Characterization of the Development of Renal Injury in Type-1 Diabetic Dahl Salt-sensitive Rats

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Running Title: Diabetic renal disease in SS rats

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Diabetic renal disease in SS rats

The present study compared the progression of renal injury in Sprague Dawley (SD) and Dahl salt-sensitive (SS) treated with streptozotocin (STZ). The rats received an injection of STZ (50 mg/kg, i.p.) and an insulin pellet (2 U/day, s.c.) to maintain the blood glucose levels between 400-600 mg/dL. Twelve weeks later, arterial pressure (143±6 vs. 107±8 mmHg) and proteinuria (557±85 vs. 81±6 mg/day) were significantly elevated in STZ-SS rats compared to the values observed in STZ-SD rats, respectively. The kidneys from STZ-SS rats exhibited thickening of glomerular basement membrane, mesangial expansion, severe glomerulosclerosis, and renal interstitial fibrosis occasional glomerular nodule formation. In additional studies, treatment with a therapeutic dose of insulin (4 U/day, s.c.) attenuated the development of proteinuria (212±32 mg/day) and renal injury independent of changes in arterial pressure in STZ-SS rats. Since STZ-SS rats developed severe renal injury, we characterized the time course changes in renal hemodynamics during the progression of renal injury. After 9 weeks of diabetes, there was a 42% increase in GFR in STZ-SS rats versus time-control SS rats with reduced RBF. These results indicate that SS rats treated with STZ develop hyperfiltration and progressive proteinuria and display renal histological lesions characteristic to those seen in patients with diabetic nephropathy. Overall, this model may be useful to study signaling pathways and mechanisms that play role in the progression of diabetes-induced renal disease and the development of new therapies to slow the progression of diabetic nephropathy.

**Keywords:** type I diabetes, STZ, hyperfiltration, glomerulosclerosis, renal fibrosis, Dahl S rats, renal hemodynamics, diabetes-associated renal disease
INTRODUCTION

Diabetes is the leading cause of end stage renal disease (ESRD), yet the mechanisms involved in the development of diabetic nephropathy remain poorly understood. A confounding factor is that not all patients with diabetes develop renal disease suggesting that hyperglycemia alone does not account for the renal injury. African Americans are at a much greater risk for the development of diabetic nephropathy (35). This suggests that environmental factors, as well as genetic susceptibility, contribute to pathogenesis of diabetic nephropathy. Clearly, there is a critical need to better understand the genetic factors and mechanisms by which diabetes triggers development of glomerulosclerosis and renal interstitial fibrosis in patients that are genetically susceptible to renal injury.

The Dahl salt-sensitive (SS) rat is a salt-dependent model of hypertension that is highly susceptible to the development of renal disease when fed a high salt (HS) diet (5, 8, 9, 23, 26-28, 31, 34). The development of glomerular disease is associated with impaired renal autoregulation contributing to altered renal hemodynamics that causes elevations in glomerular hydrostatic pressure (Pgc) that precede the development of progressive renal injury in these animals (33). The glomerular lesions that develop resemble those seen in patients with hypertension-induced nephropathy. Thus, unlike most rodent models that are highly resistant to the development of diabetic nephropathy such as the type 1 diabetic streptozotocin (STZ) treated Sprague Dawley (STZ-SD) rat, we hypothesize that the SS rat would be more susceptible to the development of glomerular injury and renal fibrosis when treated with STZ (STZ-SS). In support of our hypothesis, Korner et al. reported that SS rats treated with STZ develop hypertension, glomerular injury and albuminuria (18). Therefore, the current study compared the development of renal injury in STZ-SS rats that are susceptible to renal injury versus STZ-SD rats which are resistant to renal disease. In addition, we also determined the temporal changes in renal hemodynamics
during the progression of diabetes-induced renal injury in STZ-SS, since they develop similar renal histologic lesions to patients with diabetic nephropathy.

