

## Diabetic Peripheral Neuropathy in Spontaneously Diabetic Torii-*Lep<sup>fa</sup>* (SDT Fatty) Rats

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**ABSTRACT.** Spontaneously Diabetic Torii (SDT) rat is a hereditary model of diabetes. Although the SDT rat shows severe diabetic complications, the onset of hyperglycemia is late. SDT fatty rat, established by introducing the *fa* allele of the Zucker fatty rat to SDT rat, develops diabetes much faster than SDT rat. In the present study, diabetic peripheral neuropathy (DPN) was evaluated to show the further usefulness of this animal model. Motor nerve conduction velocity (MNCV) was delayed, and the number of sural nerve fibers was decreased in SDT fatty rat. Treatment of pioglitazone lowered blood glucose level and prevented delay of MNCV in SDT fatty rats. SDT fatty rat is a useful animal model for studies of DPN in type 2 diabetes.

**KEY WORDS:** diabetes, diabetic peripheral neuropathy, nerve conduction velocity, SDT fatty rat.

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Diabetes mellitus (DM) is a major metabolic disease, and the number of diabetics worldwide is estimated at approximately 350 million [5]. More than half of all DM patients have one or more diabetic microvascular complications such as diabetic retinopathy, diabetic nephropathy, or diabetic peripheral neuropathy (DPN). DPN is the most frequent complication, and nearly half of all diabetics suffer some type of nerve damages or symptoms [1]. Moreover, DPN causes foot ulceration, amputation, and chronic pain that reduce quality of life. Large clinical trials have proven that strict control of blood glucose level can delay the onset and progression of diabetic complications, including DPN [16, 26].

To clarify the pathophysiology of DPN, many diabetic animal models have been reported [2, 14, 30]. Spontaneously Diabetic Torii (SDT) rat is a model for non-obese type 2 diabetes [24, 25] showing pronounced hypoinsulinemia and hyperglycemia due to pancreatic  $\beta$ -cell degeneration from around 20 weeks of age [11]. SDT rat shows all three major diabetic complications in kidneys [17], nerves [21, 27], and especially in eyes [19, 22, 24, 25]. Although the SDT rat is a useful model to study diabetic complications, a late onset of hyperglycemia brings disadvantage for laboratory experiments not infrequently. To solve this problem,

the SDT fatty rat was established by introducing the *fa* allele of the Zucker fatty rat into the SDT rat genome [10]. This animal model develops diabetes from 5–6 weeks of age, and the time for progression is much earlier than that of original SDT rat (13–24 weeks, Table 1). The SDT fatty rats showed hyperinsulinemia at early stage of diabetes (4–8 weeks), but the insulin levels decreased to normal levels after 16 weeks of age. Plasma triglyceride (TG) and total cholesterol (TC) levels in SDT fatty rats were significantly higher than those in original SDT rats. These properties mitigate evaluation of diabetic complications. Although the eye and kidney complications in this animal model have been reported previously [12], nerve complications have not been examined. Therefore, in the present study, we evaluated the DPN in SDT fatty rat to show the further usefulness of this model.

Male SDT<sup>+/+</sup> (SDT) rats and SDT<sup>fa/fa</sup> (SDT-*Lep<sup>fa</sup>* or SDT fatty) rats from our colonies were used in the study. SDT fatty rats were produced by crossing heterozygous male SDT<sup>fa/+</sup> rats onto heterozygous female SDT<sup>fa/+</sup> rats. The SDT fatty rats and littermate SDT rats were selected by genotyping the *fa* locus with a PCR-restriction fragment length polymorphism (RFLP) method. Age-matched male Sprague-Dawley (SD) rats were used as control animals (Charles River Japan, Yokohama, Japan). All animal protocols used for the study were in strict compliance with our own Laboratory Guidelines for Animal Experimentation. Animals were housed in a climate-controlled room (temperature 23  $\pm$  3°C, humidity 55  $\pm$  15%, 12 hr lighting cycle) and allowed free access to basal diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

Impaired motor nerve conduction velocities (MNCV) in diabetic animals are described as markers of large myelin-

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Table 1. Biochemical parameters of non-fasted male SD, SDT, and SDT fatty rats

