RELAND FUNCTION AND HEMODYNAMICS IN RECENT ONSET TYPE 1 DIABETES MELLITUS IN SPRAGUE DAWLEY RATS

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Summary

The present study investigated the renal functional and hemodynamic changes in rats with very recent onset of type 1 diabetes mellitus (DM). Male Sprague Dawley rats were induced with experimental DM by an i.p. injection of 55 mg/kg streptozotocin (STZ). The diabetic state in rats was confirmed by hyperglycemia, polyuria, polydipsia and reduction in the body mass. Acute clearance and hemodynamic experiments were performed 7 d after the onset of DM. During the acute study, diabetic rats showed no marked alteration (all \(P>0.05\) vs. control) in the urine flow rate (UFR). Both absolute (\(U_{NaV}\)) and fractional (\(FE_{Na}\)) sodium excretions were significantly lower (all \(P<0.05\) vs. control) in diabetic rats. Kidney glomerular filtration rate (GFR), plasma sodium (\(P_{Na}\)) and plasma creatinine (\(P_{Cr}\)) were significantly higher in diabetics (all \(P<0.05\) vs. control). Mean arterial pressure (MAP) and renal blood flow (RBF) were slightly higher while renal vascular resistance (RVR) was slightly lower; however, these changes were not significantly different from the control (all \(P>0.05\)). Kidney weight was only slightly higher in diabetic rats (\(P>0.05\) vs. control) but no observable changes in renal histology were detected. These results suggest that acute renal insufficiency of a prerenal cause seems to accompany recent onset type I DM. The changes in kidney function, at least in part, are likely to be due to the associated volume depletion.

Keywords: Acute renal insufficiency; diabetes mellitus; renal function; streptozotocin

Running title: Recent onset diabetic renal disease

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Introduction

Renal disease is a regular aspect of both insulin-dependent (Type I) and noninsulin-dependent (Type II) diabetes mellitus (DM) (1, 2) in which the developed renal changes are attributed to a great extent to existing hyperglycemia (3, 4, 5). Progression of the disease process results in end-stage renal disease (ESRD) which accounts for approximately 35% of all new admissions for renal replacement therapy (1).

Most studies examining the impact of diabetes on kidney function have utilized animal models of experimental early (5, 6, 7) or full-blown (8) diabetic nephropathy; however, less attention has been focused on the renal adaptive changes accompanying the early course of diabetes. Despite the fact that these studies have provided evidence supporting a role for both metabolic and renal hemodynamic derangements as contributing factors to the development of diabetic nephropathy, there has been a lot of confounding, discrepant and controversial results. Among the major reasons for the paucity of information are the different methodological approaches used to evaluate and quantify the changes in renal function and hemodynamics in a diabetic kidney disease, metabolic control and the particular rat strain used.

Since a pivotal criterion for adequate animal models in pathological research is a close similarity to the human disease, the present study aimed to examine the renal functional and hemodynamic changes in a group of rats with a recent onset of type I DM. For this purpose, clearance and hemodynamic experiments were performed in rats with streptozotocin (STZ)-induced diabetes.

Materials & Methods

Experimental animals
Male Sprague Dawley (SD) rats weighing 250–350 g were obtained from the Animal Care Facility, Universiti Sains Malaysia (USM), Penang, Malaysia. The animals were housed in standard cages with 12:12-h artificial light cycle, fed with a standard pellet diet (Gold coin Sdn Bhd, Malaysia) and had free access to water. All experiments were approved by the institutional Animal Ethics Committee of USM.

Drugs, chemicals and solutions
Pentobarbitone sodium (Nembutal®, CAVE, France), heparin (Leo Pharmaceuticals) and cisplatin (PCH Pharmachemie) were used as commercially available injectable solutions. STZ was purchased from Sigma Chemicals Co., St. Louis, MO, USA and freshly prepared in cold 0.9% NaCl solution (9).

