



Research Article

Open Access

Investigation of Pharmacological Responses to an Anti-Diabetic Drug Pioglitazone in Female Spontaneously Diabetic Torii (SDT) Fatty Rats, A New Obese Type 2 Diabetic Rat

Murai Y¹, Miyajima K², Shinohara M³, Yamada T¹ and Ohta T^{1*}

¹Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, Niigata, Japan

²Department of Nutritional Science and Food Safety Faculty of Applied Biosciences, Tokyo University of Agriculture, Tokyo, Japan

³CLEA Japan Inc., Planning and Development Section, Tokyo, Japan

Abstract

Introduction: Rigorous glycemic control is essential to prevent the development of diabetes and onset of diabetic complications. Peroxisome proliferator-activated receptor (PPAR) γ activator pioglitazone is an anti-diabetic drug that exhibits glucose-lowering effects by enhancing insulin sensitivity in peripheral tissues. However, increases in body weight are a concerning finding associated with treatment with pioglitazone. In this study, the pharmacological effects of pioglitazone were investigated in female Spontaneously Diabetic Torii (SDT) fatty rats, a new obese type 2 diabetic rat, to verify utility of this model.

Methods: Pioglitazone was administered to SDT fatty rats from 7 to 13 weeks of age, and changes in body weight and blood chemical parameters, fat distribution using Computed tomography (CT) analysis, and histopathological analyses of the pancreas, kidney, and liver, were evaluated.

Results: Pioglitazone treatment significantly decreased blood glucose, hemoglobin A1c, and triglyceride levels. However, increases in body weight were observed. In CT analysis, both visceral and subcutaneous fat weights also increased. In SDT fatty rats, histopathological changes, such as irregular boundaries and vacuolation of pancreatic islets, renal tubular vacuolation and extension, and hepatocellular fatty changes and hypertrophy, were observed. Pioglitazone treatment improved histopathological abnormalities.

Conclusion: In female SDT fatty rats, the sub chronic administration of pioglitazone improved hyperglycemia, hypertriglyceridemia, and histopathological lesions in the pancreas, kidney, and liver. However, treatment induced increases in the body weight. Female SDT fatty rats are useful for the development of new anti-diabetic drugs which show potential to enhance insulin sensitivity.

Keywords

SDT fatty rat, Pioglitazone, Blood chemical parameters, Fat distribution, Histopathological changes

Abbreviations

CT: Computed Tomography; DPP: Dipeptidyl Peptidase; HbA1c: Hemoglobin A1c; HE: Hematoxylin and Eosin; NASH: Nonalcoholic Steatohepatitis; PPAR: Peroxisome Proliferator-activated Receptor; SDT: Spontaneously Diabetic Torii; TC: Total Cholesterol; TG: Triglyceride; VEGF: Vascular Endothelial Growth Factor; V/S: Visceral/Subcutaneous; ZDF: Zucker Diabetic Fatty

Introduction

Metabolic disorders, such as obesity and diabetes, have become global health problems, and this population of patients is rapidly increasing all over the world [1-3]. The growing population of patients with metabolic disorders has resulted in an increase in the number of patients with diabetic complications, such as neurop-

thy, retinopathy, and nephropathy [4-6]. In addition to adverse effects on the quality of life of such patients, the growing number of patients contributes to increasing medical costs [7]. The onset of diabetic complications is affected by various factors, including insulin resistance, hyperglycemia, and dyslipidemia and, in particular, chronic glycemic control is important for preventing

Copyright: © 2017 Murai Y, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

the onset of these complications. Epidemiological mega-studies, such as the Diabetes Control and Complications trial and the UK Prospective Diabetes Study, have demonstrated significant benefit with long-term intensive control of blood glucose levels [8,9]. Since there are various critical factors causing diabetic patients, including genetic and environmental factors, several anti-diabetic drugs with differing mechanisms have been developed [10]. Peroxisome proliferator-activated receptor (PPAR) γ activator pioglitazone is an anti-diabetic drug that exhibits glucose-lowering effects by enhancing insulin sensitivity in peripheral tissues, thereby widely contributing to diabetes management. Furthermore, a prior meta-analysis showed that pioglitazone reduced the risk of myocardial infarction, stroke and death in patients with type 2 diabetes [11].

