Abnormal autoregulation and tubuloglomerular feedback in prediabetic and diabetic OLETF rats

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kushima Otsuka (LETO) rats, whereas a marked diuresis and reduced osmotic urine concentration prevail in OLETF rats. Although some studies have attempted to clarify the underlying mechanisms, a number of questions have remained. For example, it is unclear whether physiological or pathological abnormalities exist already in the prediabetic stage.

The present study demonstrates the presence of hemodynamic abnormalities in OLETF rats at both prediabetic (~11 wk of age) and diabetic stages (~40 wk of age) stages. In addition, OLETF rats exhibit pathological features of renal injury similar to those of human type 2 diabetes (18, 25). These data suggest that renal vascular dysregulation precedes and accompanies diabetic nephropathy.

METHODS

All studies were approved by and performed in compliance with the guidelines and practices of Hokkaido University Graduate School of Medicine.

Animals and preparation. Male 4-wk-old OLETF rats and LETO rats (genetic control of OLETF rats) were supplied by Otsuka Pharmaceutical (Tokushima, Japan). Rats were kept on standard rodent chow and tap water. Systolic blood pressure was measured in conscious rats by tail-cuff plethysmography (BP-98A; Softron, Tokyo, Japan). Blood glucose was measured by Medisafe mini GR 102 (TERUMO, Tokyo, Japan). Rats were anesthetized with 100 mg/kg thiobutabarbital (Inactin) intraperitoneally. Body temperature was monitored in the lower abdominal aorta (MD-100; Crystal Biotech, Northborough, MA) and arterial blood pressure was measured in conscious rats by tail-cuff plethysmography (BP-98A; Softron, Tokyo, Japan). Blood glucose was measured by Medisafe mini GR 102 (TERUMO, Tokyo, Japan). Rats were anesthetized with 100 mg/kg thiobutabarbital (Inactin) intraperitoneally.

Body temperature was maintained at 38.0°C by placing the animals on an operating table with a servo-controlled heating plate. The trachea was cannulated, and a stream of 100% oxygen was blown toward the tracheal tube throughout the experiment. Cannulas were placed in the trachea, in the femoral artery for measurement of arterial blood pressure, and in the femoral vein for an intravenous saline infusion at a rate of 1.0 ml·h⁻¹·100 g body wt⁻¹.

Measurements of total renal blood flow and superficial and deep cortical renal blood flow. Experiments were performed in male OLETF and LETO rats both in the prediabetic (10–12 wk) and diabetic (30–40 wk) age range. The femoral artery was catheterized for measurement of arterial blood pressure and blood withdrawal. Mean arterial pressure monitored in the lower abdominal aorta was regarded as arterial perfusion pressure (APP). RPP was set to the desired level by a manual clamp placed above the branching sites of both renal arteries. RPP was reduced in two stages by tightening the clamp mildly or more severely. The left renal artery was approached from a flank incision and carefully dissected free to permit placement of a Doppler blood flow transducer (internal diameter, 1.0 mm; HDP-10, Crystal Biotech, Northborough, MA) to connect to a 20-MHz module (PD-20; Crystal Biotech) and dedicated amplifier (VF-1; Crystal Biotech). Because the preparation of the renal artery may interfere with renal nerve integrity, we complemented determinations of total renal blood flow (RBF) with measurements of superficial blood flow (SBF) and deep cortical blood flow (DBF), often in the same rats, but also in independent groups of animals. Regional blood flow of the left kidney was monitored with two glass fiber probes connected to a real-time dual laser Doppler flowmeter (PeriFlux System 5000; Perimed, Stockholm, Sweden). For recordings of superficial and deep cortical flow signals, the probes were placed in the cortical flow signals, the probes were held in place at the surface and at a depth of ~3 mm, respectively, and regarded to register SBF and DBF. RBF, SBF, and DBF signals were digitized and analyzed using Labchart software (AD Instruments, Colorado Springs, CO).

