

Spontaneously Diabetic Torii *Lepr^{fa}* (SDT Fatty) Rat: A Novel Model of Obese Type 2 Diabetes

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Abstract: Diabetic animal models play critical roles in the elucidation of the mechanisms of diabetes mellitus and the complications, and in the development of novel drugs as treatments. Spontaneously Diabetic Torii *Lepr^{fa}* (SDT fatty) rat, established by introducing the *fa* allele of the Zucker fatty rat into SDT rat genome, is a new model of obese type 2 diabetes. We have investigated the characteristics of SDT fatty rats. Both male and female SDT fatty rats show overt obesity, and hyperglycemia and hyperlipidemia are observed at a young age as compared with SDT rats. With early incidence of diabetes mellitus, diabetic complications in SDT fatty rats were seen at younger ages than those in the SDT rats. Furthermore, we evaluated the pharmacological effects of anti-diabetic drugs, such as metformin, pioglitazone, and dipeptidyl peptidase-4 inhibitor on SDT fatty rats. SDT fatty rat is a useful model for analysis of metabolic disease and the evaluation of drugs related to metabolic disease.

Keywords: Diabetes, diabetic complication, dipeptidyl peptidase-4, SDT fatty rat, SDT rat.

ESTABLISHMENT OF A NEW DIABETIC MODEL

Type 2 diabetes mellitus is a polygenic disorder that is caused by a metabolic and/or hormonal imbalance between insulin secretion from β cells and insulin sensitivity in peripheral tissues, both of which might be modified by genetic and environmental factors [1]. The decreased sensitivity to insulin leads to an increased requirement for insulin, and is often associated with obesity in which metabolic disturbances are marked in insulin-target organs, such as the liver, muscle and adipose tissues [2]. Obesity plays key roles in the pathophysiology of several metabolic diseases and is a risk factor for diabetes mellitus or dyslipidemia. Based on the above concept, a novel model of obesity-related diabetes was established by Masuyama *et al.* [3]. They established a congenic line of the Spontaneously Diabetic Torii (SDT) rat by introducing the *fa* allele of the Zucker Fatty rat into the SDT rat genome *via* the Speed Congenic Method using a PCR technique with DNA markers. This congenic strain has been maintained by intercrossing between *fa*-heterozygous littermates.

PATHOPHYSIOLOGICAL FEATURES

The *fa/fa* (SDT fatty) rats of both sexes became overtly obese and were distinguishable from wild-type (+/+) (SDT) and heterozygous (+/*fa*) lean rats, and showed a significant polyphagia. Also, the BMI (body mass index) was greater in SDT fatty rats (mean value, 0.91 and 0.87 g/cm² in males and females, respectively) than in lean rats (0.75 and 0.58 g/cm² in males and females, respectively) at 14 weeks of age [3]. Retroperitoneal and interscapular fat pads were

markedly heavier in SDT fatty rats (26.2 and 10.1 g, respectively, in males and 29.1 and 16.6 g, respectively, in females) than in SDT rats (11.0 and 1.9 g, respectively, in males and 8.2 and 1.5 g, respectively, in females).

Metabolic disorder in SDT fatty rats was obviously promoted as compared with SDT rats [4, 5]. In male SDT rats, an incidence of diabetes was admitted after 20 weeks of age, and the cumulative incidence increased to 100 % in 40 weeks of age. In female rats, diabetes mellitus was observed after 40 weeks of age, and the cumulative incidence of diabetes increased to 33 % in 65 weeks of age [6]. Serum glucose levels in SDT fatty rats of both sexes were elevated from 6 weeks, and lipid parameters such as serum triglyceride and total cholesterol levels in the rats were elevated from 4 weeks of age. The hyperglycemia and hyperlipidemia were sustained for a long time afterwards (Fig. 1). As a result of continued hyperglycemia, an increase in body weights of SDT fatty rats was inhibited after 16 weeks of age. The male SDT fatty rats showed hyperinsulinemia from 4 to 8 weeks of age, but after 16 weeks their insulin levels decreased to levels similar to those in SDT rats. In the female rats, hyperinsulinemia was shown from 4 to 12 weeks of age, and the insulin levels decreased gradually. Also, a remarkable rise in renal parameters such as urine volume and urine protein was shown in SDT fatty rats of both sexes.