Diabetic animal models are essential for obtaining a better understanding of diabetes mellitus and developing novel anti-diabetic drugs. The Spontaneously Diabetic Torii (SDT) fatty rat was established by introducing the *fa* allele of the Zucker fatty rat into the SDT rat genome [12]. The male SDT fatty rat presents with obesity, hyperglycemia and dyslipidemia at a young age compared with the male SDT rat. Furthermore, with the early incidence of diabetes mellitus, diabetes-associated complications in the SDT fatty rat are seen at younger age compared with the SDT rat [13]. The female SDT fatty rat also develops diabetes and its complications at a young age and has the potential to become an important animal model for obese type 2 diabetes, especially for women, for which few models currently exist [14]. In fact, the SDT fatty rat is considered to be a suitable model to help understand the properties of type 2 diabetes with obesity. Furthermore, in addition to pathophysiological analyses of diabetes and its complications in the diabetic model, investigating the pharmacological effects of anti-diabetic drugs to elucidate the properties of these animals as a diabetic animal model is important. In this study, pioglitazone was repeatedly administered to female SDT fatty rats, and the effects of pioglitazone were investigated.

Material and Methods

Female SDT fatty rats were purchased from CLEA Japan Inc. (Tokyo, Japan). Rats were housed in suspended bracket cages and given a standard laboratory diet (CRF-1, Oriental Yeast Co., Ltd. Tokyo, Japan) and water *ad libitum* in a controlled room for temperature, humidity and lightning. Pioglitazone (1, 10 mg/kg) was administered to SDT fatty rats from 7 to 13 weeks of age ($n = 5$). The drug, suspended in 0.5% methyl cellulose (MC) solution, was administered orally by means of a stomach tube at a volume of 5 mL/kg.

Body weights, and non-fasting serum biochemical pa-

rameters, such as glucose, insulin, triglyceride (TG), and total cholesterol (TC) levels, were evaluated every two weeks. Hemoglobin A1c (HbA1c) levels were also determined at 13 weeks of age. Glucose, TG, TC, and HbA1c levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin levels were measured using rat-insulin enzyme-linked immunosorbent assay (ELISA) kits (Morinaga Institute of Biological Science, Yokohama, Japan).

Computed tomography (CT) analyses were taken at 13 weeks of age, and visceral fat, subcutaneous fat and lean body mass weights were determined. Weights were measured using a laboratory X-ray CT device (LA Theta, ALOKA Co., LTD., Osaka, Japan). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital (Tokyo Chemical Industry, Tokyo, Japan), and approximately 20 CT photographs in a rat were taken at 5 mm intervals between the diaphragm and lumbar vertebrae of the rats. Total fat weight and visceral/subcutaneous (V/S) ratios were calculated based on visceral and subcutaneous fat weights.

Necropsies were performed at 13 weeks of age. After measuring the weights of kidneys and livers, the organs including the pancreas were fixed in 10% neutral buffered formalin. After resection, the tissues were paraffin-embedded using standard techniques and thin-sectioned (3 to 5 μ m). The sections were stained with hematoxylin and eosin (HE).

Results of biological parameters were expressed as means \pm standard deviation. A statistical analysis of differences between mean values in the control group and pioglitazone-treatment groups was performed using a One-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test.