		8 weeks	24 weeks	40 weeks
Body weight (g)	SD	311 ± 7	658 ± 34	731 ± 35
	SDT	300 ± 6	487 ± 13**	445 ± 9**
	SDT fatty	387 ± 16**, ††	617 ± 30††	558 ± 14**, ††
Glucose (mg/dl)	SD	123 ± 3	144 ± 12	128 ± 1
	SDT	113 ± 2*	454 ± 82**	850 ± 42**
	SDT fatty	376 ± 91*, †	574 ± 17**	863 ± 57**
Insulin (ng/ml)	SD	1.90 ± 0.31	1.90 ± 0.34	2.78 ± 1.18
	SDT	1.36 ± 0.20	0.82 ± 0.54	0.07 ± 0.02
	SDT fatty	16.84 ± 4.11*, †	1.91 ± 0.45	1.03 ± 0.24††
TG (mg/dl)	SD	137 ± 14	194 ± 23	248 ± 32
	SDT	111 ± 12	195 ± 22	1,060 ± 202*
	SDT fatty	847 ± 168*, †	352 ± 36**, ††	1,095 ± 274*
TC (mg/dl)	SD	77 ± 3	96 ± 5	100 ± 5
	SDT	76 ± 2	83 ± 3	144 ± 12*
	SDT fatty	118 ± 8*, ††	141 ± 6**, ††	242 ± 22**, ††

Plasma glucose, TG, and TC levels were measured with commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Plasma insulin levels were measured with enzyme-linked immunosorbent assay kits (Morinaga Institute of Biological Science, Yokohama, Japan). Each value represents mean ± SE (n=4–6). \* $P<0.05$ , \*\* $P<0.01$  vs. age-matched SD rats, † $P<0.05$ , †† $P<0.01$  vs. age-matched SDT rats (unpaired *t*-test).

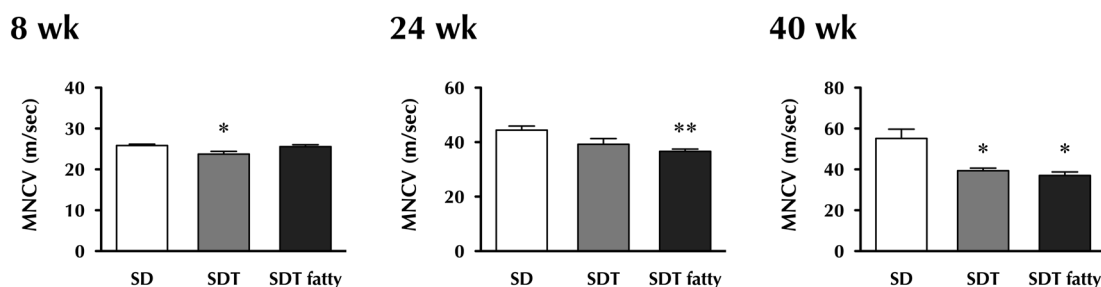


Fig. 1. Tail motor nerve conduction velocity (MNCV) at 8, 24, and 40 weeks of age. MNCV in male SDT fatty rats decreased by 18% compared to that of normal SD rats at 24 weeks of age, and decreased by 33% at 40 weeks of age. The delay of MNCV in SDT rats was not significant at 24 weeks, but was at 40 weeks. The tail MNCV was measured as described previously [23]. Briefly, tail nerve was stimulated with an electronic stimulator through a bipolar electrode under halothane anesthesia. Muscle action potentials were amplified and displayed with an amplifier oscilloscope. The MNCV was calculated from delta latency between two recording electrodes divided by a distance. Each value represents mean ± SE (n=5). \* $P<0.05$ , \*\* $P<0.01$  vs. age matched normal SD rats (unpaired *t*-test).

