Diabetic Complications in a New Animal Model (TSOD mouse) of Spontaneous NIDDM with Obesity

Seiichi IIZUKA1), Wataru SUZUKI1), Masahiro TABUCHI1,2), Mitsunobu NAGATA1), Sachiko IMAMURA1), Yujiro KOBAYASHI1), Masanao KANITANI1), Toshihiko YANAGISAWA1), Yoshio KASE1), Shuichi TAKEDA1), Masaki ABURADA1,2), and Kazuaki W. TAKAHASHI3)

1) Research Division, Tsumura & Co. 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, 2) Musashino University 1–1–20 Shinmachi, Nishitokyo-shi, Tokyo 202-8585, and 3) Department of Laboratory Animal Science, Nippon Veterinary & Animal Science University 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180-0023, Japan

Abstract: The TSOD mouse has been established as an inbred strain with spontaneous development of diabetes mellitus as the first clinical signs of diabetes. Polydipsia and polyuria are observed at about 2 months old only in male mice, after which hyperglycemia and hyperinsulinemia are detected. Following these symptoms obesity gradually develops until about 12 months old. In histopathological examination of the pancreas, severe hypertrophy of pancreatic islets was observed due to proliferation and swelling of B cells. In the kidney, thickening of the basement membrane in glomeruli and an increase of the mesangial area were observed at 18 months old. Motor neuropathy in TSOD mice began to appear at 14 months old and most male mice at 17 months old showed weakness of front and hind paws caused by neuron degeneration in the peripheral nerve. In sensory neuropathy, the threshold in the tail pressure test decreased significantly at 12 months old. Light microscopic and electron microscopic examination of sciatic nerves showed a decrease in the density of nerve fibers by the endoneural fibrosis and loss of these fibers. Degenerative changes of myelinated fibers, separation of myelin sheaths with intralamellar edema and remyelination were frequently observed. In the severely affected nerve fibers, the lamellar structure was completely destroyed and macrophages migrated around the myelin sheath or invaded the intramyelin space. Considering these findings similar to non-insulin dependent diabetes mellitus (NIDDM) in humans, the TSOD mouse should be a useful model for the pathogenic study of diabetic complications, especially of peripheral neuropathy.

Key words: diabetes mellitus, hyperinsulinemia, peripheral neuropathy, TSNO mouse, TSOD mouse

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Address corresponding: S. Iizuka, Research Division, Tsumura & Co. 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan
Introduction

Many animal models of spontaneous diabetes have contributed to the elucidation of different aspects of human diabetic syndromes and associated genetic factors. The TSOD (Tsumura, Suzuki, Obese Diabetes) mouse was established as an inbred line in 1992 and some of the clinical diabetic characteristics have been described by Suzuki et al. [25]. The TSOD mouse showed spontaneous obesity, glucosurea, hyperglycemia, and hyperinsulinemia only in male mice [25]. Miura et al. reported that the insulin-stimulated translocation of glucose transporter (GLUT) 4 from low-density microsomal membranes to plasma membrane was reduced in both skeletal muscle and adipose tissue of TSOD mice, and that the reduced insulin sensitivity was presumably due to this impaired GLUT4 translocation in both skeletal muscles and adipocytes [15]. Using a whole genome scan of quantitative trait loci affecting body weight, blood glucose and insulin concentrations, Hirayama et al. identified four major loci of the TSOD mouse meeting the rigorous criteria for linkage [11]. On the other hand, the function and the morphological evidence of the pancreas and the complications in TSOD mice have not been researched in detail. So we report here the biochemical characterization and morphological changes of the pancreas, the peripheral neuropathy and other complications in the spontaneously diabetic TSOD mouse.