Results

Changes in body weight and biochemical parameters are shown in Figure 1. Body weight in the pioglitazone 10 mg/kg group significantly increased after 11 weeks of age (Figure 1A). Serum glucose and TG levels in the piogli-

***Corresponding author:** Takeshi Ohta, Ph.D., Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, 2-8050 Ikarashi, Nishi-ku, Niigata 950-2181, Japan, E-mail: takeshi.ota@jt.com

Received: March 30, 2017; **Accepted:** April 25, 2017;

Published online: April 27, 2017

Citation: Murai Y, Miyajima K, Shinohara M, et al. (2017) Investigation of Pharmacological Responses to an Anti-Diabetic Drug Pioglitazone in Female Spontaneously Diabetic Torii (SDT) Fatty Rats, A New Obese Type 2 Diabetic Rat. Clin Diabetes Res 1(1):8-13

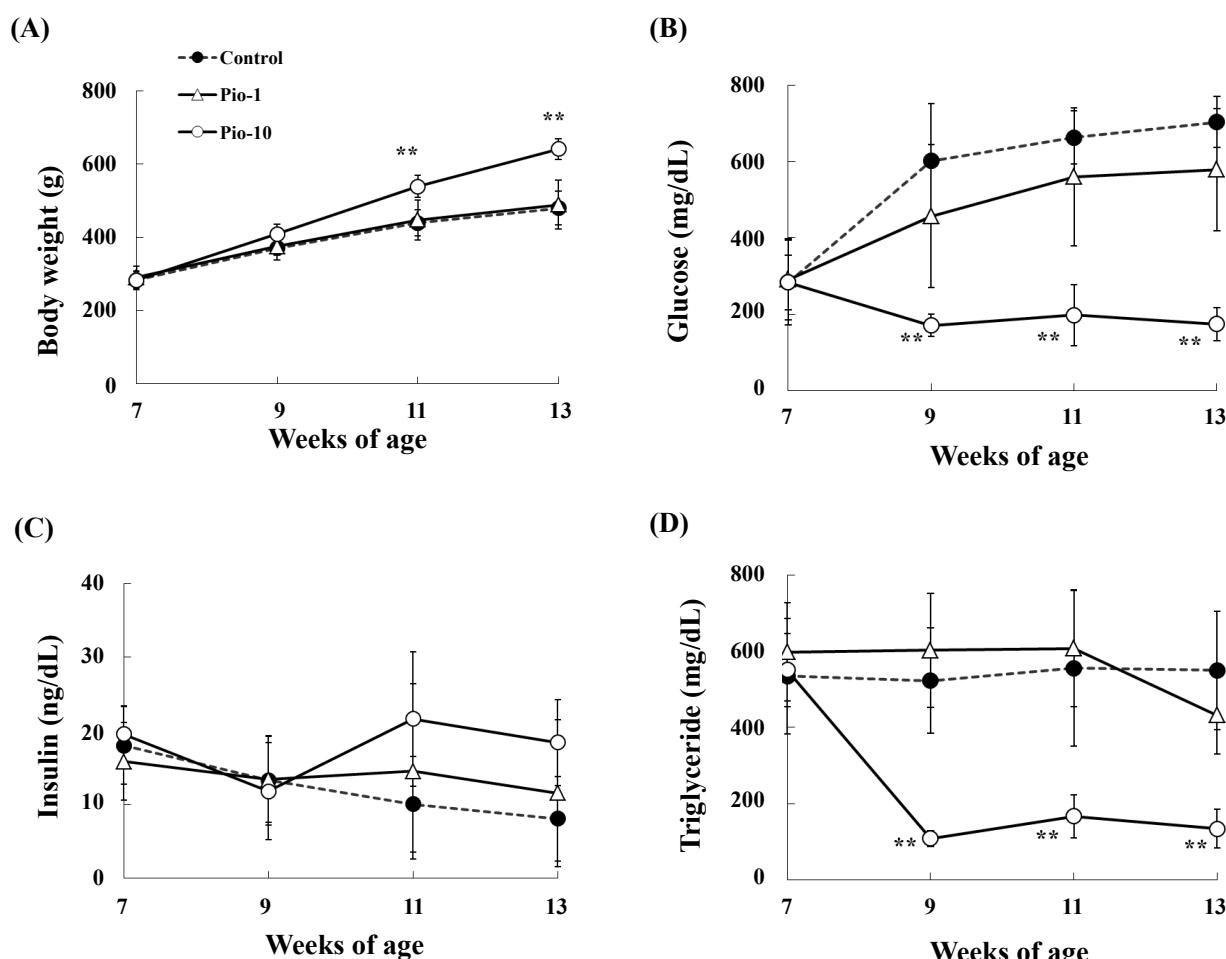


Figure 1: Effects of pioglitazone on body weight A), serum glucose B), insulin C), and TG D) levels in female SDT fatty rats. Data represent mean \pm standard deviation ($n = 5$). ** $p < 0.01$; significantly different from the control.

Table 1: Effects of pioglitazone on fat tissue weights in female SDT fatty rats at 13 weeks of age.

	Visceral fat (g)	Subcutaneous fat (g)	Total fat (g)	V/S ratio	Lean body mass (g)	Fat rate (%)
Control group	62.7 \pm 5.8	56.7 \pm 17.8	119.3 \pm 23.3	1.17 \pm 0.25	151.1 \pm 7.2	45.3 \pm 4.3
Pioglitazone 1 mg/kg group	61.2 \pm 9.7	70.3 \pm 20.3	131.5 \pm 29.5	0.90 \pm 0.13*	137.9 \pm 6.8*	48.3 \pm 4.9
Pioglitazone 10 mg/kg group	84.8 \pm 4.4**	129.7 \pm 3.0**	214.4 \pm 6.9**	0.65 \pm 0.03**	135.0 \pm 5.8**	61.4 \pm 0.4**

Data represent mean \pm standard deviation ($n = 5$). * $P < 0.05$, ** $P < 0.01$: significantly different from Control group.