Glomerular capillary pressure. Glomerular capillary pressure (Pgc) was measured by direct puncture of glomeruli exposed by partial corticotomy using the method of Aukland et al. (1). Although partial kidney cortex removal was obviously traumatic, compression of the cut surface usually caused the bleeding to stop within 15 min, and it was not excessive as judged from unaltered arterial blood pressure. An intravenous injection of FD&C no. 3 green dye was given to identify individual glomeruli as rapidly clearing green spheres. Glomeruli slightly below the surface were chosen for micropuncture to reduce

Table 1. Profile of OLETF and LETO rats

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Left Kidney Wt, g</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Blood Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>LETO (G1)</td>
<td>331±13</td>
<td>1.1±0.07</td>
<td>110±3</td>
<td>106±6</td>
</tr>
<tr>
<td>OLETO (G1)</td>
<td>400±29*</td>
<td>1.5±0.13*</td>
<td>111±2</td>
<td>113±8</td>
</tr>
<tr>
<td>LETO (G2)</td>
<td>532±15</td>
<td>1.21±0.03</td>
<td>117±2</td>
<td>118±11</td>
</tr>
<tr>
<td>OLETO (G2)</td>
<td>678±11*</td>
<td>2.34±0.15*</td>
<td>139±3*</td>
<td>426±28*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LETO, Long-Evans Tokushima Otsuka; OLETO, Otsuka Long-Evans Tokushima Fatty; G1, 11–13 wk or age; G2, 30–40 wk of age. Body wt, kidney wt, systolic blood pressure, and blood glucose at G1 and G2 are shown. *P < 0.05, OLETF vs. LETO rats.
Fig. 1. Experiments were performed in rats of two age groups, 10–12 and 30–40 wk of age. The left kidney was fixed in a Lucite cup with 2% agar, and the kidney surface was immersed in warm saline (37°C). Tubules for study were identified by injecting a small amount of saline colored with lissamine green in a randomly chosen proximal tubular segment with a micropipette. At the identification site, the proximal tubule was then completely blocked by solid wax (Merck, Darmstadt, Germany) injected by a hydraulic pressure system (Effenberg, Attel, Germany). A widened proximal segment upstream from the wax block was gently punctured with a micropipette (OD, 2 μm) filled with 2 M NaCl solution stained with lissamine green and mounted in a servo-null micropressure system (900A; WPI, Sarasota, FL). Measurements of stop flow pressure (Psf) were performed during loop of Henle perfusion to assess TGF function. When Psf had stabilized, loop of Henle perfusion rate was altered between 40 and 0 nL/min in a random fashion. The tubular perfusion solution was an artificial tubular fluid containing 140 mM NaCl, 140 mM Cl−, 4 mM K+, 4 mM Ca2+, 8 mM HCO3−, 7.5 mM urea, and 100 mg/100 ml FD&C green.

Glomerular pathology. All rats were killed at 30–40 wk of age. The kidneys of OLETF rats (n = 5) and LETO rats (n = 5) were removed and fixed in 10% formalin solution, embedded in paraffin, and sliced into 1-mm sections in the transverse plane. Two sections from each rat were stained with periodic acid-Schiff. The renal cortex was equally divided into three zones, and the glomeruli in the outer or the inner zone were regarded as superficial or deep glomeruli, respectively. Fifty glomeruli per rat (25 in superficial cortex and 25 in juxtamedullary cortex) were evaluated with a computer-assisted quantification method using NIH-image software, and average values were obtained for each rat. Total 250 glomeruli of each strain of rats were equally divided into three zones, and the glomeruli in the outer cortex were stained with periodic acid-Schiff. The renal cortex was equally divided into three zones, and the glomeruli in the outer cortex were stained with periodic acid-Schiff.

Fig. 2. Renal blood flow (RBF) and percent change from baseline (ΔRBF) during steady state and following a change in renal perfusion pressure (RPP) in 30- to 40-wk Long-Evans Tokushima Otsuka (LETO) and Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Left: effect of stepwise reductions in RPP on RBF. Right: RBF and ΔRBF during experimental periods [baseline (B), time control (C), mild clamp (E1), and severe clamp (E2)] were significantly altered by the change in RPP in OLETF rats (C: n = 7) compared with LETO rats (C: n = 7). P < 0.05 vs. control (*) and vs. LETO (#).
RBF of OLETF rats (9.8 ± 0.43 ml/min) and LETO rats (8.6 ± 0.44 ml/min) under basal conditions, although there was a tendency for a higher RBF in the OLETF group. RBF of OLETF rats fell significantly at the E1 (to 8.2 ± 0.35 ml/min) and E2 (to 7.0 ± 0.55 ml/min) stage. In contrast, RBF of LETO rats did not change significantly at the E1 stage (7.9 ± 0.4 ml/min) but fell significantly at the E2 stage (to 7.6 ± 0.4 ml/min). Expressed as percent change from baseline, RBF of OLETF rats fell to 84.0 ± 4.3% at E1 and to 71.3 ± 4.8% at E2. RBF in LETO rats at E2 fell to 88.5 ± 2.5% of baseline. Autoregulation curves are shown in Fig. 2.