DIABETIC COMPLICATIONS

With early incidence of diabetes mellitus, diabetes-associated complications in SDT fatty rats were seen at younger ages than those in the SDT rats. In male SDT fatty rats, histopathological examination of the kidneys revealed changes in the glomeruli from 16 weeks, and in the renal tubules from 8 weeks of age [5]. In the glomeruli, glomerulosclerosis was observed from 16 weeks of age, and

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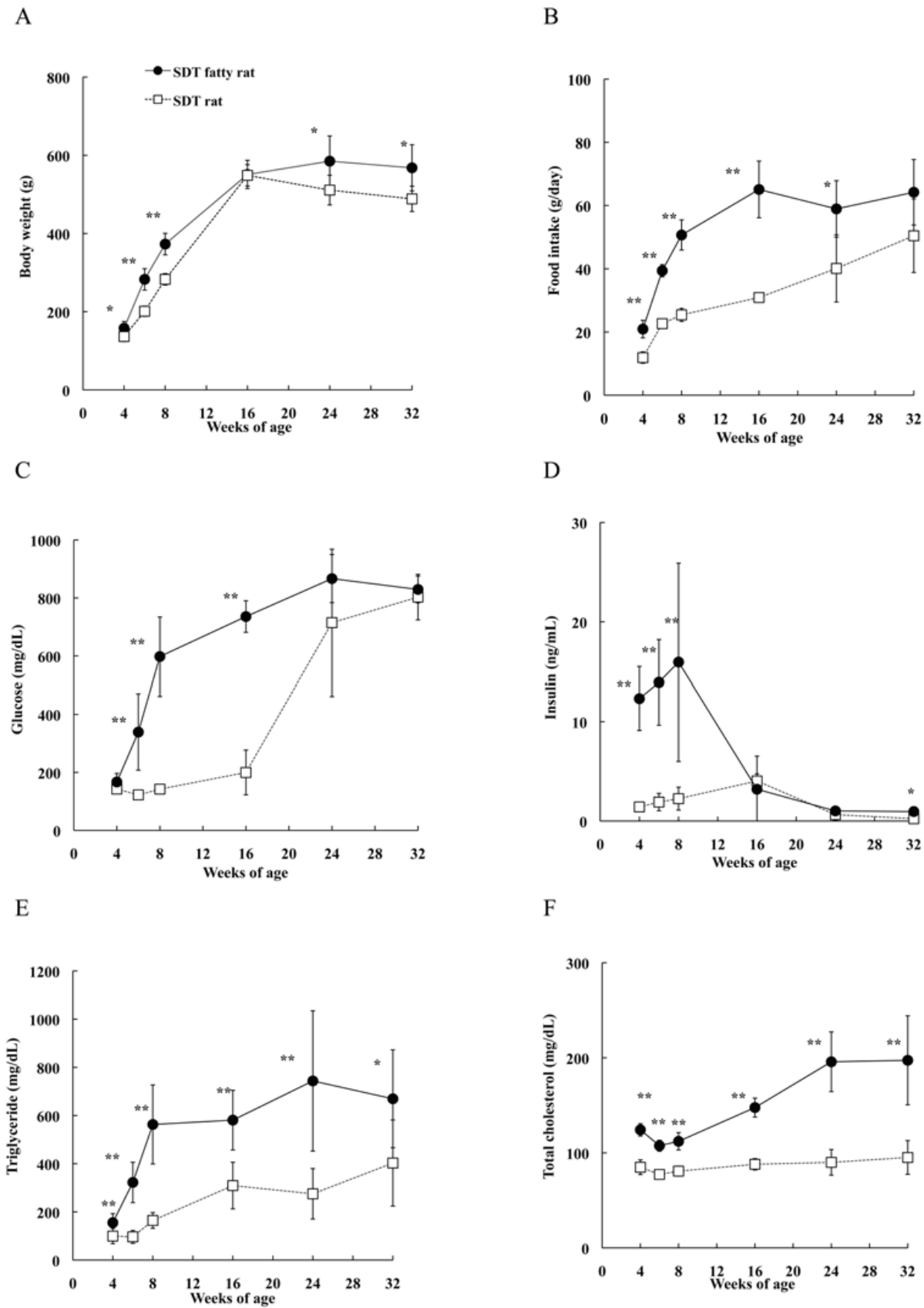


Fig. (1). Changes of biological parameters (A, Body weight; B, Food intake; C, Glucose; D, Insulin; E, Triglyceride; F, Total cholesterol) in male SDT fatty rats and SDT rats [5]. Data shown as mean \pm SD (n=5-9). *P<0.05, **P<0.01; significant difference from SDT rat.