Induction of diabetes mellitus and metabolic cage experiments
The rats were randomly allocated into non-diabetic control and diabetic groups (all n=5–7). The animals were caged individually in custom-built stainless steel metabolic cages and acclimatized for at least 3 d before the induction of DM with STZ. Baseline physiological data (body weight, 24 h water intake, and 24 h urine output) were recorded on day 1. Subsequently, DM was induced by a single i.p. injection of STZ (55 mg/kg) after at least 12 h of food deprivation (10). Control littersmates, on the other hand, were not treated with STZ. Further physiological data were collected twice (on d 4 and 7) prior to the use of animals in the acute renal functional and hemodynamic studies on d 8. The kidney index (KI) was calculated as 100 × kidney weight/body weight (15-17) at the end of the acute protocol.
Rats were included in the diabetic group if fasting blood glucose (FBG) levels, which were measured 3 d after STZ injection in capillary tail blood samples, were ≥250 mg/dL (5). Blood was withdrawn from the tail (between 9:00–9:30 am) and tested for glucose level using a glucometer (ApexBio, Taiwan). Apart from elevated blood glucose, changes in other physiological parameters, such as polyuria, polydipsia and a reduction in the body weight, were also considered in selecting the diabetic animals.

Surgical preparation of renal functional and hemodynamic studies

Animals were starved overnight and anesthetized with an i.p. injection of 60 mg/kg sodium pentobarbitone (Nembutal®, CAVE, France). The trachea was cannulated to provide a clear airway passage. The left jugular vein was cannulated to enable the administration of an i.v. maintenance infusion of saline (0.9 g/L NaCl infused at a rate of 6 mL/h) and also to allow supplementary injections of anesthetic (sodium pentobarbitone diluted 1:1 in 150 mM NaCl) to be given as required using bolus doses of 0.05–0.1 mL. The right carotid artery was cannulated for blood sample collection and the measurement of systemic mean arterial pressure (MAP) using a pressure transducer (P23 ID Gould, Statham Instrument, Nottingham, UK) connected to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia). The left kidney was exposed via a midline abdominal incision and the abdominal contents were carefully moved to the right. The left renal artery was cleared of connective tissue so that an electromagnetic flowmeter probe (EP100 series probe connected to a Squarewave Electromagnetic flowmeter, Carolina Medical Electronics Model FM501 King, NC) could be fitted for measurement of renal blood flow (RBF) and subsequently calculating the renal vascular resistance (RVR). The left ureter was cannulated to enable collection of urine. Upon completion of the surgical procedure, 2 mL of saline (i.v.) were given via the jugular vein cannula, after which the animal was stabilized for 1 h before the experimental protocol was begun.

Experimental protocol

MAP, RBF and RVR were continuously recorded throughout the experiment. The clearance study comprised six 20 min urine collections to calculate urine flow rate (UFR), absolute sodium excretion (U\textsubscript{Na}V), fractional sodium excretion (FE\textsubscript{Na}) and glomerular filtration rate (GFR). Blood samples were collected at the same time intervals for measurement of plasma sodium (P\textsubscript{Na}) and creatinine (P\textsubscript{Cr}) and then calculating FE\textsubscript{Na} and GFR, respectively. At the conclusion of the experiment, the animals were killed using an overdose of anesthetic and the left kidney was removed and immediately cleared of any connective tissue, blotted on tissue paper and weighed to calculate K\textsubscript{I}. Subsequently, the animals were disposed of in accordance with the guidelines of the Animal Ethics Committee of USM, Penang, Malaysia.

Biological samples and biochemical analysis

Urine samples were collected in microcentrifuge tubes (Eppendorf, Hamburg, Germany) and the volumes obtained were gravimetrically quantified. Blood samples were collected (0.5 mL) from the right carotid artery into a pre-cooled heparinized syringe, centrifuged (3000 rpm, 1 min) and the clear plasma was separated. The blood cells were resuspended in normal saline at an equal volume to the plasma obtained and reinfused into the animal immediately. Plasma and urine samples were stored at −4 °C until assayed for sodium and creatinine. Sodium levels in urine and plasma were quantified using a standard flame emission photometry while creatinine content in these biological samples was determined using a standard spectrophotometric analysis.

Histopathological study of renal tissue

The tissues were fixed in 10% formalin before being processed using Citadel 1000 histokinette (Shandon Scientific Ltd., Cheshire, UK). After processing, the tissues were embedded in paraffin with Histo-Center II-N (Barnstead/Thermolyne Corp., Dubuque, IA) and
sectioned to a thickness of 5 µm using a Reichert-Jung Histocut 820 II (Cambridge Instrument GmbH, Nussloch, Germany). The sections were stained with hematoxylin and eosin and examined under light microscope.