METHODS

General. Experiments were performed on 96, 8-21 week-old male SD and SS rats. SD rats were purchased from Taconic Farms (Germantown, NY). SS rats were obtained from in house colonies of SS/Jr rats that has been maintained by brother sister mating for 20 years from breeder pairs originally obtained from Dr. John Rapp at the University of Toledo. The rats are housed in the Laboratory Animal Facility at the University of Mississippi Medical Center, which is approved by the American Association for the Accreditation of Laboratory Animal Care. The rats had free access to food and water throughout the study. All protocols were approved by the Animal Care Committee of the University of Mississippi Medical Center.

Protocol 1. Comparison of the development of diabetes-induced renal disease in STZ-treated Sprague Dawley (SD) and Dahl salt-sensitive (SS) rats. Experiments were performed on nine week-old SD and SS rats that were treated with streptozotocin (STZ, 50 mg/kg, i.p.) to induce diabetes and given one long-acting insulin implant (low dose, 2 U/day, s.c., recombinant human insulin, Linshin Canada, Ontario, Canada) to maintain blood glucose levels between 300-500 mg/dL. The rats were fed a 0.3% NaCl diet (Harlan Teklad 7034, Harlan Laboratories, Madison, WI) to minimize the development of hypertension. A catheter of a telemetry unit (Model TA11PA-C40, Data Sciences International, St. Paul, MN) was implanted in the femoral artery and the unit was placed under the skin on the back at 8 weeks of age for the measurement of mean arterial pressure (MAP). After one week of recovery, MAP was recorded for three consecutive days to obtain a baseline MAP at 9 weeks of age, and then, the rats were injected with STZ. One week after STZ injection, diabetes was confirmed by the measurement of blood
glucose levels and the insulin pellets were implanted subcutaneously. MAP was measured in 3 week intervals throughout the study for 12 weeks. At each time point, urine was collected overnight to determine protein excretion and blood was collected from the tail vein to measure blood glucose levels. At the end of the study, the kidneys were collected, weighed and fixed in a 10% buffered formalin solution. Paraffin sections (3 μm) were prepared and stained with periodic acid-Schiff and Masson’s Trichrome to assess the degree of glomerular injury and renal fibrosis, respectively, on approximately 30 images per section. Thirty glomeruli per section were scored in a blinded fashion on a 0-4 scale with 0 representing a normal glomerulus, 1 representing a 25% of loss, 2 representing a 50% loss, 3 representing a 75% loss, and 4 representing >75% loss of capillaries in the tuft. Five glomerular images at 100X were taken from each section to determine the thickness of the glomerular basement membrane (GBM) by using the NIS-Elements D 3.0 software. Additional analysis was performed to determine the degree of renal fibrosis. Images were captured using a Nikon Eclipse 55i microscope equipped with a Nikon DS-Fi1 color camera (Nikon Inc., Melville, NY) and analyzed for the percentage of the image stained blue (primarily collagen) in the Mason trichrome stained sections using the software mentioned previously. Fifteen to twenty representative fields were analyzed per section.

**Protocol 2. Effect of controlling blood glucose levels with insulin in STZ-treated Dahl salt-sensitive (SS) rats on the development of proteinuria and renal injury.** We next determined whether controlling blood glucose levels with a therapeutic dose of insulin would prevent the development of renal disease in STZ-SS rats. Experiments were performed on two groups of 9 week-old SS rats implanted with telemetry transmitters at 8 weeks of age and treated with either vehicle or STZ. STZ-SS rats were separated into two groups and given either one insulin implant (low dose, 2 U/day, s.c., recombinant human insulin) to maintain hyperglycemia at 300-500
mg/dL or given two implants (therapeutic dose, 4 U/day, s.c., STZ-insulin-SS) that normalizes plasma glucose levels. Arterial pressure and protein excretion were measured every 3 weeks until 12 weeks of diabetes was completed. At each time point, blood was collected for the measurement of blood glucose levels. At the end of the experiment, a final blood sample was collected to determine the plasma concentration of insulin (Mercodia Rat Insulin ELISA, Uppsala, Sweden). The kidneys also were weighed, collected and fixed in a 10% buffered formalin solution for the assessment of renal injury as described above.