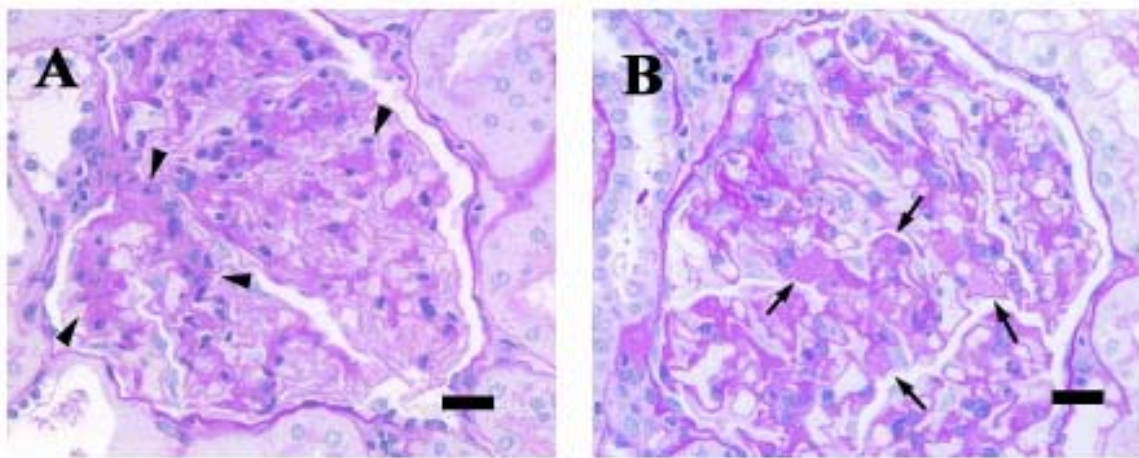


Fig. (2). Histological analysis of glomeruli. PAS stain. **A**, SDT fatty rats (Male, 24 weeks of age); **B**, SDT fatty rats (Male, 40 weeks of age) [5]. Bar = 20 µm. Diffuse glomerulosclerosis (arrowheads). Nodular lesions (arrows).

the sclerosis progressed with aging. Nodular lesions were observed at 40 weeks of age (Fig. 2). In the renal tubules, glycogen deposition in the tubular epithelium (Armanni-Ebstein lesions) and tubular dilation were noted from 8 weeks of age, and the change progressed from 8 to 16 weeks of age (Fig. 3). In female SDT fatty rats, a qualitatively equal change was observed in histopathological findings of kidneys [4]. The female rats revealed changes in the glomeruli from 32 weeks of age, and in the renal tubules from 16 weeks, and the changes progressed with aging. Renal enlargement is observed in diabetes mellitus, and in particular glomerular enlargement is observed from early stage in diabetic nephropathy. Although the precise mechanisms linking glomerular enlargement and glomerular injury are speculative, their association in several renal diseases, including diabetes, is intriguing [7]. In male SDT fatty rats, the glomerular size was increased from 16 to 32 weeks of age, as compared with that in SDT rats (Fig. 4A). Moreover, the glomerular size in female SDT fatty rats was elevated from 8 weeks of age, as compared with that in SDT rats or Sprague-Dawley (SD) rats (Fig. 4B).

Histopathological findings in lens, including hyperplasia of epithelium, vacuolation of fiber, and occurrence of

Morgagnian globules, were observed from 8 weeks of age in male SDT fatty rats, and these changes progressed with aging (Fig. 5). The female rats showed similar changes from 16 weeks of age.

PATHOPHYSIOLOGICAL CHANGES ON FOOD RESTRICTION

Effect of food restriction in SDT fatty rats was investigated [8, 9]. SDT fatty rats were subjected to pair-feeding with SDT rats from 6 to 26 weeks of age. Body weights of the pair-fed rats were similar with those of SDT rats. Improvement of hyperglycemia or hypertriglyceridemia was observed, but hypercholesterolemia was not entirely improved. Moreover, the incidence or progression of diabetic complications, such as renal lesions and cataract, was reduced. The changes in adipose tissue were interesting. The ratio of visceral fat weight to subcutaneous fat weight (V/S) decreased in the pair-fed rats, although the total fat (visceral fat and subcutaneous fat) weight did not change. Cell size of the epididymal fat in the pair-fed rats tended to decrease, and glucose oxidation level in epididymal fat in the pair-fed rats was recovered to a similar level with that in SDT rats.