**Calculations**

Urine flow rate was calculated by the following formula: UFR = UV / T x BW. Here, UFR is the urine flow rate, UV is the urine volume, T is the time and BW is the body weight of the rat.

Absolute excretion of sodium was calculated using the equation: $U_{Na}V = U_{Na} \times UFR$. Here, $U_{Na}V$ is the absolute urinary excretion of sodium and $U_{Na}$ is the urine concentration of sodium.

Clearances were calculated using the usual formula: $C_x = U_x \times UFR / P_x$, where, $C_x$ is the clearance of substance $x$ and $P_x$ is the plasma concentration of $x$. Glomerular filtration rate (GFR) was determined by clearance of creatinine. Fractional excretion of sodium (FE$_{Na}$) was calculated by $C_{Na}/$GFR, where $C_{Na}$ is the clearances of sodium.

Renal vascular resistance was calculated by the equation: RVR = MAP/RBF, where RVR is the renal vascular resistance, MAP is the mean arterial pressure and RBF is the renal blood flow.

**Statistical analysis**

The response variables are the average values calculated from individual animals and are given as mean ± S.E.M. The statistical analysis of the data was done using one- and two-way ANOVA followed by Bonferroni-Dunn (all means) post-hoc test. The differences between the means were considered significant at 5% level. All statistical analysis were done using SuperANOVA statistical package (Abacus Inc., Barkley, CA, USA).

**Results**

**Metabolic study observations**

In the metabolic cage experiments in the diabetic rats there was a marked loss in body mass, hyperglycemia, polydipsia and polyuria (all $P<0.05$ vs baseline measurements prior to STZ administration). Though it tended to become higher, no significant ($P>0.05$) differences in the KI of the diabetic rats were observed as compared to their respective control group (Table 1).

**Acute study observations**

DM did not lead to a significant change ($P<0.05$ vs control) in UFR in surgically instrumented rats kept on continuous i.v. saline infusion (Fig. 1A).

In contrast, there was a significant reduction (all $P<0.05$) in sodium excretion, both in absolute terms (Fig. 1B) and as a fraction of the filtered load (Fig. 1C), in diabetic rats as compared to rats not subjected to STZ-induced DM. The GFR, however, was significantly higher ($P<0.05$) in diabetics compared with the control group (Fig. 1D).

Interestingly, a pronounced proportional rise ($P<0.05$ vs. control) in $P_{Na}$ levels of diabetic rats, which was consistent with the observed reduction in sodium excretion, was seen (Fig. 1E).

Similarly, a marked increase ($P<0.05$) in $P_{CO}_2$ levels was observed in rats induced with DM as compared to non-diabetic control rats (Fig. 1F). Although diabetic rats showed slightly higher MAP and RBF and concomitantly lower RVR compared to the control, these differences were not statistically significant (all $P>0.05$ vs control) throughout the acute protocol (Table 2).
Renal histology

No remarkable histopathological changes were identified in the renal tissue of diabetic animals compared to those renal specimens obtained from control SD rats. The light microscopy of the hematoxylin and eosin-stained renal slides showed almost intact glomeruli, renal tubules, collecting ducts and renal interstitium (Fig. 2).

Table 1. Body weight, water intake, urine flow, fasting blood glucose and kidney index in control and diabetic Sprague Dawley rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Day</th>
<th>BW (g)</th>
<th>WI (mL/d)</th>
<th>UFR (µL/min/kg)</th>
<th>FBG (mg/dL)</th>
<th>KI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>Day 1</td>
<td>281.0±4.9</td>
<td>41.7±2.8</td>
<td>15.1±2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>291.3±4.7</td>
<td>41.5±3.8</td>
<td>14.1±2.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>293.8±5.9</td>
<td>38.7±4.1</td>
<td>13.9±2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>Day 1</td>
<td>321.5±4.8</td>
<td>36.3±2.5</td>
<td>33.7±8.3</td>
<td>92.5±5.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>401.6±30.3*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>292.7±3.9*</td>
<td>95.3±11.2*</td>
<td>151.0±20.0*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>285.5±5.2*</td>
<td>144.7±7.0*</td>
<td>274.8±16.9*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.45±0.02</td>
</tr>
</tbody>
</table>

* Results are given as mean ± S.E.M. (all n=5–7). Data were analyzed by one-way ANOVA followed by Bonferroni-Dunn (all mean) post hoc test.