Materials and Methods

Animals

The TSOD male mice used in this study were males derived from the breeding stock of the Medical Evaluation Laboratories of the Tsumura Research Institute (Ibaraki). Two to five mice were housed in plastic cages in a non-barrier-sustained animal room maintained at 23 ± 2°C with 50 ± 10% relative humidity and a 12/12 h light/dark cycle. They were maintained on a basal diet MF (Oriental Yeast Co., Ltd., Tokyo) and chlorinated water ad libitum before autopsy. Age- and sex-matched non-diabetic TSNO mice (Tsumura, Suzuki, Non Obesity) served as controls. All of the animal experiments were carried out following the Guideline for Animal Experimentation of TSUMURA & CO.

Tissue sampling

All animals were sacrificed by exsanguination from the inferior vena cava and infused with 2.5% phosphate-buffered glutaraldehyde under deep ether anesthesia. Sciatic nerves and pancreata of 5 male TSOD mice and 5 male TSNO mice at 6, 12, 18 and 22 months of age respectively, were prefixed in 2.5% glutaraldehyde solution for 2 h, fixed in 1% osmium tetroxide solution for 2 h, then dehydrated and embedded in epon. Semi-thin sections stained with toluidine blue were used for light microscope examinations (Olympus Optical Co., Ltd., Tokyo) and the morphometric analysis of myelinated fibers. Ultra-thin sections of the sciatic nerve and pancreas were stained with uranyl acetate and lead citrate, and then examined by electron microscope (JEOL Ltd., Tokyo).

Samples for teased nerve fibers from the sciatic nerve in 5 male TSOD mice at 22 months of age were fixed in 2.5% phosphate-buffered glutaraldehyde for 30 min, osmicated for 2 h, and then carried through 50% and 100% glycerin solutions each for 24 h at room temperature. Single nerve fibers were isolated with dissecting needles under a stereoscope in glycerin solution.

For the light microscopic, immunohistochemical or morphometric examinations, pancreata and kidneys in 5–11 male TSOD mice and 5–8 male TSNO mice at 1–22 months of age were dissected and fixed in 10% phosphate-buffered formalin, and then embedded in paraffin wax in routine processing and sectioned at 3 µm thickness.

Biochemical Measurements

Blood samples were collected from fed male TSOD and male TSNO mice at 6, 12 and 18 months of age by cardiocentesis. The concentrations of glucose, triglyceride, total cholesterol in the serum and hemoglobin A1c (HbA1c) were measured using a biochemical analyzer (TBA-40FR, TOSHIBA Co., Tokyo). HbA1c was measured by a turbidimetric inhibition immunoassay (TINIA) (Roche Diagnostics K. K., Tokyo) for hemolyzed whole blood. Glucose concentration was measured enzymatically using hexokinase and glucose-6-phosphate dehydrogenase. Triglyceride concentration was measured enzymatically using lipase, glycerol kinase, pyruvate kinase, and lactate dehydrogenase. Cholesterol concentration was measured enzymatically...
using cholesterol esterase, cholesterol oxidase, and peroxidase. The concentration of insulin in the plasma was determined using the enzyme linked immunosorbent assay (ELISA) kit for mouse (Shibayagi Co., Ltd., Gunma).

Urine samples from 10–23 male TSOD mice and 10–22 male TSNO mice at 6, 12 and 18 months of age were collected by cystocentesis of the urinary bladder at necropsy. Glucose and ketones of the urine were analyzed using multisticks (Miles Sankyo Co., Ltd., Tokyo).

**Immunohistochemistry**

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections (3 \( \mu \)m thick) of kidney and sciatic nerve in 5 male TSOD and 5 male TSNO mice at 6, 12 and 18 months of age. Advanced glycation end products (AGE) accumulation within tissues was stained by an immunoperoxidase technique using the anti-AGE, N’-(carboxyethyl)-L-lysine (CEL), N’-(carboxymethyl)-L-lysine (CML), 3-deoxyglucosone derived imidazolone and pyrraline monoclonal antibodies, purchased from Trans Genic Inc., (Kumamoto). Sections were rehydrated and treated with 1% H\(_2\)O\(_2\)/methanol, then washed with PBS and incubated with primary antibodies for 2 h at room temperature followed by biotinylated anti-rabbit immunoglobulin and peroxidase conjugated streptavidin. Localization of peroxidase conjugates was revealed using diaminobenzidine tetrahydrochloride (DAB).