**Protocol 3. Time course of changes in renal hemodynamics in control and STZ-treated Dahl salt-sensitive (SS) rats.** Measurement of renal hemodynamics was performed on two groups of SS rats treated with either vehicle (Control-SS) or STZ (STZ-SS) with a low dose of insulin (2 U/day, s.c.) to maintain blood glucose levels between 300-500 mg/dL during the control period and 3, 9, and 12 weeks after induction of diabetes. On the day of the experiment, the rats were anesthetized with Ketamine (30 mg/kg, *i.m.*, Phoenix Pharmaceutical Co., St. Joseph, MO) and Inactin (50 mg/kg, *i.p.*, Sigma, St. Louis, MO) and catheters were placed in the femoral artery and vein for the measurement of arterial pressure and the infusion of a 2% BSA solution containing FITC-labeled inulin (2 mg/mL, Sigma, St. Louis, MO) in a 0.9% NaCl solution at a rate of 6 mL/hr. Renal blood flow (RBF) was measured using a flowmeter (Transonic System, Ithaca, NY) and GFR was measured from the clearance of FITC-labeled inulin. After a 30 minute equilibration, urine and plasma samples were collected during a 30-minute collection period. At the end of the experiment, the left kidney was removed and weighed and the concentrations of inulin in the urine and plasma samples were determined with a microplate fluorometer (Bio Tek Instruments, Winooski, VT).
**Statistical analysis.** Mean values ± SEM are presented. The significance of differences in control and experimental values within the same animal were determined by a paired t test. The significance of differences in the mean values between groups was determined by either an one-way or two-way repeated measures ANOVA followed by Holm-Sidak test. \( P < 0.05 \) was considered to be significant.

**RESULTS**

**Protocol 1. Comparison of the development of diabetes-induced renal injury in STZ treated SD and SS rats.** A comparison of development of renal injury in STZ treated SD and SS rats is presented in **Figure 1**. Baseline blood glucose levels were similar in SD and SS rats (99±4 and 96±7 mg/dL, respectively) (**Figure 1A**). After 12 weeks of diabetes, blood glucose levels increased to 500±7 and 469±35 mg/dL, respectively, in STZ treated SD and SS rats. After 12 weeks of diabetes, MAP rose from 117±1 to 143±6 mmHg in STZ-SS rats while it remained unaltered in STZ-SD rats (107±8 mmHg) (**Figure 1B**). Over the course of the study, proteinuria increased to 557±85 mg/day in STZ-SS rats but only increased to 81±10 mg/day in STZ-SD rats (**Figure 1C**).

A comparison of the degree of renal injury in STZ-SD and STZ-SS rats is represented in **Figure 2**. The kidneys from STZ-SS rats exhibited severe renal injury with thickening of the glomerular basement membrane, mesangial expansion, severe glomerulosclerosis and renal interstitial fibrosis (**Figure 2B and 2D**). We also observed occasional formation of glomerular nodules (Figure 2B, arrow) in the kidneys of STZ-SS rats. In contrast, kidneys from STZ-SD rats exhibited only a minor expansion of the mesangial matrix in the glomerulus (**Figure 2A**) and little if any renal interstitial fibrosis (**Figure 2C**). The glomerular injury score was significantly
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greater in STZ-SS rats compared to STZ-SD rats (Figure 2E). The percentage of renal fibrosis (blue staining) (Figure 2G) and GBM thickness (Figure 2H) were significantly elevated in STZ-SS rats compared to values seen in STZ-SD rats after 12 weeks of diabetes. The glomerular injury score in STZ-SS rats averaged 2.7 indicating that approximately 66% of the glomerular capillary area available for filtration was lost. This could be due to focal glomerular sclerosis with very severe injury to some glomeruli and less involvement in others or global injury to most glomeruli. To address the issue we measured the percent of the glomeruli with severe injury (score >3) and found that 66% of the glomeruli were severely injured. This indicates that the STZ-SS rats exhibit global rather than focal glomerulosclerosis after 12 weeks of diabetes in (Figure 2F).