BW = body weight, WI = water intake, UFR = urine flow rate, FBG = fasting blood glucose and KI = kidney index.

Kidney index (%) was calculated from the weight of the kidney (g) collected following termination of the experiment and the animal fasting body weight (g).

*P<0.05 vs baseline value on day 1 in the same experimental group.
Fig. 1. Renal functional responses in control (○) and diabetic (▲) Sprague Dawley (SD) rats. (A) urine flow rate (UFR), (B) absolute sodium excretion (U_NaV), (C) fractional sodium excretion (FE_Na), (D) glomerular filtration rate (GFR), (E) Plasma sodium (P_Na) and (F) Plasma creatinine (P_Cr). Data presented as mean ± S.E.M (n=5–7). * indicates P<0.05: significant difference between diabetic rats and control rats. Data were analyzed by two-way ANOVA followed by Bonferroni-Dunn (all mean) post-hoc test.
Table 2. Mean arterial pressure, renal blood flow and renal vascular resistance in control and diabetic Sprague Dawley rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MAP (mmHg)</th>
<th>RBF (mL/min/kg)</th>
<th>RVR (mmHg/mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>108.4±1.9</td>
<td>17.6±1.2</td>
<td>23.0±2.6</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>113.9±2.6</td>
<td>19.1±1.7</td>
<td>20.2±2.0</td>
</tr>
</tbody>
</table>

a Results are given as mean ± S.E.M. (all n=5–7). Data were analyzed by one-way ANOVA followed by Bonferroni-Dunn (all mean) post hoc test. MAP = mean arterial pressure, RBF = renal blood flow and RVR = renal vascular resistance.

Fig. 2. Light microscopy of renal tissue (5 µm) from (A) control and (B) diabetic Sprague Dawley rats. Hematoxylin and eosin staining (x200). The slides show almost no marked histopathological changes between experimental groups.

Discussion

The possible existence of prerenal acute renal failure in rats with a recent onset type I DM has not been thoroughly described in the literature. Thus, the major striking finding of the present study is the demonstration that the early course of uncontrolled diabetic renal disease is likely to be accompanied by acute renal dysfunction of a prerenal cause whereby the latter represents one of the hallmarks towards progression to an intrinsic acute renal insufficiency and hence an established diabetic nephropathy.

Surprisingly, during the acute study, the urine flow rate showed a tendency to be lower in the diabetic rats than in the control cohorts. The absence of polyuria in surgically instrumented
anesthetized diabetic rats is likely to be a consequence of volume depletion since the rats in the diabetic and control groups were maintained on the same rate of continuous fluid input (6 mL/h) and the diabetic animals had no access to drinking water during the acute protocol to counteract for diabetes-induced polydipsia. Another possible explanation for this effect is that the diabetic rats might be more stressed than the control. Previous investigations in rats with STZ-induced diabetes have reported an increase in the urinary excretion of catecholamines (11, 12, 13), and therefore a certain degree of stress in the diabetic rats cannot be excluded.

Diabetes is also regarded as a major contributor to renal disease in terms of renal imbalance of electrolytes, mainly in the form of sodium retention (14). We have observed markedly high \( P_{Na} \) along with low urinary sodium in the experimental diabetic rats, indicating an apparent renal impairment of sodium handling. The development and progression into an established diabetic nephropathy is dependent on the glycemic status along with several other factors including markedly altered renal handling of sodium, a consistent finding observed in both type I and type II diabetes (15). The possible mechanism of sodium retention includes increased glomerular filtration of glucose leading to enhanced proximal tubular sodium-glucose counter-transport and an extra-vascular shift of fluid with sodium (15). The observed changes in sodium handling can further be explained in terms of changes in \( \text{Na}^{+}-\text{K}^{+}/\text{ATPase} \), one of the fundamental enzyme systems involved in the maintenance of sodium homeostasis. Indeed, it is reported that the development of diabetic renal disease involved changes in \( \text{Na}^{+}-\text{K}^{+}/\text{ATPase} \) activity (15).