**Quantitation of endocrine cells in the pancreas**

Formalin-fixed, paraffin-embedded pancreata of 4–11 male TSOD and 5–8 male TSNO mice at 6, 9, 12 and 18 months of age were serially sectioned (3 \( \mu \)m thick). On adjacent sections, islet cells were localized by the immunohistochemical technique for insulin, glucagon, somatostatin and pancreatic polypeptide antibody. Then, the number of the respective positive cells in the pancreatic islets was counted under the light microscope. The insulin positive cell (B cell area) was measured by the light microscopic image analysis system (SUMIKA TECHNO SERVICE Co., Osaka) and the ratio of the B cells occupancy was calculated.

**Pain test**

The tail pressure test was performed on 6 male TSOD mice at 6 and 12 months of age, and 5 age-matched male TSNO mice. Nociceptive threshold, expressed in grams, was measured using a Ugo Basile analgesymeter (Ugo Basile Biological Research Apparatus, Comerio VA) by applying increasing pressure to the tail until withdrawal [5, 11].

**Morphometry of the sciatic nerve**

Semi-thin cross sections of sciatic nerve and tibial nerve in 3 male TSOD and 3–4 male TSNO mice at 18 and 22 months of age were examined by a light microscopic image analysis system (SUMIKA TECHNO SERVICE Co., Osaka). The total fascicular area and each myelinated nerve fiber area were measured using this image analysis system. The total fiber area was a total of each myelinated nerve fiber area. Fiber occupancy was calculated as the percentage of endoneural area occupied by myelinated fiber area.

**Statistical analysis**

The significance of difference of values was tested by the uniformity of variance (F-test), and analyzed by Student’s t-test. Histograms of nerve fiber size distributions were compared by the Chi-square test. A value of P<0.05 was considered statistically significant.

**Results**

**General and biochemical characterization**

Body weights of TSOD mice at 6, 12 and 18 months of age were significantly greater than those of non-diabetic TSNO mice at each matched age, and pancreatic weights of TSOD mice at 6, 12 and 18 months of age were significantly higher than those of TSNO mice at each matched age (Table 1). Visceral fat in TSOD mice had a marked increase (data not shown).

Blood glucose concentrations of TSOD mice at 6 and 12 months of age were significantly higher than those of TSNO mice. Non-fasting insulin concentrations of TSOD mice at 6, 12 and 18 months of age were significantly higher than those of TSNO mice. HbA\(_{1c}\) levels in TSOD mice at 6 and 12 months of age was markedly higher than those of TSNO mice. The total cholesterol and triglyceride concentrations of TSOD mice were also significantly higher than TSNO mice. Glucosuria was detected in all TSOD mice at the
three ages, although ketonuria was not detected (data not shown). No glucose or ketones in urine were detected in TSNO mice (data not shown). Urinary volumes of TSOD mice at 6, 12 and 18 months of age were significantly higher than those of TSNO mice at each matched age.

**Light microscopy of pancreas, kidney and sciatic nerve**

Light microscope observations of pancreata stained with hematoxylin-eosin and anti-insulin monoclonal antibodies immunohistochemically revealed severe hypertrophy of pancreatic islets in TSOD mice at 6, 12 and 18 months of age (Fig. 1a), due to proliferation and swelling of B cells (Fig. 2a), but it was not observed in TSNO mice (Figs. 1b and 2b).

In the kidney of TSOD mice, several histological abnormalities, which were thickening of basement membrane in glomeruli, increase of mesangial area (Fig. 3a), dilatation of pelvic cavity and urinary tubules were observed remarkably at 18 months of age (Table 2).