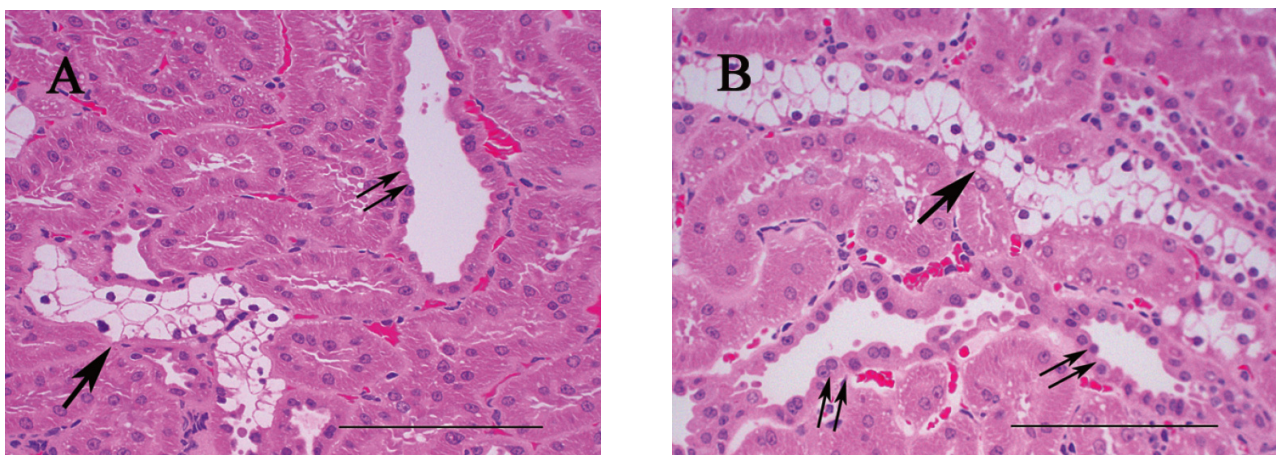


Fig. (3). Histological analysis of renal tubules. HE stain. **A**, SDT fatty rats (Male, 16 weeks of age); **B**, SDT fatty rats (Male, 24 weeks of age). Bar = 100 µm. Armanni-Ebstein change (single arrows). Tubular dilation (double arrows).

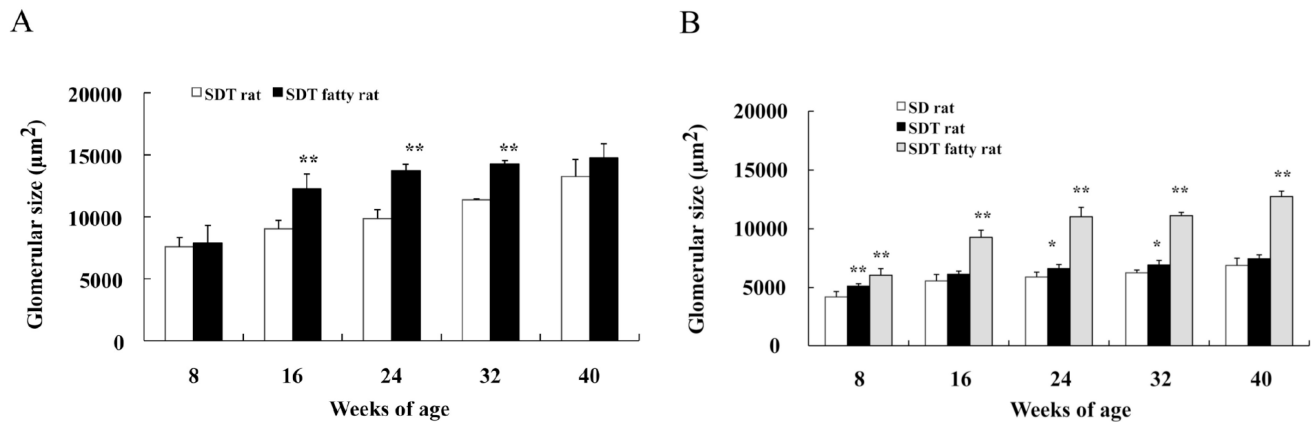


Fig. (4). Changes of glomerular size in male rats (A) and female rats (B). Glomerular size in male SDT fatty rats was increased from 16 to 32 weeks of age and that in female SDT fatty rats was elevated from 8 weeks of age, as compared with that in SDT rats or SD rats. Data shown as mean \pm SD (male rats;n=3-4, female rats;n=5). * $P < 0.05$, ** $P < 0.01$; significant difference from SDT rat (male rats) or SD rats (female rats).