ated fiber dysfunction [4, 9]. Tail MNCV in original SDT rats was delayed around 30 weeks of age [21, 27]. In the present study, tail MNCV in male SDT fatty rats was significantly decreased by 18% from that of normal SD rats at 24 weeks of age, and decreased by 33% at 40 weeks of age (Fig. 1). The delay of MNCV in SDT rats was not significant at 24 weeks, but was significant at 40 weeks. At 8 weeks of age, SDT rats showed significant delay in MNCV compared to SD rats; however, this may be not a meaningful change. Regularly, delayed MNCV was not observed in SDT rats at this age [21, 27]. In our preliminary observation, SDT fatty rats also showed delay in sensory nerve conduction velocity (SNCV). Decreased caudal nerve blood flow and accumulation of sorbitol in sciatic nerve were also found (data not shown). The severity of nerve dysfunction is thought to be

proportionate to duration of hyperglycemia. However, further studies should be conducted to clarify the characteristics of small fibers (e.g. A $\delta$  and C-fiber) in terms of how they mediate sensation of temperature and pain, and autonomic nerve functions in this animal model as revealed in original SDT rats [13, 21, 22, 31].

The histopathological characteristics of sural nerve in SDT rats were described previously [21, 27]. Morphologically, the number of sural nerve fibers was slightly decreased in 24 weeks SDT fatty rats. At 40 weeks of age, fiber number was significantly decreased in both SDT and SDT fatty rats (Figs. 2 and 3). Furthermore, SDT fatty rats revealed significant atrophy in myelinated nerve at 40 weeks. Cross-sectional area of sural nerve in 40 week-old SDT rats was decreased to 80.1% that of normal SD rats, and further de-

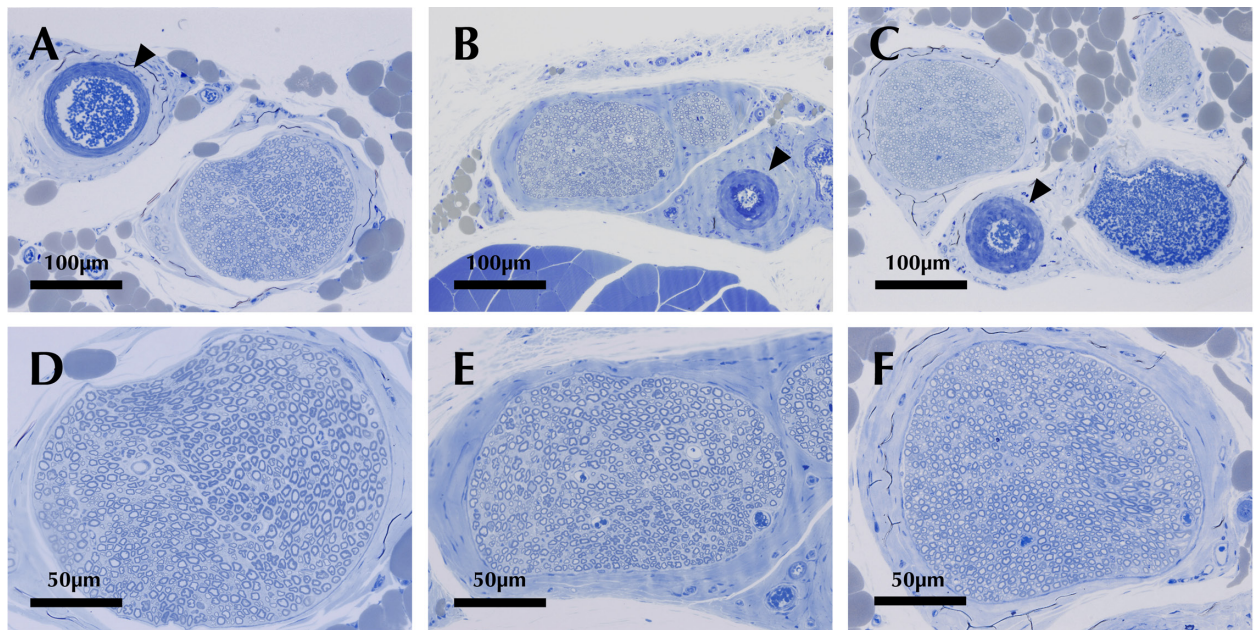


Fig. 2. Typical microphotographs of low (A–C) and high (D–F) magnification of sural nerve in normal SD rats (A, D), original SDT rats (B, E), and SDT fatty rats (C, F) at 40 weeks of age. Thickened epineurial arterioles were frequently found in both SDT rats and SDT fatty rats (arrowhead). Sural nerve samples were fixed in 2.5% glutaraldehyde overnight. Semithin sections were cut and stained with toluidine blue.