Protocol 2. Effect of effective control of blood glucose levels with chronic insulin therapy on the development of renal injury in STZ-SS rats. Plasma insulin levels were significantly lower in STZ-SS rats treated with the low dose of insulin compared to control SS rats (0.59±0.04 vs. 0.95±0.05 ng/mL, respectively). Increasing the dose of insulin normalized plasma insulin levels in the STZ-insulin-SS rats to 1.04±0.08 ng/mL. The effects of controlling blood glucose levels with a therapeutic dose of insulin on the development of hypertension and proteinuria in STZ-SS rats are presented in Figure 3. Baseline blood glucose levels were similar in all groups (89±4 mg/dL) (Figure 3A). One week after STZ injection, blood glucose levels increased to the same extent in STZ-SS and STZ-insulin-SS groups (516±34 mg/dL) while blood glucose levels remained normal for the duration of the study in control SS rats (86±4 mg/dL). Administration of a therapeutic dose of insulin effectively controlled blood glucose levels in the STZ-insulin-SS rats to the same levels seen in control SS rats. The induction of diabetes in STZ-SS rats caused proteinuria to increase to 544±60 mg/day whereas proteinuria only increased to 248±38 mg/day
in control SS rats. Normalization of glucose levels with a therapeutic dose of insulin prevented the increase in proteinuria seen in STZ-SS rats (Figure 3C).

A comparison of the degree of renal injury in control, STZ and STZ-insulin SS rats treated for 12 weeks is presented in Figure 4. The kidneys from STZ-SS rats exhibited severe glomerulosclerosis, renal fibrosis, tubular necrosis, and inflammation (Figure 4A). The glomerular injury score (Figure 4B) and the percentage of renal fibrosis (Figure 4C) was significantly elevated in STZ-SS rats as compared to values observed in control SS rats. Administration of a therapeutic dose of insulin prevented the increase in the glomerular injury score but was only partially effective in reducing the degree of renal interstitial fibrosis in STZ-insulin-SS rats. We observed a significant increase in kidney weight (renal hypertrophy) in STZ-SS rats versus the values seen in control SS rats (2.30±0.12 vs. 1.41±0.04 g, respectively) (data not shown). Treatment with a therapeutic dose of insulin reduced the degree of renal hypertrophy in STZ-insulin-SS by 26% (1.70±0.11 g).


The results of these experiments are represented in Figure 5. We did not observe any differences in MAP (measured under Inactin anesthesia) between control SS and STZ-SS rats and both groups averaged 145±5 mmHg by the end of the study (data not shown). Left kidney weight to body weight ratio (LKW/BW) was similar at baseline between the two groups (data not shown). However, after 3 weeks of diabetes, LKW/BW ratio rose by 55% in STZ-SS rats compared to values observed in control SS rats and remained at this elevated level for the duration of the study. After 3 weeks of hyperglycemia, RBF increased by 35% increased versus the values observed in control SS rats. However, when normalized for the increase in kidney weight, there was no difference in RBF in STZ-SS rats and SS rats at 3 weeks of age indicating
that the rise in total renal blood flow was secondary to renal. Normalized RBF decreased significantly over the remainder of study in STZ-SS rats compared to time-control SS rats (Figure 5A). Similarly, there was a 40% increase in total GFR in the first 3 weeks after induction of diabetes in the STZ-SS rats that was secondary to the initial renal hypertrophy but GFR factored per gram kidney weight was not altered. Normalized GFR increased by 42% increase in STZ-SS rats that were diabetic for 9 weeks compared to values seen in control SS rats of the same age (Figure 5B). Thereafter, GFR was reduced by 44% in STZ-SS rats versus control SS rats after 12 weeks of diabetes which likely reflects the progression of glomerulosclerosis and loss of filtration area and the increase in renal vascular resistance (Figure 5D). Filtration fraction (FF) was the same in both groups at baseline and after 3 weeks of diabetes (Figure 5C). FF was significantly elevated in STZ-SS rats versus control SS rats when animals reached 9 weeks of diabetes. However, after 12 weeks of diabetes, FF was similar in both groups.