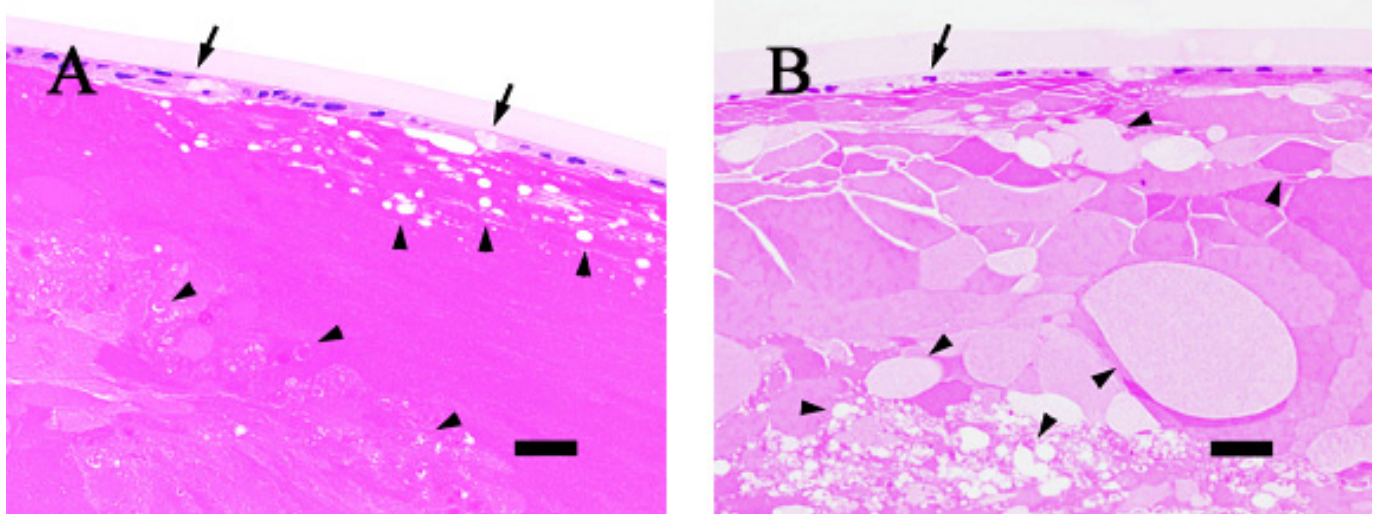


Fig. (5). Histological analysis of lens. HE stain. A, SDT fatty rats (Male, 16 weeks of age); B, SDT fatty rats (Male, 32 weeks of age) [5]. Bar = 20 μ m. Hypertrophy of epithelial cells (arrows). Degenerative lesions such as vacuolation of lens fiber and Morgagnian globules (arrowheads).

DRUG THERAPY

We investigated effects on anti-diabetic drugs in SDT fatty rats. For male SDT fatty rats, pioglitazone (3 mg/kg) was given as a dietary admixture in the powdered diet. The drug was administered from 6 to 12 weeks of age in the rats. Pioglitazone showed a hypoglycemic effect and good glycemic control during the experimental period (Fig. 6C, D). Blood insulin levels in SDT fatty rats were decreased with aging, but the decrease was inhibited by pioglitazone-treatment (Fig. 6E). Moreover, pioglitazone decreased the blood triglyceride level to the same level as the control rat (Fig. 6F). However, pioglitazone showed a significant increase of body weight (Fig. 6A). It is reported that peroxisome proliferator-activated receptor- γ (PPAR γ) agonists, such as pioglitazone and troglitazone, induce obesity in other diabetic models [10, 11].

We also performed an insulin therapy in female SDT fatty rats. Insulin pellets were implanted subcutaneously from 11 to 16 weeks of age. Blood glucose and HbA1c levels in the insulin-treated group decreased at 16 weeks of

age as compared with those in the control group (blood glucose level, mean value \pm SD, control: 629.0 ± 71.8 mg/dL vs insulin: 448.4 ± 141.0 mg/dL, HbA1c level, control: $8.17 \pm 0.91\%$ vs insulin: $5.80 \pm 0.71\%$). Blood samples were collected from the tail veins of non-fasted rats. Although an insulin therapy was performed in male SDT fatty rats, it was difficult to maintain good glycemic control.

We evaluated effects of metformin on SDT fatty rats. A single dose of metformin (1000 mg/kg) showed a hypoglycemic effect on male SDT fatty rats at 9 weeks of age. The rats showed significant decrease of blood glucose levels at 2 and 3 hours after the single dose. Moreover, chronic effects of metformin were examined. Metformin (300 mg/kg) was given as a dietary admixture in the powdered diet. The drug was treated to male SDT fatty rats for 4 weeks from 6 to 10 weeks of age. Metformin significantly decreased the blood glucose levels at 4 weeks after administration (blood glucose level, control: 739.2 ± 82.5 mg/dL vs metformin: 627.6 ± 53.2 mg/dL). Body weight and food intake in metformin-treated group did not change during the experimental period.