**Table 1.** General and biochemical characterization of TSOD and TSNO mice at 6, 12 and 18 months

<table>
<thead>
<tr>
<th></th>
<th>6 months</th>
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<th>12 months</th>
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<th>18 months</th>
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<tbody>
<tr>
<td></td>
<td>n TSOD</td>
<td>n TSNO</td>
<td>n TSOD</td>
<td>n TSNO</td>
<td>n TSOD</td>
</tr>
<tr>
<td></td>
<td>(diabetic)</td>
<td>(non-diabetic)</td>
<td>(diabetic)</td>
<td>(non-diabetic)</td>
<td>(diabetic)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>16 60.6±1.4*** 14 36.2±0.6</td>
<td>23 62.0±1.3*** 22 40.5±0.7</td>
<td>10 50.8±1.7*** 10 40.4±0.7</td>
<td></td>
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<tr>
<td>Pancreatic weight (mg)</td>
<td>16 589±28*** 14 422±20</td>
<td>23 667±23*** 22 439±11</td>
<td>10 670±53*** 10 499±13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fasting blood glucose (mg/dl)</td>
<td>10 414±62*** 10 175±7</td>
<td>10 337±49*** 10 167±6</td>
<td>10 133±6 10 134±11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fasting plasma insulin (ng/ml)</td>
<td>10 23.47±7.62*** 10 1.05±0.12</td>
<td>10 32.88±0.16*** 10 1.24±0.16</td>
<td>10 4.82±1.12** 10 2.20±0.15</td>
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<tr>
<td>HbA1c (%)</td>
<td>5 8.60±0.94** 5 3.80±0.20</td>
<td>8 6.13±0.36*** 8 2.96±0.66</td>
<td>8 3.76±0.61 8 2.96±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fasting blood total cholesterol (mg/dl)</td>
<td>10 227±11*** 10 114±4</td>
<td>10 204±12*** 10 117±5</td>
<td>10 104±4 10 88±7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fasting blood triglycerides (mg/dl)</td>
<td>10 265±27*** 10 81±15</td>
<td>10 282±29*** 10 50±12</td>
<td>10 89±17** 10 51±10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary volume (µl)</td>
<td>16 306±39*** 14 32±12</td>
<td>23 691±108*** 22 23±9</td>
<td>10 2,750±780** 10 35±18</td>
<td></td>
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</table>

**:** p<0.01, ***: p<0.001 compared with the value of age-matched TSNO mice. Values are mean±SE.

![Fig. 1.](image) Light microscopic features of pancreas. H&E stain, × 25. (a) 12-month-old TSOD mouse. Hyperplastic islet. (b) 12-month-old TSNO mouse.
Nodular glomerular changes were rarely observed in long-term diabetic TSOD mice over 18 months of age (Fig. 3b). These light microscopical findings of the kidney were not observed in age-matched TSNO mice (Fig. 3c).

Teased nerve fiber preparations of the sciatic nerve are shown in Fig. 4. The most common finding was focal swelling of the myelin sheath (Fig. 4A). Segmental disappearance of the myelin sheath and accumulation of myelin debris were seen in severely affected fibers (Figs. 4B, C and D).

In semi-thin toluidine blue stained transverse and longitudinal sections of the sciatic nerve, the density of nerve fiber as shown in Fig. 5 decreased due to endoneural fibrosis and the loss of nerve fibers (Table 3, Fig. 5a). The main changes of myelinated nerve fibers were myelin corrugation, distention and denudation of axons. Myelin debris and irregular folding of the myelin sheath were common, and thinly remyelinated fibers and onion bulb formation were also found. These changes were diffusely observed in the sciatic nerve and marked endoneural fibrosis among the affected fibers was constantly detected. The median nerve in the forelimbs showed the same changes in the sciatic nerve (data not shown). However, similar microscopic changes in median and sciatic nerve were not observed in age-matched non-diabetic TSNO mice (Fig. 5b).

**Electron microscopy of the pancreas and sciatic nerve**

In TSOD mice, the B cells were characterized by their electron-dense granules surrounded by loosely fitting sacs. Most of the B cells in pancreata of TSOD mice had many vesicles without electron-dense granules as shown in Fig. 6. The cytoplasm contained a well-developed Golgi complex and a smooth-surfaced endoplasmic reticulum. The mitochondria in many B cells were variable in shape and were swollen with
some vacuoles or regular transverse cristae (data not shown). The A, D and PP cells of the pancreas did not exhibit any electron microscopical changes unlike the B cells in TSOD mice (data not shown).