DISCUSSION

The SS rat has been commonly used to investigate mechanisms involved in the development of hypertension-induced renal disease. Williams et al. observed that the development of renal injury in SS rats fed a high salt diet was associated with a marked elevation in Pgc (33) suggesting they exhibit an impairment in long-term autoregulation of RBF and GFR. This was further supported by preliminary studies by Roman and colleagues demonstrating that SS rats an impaired myogenic response in afferent arterioles of SS rats (14). Therefore, we hypothesized that they might be more susceptible to the development of proteinuria and glomerulosclerosis following induction of hyperglycemia unlike other strains of rats and mice (i.e. SD rat). The present study compared the development of diabetes-induced renal injury in
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SD and SS rats treated with STZ. Administration of STZ to SD and SS rats raised blood glucose levels to a similar extent; however, hyperglycemia stimulated the development of hypertension, progressive proteinuria and severe glomerular disease in STZ-SS rats following 12 weeks of diabetes but had little effect in SD rats treated with STZ. Normalizing blood glucose levels by administration of a therapeutic dose of insulin prevented the development of proteinuria and the degree of glomerular injury in STZ-SS rats indicating the changes seen were dependent on hyperglycemia and not due to a nephrotoxic effect of STZ. Since we observed that STZ-SS developed most of the characteristics of human diabetic nephropathy including renal hypertrophy, thickening of the glomerular basement membrane, expansion of the mesangial matrix, severe glomerulosclerosis and renal interstitial fibrosis, we next examined the time course changes in renal hemodynamics during the progression of renal injury. We observed hyperfiltration at 9 weeks of diabetes that was associated with the development of progressive proteinuria and renal injury in STZ-SS rats. Later, after 12 weeks of diabetes, GFR declined and this is consistent with the progression of severe glomerulosclerosis and loss of filtration area seen in a large percentage (>66%) of glomeruli 12 weeks after the onset of diabetes in SS-STZ rats.

Several factors have been reported to contribute to the progression of renal injury in diabetic nephropathy including hyperglycemia, hypertension, proteinuria and genetic background (17). The ethnic background plays a major role as well because African American patients are susceptible to hypertension, diabetic nephropathy and ESRD (13). In the present study, we compared the progression of diabetes-induced renal injury in SS rats that are more susceptible to renal injury to SD rats which are resistant to renal disease when treated with STZ. We found that proteinuria was 5 times higher in STZ-SS rats compared to STZ-SD rats after 12 weeks of diabetes. The kidneys from STZ-SS displayed histological lesions that characteristic of those
patients with diabetic nephropathy. We believe the increase in susceptibility in African
Americans and SS rats may due to alterations in renal hemodynamics in which the autoregulation
of either RBF or GFR in response to increases in arterial pressures is impaired. In response to
elevations in arterial pressure by either increased salt intake (19) or norepinephrine infusion (24),
GFR increases substantially more in African Americans compared to their Caucasian
counterparts. Similarly, previous studies have shown that the kidneys of SS fed a high salt diet
for a week or so exhibit elevations in Pgc caused by reductions in afferent arteriolar resistance
which allows greater transmission of systemic pressure to the glomerulus to initiate the
development of proteinuria and renal injury (2). Previous studies by Hayashi et al. using the
hydronephrotic kidney preparation (15) and more recent preliminary studies by Ge et al. (29)
have suggested that the myogenic response of the afferent arteriole is impaired in SS rats fed
either a low salt (LS) or HS diet. These data suggest that glomerular hyperfiltration in response
to hyperglycemia may be a mechanism contributing to the increased susceptibility to develop
diabetic nephropathy.

