CONNOR DT, COHEN IM, STONE RA: Hypertension in adult onset diabetes mellitus: Abnormal renal hemodynamics and endogenous regulatory factors. *Am J Kidney Dis* 2:271–279, 1982
13. BAROSI G, BARALDI A, BELLOMO G, CAZZOLA M, MARTINO AP, PALESTRA P, SPRIANO P, FRANTINO P: Increased whole body hematocrit: Venous hematocrit ratio in diabetes mellitus, evidence of microcirculatory hemoconcentration. *Acta Diabetol Lat* 21:257–262, 1984
14. GELLAI M, VALTIN H: Chronic vascular constrictions and measurements of renal function in conscious rats. *Kidney Int* 15:419–427, 1979
15. TUCKER BJ, MUNDY CA, ZIEGLER MG, BAYLIS C, BLANTZ RC: Head-down tilt and restraint on renal function and glomerular dynamics in the rat. *J Appl Physiol* 63:505–513, 1987
16. DALTON RN, WISEMAN MJ, TURNER C, VIBERTI G: Measurement of urinary para-aminohippuric acid in glycosuric diabetics. *Kidney Int* 34:117–120, 1988
17. STERLING K: Radioactive chromium technic for circulation red cell volume. *Meth Med Res* 8:69–76, 1960
18. STERLING K, GRAY SJ: The tagging of red blood cells and plasma protein with radioactive chromium. *J Clin Res* 29:1604–1609, 1950
19. TUCKER BJ, BLANTZ RC: Effect of furosemide administration on glomerular and tubular dynamics in the rat. *Kidney Int* 26:112–121, 1984
20. BLANTZ RC, TUCKER BJ: Measurement of glomerular dynamics, in *Methods of Pharmacology, Renal Pharmacology*, edited by M. MARTINEZ-MALDONADO, New York, Plenum Press, 1978, vol. 4B, p. 141–163
21. BAYLIS C, BADR KF, COLLINS RC: Effect of chronic prolactin administration on renal hemodynamics in the rat. *Endocrinology* 117:722–729, 1985
22. LOWRY OH, ROSENBROUGH NH, FARR AI, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
23. DURRETT LR, ZIEGLER MG: A sensitive radioenzymatic assay for catechol drugs. *J Neurosci Res* 5:587–598, 1980
24. ZAR JH: *Biostatistical Analysis*. Englewood Cliffs, N.J., Prentice-Hall, 1984
25. FELDT-RASMUSSEN B, MATHESEN E, DECKERT T: Effect of two years of strict metabolic control on the progression of incipient nephropathy in insulin-dependent diabetes. *Lancet* 2:1300–1304, 1986
26. MADDOX DA, PRICE DC, RECTOR FC: Effects of surgery on plasma volume and salt and water excretion in rats. *Am J Physiol* 233: F600–F606, 1977
27. CARNEY SH, WONG NL, DIRKS JH: Acute effects of streptozotocin diabetes on rat renal function. *J Lab Clin Med* 93:950–961, 1979