Measurements of regional renal hemodynamics are summarized in Fig. 3. With baseline values set at 100%, there were no significant changes of SBF at the E1 level of clamping. At the E2 level, SBF fell in both OLETF (to 94 ± 2.6%) and LETO (to 91 ± 3%) rats, with differences between both rat strains not being significant. In contrast, DBF of OLETF fell significantly to 92.2 ± 1.8% at the E1 stage and to 84 ± 1.7% at the E2 stage, whereas DBF of LETO rats only fell modestly at the E2 level (to 95.0 ± 3.7%). The fall of DBF at the E2 level was significantly greater in OLETF rats than LETO rats.

Total, SBF, and DBF at the prediabetic stage. RPP at baseline was not significantly different between OLETF rats and LETO rats (OLETF rats: 100.5 ± 0.6 mmHg, LETO rats: 99.5 ± 2.6 mmHg). RPP fell by ~10 mmHg (OLETF rats: 11.0 ± 0.7 mmHg, LETO rats: 11.7 ± 2.0 mmHg) with mild clamping of the abdominal aorta (E1) and by ~20 mmHg (OLETF rats: 20.7 ± 0.9 mmHg, LETO rats: 21.7 ± 1.3 mmHg) with moderate clamping (E2) in both groups of rats, changes of RPP similar to those induced in the older animals. There were no significant differences between RBF of OLETF rats (5.8 ± 0.26 ml/min) and LETO rats (5.1 ± 0.29 ml/min) under basal conditions (Fig. 4). RBF of OLETF rats fell significantly at the E1 (to 4.6 ± 0.36 ml/min) and E2 (to 4.0 ± 0.38 ml/min) stage. In contrast, RBF of LETO rats did not change significantly at either the E1 stage (5.0 ± 0.31 mmHg) and E2 stage (to 4.9 ± 0.29 ml/min). Expressed as percent change from baseline, RBF of OLETF rats fell to 80.0 ± 3.9% at E1 and to 69.0 ± 5.2% at E2. RBF in LETO rats at E2 fell only to 95.3 ± 2.0% of baseline (P > 0.05).

Measurements of regional renal hemodynamics are summarized in Fig. 5. With baseline values set at 100%, there were no significant changes of SBF at the E1 level of clamping. At the E2 level, SBF fell significantly in OLETF (to 92 ± 4.5%), whereas there was no significant reduction of SBF in LETO rats (to 97 ± 3.0%). DBF of OLETF fell significantly to 89 ± 3.7% at the E1 stage and to 80 ± 4.8% at the E2 stage, whereas DBF of LETO rats only fell modestly at the E2 level (to 95 ± 5.4%). The fall of DBF at the E2 level was significantly greater in OLETF rats than LETO rats.

Pgc. With the use of the method of partial corticotomy, measurements of Pgc were performed in 9 superficial and 12 deep cortical glomeruli in 4 OLETF and 4 LETO rats (Fig. 6). Blood pressure did not show a significant change before and after partial corticotomy. Pgc in OLETF rats was significantly higher than in LETO rats in both superficial and deep renal cortex (deep cortex: 78.5 ± 2.7 vs. 58.5 ± 1.6 mmHg; superficial cortex: 57.5 ± 4 vs. 48.2 ± 2 mmHg). In addition, deep cortical Pgc was higher than superficial Pgc in both strains of rats.

TGF. Micropuncture evaluation of TGF was conducted in four OLETF and four LETO rats at 10–12 wk of age and in four OLETF and four LETO rats at 30–40 wk of age. Thirteen nephrons were studied in each of the four groups. At 10–12 wk, arterial blood pressure averaged 106 ± 1.0 mmHg in OLETF and 107 ± 1.3 mmHg in LETO rats. Pcg of OLETF rats was 41.0 ± 2.3 mmHg, not significantly different from the average of 41.6 ± 1.9 mmHg in LETO rats. During perfusion of the loop of Henle with artificial tubular fluid at 40 nl/min, Pcg fell to 37.7 ± 2.1 mmHg in OLETF rats (a reduction of 7.2 ± 1.8% compared with zero perfusion) and to 30.7 ± 1.3 mmHg in LETO rats (a reduction of 25.0 ± 2.3%). Thus TGF responses were significantly attenuated in prediabetic rats (Fig. 7). Identical experiments were performed in OLETF and LETO rats.