Previous investigators have reported that renal vasodilation and hyperfiltration precedes
the development of proteinuria and diabetic nephropathy whereas in later stages, diabetic
patients develop chronic kidney disease and hypertension (36). However, most rodent models of
diabetic nephropathy especially mouse and rat models treated with STZ do not develop
progressive proteinuria or severe glomerular injury. Moreover, the characterization of the early
changes in renal hemodynamics in most of these models has not been well described. In the
current study, we found that the development of renal injury in STZ-SS rats was associated with
first with a rise in RBF and GFR during the first 3 weeks of induction of diabetes. However,
RBF and GFR were not elevated when normalized for kidney weight and these early increases in
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RBF and GFR likely reflect the adaptive hypertrophy of the kidney to hyperglycemia. After the initial renal hypertrophy, RBF declined over the course of the experiment but there was a two-fold increase in GFR (normalized for kidney weight) after 9 weeks of diabetes compared to the values seen in time control SS rats of the same age indicating that the STZ-SS rats developed a glomerular hyperfiltration between 3 and 9 weeks of age. This may be due to the hyperglycemia-mediated stimulation of the renin angiotensin system constrict the efferent since we found that that filtration fraction was elevated after 9 weeks of diabetes in STZ-SS rats compared to control SS rats. Thereafter, STZ-SS rats displayed a decline in renal function and GFR which likely reflects the progressive loss of capillary filtration area in most of the glomeruli of the kidney that eventually overcomes the initial renal vasodilation and glomerular hypertrophy. Overall, the current study is one the first studies that shows early increases and sequential changes in renal hemodynamics in a rodent model of diabetes-induced renal injury. These data suggest that the initial changes in renal hemodynamics during the early phase of diabetes is due to adaptive renal hypertrophy that is later followed an increase in GFR (hyperfiltration) leading to the development of renal injury and chronic kidney disease.

STZ has been extensively used to induce diabetes in many strains of rats including Sprague Dawley (SD) (1, 20, 21, 25), Wistar-Kyoto (WKY) (6, 32) and Spontaneously hypertensive (SHR) strains (6, 7, 10-12, 16). Previous studies have demonstrated that the development of renal injury is greater in STZ-treated SHR rats as compared to WKY rats. This likely is secondary to the hypertension in SHR rats that is transmitted to the glomerulus after the onset of diabetes (6). However, even in the SHR, the development of the renal disease occurs very gradually over an 8 month period and it remains to be determined whether the renal injury in this model truly reflects diabetic nephropathy and can be prevented by normalizing
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glucose levels or blood pressure. In an attempt to enhance the development of diabetes induced renal injury other investigators have induced diabetes with STZ in uninephrectomized strains of rats and mice (16, 32). However, the time frame for the development of renal histological lesions varies among the strains from 4 to 8 months after STZ administration. In the current study, proteinuria increased in the first 3 weeks following administration of STZ to SS rats. Over the course of study, hyperglycemia increased arterial pressure by 20 mmHg and augmented the development of renal injury in this model. By 12 weeks, the kidneys from STZ-SS rats show histological evidence of renal injury that is at least equal to and in most cases far greater than that reported in any other strain of rats or mice treated with STZ. Similar results are observed in the two-kidney, one-clipped diabetic model in which the combination of diabetes and elevations in arterial pressure exacerbates renal disease (22). These data indicate that the SS rats are more susceptible to the development of renal injury than other strains and they may be a useful model to study therapeutic interventions to slow the progression of diabetic nephropathy.