V/S ratio: Visceral fat weight/Subcutaneous fat weight ratio.

zone 10 mg/kg group significantly decreased from 9 to 13 weeks of age (Figure 1B and Figure 1D), and insulin levels in the pioglitazone 10 mg/kg group tended to increase at 13 weeks of age (Figure 1C). Serum TC levels did not change across each group (data not shown). HbA1c level in the pioglitazone 10 mg/kg group significantly decreased at 13 weeks of age (control group, $8.91 \pm 0.98\%$; pioglitazone 1 mg/kg group, $7.84 \pm 2.12\%$; pioglitazone 10 mg/kg group, $4.30 \pm 0.42\%$). The biochemical parameters in the pioglitazone 1 mg/kg group did not change significantly compared with those in the control group.

The result in CT analysis of fat weights is shown in Table 1. Visceral and subcutaneous fat weights in the pioglitazone 10 mg/kg group increased significantly com-

pared with those in the control group. The fat rate also increased; however, the V/S ratio in the pioglitazone 1 and 10 mg/kg groups decreased. Lean body mass in the pioglitazone 1 and 10 mg/kg groups decreased significantly compared with that in the control group.

Histopathological changes in the pancreas, kidney, and liver are shown in Figure 2, Figure 3 and Figure 4. Since the biochemical parameters in the pioglitazone 1 mg/kg group were not improved, the histopathological analysis was not performed. Histopathological abnormalities, such as irregular boundaries, atrophy, and vacuolation of islets, were observed in the pancreas of animals in the control group. These changes in the pancreas were inhibited with pioglitazone treatment at a dose of