The sciatic nerve in TSOD mice showed conspicuous degenerative changes of myelinated fibers (Fig. 7). Separation of myelin sheaths with intralamellar edema was frequently found. In the severely affected nerve fibers, the lamellar structure was completely destroyed and macrophages migrated around the myelin sheath or invaded intramyelin space (Table 3). Remyelination as thinly myelinated axons surrounded by cytoplasmic process of Schwann cells were frequently observed. In the endoneurium, the collagen fibers increased markedly.

**Quantitation and immunohistochemistry of endocrine cells in the pancreas**

On immunostained sections, endocrine cells in TSOD mice showed positive reaction with insulin, glucagons, somatostatin or pancreatic polypeptide. B cells were the most numerous and were located in the center of the islets. In the quantitative investigations of pancreatic endocrine cells in TSOD and TSNO mice, the B cell area in the pancreatic islets increased markedly with age and there were significant differences between TSOD and age-matched TSNO mice more than 6 months old (Fig. 8). The A, D and PP cells of TSOD mice were found in appreciably smaller numbers than the B cells, and the A cells were localized predominantly along the periphery of the islets. A few D and PP cells were randomly present. The locations of the

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**Fig. 3.** Light microscopic features of kidney. H&E stain, ×100. (a) 18-month-old TSOD mouse. Increase of mesangial area in glomerulus. (b) 18-month-old TSOD mouse. Nodular lesions in glomerulus. (c) 18-month-old TSNO mouse.

**Fig. 4.** Teased nerve-fiber preparations of sciatic nerves. (A), (B), (C), (D) are single teased fibers from 22-month-old TSOD mouse, which show beaded appearance (arrows) due to varied swelling of the myelin sheath (A, B) and demyelination (arrowheads) in affected nerve fibers (C, D). (E) shows a single teased fiber from an age-matched TSNO mouse.
Table 3.  Histopathological characteristics of sciatic nerve in TSOD mice at 18 and 22 months

<table>
<thead>
<tr>
<th>Findings</th>
<th>18 months (n=5)</th>
<th>22 months (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Decrease of myelinated fibers</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Endoneural fibrosis</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Distended myelin sheaths</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Myelin destruction or splitting of myelin sheaths</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Degenerated myelin debris</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Proliferation of macrophages</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Remyelination</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Thickening of endothelial cells in blood vessels</td>
<td>1</td>
<td>4</td>
</tr>
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</table>

Each abnormality was divided into three grades from + to ++++, mild, moderate and severe, qualitatively.

(a) (b)

Fig. 5. Light microscopic features of sciatic nerve. Toluidine blue stain, ×100. (a) 22-month-old TSOD mouse. Reduction of myelinated fibers, endoneural fibrosis and distention of fibers were observed. Many small-sized nerve fibers had thinly myelinated axons. (b) 22-month-old TSNO mouse.

B, A, D and PP cells in TSOD mice were similar to those of non-diabetic TSNO mice (data not shown). The number of these cells at 9 months old in TSOD mice was significantly more than age-matched TSNO mice (Table 4).

Detection of AGE in the sciatic nerve and kidney

The predominant site of AGE immunohistochemical staining within the sciatic nerve was endothelial cells of vessels in TSOD mice at 18 months of age (Fig. 9). The immunostain using anti-AGE, CML, CEL, pyrraline and 3-deoxyglucosone derived imidazolone antibodies demonstrated that AGE proteins were detected in the endothelial cells of vessels in the sciatic nerve of TSOD mice. The AGE proteins were also observed diffusely in degenerated nerve fibers of TSOD mice at 18 months of age, although these findings in the sciatic nerve were not observed in age-matched, nondiabetic TSNO mice.