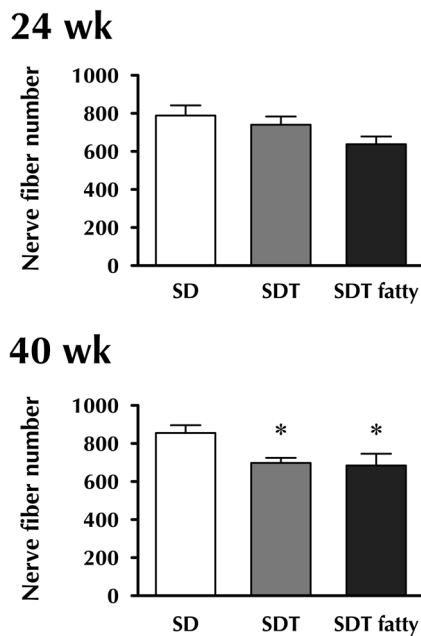


Fig. 3. Number of sural nerve fibers in normal SD rats, original SDT rats, and SDT fatty rats. At 24 weeks, nerve fiber number was slightly decreased in SDT fatty rats. At 40 weeks of age, fiber number was significantly decreased in both SDT and SDT fatty rats. Morphometric analysis was carried out using a microscope (BX50; Olympus, Tokyo, Japan) connected to a CCD camera (Penguin 600CL; Pixera, CA, U.S.A.) and analysis software WinROOF ver. 5.01 (Mitani corporation, Fukui, Japan). Each value represents mean  $\pm$  SE ( $n=5$ ). \* $P<0.05$  vs. normal SD rats (unpaired  $t$ -test).

crease was observed in SDT fatty rats (71.0% of SD rats). The number of small fiber seemed to increase relatively in SDT fatty rats due to atrophy (Fig. 2) as reported in SDT rats [21, 27]. The number of endoneurial blood vessels in SDT rats was comparable with that in normal rats; however, occluded/thickened epineurial arterioles were found in SDT rats [21, 27]. In the present study, the thickened epineurial arterioles were frequently observed in both SDT rats and SDT fatty rats (Fig. 2). In diabetics, the increased intima in epineurial arterioles primarily due to proliferation of intima cells is reported [8]. The increased intima possibly results decrease of nerve perfusion and may contribute to development of DPN. However, considering that the main pathological features in diabetic nerves consist of progressive fiber loss and endoneurial microangiopathy [29], further histopathological investigation is needed to characterize the DPN in SDT fatty rat.

Multiple mechanisms have been implicated in diabetic complications, including DPN: polyol pathway, hexosamine pathway, diacylglycerol/protein kinase C (DAG/PKC) pathway, and advanced glycation endproducts (AGEs) are well-known factors. Similarly, as in other diabetic animal models, DPN in original SDT rats was clearly ameliorated by treatment with PKC $\beta$  inhibitor JTT-010 [20], aldose reductase inhibitor (ARI) ranirestat [7], or vitamin B1 derivative benfotiamine (activator of transketolase, a thiamine-dependent pentose phosphate pathway enzyme) [21]. Although current anti-hyperglycemic drugs have not completely prevented the development of diabetic complications, correction of hyperglycemia is still a primary treatment modality of DPN [16, 26]. In fact, treatment of insulin lessened DPN progression