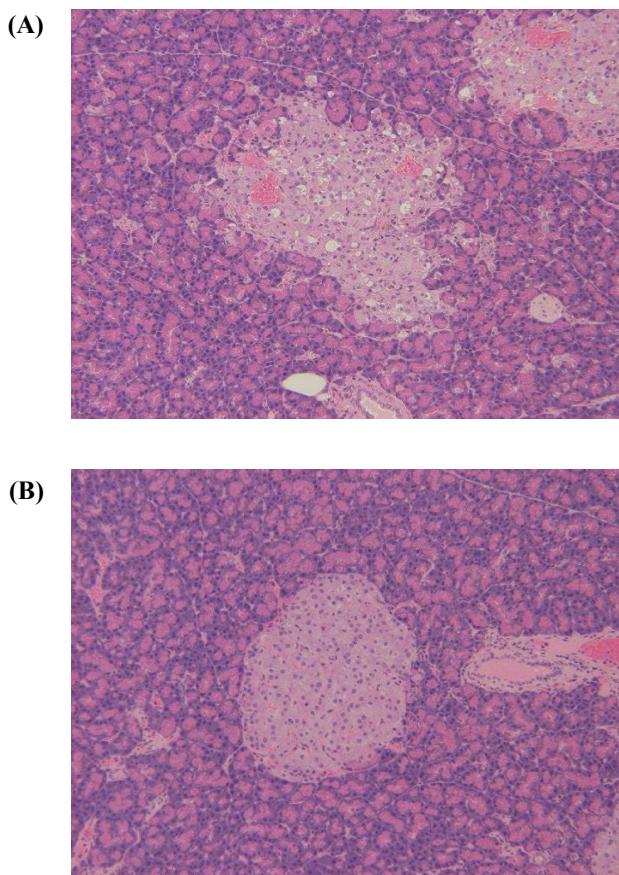


Figure 2: Histopathological analysis of pancreas A) Female SDT fatty rat at 13 weeks of age and B) Pioglitazone 10 mg/kg-treated SDT fatty rat. Original magnification $\times 200$ HE stain.

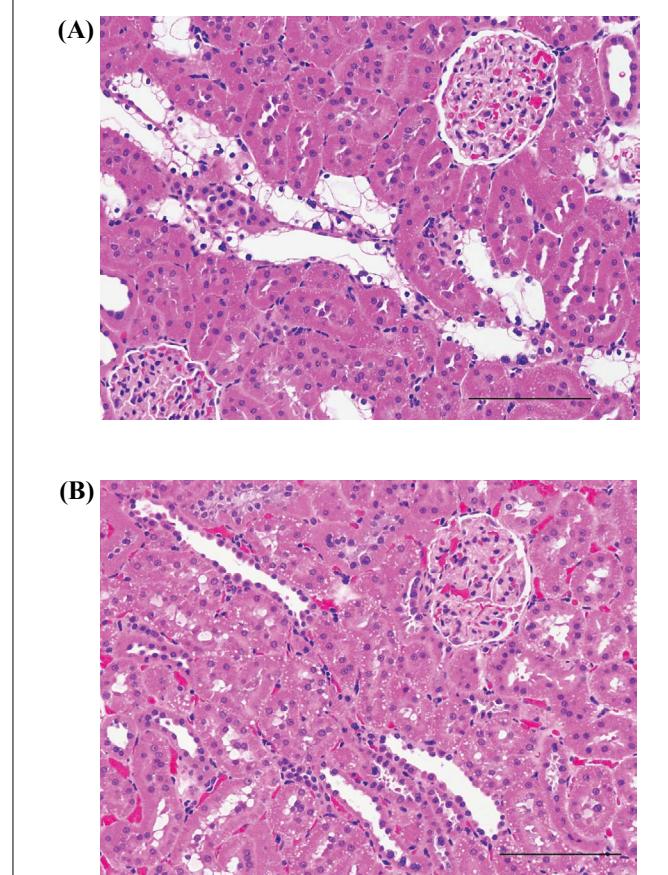


Figure 3: Histopathological analysis of kidney A) Female SDT fatty rat at 13 weeks of age and B) Pioglitazone 10 mg/kg-treated SDT fatty rat. Bar = 100 μ m HE stain.

Table 2: Effects of pioglitazone on organ weights in female SDT fatty rats at 13 weeks of age.