The localization of AGE, CML, CEL, pyrraline and 3-deoxyglucosone derived imidazolone in endothelial cells of glomeruli were demonstrated in the kidney of the TSOD mice at 6, 12 and 18 months of age (Fig. 9). Especially, in TSOD mice at 18 months of age, the AGE proteins reacted in the increased mesangium area.
In the nodular lesions in glomeruli of TSOD mice at 18 months of age, the accumulation of AGE proteins was observed. These findings in the kidney were not observed in age-matched, non-diabetic TSNO mice. Some macrophages in the sciatic nerve and kidney were also weakly stained in TSOD and TSNO mice at 6, 12 and 18 months of age.

Pain test

At 6 months of age, the nociceptive threshold in the tail pressure test did not differ between TSOD mice and TSNO mice (Fig. 10). The nociceptive threshold of TSNO mice at 12 months of age was unchanged compared with at 6 months of age. At 12 months of age, the nociceptive threshold in TSOD mice was significantly lower than in age-matched TSNO mice.

Morphometry of the sciatic nerve

Morphometric analysis revealed marked reduction of fiber occupancy in TSOD mice at 18 and 22 months of age compared with those in non-diabetic TSNO mice (Table 5). Light microscopically, endoneural fibrosis among myelinated fibers was constantly observed.

**Table 4.** Number of A, D and PP cells in pancreatic islet of TSOD and TSNO mice

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>n</th>
<th>A cell</th>
<th>D cell</th>
<th>PP cell</th>
<th>n</th>
<th>A cell</th>
<th>D cell</th>
<th>PP cell</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>11</td>
<td>8.4 ± 1.0</td>
<td>4.6 ± 0.8</td>
<td>7.3 ± 0.8</td>
<td>8</td>
<td>5.8 ± 0.9</td>
<td>3.3 ± 0.4</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>6.4 ± 0.8**</td>
<td>3.7 ± 0.6</td>
<td>5.8 ± 0.6**</td>
<td>5</td>
<td>16.0 ± 2.3</td>
<td>4.0 ± 0.3</td>
<td>9.8 ± 0.3</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>7.6 ± 1.1</td>
<td>3.9 ± 0.6</td>
<td>7.6 ± 0.6</td>
<td>5</td>
<td>13.7 ± 3.4</td>
<td>3.7 ± 0.7</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>7.4 ± 0.7</td>
<td>4.3 ± 0.8</td>
<td>6.0 ± 0.8</td>
<td>5</td>
<td>8.3 ± 0.8</td>
<td>3.0 ± 0.4</td>
<td>6.4 ± 0.4</td>
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**: p<0.01 compared with the value of age-matched TSNO mice. Values are mean ± SE.
throughout the whole endoneurium. The fiber size in TSOD mice was not significantly different from age-matched TSNO mice (Fig. 11).

Discussion

The TSOD mouse has been established as an inbred strain of mouse with spontaneous development of diabetes mellitus, by selective breeding for body weight and glucosuria at 2 months of age, and male mice obtained in this colony are used as diabetic model animals [25]. The incidence of diabetes mellitus in TSOD mice is very high in males, although the symptom doesn’t appear in the female at all [25]. A higher incidence of diabetes in males than in females is also reported in other animal models of NIDDM [20]. The main clinical signs in TSOD mice, obesity, polydipsia and polyurea, are first detected at about 2 months of age and thereafter, hyperglycemia and hyperinsulinemia are also detected [25]. Although insulin sensitivity was not measured in the present study, hyperinsulinemia in TSOD mice suggests that insulin resistance as well as impaired insulin secretion contributes to the deterioration of glucose tolerance. HbA1c levels in TSOD mice at 6 and 12 months of age were markedly higher than those of TSNO mice. Estimations of HbA1c levels are used to assess long-term glycemic control in general. We think that the deterioration of glucose tolerance in TSOD mice is long term, beginning in the young, and that the high level of HbA1c seen in TSOD mice induces the morphological changes of the kidney and peripheral nerves. Following these symptoms obesity gradually develops until about 12 months of age due to accumulation of visceral fat. These characteristics are similar to those observed in human NIDDM in which visceral fat accumulation is a risk factor for glucose intolerance in patients with obesity [4].