To determine whether the development of renal injury was due to elevations in blood glucose levels, STZ-SS rats were treated with a therapeutic dose of insulin. The tight control of blood glucose levels with insulin following the initial onset of diabetes prevented the development of renal injury in STZ-SS rats. There are two possible mechanisms by which treatment with insulin could have a renoprotective effect other than reducing blood glucose levels: (1) preventing the hyperglycemia-mediated increase in GFR and (2) reducing renal hypertrophy. Chang et al. recently reported the prevention of hyperglycemia with insulin normalized GFR and inhibited the development renal injury in diabetic mice (4). This may suggest that insulin by restoring normoglycemia prevents the increased glucose-stimulated hyperfiltration in diabetes by blunting tubuloglomerular feedback and causing dilation of the
afferent arteriole (30). The inhibition of increased GFR decreases the reabsorption of filtered protein contributing to renal hypertrophy. While treatment with a therapeutic dose of insulin prevented most signs of renal injury, it had a minimal effect reducing renal interstitial fibrosis in STZ-SS rats. This may be due to an inflammatory response by exogenous r-human insulin used in the current study that causes fibrosis for 12 weeks. Overall, these data indicate that treating diabetes early with insulin prevents hyperglycemia, elevations in GFR (hyperfiltration) and inhibits renal hypertrophy which inhibits the progression of diabetes-associated renal disease.

Patients with type 1 diabetes are at higher risk to develop diabetic nephropathy than type 2 diabetic patients. Currently, there are no rodent models of type 1 diabetic complications that express all the characteristics of diabetic nephropathy that are observed in patients. Recently, the Animal Models of Diabetic Complications Consortium (AMDCC) defined characteristics of validating rodent models for diabetic nephropathy which are 1) progressive proteinuria with a decline in renal function compared to control animals of the same age, 2) glomerulosclerosis with thickening of the glomerular basement membrane, 3) mesangial matrix deposition, 4) development of hypertension, and 5) formation of nodules (3). The current study demonstrated that the STZ-SS model meets mostly all the criteria for diabetic nephropathy (Table 1). STZ-SS rats developed progressive proteinuria, thickening of the glomerular basement membrane, expansion of the mesangial matrix, renal and glomerular hypertrophy, severe glomerulosclerosis and renal interstitial fibrosis and occasional formation of glomerular nodules. Moreover, proteinuria develops faster and the degree of renal injury is more severe in STZ-SS rats than in most other strains of rats and mice treated with STZ (i.e. STZ-SD rats). The mechanism by which renal disease develops in this model remains to be determined but it is associated with early renal vasodilation and hyperfiltration characterized by a marked increase in GFR. Over
time, STZ-SS rats develop hypertension and severe renal injury which eventually leads to a fall in RBF and GFR when taking into account for renal hypertrophy. Overall, this model may be useful to study signaling pathways and mechanisms that play role in the progression of diabetes-induced renal disease and the development of new therapies to slow the progression of diabetic nephropathy.

ACKNOWLEDGMENTS

This work was supported in part by a National Institutes of Health grant HL094446 and the Robert M. Hearin Foundation awarded to M. Garrett, National Institutes of Health grants HL36279, HL29587 and HL2958727S1 awarded to R. Roman and an American Heart Association Scientist Development Grant and a PhRMA Foundation Starter Grant awarded to J. Williams.
FIGURE LEGENDS

Figure 1. Time course measurements of blood glucose (Panel A), mean arterial pressure (MAP) (Panel B), and proteinuria (Panel C) in streptozotocin (STZ, 50 mg/kg, i.p.) treated Sprague Dawley (SD) and Dahl salt-sensitive (SS) rats with a low dose of insulin (2 U/day, s.c.,) during 12 weeks of diabetes. Numbers in parentheses indicate the number of rats studied per group. Values are means ± SE. * indicates a significant difference from the corresponding value within the same strain at week 0 and † indicates a significant difference from the corresponding value in STZ-SD rats at the same time period.

Figure 2. Comparison of the renal histopathology: Periodic acid-Schiff (PAS) staining (Panels A and B), Masson’s Trichrome (Panels C and D), glomerular injury score (Panel E), percentage of severe glomerulosclerosis (Panel F), renal fibrosis (Panel G) and glomerular membrane thickness (GBM) (Panel H) in streptozotocin (STZ, 50 mg/kg, i.p.) treated Sprague Dawley (SD) and Dahl salt-sensitive (SS) rats with a low dose of insulin (2 U/day, s.c.) after 12 weeks of diabetes. Arrow indicates areas of glomerular nodule formation. Numbers in parentheses indicate the number of glomeruli and rats studied per group. Values are means ± SE. † indicates a significant difference from the corresponding value in STZ-SD rats at the same time period.