Fig. 4. RBF and ΔRBF during steady state and following a change in RPP in 10- to 12-wk LETO rats and OLETF rats. Left: effect of stepwise reductions in RPP on RBF. Right: RBF and ΔRBF during experimental periods were significantly altered by the change in RPP in OLETF rats (●; n = 6) compared with LETO rats (●; n = 7). ΔRBF was more valuable than ΔSBF in OLETF rats. P < 0.05 vs. control (*) and vs. LETO (#).

Fig. 5. Percent change from baseline of ΔSBF and ΔDBF during steady state and change in RPP in 10- to 12-wk LETO rats and OLETF rats. During experimental periods, the regional blood flow was significantly affected by the change in RPP in OLETF rats (●; n = 6) compared with those in LETO rats (●; n = 7). ΔDBF was more valuable than ΔSBF in OLETF rats. P < 0.05 vs. control (*) and vs. LETO (#).
rats at 30–40 wk of age (Fig. 8). Although mean arterial pressure did not significantly change in the older rats, \( P_{sf} \) of OLETF rats (42.0 ± 2.0 mmHg) was significantly higher than that of LETO rats (36.0 ± 1.7 mmHg). Maximum activation of TGF caused \( P_{sf} \) at 40 nl/min in OLETF and LETO rats to fall to 39.4 ± 2.5 and to 29.0 ± 1.5 mmHg, respectively. Percent changes of zero perfusion values averaged –6.7 ± 3.1% in OLETF rats and –19.7 ± 1.5% in LETO rats (\( P < 0.05 \)).

Renal pathology. Measurements of glomerular volume and sclerosis index for both strains of rats at 30–40 wk of age are summarized in Fig. 9. Although the tendency of a larger glomerular volume in the superficial cortex of OLETF rats did not reach significance, glomerular volume of juxtamedullary glomeruli was significantly greater in OLETF (2.17 ± 0.36 \( \times 10^6 \mu m^3 \)) than LETO (1.27 ± 0.06 \( \times 10^6 \mu m^3 \)) rats. Sclerosis index was significantly greater in OLETF than LETO rats in both surface (1.31 ± 0.12 vs. 0.68 ± 0.92) and deep cortical glomeruli (1.84 ± 0.12 vs. 0.99 ± 0.09). Moreover, regional comparisons showed significantly higher sclerosis index in deep cortical than in surface glomeruli in both strains of rats.

**DISCUSSION**

The present study was performed to assess autoregulatory and TGF responses of superficial and deep nephrons in type 2 diabetic OLETF rats. One of the important observations in the present study is that the ability of RBF autoregulation appears to be markedly reduced in the diabetic OLETF rats. RBF of OLETF rats fell almost linearly with a reduction of RPP, whereas an identical decrease of RPP had little effect on RBF in control LETO animals. The hemodynamic dysregulation of OLETF rats was more pronounced in the deep region of the renal cortex. Furthermore, impairment of autoregulation in OLETF rats was observed before the development of overt hyperglycemia, consistent with the notion that it may be a causal factor in diabetic nephropathy.

Autoregulation of RBF and glomerular filtration rate is thought to be mediated by at least two distinct mechanisms. One is a myogenic reaction that is intrinsic to all resistance vessels, whereas the second is a kidney-specific mechanism called TGF. Our present observations show that TGF abnormalities exist in OLETF rats and that they precede the rise of blood sugar and the development of diabetes. This raises the possibility that an abnormal TGF may be causally connected with the development of the hemodynamic abnormalities in the diabetes state. Abnormal TGF responsiveness has been reported previously in diabetes, especially in insulin-deficient states (8, 27). In addition, a dysfunction of voltage-dependent \( Ca^{2+} \) channels in vascular smooth muscle cells or an increased activation of ATP-dependent \( K^+ \) channels has been reported, suggesting that myogenic reactivity may be abnormal in diabetes as well (3). The diminished stability of RBF in the face of changes of RPP in STZ-induced diabetes in rats has been found to be improved by treatment with insulin (2, 5). Therefore, it is thought that not only insulin resistance but also hyperglycemia per se may affect autoregulation.