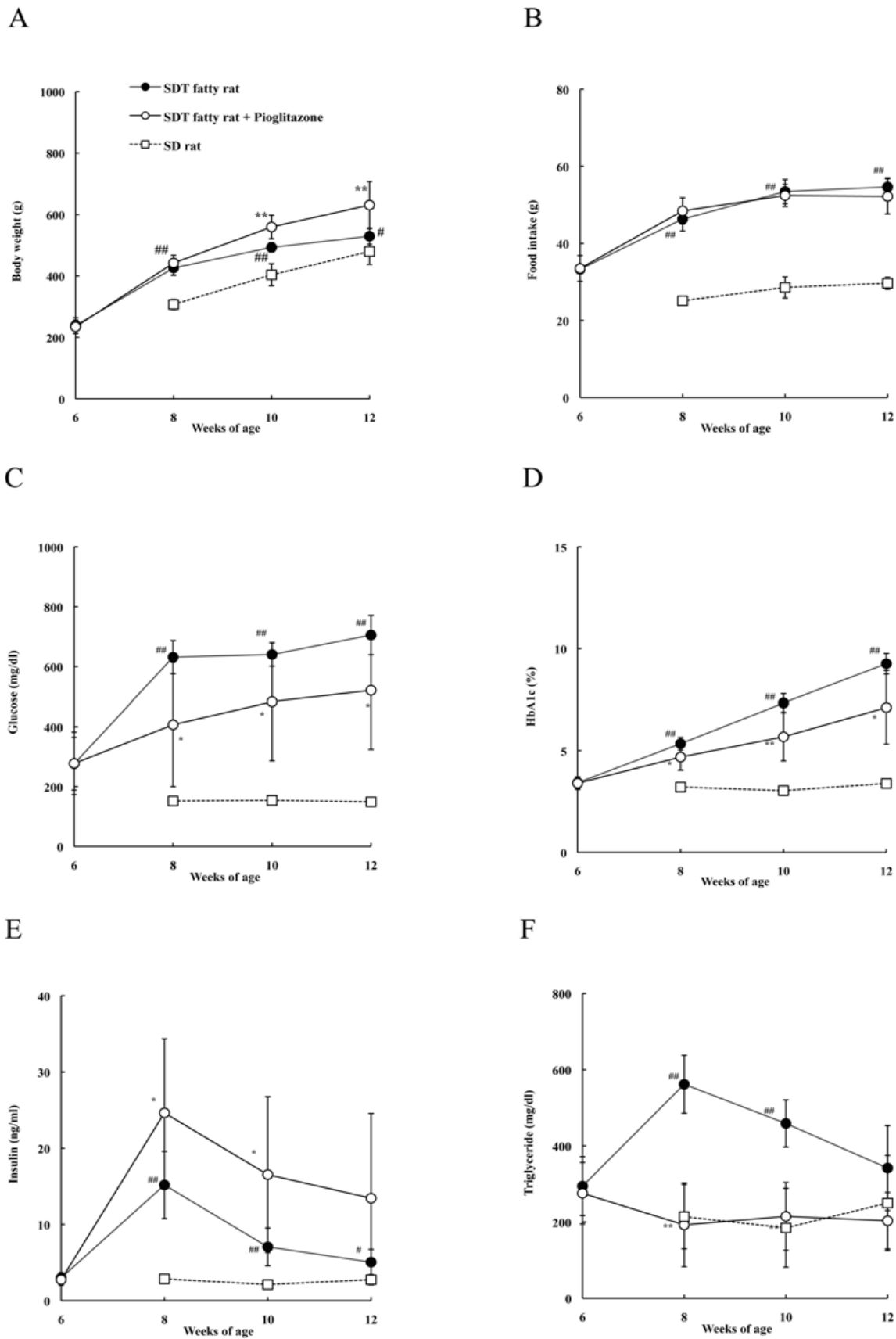


Fig. (6). Effect of pioglitazone on body weight (A), food intake (B), blood glucose (C), HbA1c (D), insulin (E), and triglyceride (F), levels in male SDT fatty rats. Pioglitazone showed good glycemic control, but the body weight was increased. Data shown as mean \pm SD (n=5-8). *P<0.05, **P<0.01; significant difference from SDT fatty rat. #p<0.05, ##p<0.01; significant difference from SD rat.