This study has also shown a marked increase in GFR calculated from clearance of creatinine in the diabetic rats that can be explained in terms of hyperfiltration in these animals. It is worth mentioning that renal injury in diabetes is mainly caused by hemodynamic alterations such as hyperfiltration and hyperperfusion (16, 17). Further support of this glomerular hyperfiltration came from markedly increased GFR in these rats as described in several previous investigations (5, 6, 7, 18). The mechanisms involved in glomerular hyperfiltration are heavily debated. Researchers and investigators have characterized the functional effects of diabetes on the various segments of glomerular microvasculature. Many substances have also been invoked as humoral mediators of vasodilation in the diabetic glomerulus. This was to degree supported by the tendency towards increased RBF and reduced RVR in diabetic rats as compared to the non-diabetic control counterparts. A prior increase in proximal reabsorption capacity, with a subsequent reduction in tubuloglomerular feedback response, has been implicated as a cause for the increased GFR (19). Studies have further shown that between 25 and 40% of patients with type I DM have a GFR above the normal range of age-matched healthy subjects (20). Glomerular hyperfiltration is particularly pronounced in patients with newly diagnosed type I DM and during intervals of poor metabolic control (20), an effect which was readily reproducible in our experimental setting.

Practically noteworthy was the observation that a significant increase in \( P_{Cr} \) was observed in diabetic animals indicating possible renal impairments (21). The increased \( P_{Cr} \) observed in diabetic rats was in agreement with several earlier reports (22, 23). A non-significant increase of \( P_{Cr} \) has also been reported in the STZ-induced diabetic rats (5, 21). Together, the findings strongly suggest that glomerular hyperfiltration, sustained elevation in \( P_{Cr} \) and \( P_{Na} \) and concomitant reduction in urinary sodium excretion, mostly agreed with the likely occurrence of prerenal azotemia. It has been hypothesized that during prerenal azotemia the functional ability of proximal renal tubules remains intact and sodium reabsorbing capabilities are markedly enhanced (\( \text{FE}_{Na} < 1\% \)) because of the effects of circulating vasopressin and activation of renin-angiotensin system (RAS) (24, 25). Our findings are in agreement with the stated hypothesis since no evident tubular damage or any other structural changes were observed during the histological assessment of renal tissues of diabetic rats and \( \text{FE}_{Na} \) was far below 1%.
It is important to highlight the fact that the kidney size, indexed by the kidney weight to body weight ratio, tended to be somewhat higher in diabetic rats compared to the control. These observations suggested a tendency towards increased renal growth and thus kidney hypertrophy 7 days after STZ treatment. Several growth factors have been suggested as humoral mediators of kidney growth in the diabetic glomerulus, particularly growth hormone and insulin-like growth factor I (26). It is also believed that this growth is probably due to adaptive changes in tubular function, which prevents the urinary loss of water and electrolytes (27). Within the timeframe of our experimental setting, the slight but statistically insignificant increase in KI, which was associated with marked glomerular hyperfiltration, is most likely due to the observation that glomerular enlargement in diabetic rats may occur without detectable changes in kidney weight as the glomeruli account for ≈2% of total renal weight (28).

With respect to MAP, there was no significant difference in MAP readings of diabetic and control rats despite a few mmHg increments in diabetics. This observation is in agreement with previous reports on the effect of diabetes on the control of MAP (29). One might expect to see a significantly higher MAP values since the data reflected a marked elevation in $P_{Na}$ levels. However, it is unlikely to see such effect since diabetic animals are highly prone to dehydration and reduced blood volume. It also important to emphasize that the differences in excretion of fluid and sodium is unlikely to be due to significant differences in the MAP as it was not significantly affected by the disease state or by saline volume loading in any of the groups.

In summary, acute renal insufficiency of a prerenal cause seems to accompany recent onset type I DM. The changes in kidney function, at least in part, may be a consequence of the associated volume depletion.

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Conflict of Interest

We have no financial, consultant, institutional and other relationships that might lead to bias or conflict of interest.

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