Fig. 9. (a) Light microscopic features of the sciatic nerve of an 18-month-old TSOD mouse. AGE proteins were detected in the endothelial cells of blood vessels. Immunostaining for pyrraline, ×100. (b) Light microscopic features of the kidney of an 18-month-old TSOD mouse. AGE proteins were detected in the endothelial cells of blood vessels in the glomerulus. Immunostaining for AGE, ×100. (c) Light microscopic features of kidney of an 18-month-old TSOD mouse. CEL was detected in the nodular lesion of the glomerulus. Immunostaining for CEL, ×100.
Pancreatic islets at 6 months of age showed severe hypertrophy due to B cell proliferation, although islet B cells in aged mice were not destroyed by hormonal hypersecretion in the long term. In these clinical and histopathological findings, the diabetic conditions of TSOD mice are similar to NIDDM in humans [2, 17, 22].

Non-enzymatic glycation involves the condensation of free aldehyde groups of glucose with the amino groups of proteins. This reaction occurs very slowly and results in the formation of cross-linked, modified long-lived proteins referred to as AGE [18]. The accumulation of AGE have been reported to be associated with diabetic nephropathy [14, 18]. In the light microscopy examination of the kidney in long-term diabetic TSOD mice, thickening of basement membrane in glomeruli, increase of mesangial area and nodular glomerular changes were observed. The anti-AGE, CML, CEL, pyrraline and 3-deoxyglucosone derived imidazolone antibodies reacted in these increased mesangial areas

**Table 5.** Morphometric analysis of sciatic nerve in TSOD and TSNO mice

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Total fascicular area (µm²)</th>
<th>Total fiber area (µm²)</th>
<th>Fiber occupancy (%)</th>
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<tr>
<td><strong>At 18 months</strong></td>
<td></td>
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<td></td>
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<tr>
<td>TSOD mice (diabetic)</td>
<td>3</td>
<td>387,538 ± 110,966</td>
<td>167,425 ± 52,751</td>
<td>42.0 ± 2.2**</td>
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<tr>
<td>TSNO mice (non-diabetic)</td>
<td>3</td>
<td>113,561 ± 46,957</td>
<td>64,395 ± 25,169</td>
<td>57.8 ± 1.4</td>
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<tr>
<td><strong>At 22 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSOD mice (diabetic)</td>
<td>3</td>
<td>213,291 ± 54,751</td>
<td>81,360 ± 17,796</td>
<td>39.9 ± 4.3**</td>
</tr>
<tr>
<td>TSNO mice (non-diabetic)</td>
<td>4</td>
<td>55,173 ± 6,265</td>
<td>31,421 ± 4,014</td>
<td>56.9 ± 0.7</td>
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****: p<0.01 compared with the value of age-matched TSNO mice. Values are mean ± SE.
and nodular lesions of glomeruli. Makino et al. demonstrated the immunostaining of AGE in the mesangium and nodular lesions of advanced diabetic nephropathy [14]. Our findings in TSOD mice are consistent with their results.