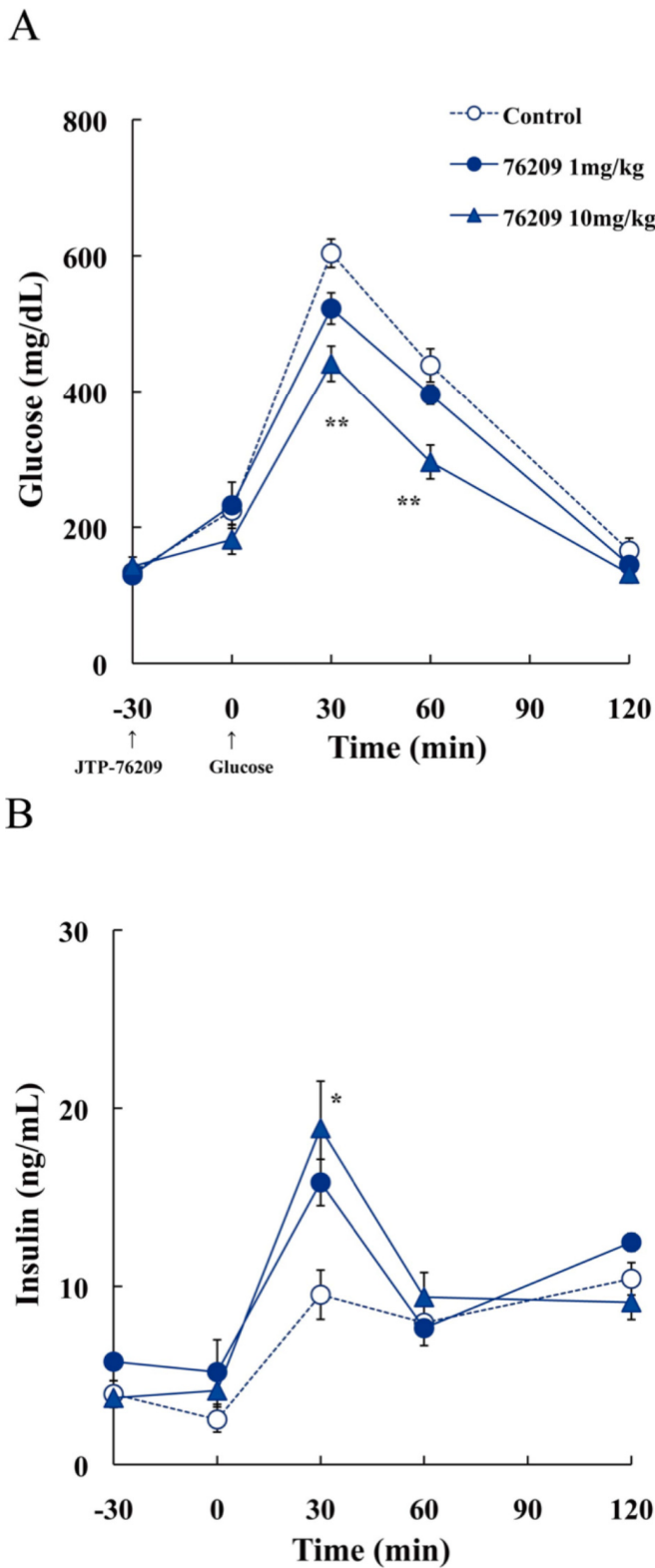


Fig. (7). Effect of JTP-76209 on blood glucose (A) and insulin (B) levels in glucose-loaded SDT fatty rats. JTP-76209 was administered orally 30 min before glucose-loading (1 g/kg). A single dose of JTP-76209 improved the glucose tolerance and enhanced the glucose-stimulated insulin secretion in dose dependent manner. Data shown as mean \pm SD (n=5). *P<0.05, **P<0.01; significant difference from the control.