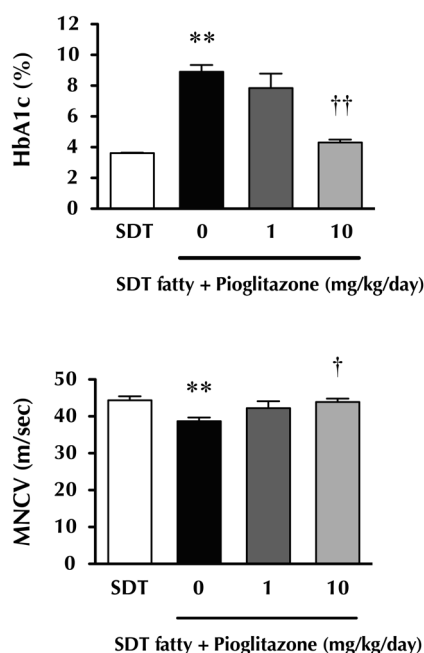


Fig. 4. Effect of pioglitazone on HbA1c level and sciatic MNCV in female SDT fatty rats. Shown are HbA1c levels in SDT fatty rats treated with vehicle, pioglitazone 1, and pioglitazone 10 mg/kg/day. HbA1c levels were decreased significantly by treating with 10 mg/kg/day of pioglitazone as food admixture for 6 weeks (7–13 weeks of age). MNCV was also significantly improved compared to vehicle-treated SDT fatty rats. HbA1c levels were measured with commercial kits (Roche Diagnostics) and an automatic analyzer. Sciatic MNCV was measured in accordance with described methods [23]. Briefly, sciatic nerve was stimulated at the sciatic notch and the Achilles tendon by adequate intensity under halothane anesthesia. Action potentials in muscle (M-waves) were recorded using a needle electrode. MNCV was calculated from the delta latency between M-wave peaks divided by the distance of the nerve length measured. Each value represents mean  $\pm$  SE ( $n=5$ ). \*\* $P<0.01$  vs. age-matched (pre-diabetic) SDT rats (unpaired  $t$ -test), † $P<0.05$ , †† $P<0.01$  vs. vehicle treated SDT fatty rats (Dunnett's test).

in SDT rats [21]. Therefore, in the present study, we next treated SDT fatty rats with peroxisome proliferator-activated receptor (PPAR)- $\gamma$  agonist pioglitazone, an oral anti-diabetic drug works as an insulin sensitizer, to lower blood glucose level and evaluate the effect on DPN. Six-week administration of pioglitazone to 7 weeks old female SDT fatty rats lowered HbA1c level and prevented delay of sciatic MNCV (Fig. 4). The same tendency was seen in SNCV (data not shown). However, pioglitazone promoted obesity by stimulating PPAR- $\gamma$  in adipose tissue. Body weight of SDT fatty rats treated vehicle, pioglitazone 1, and pioglitazone 10 mg/kg/day at 6 weeks was  $480.3 \pm 20.8$ ,  $489.7 \pm 29.8$  and  $641.3 \pm 12.8$  g, respectively. In our preliminary data, metformin, a first-line drug for type 2 diabetes, also prevented decreasing MNCV in SDT fatty rats (unpublished data). The results suggest that the peripheral neuropathy in SDT fatty rats is caused by marked hyperglycemia, and therefore this animal

is workable model to evaluate drugs for DPN in obese type 2 diabetes.

In addition to hyperglycemia, dyslipidemia and hypertension are key factors of metabolic syndrome. Relationships between these factors and peripheral neuropathy have also been pointed out. Correlation between blood TG and myelinated fiber density loss was reported in a clinical trial [28]. The correlation was independent of diabetes duration, age, and diabetes control. Mice fed a high-fat diet, a model of pre-diabetes and alimentary obesity, also showed the peripheral nerve functional abnormalities [15]. Therefore, the dyslipidemia is an independent risk factor for the DPN. Concerning hypertension, the DEMAND study indicated that lowering of blood pressure by angiotensin-converting enzyme (ACE) inhibitor and  $\text{Ca}^{2+}$  channel blocker reduced the risk of DPN in hypertensive patients with type 2 diabetes [18]. An ACE inhibitor also improved autonomic imbalances in diabetic hypertensive *db/db* mice [3]. Because SDT fatty rat shows both marked dyslipidemia (Table 1) and hypertension [6], these two factors may be deeply involved in the DPN of this animal model. Clarifying the participation of these factors in neuropathies will provide critical information for the development of new drugs for DPN.

In conclusion, neuropathies in SDT fatty rats are caused by sustained hyperglycemia, and therefore this rat is a useful diabetic animal model for studying peripheral neuropathy in obese type 2 diabetes and for the development of new drugs and therapies for DPN. Two additional characteristics (hyperlipidemia and hypertension) as metabolic syndrome will work to the advantage of SDT fatty rats compared to other animal models.

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