There are many reports about diabetic neuropathy in several animal models of streptozotocin or alloxan-induced diabetes [1, 33], transgenic mice which developed islet cell adenoma [8], spontaneous IDDM [23, 30, 31] and NIDDM [3, 7, 9, 10, 29, 30]. To elucidate the mechanisms of peripheral nerve damage in diabetic neuropathy, various animal models have been studied [1, 8, 16, 23, 29, 32, 34], whereas there are few accounts in the literature of spontaneous animal models of peripheral neuropathy with progressive NIDDM [30]. The db/db mouse showed fiber atrophy in both somatic and autonomic nerves [30], and the GK rat showed myelinated fiber atrophy without apparent fiber loss [30]. In TSOD mice, the motor neuropathy began to show at 14 months of age and was observed in most male mice at 17 months. The characteristic features in aged TSOD mice were dysfunction of the fore-limb and hind-limb. By approximately 14 months of age, TSOD mice developed progressive front and hind paw weakness. This weakness was detected by a weak grip reflex when the mouse was held suspended by its tail and allowed to grasp a grid bar. In addition, TSOD mice occasionally showed loss of withdrawal reflex when lifted by their tails. These clinical signs of paw weakness and loss of withdrawal reflex would demonstrate myelinated fiber loss in the sciatic nerve by disease progression in aged TSOD mice. In mice with severe hind-limb weakness, the characteristic signs of distension of urinary bladder, dilatation of pelvis, denudation and smudge of penis were observed. Similar symptoms of the urinary bladder were also described in Chinese Hamster with diabetes [6] and streptozotocin-induced diabetic rats [13, 24]. In Chinese Hamster, aberrant myelination was found in the pelvic plexus and urinary bladder [6]. It has been suggested that abnormality of pelvic visceral nerves is associated with urinary bladder dysfunction in diabetic hamsters [6]. Severely distended urinary bladders of TSOD mice were often observed at 18 months of age and the mean of urinary volume was markedly higher in TSOD mice than in age-matched TSNO mice. We consider that these symptoms in TSOD mice may also be related to abnormality of the autonomic nerve with morphological changes induced by metabolic abnormalities.

In sensory nerves of TSOD mice, the mean of nociceptive thresholds was significantly lower at 12 months of age. These results are similar to those obtained in streptozotocin or alloxan-induced diabetic rats with the same test [5, 12], and may correspond to hyperalgesia which is encountered in painful diabetic neuropathy of humans.

The most common histopathological findings in the sciatic nerves of TSOD mice were myelin distension, denudation of axons, remyelination and axonal degenerative changes. In some mice, these changes were particularly numerous around endoneural vessels, where they were accompanied by endoneurial fibrosis. Immunostaining with anti-AGE, CML, CEL, pyrraline and 3-deoxyglucosone derived imidazolone antibodies demonstrated that AGE proteins were detected in the endothelial cells of vessels in the sciatic nerve of TSOD mice. The function of blood vessels in TSOD mice might have been affected by the accumulation of AGE proteins.

Morphometric examination detected significant reductions of myelinated fiber occupancy in the sciatic nerve. This may be mainly due to myelinated fiber loss and endoneurial fibrosis was not likely to represent areas of edema morphologically.

We consider that this neuropathy in TSOD mice is different from age-dependent morphological changes in peripheral nerves of aged mice [16], uremic neuropathy [16, 26], radiculoneuropathy and pressure neuropathy due to housing in wire-mesh cages [16, 19, 21, 27], because it was not observed in age-matched non-diabetic TSNO mice, kept under the same conditions. In addition, severe chronic renal failure was not seen and the same morphological changes such as neuropathy in the sciatic nerve were observed in the median nerve of TSOD mice. In general, the median nerve is not affected by pressure neuropathy and radiculoneuropathy anatomically. These results indicate that the peripheral neuropathy of TSOD mice is not uremic neuropathy, radiculoneuropathy or pressure neuropathy.

The main pathological findings in human diabetic nerves consist of progressive fiber loss and endoneurial microangiopathy [30]. In WBN/kob rat, myelinopathy and axonopathy with myelinated fiber loss caused by subsequent chronic pancreatitis and hyperglycemia have
also been reported [16, 19, 28, 33]. We consider that continuous hyperglycemia or hyperinsulinemia from a young age in TSOD mice might affect the peripheral nerves metabolically [34]. In addition, the clinical signs in the urinary bladder and sciatic and median nerve changes suggest that the peripheral nerves are affected widely by long-standing metabolic abnormality. These characteristic findings of peripheral nerves in TSOD mice are similar to NIDDM in humans [2, 17, 22].

The TSOD mouse will be a new and useful model for studies of diabetic complications on the pathogenesis of human NIDDM.

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