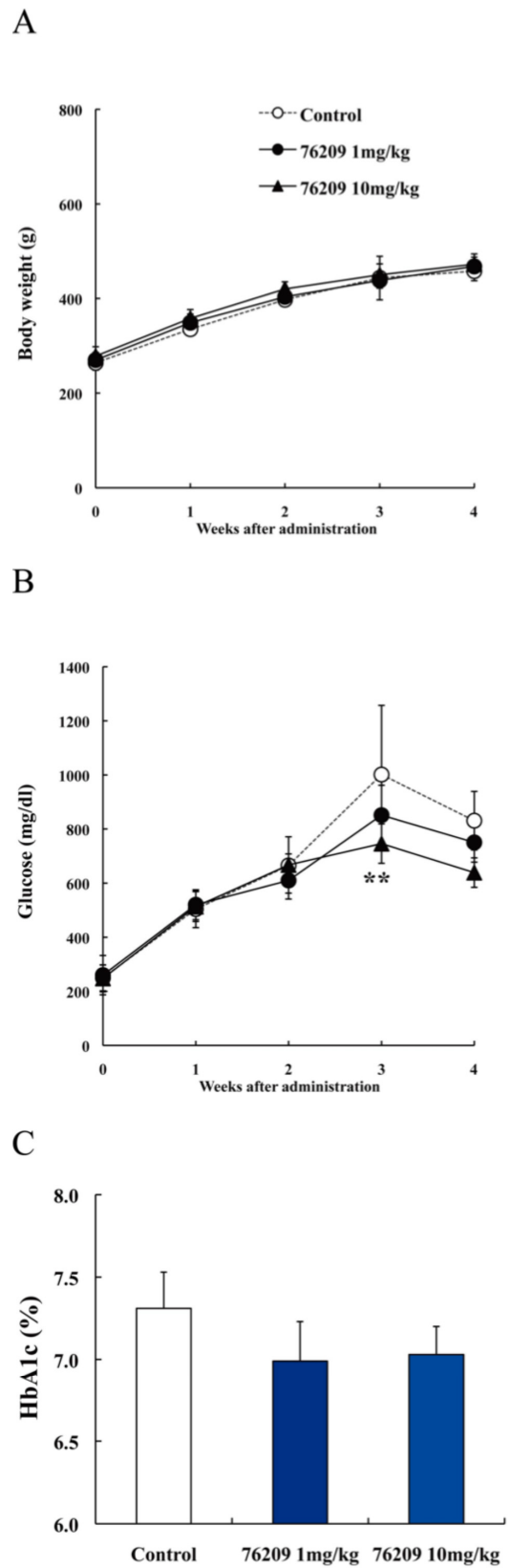


Fig. (8). Chronic effect of JTP-76209 on body weight (A), blood glucose (B), and HbA1c (C) levels in SDT fatty rats. JTP-76209 (1, 10 mg/kg) was administered as a dietary admixture for 4 weeks. JTP-76209 showed good glycemic control without increasing body weight. Data shown as mean \pm SD (n=5). **P<0.01; significant difference from the control.

Much recent attention has focused on glucagon-like peptide-1 (GLP-1) as a potential target for antidiabetic drugs [12, 13]; however, the use in antidiabetic drugs was impractical due to the short half-life as result of rapid inactivation by a protease called dipeptidyl peptidase type-4 (DPP-4) [14]. Thus, it is essential to design drugs that inhibit the action of DPP-4. We developed a DPP-4 inhibitor (JTP-76209) and evaluated the effects on SDT rats. JTP-76209 showed the DPP-4 inhibitory activity for a long time (6 hours or longer), and showed an increase of glucose stimulated-insulin secretion and an improvement of glucose tolerance [15]. Also, we investigated the pharmacological effects on SDT fatty rats. Male SDT fatty rats at 9 weeks of age were used after overnight fasting. The effect of drug on blood glucose and insulin levels in glucose-loaded animals was examined by means of an oral glucose tolerance test (1g glucose/kg) 30 min after single oral administration of JTP-76209. When JTP-76209 was administered at doses of 1 and 10 mg/kg, the impaired glucose tolerance was improved dose dependently and insulin secretion was enhanced (Fig. 7). Furthermore, we investigated the chronic effect of JTP-76209 in male SDT fatty rats. JTP-76209 (1, 10 mg/kg) was given as a dietary admixture in the powder diet for 4 weeks. Non-fasted blood glucose levels decreased dose-dependently after 3 weeks of administration with JTP-76209, and the hemoglobin A1c (HbA1c) levels at 4 weeks after the administration tended to decrease (HbA1c level, control: $7.31 \pm 0.22\%$, JTP-76209 1 mg/kg: $6.99 \pm 0.24\%$, JTP-76209 10 mg/kg: $7.03 \pm 0.17\%$) (Fig. 8). There was no change in body weights during the experimental period. JTP-76209, DPP-4 inhibitor, is expected to control postprandial hyperglycemia in patients with type 2 diabetes mellitus without increasing body weight.

CONCLUSION

Diabetes mellitus and diabetic complications in SDT fatty rats were found at a younger age than those in SDT rats. The early onset of diabetes or diabetic complication has advantages for the use of SDT fatty rats in diabetes research. Furthermore, not only the male rats but the female rats developed diabetes mellitus at a young age. Female SDT fatty rat has the potential to become an important animal model of type II diabetes mellitus with obesity, especially in women, where few models currently exist. Moreover, we have shown the pharmacological

effects of anti-diabetic drugs, such as metformin, pioglitazone, and dipeptidyl peptidase-4 inhibitor on SDT fatty rats. Use of SDT fatty rats will assist in the further elucidation of the pathogenesis of human diabetes mellitus and in discovering new drugs.

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