	Absolute kidney weight (mg)	Absolute liver weight (g)	Relative kidney weight (mg/g)	Relative liver weight (g/g)
Control group	3754 \pm 194	29.1 \pm 11.0	7.882 \pm 0.922	0.0594 \pm 0.0172
Pioglitazone 1 mg/kg group	3442 \pm 361	27.1 \pm 9.8	7.121 \pm 1.083	0.0541 \pm 0.0126
Pioglitazone 10 mg/kg group	3083 \pm 175*	26.3 \pm 3.4	4.805 \pm 0.072**	0.0411 \pm 0.0056

Data represent mean \pm standard deviation ($n = 5$). * $P < 0.05$, ** $P < 0.01$: significantly different from Control group.

10 mg/kg (Figure 2). Histopathological abnormalities, such as vacuolation of the tubular epithelium (Armanini-Ebstein lesion) and tubular dilation, were observed in the kidneys of animals in the control group. These changes were not observed in the pioglitazone 10 mg/kg group (Figure 3). Glomerular lesions including glomerulosclerosis were not observed in the female SDT fatty rats at 13 weeks of age (Figure 3A). Hepatosteatosis and hypertrophy of hepatocytes were observed in the livers of animals in the control group. However, these changes were inhibited in the pioglitazone 10 mg/kg group (Figure 4). Nonalcoholic steatohepatitis (NASH)-like hepatic lesions, such as inflammation and fibrosis, were not observed in the female SDT fatty rats at 13 weeks of age (Figure 4A). In measurements of organ weights of kidneys and livers, absolute and relative kidney weights de-

creased significantly in the pioglitazone 10 mg/kg group, and the relative liver weight in the pioglitazone 10 mg/kg group showed a tendency to decrease (Table 2).

Discussion

The pathogenesis of type 2 diabetes is heterogeneous, and hyperglycemia and dyslipidemia in these patients are caused by defects in insulin secretion and/or insulin sensitivity [15,16]. Accordingly, improving both insulin deficiency in pancreatic β cells and enhancing insulin sensitivity are necessary to achieve tight blood glucose control. Numerous drugs have been developed to treat type 2 diabetic patients, e.g. sulfonylureas and dipeptidyl peptidase (DPP)-4 inhibitors to improve insulin deficiency, biguanides and thiazolidinedione-based agents to enhance insulin sensitivity [15,17]. The most

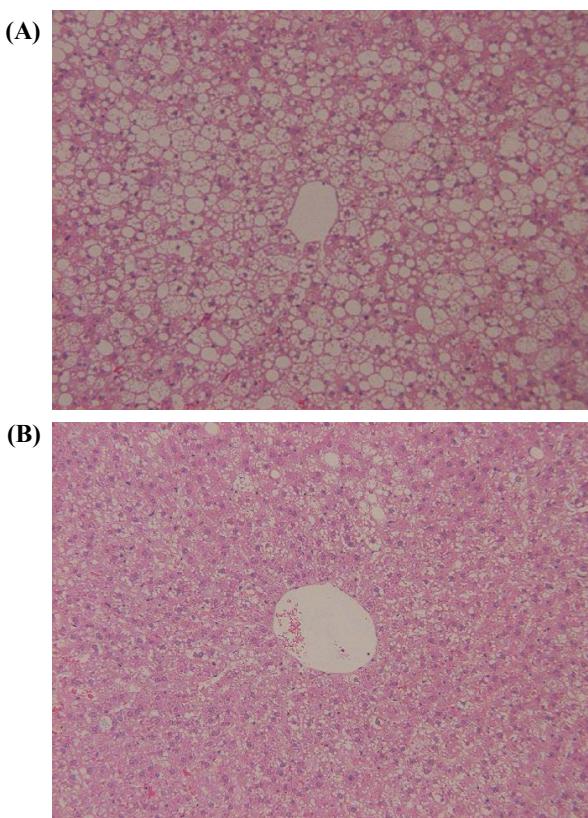


Figure 4: Histopathological analysis of liver A) Female SDT fatty rat at 13 weeks of age and B) Pioglitazone 10 mg/kg-treated SDT fatty rat. Original magnification $\times 200$ HE stain.

well-known thiazolidinedione-based agents are PPAR γ activators, and pioglitazone contributes to the treatment of type 2 diabetic patients with insulin resistance. In this study, the pharmacological effects of pioglitazone were investigated to elucidate the properties of female SDT fatty rats, a new obese type 2 diabetic model.