Figure 3. Comparison of blood glucose levels (Panel A), mean arterial pressure (MAP) (Panel B) and proteinuria (Panel C) in Control and streptozotocin (STZ, 50 mg/kg, i.p.) treated Dahl salt-sensitive (SS) rats with either a low dose (2 U/day, s.c., STZ-SS) or therapeutic dose (4 U/day, s.c., STZ-insulin-SS) of insulin during 12 weeks of diabetes. Numbers in parenthesis indicate the number of rats studied per group. Values are means ± SE. * indicates a significant difference from the corresponding value within the same strain at week 0, † indicates a significant difference from the corresponding value in Control SS rats at the same time period.
and # indicates a significant difference from the corresponding value in STZ-SS rats at the same
time period.

Figure 4. Comparison of the renal histopathology (Panel A), glomerular injury scores (Panel B),
and renal fibrosis (Panel C) in Control and streptozotocin (STZ, 50 mg/kg, i.p.) treated Dahl salt-
sensitive (SS) rats with either a low dose (2 U/day, s.c., STZ-SS) or therapeutic dose (4 U/day,
s.c., STZ-insulin-SS) of insulin after 12 weeks of diabetes. Numbers in parentheses indicate the
number of glomeruli and rats studied per group. Values are means ± SE. * indicates a significant
difference from the corresponding value in Control SS rats. † indicates a significant difference
from the corresponding value in STZ-SS rats.

Figure 5. Temporal changes in renal hemodynamics in Control and streptozotocin (STZ, 50
mg/kg, i.p.) treated Dahl salt-sensitive (SS) rats with a low dose (2 U/day, s.c.) of insulin during
12 weeks of diabetes: renal blood flow (RBF, mL/min/gkw) (Panel A), glomerular filtration rate
(GFR, μL/min/gkw) (Panel B), filtration fraction (FF, %), (Panel C) and renal vascular resistance
(RVR, mmHg/mL/min/gkw) (Panel D). The number of rats studied was 5-8 animals per group.
Values are means ± SE. * indicates a significant difference from the corresponding value within
the same group at week 0 and † indicates a significant difference from the corresponding value in
Control SS rats within the same time period.
REFERENCES


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Figure 1. Diabetic renal disease in SS rats

A. Blood glucose (mg/dL)

B. MAP (mmHg)

C. Proteinuria (mg/day)

Weeks after STZ administration
Figure 2.

Diabetic renal disease in SS rats

E. Glomerular injury score

STZ-SD: (120,4) STZ-SS: (150,5)

F. % of severe glomerulosclerosis

STZ-SD STZ-SS

G. Renal fibrosis (% of blue staining)

STZ-SD: (60,4) STZ-SS: (97,7)

H. GBM thickness (μm)

STZ-SD: (30,6) STZ-SS: (35,5)
Figure 3. Diabetic renal disease in SS rats

A

Plasma glucose (mg/dL)

B

MAP (mmHg)

C

Proteinuria (mg/day)

Weeks after STZ administration

- Control SS (10)
- STZ-SS (10)
- STZ-insulin-SS (11)
Figure 4.

A

Diabetic renal disease in SS rats

B

Glomerular injury score

C

Renal fibrosis (% of blue staining)
Figure 5. Diabetic renal disease in SS rats

A. Renal blood flow (mL/min/gkW)

B. Glomerular filtration rate (mL/min/gkW)

C. Filtration fraction (%)

D. Renal vascular resistance (mmHg/mL/min/gkW)
### Table 1. Comparison of the criteria for diabetic nephropathy in human patients and streptozotocin (STZ) treated Dahl salt-sensitive (SS) rats

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Human</th>
<th>STZ-SS</th>
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<tbody>
<tr>
<td>Early increase in GFR (hyperfiltration)</td>
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<td>Progressive proteinuria</td>
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<td>✓</td>
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</tbody>
</table>