Glomeruli located close to the kidney surface differ in structure and function from those in the juxtamedullary region (8). Studies of regional hemodynamics have frequently used the Laser Doppler technology, although this approach only permits measurements of relative blood flow changes. Nevertheless, the Laser Doppler approach yields a continuous recording of blood flow velocity and is associated with comparatively minor disturbance of function. In the present study, we used this technique to evaluate autoregulation in the superficial and juxtamedullary regions of the kidney. In LETO rats, both surface and deep cortical blood flow was well regulated during an ~20 mmHg arterial pressure change, confirming previous reports using the same method (14–16). Whereas superficial cortical blood flow of OLETF rats was also well regulated, our data show for the first time that there is an autoregulation defect in the deep cortex in this type 2 diabetes model. The causes for the regional differences in autoregulatory abnormalities are unclear but could be related to changes of extracellular fluid volume and the renin-angiotensin system (16). It is also possible that distension of renal vessels resulting from the diabetic state may cause the autoregulation disability in the deep cortex.

![Fig. 6. Glomerular capillary pressure in superficial and juxtamedullary glomeruli in 30-wk LETO rats (n = 4) and OLETF rats (n = 4). \( P < 0.05 \) vs. LETO (*) and vs. superficial (#).](attachment:fig6.png)

![Fig. 7. Stop flow pressure (\( P_{sf} \)) and change in \( P_{sf} \) (\( \Delta P_{sf} \)) in OLETF and LETO rats at 10–12 wk. Left: relationship between \( P_{sf} \) and rate of loop perfusion rate (0, 40 nl/min) with ATP in OLETF rats (●, n = 13) compared with those in LETO rats (●, n = 13). Right: % change of \( P_{sf} \) in OLETF rats (●, n = 13) compared with those in LETO rats (●, n = 13). \( P < 0.05 \) vs. \( P_{sf} \) at 0 nl/min (*) and vs. LETO (#).](attachment:fig7.png)
Glomeruli in rats, including those of the Long-Evans strain, and in other mammals are usually not found on the kidney surface. Previous direct measurements of $P_{gc}$ in diabetes have been performed in STZ-treated Munich-Wister rats in which surface glomeruli can be found with regularity (7). In the present study, we used the method of partial cortical ablation, first introduced by Aukland et al. (1), to access glomerular structures and to compare $P_{gc}$ in surface and deep nephron in LETO and OLETF rats. We found that the bleeding was well controllable and that the tissue damage appeared to be relatively minor. We observed in these studies that $P_{gc}$ was higher in deep cortical than surface glomeruli in both nondiabetic and diabetic animals. Furthermore, $P_{gc}$ of OLETF rats at 30 wk of age exceeded that of control rats in both regions of the kidney. Using the same method, Iversen et al. (10) reported that the pressure in surface glomeruli of spontaneously hypertensive rats at 10 or 70 wk of age was higher in deep cortical than surface glomeruli. One of the causes of the raised $P_{gc}$ may be the suppressed TGF mechanism that was found by micropuncture examination of superficial nephrons.

Pathological examination of both surface and deep cortex was performed in LETO and OLETF rats at 40 wk of age to determine whether the functional disorder was associated with structural abnormalities. The area of deep cortical glomeruli was larger than that of surface glomeruli in both groups of rats. Similar differences have also been reported in humans at an older age (23). Comparison between LETO and OLETF rats revealed no significant difference in the area of surface glomeruli. In contrast, deep cortical glomeruli of OLETF rats were significantly larger than those of LETO rats. Furthermore, increased glomerular disease in deep nephrons of OLETF rats is indicated by the sclerosis index. These observations correspond remarkably well with the rise of glomerular pressure found in this nephron population. Toyota et al. (24) recently observed increased variations of glomerular volumes in 26- to 27-wk-old OLETF rats without systematic regional differences using computer microtomography. Numerous previous reports have supported a causal connection between glomerular pressure and glomerular sclerosis. Periodic distension of mesangial cells in culture causes increased production of cellular matrix, and this effect is markedly enhanced by a high glucose concentration (4, 20).

In summary, our study reveals profound dysfunction of autoregulation and TGF that is associated with higher $P_{gc}$ and augmented glomerular pathology in a type 2 model of diabetes. Hyperfiltration and renal hemodynamic abnormalities were found to precede the rise of serum glucose levels, consistent with the notion that an inefficient autoregulation may contribute to the development of diabetic renal disease.

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GRANTS

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REFERENCES