Female SDT fatty rats exhibited significant glucose and TG lowering effects with pioglitazone treatment. However, the body weight of animals increased, suggesting that pioglitazone treatment may promote obesity with glycemic control. In diabetic patients, pioglitazone treatment reportedly induces increases in body weight, and combining diet therapy with pioglitazone treatment is considered essential [18]. In other diabetic models, such as the KK-Ay mouse and Zucker diabetic fatty (ZDF) rat, pioglitazone treatment also caused increases in body weights due to hypoglycemic effects [19]. Furthermore, pioglitazone reportedly increases plasma vascular endothelial growth factor (VEGF) levels, possibly one of the causes of drug-induced fluid retention and edema, leading to obesity [20].

The increase in body weight with pioglitazone treatment was supported by results in CT analysis. Pioglitazone treatment increased both visceral and subcutaneous fat weights and, in particular, an increase in subcu-

taneous fat weight was noted (rate of increase compared with the control group: 84% increase in subcutaneous fat vs. 39% increase in visceral fat); however, the V/S ratio decreased. Pioglitazone reportedly increases fat masses by promoting adipocyte differentiation via PPAR γ activation [21,22]. It is considered that the increase in body weight with pioglitazone treatment is caused by retention of fluid and increase in fat mass, and the increase in fat weight is induced by activating adipocyte differentiation.

The vacuolation of islets and the tubular epithelium are considered to be caused by glycogen accumulation associated with sustained hyperglycemia in SDT fatty rats, and these changes improved with glycemic control observed after pioglitazone treatment. The HbA1c level in the pioglitazone group also decreased. The histopathological abnormalities including irregular boundaries and atrophy in islets improved with glycemic control, and the improvements are considered to induce increases in blood insulin levels. The tubular dilation in the pioglitazone groups is considered to be inhibited by decrease of urine volume with an improvement of hyperglycemia. Moreover, nephromegaly is reportedly observed in type 2 diabetic rat models [23]. The kidney weights of pioglitazone-treated rats were reduced by satisfactory glycemic control. Decreases of lean body mass in the pioglitazone groups may be related with the decreases of organ weights, such as kidney and liver.

Female SDT fatty rats reportedly present with NASH-like hepatic lesions, such as inflammation and fibrosis [24]. In this study, pioglitazone treatment for 6 weeks improved fatty livers in SDT fatty rats, and longer treatment is expected to also improve NASH-like lesions in female SDT fatty rats.

In female SDT fatty rats, the repeated administration of pioglitazone led to improvements in glucose/lipid metabolic abnormalities and pathological lesions in the pancreas, kidney, and liver. However, pioglitazone treatment also induced increases in body weight. The female SDT fatty rat is useful for the development of new anti-diabetic drugs which show potential to enhance insulin sensitivity.

References

1. DeFronzo RA, Ferrannini E, Groop L, et al. (2015) Type 2 diabetes mellitus. Nat Rev Dis Primers 1: 15019.
2. Yoon KH, Lee JH, Kim JW, et al. (2006) Epidemic obesity and type 2 diabetes in Asia. Lancet 368: 1681-1688.
3. Szoke E, Shrayef MZ, Messing S, et al. (2008) Effect of aging on glucose homeostasis: accelerated deterioration of beta-cell function in individuals with impaired glucose tolerance. Diabetes Care 31: 539-543.
4. Cortez M, Singleton JR, Smith AG (2014) Glucose intolerance, metabolic syndrome, and neuropathy. Handb Clin Neurol 126: 109-122.

5. Singh R, Kaur N, Kishore L, et al. (2013) Management of diabetic complications: a chemical constituents based approach. *J Ethnopharmacol* 150: 51-70.
6. Sivaprasad S, Gupta B, Crosby-Nwaobi R, et al. (2012) Prevalence of diabetic retinopathy in various ethnic groups: a worldwide perspective. *Surv Ophthalmol* 57: 347-370.
7. Calcutt NA, Cooper ME, Kern TS, et al. (2009) Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials. *Nat Rev Drug Discov* 8: 417-429.
8. Ginsberg BJ, Mazze R (1994) Clinical consequences of the Diabetes Control and Complications Trial. *N J Med* 91: 221-224.
9. Turner RC (1998) The U.K. Prospective Diabetes Study. A review. *Diabetes Care* 3: 35-38.
10. Zaccardi F, Webb DR, Yates T, et al. (2016) Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. *Postgrad Med J* 92: 63-69.
11. Schernthaner G, Currie CJ, Schernthaner GH (2013) Do we still need pioglitazone for the treatment of type 2 diabetes? A risk-benefit critique in 2013. *Diabetes Care* 36: S155-S161.
12. Masuyama T, Katsuda Y, Shinohara M (2005) A novel model of obesity-related diabetes: introgession of the Lepr(fa) allele of the Zucker fatty rat into nonobese Spontaneously Diabetic Torii (SDT) rats. *Exp Anim* 54: 13-20.
13. Matsui K, Ohta T, Oda T, et al. (2008) Diabetes-associated complications in Spontaneously Diabetic Torii fatty rats. *Exp Anim* 57: 111-121.
14. Ishii Y, Ohta T, Sasase T, et al. (2010) Pathophysiological analysis of female Spontaneously Diabetic Torii fatty rats. *Exp Anim* 59: 73-84.
15. Meece J (2007) Pancreatic islet dysfunction in type 2 diabetes: a rational target for incretin-based therapies. *Curr Med Res Opin* 23: 933-944.
16. Blair M (2016) Diabetes Mellitus Review. *Urol Nurs* 36: 27-36.
17. Wilding JP (2012) PPAR agonists for the treatment of cardiovascular disease in patients with diabetes. *Diabetes Obes Metab* 14: 973-982.
18. Chalmers J, Hunter JE, Robertson SJ, et al. (2007) Effects of early use of pioglitazone in combination with metformin in patients with newly diagnosed type 2 diabetes. *Curr Med Res Opin* 23: 1775-1781.
19. Shibata T, Matsui K, Nagao K, et al. (1999) Pharmacological profiles of a novel oral antidiabetic agent, JTT-501, an isoxazolidinedione derivative. *Eur J Pharmacol* 364: 211-219.
20. Baba T, Shimada K, Neugebauer S, et al. (2001) The oral insulin sensitizer, thiazolidinedione, increases plasma vascular endothelial growth factor in type 2 diabetic patients. *Diabetes Care* 24: 953-954.
21. Koenen TB, Tack CJ, Kroese JM, et al. (2009) Pioglitazone treatment enlarges subcutaneous adipocytes in insulin-resistant patients. *J Clin Endocrinol Metab* 94: 4453-4457.
22. Kajita K, Mori I, Hanamoto T, et al. (2012) Pioglitazone enhances small-sized adipocyte proliferation in subcutaneous adipose tissue. *Endocr J* 59: 1107-1114.
23. Katsuda Y, Ohta T, Miyajima K, et al. (2014) Diabetic complications in obese type 2 diabetic rat models. *Exp Anim* 63: 121-132.
24. Ishii Y, Motohashi Y, Muramatsu M, et al. (2015) Female spontaneously diabetic Torii fatty rats develop nonalcoholic steatohepatitis-like hepatic lesions. *World J Gastroenterol* 21: 9067-9078.