Pathophysiological Changes in Pre-Diabetic Stage of Spontaneously Diabetic Torii (SDT) Rats

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Abstract: The Spontaneously Diabetic Torii (SDT) rat is a new model of non-obese type 2 diabetes. In the present study, researchers investigated pathophysiological changes of male SDT rats in pre-diabetic stage from 8-16 weeks of age. In oral glucose tolerance test at 12 weeks of age, SDT rats showed glucose intolerance and decrease of glucose-stimulated insulin secretion. Different doses of Streptozotocin (STZ) were administered to SDT rats or SD rats and the pancreatic weakness was examined. SDT rats treated with STZ at a dose level of 20 mg kg^{-1} were diabetic within 1 week after treatment whereas SD rats got the diabetes after 30 mg kg^{-1} STZ was received. Insulin tolerance test was performed at 8, 16 and 24 weeks of age in order to examine the insulin sensitivity and the K value was calculated. Interestingly in SDT rats at 16 weeks of age, insulin sensitivity was increased as compared with that in SD rats. Pathohistological changes in pancreas such as atrophy of islets, multiple irregular projection of islets and islet fibrosis were observed in SDT rats at 15 weeks of age. In this study, SDT rat is a useful model for the analysis of the progression of pre-diabetes and the onset of type 2 diabetes.

Key words: Pancreas, pre-diabetes, SDT rat, STZ, insulin, diabetes

INTRODUCTION

The presence of both Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) increases the risk of progression to diabetes (Bloomgarden, 2008). During the phase of abnormal glucose tolerance before development of diabetes, insulin resistance occurs in addition to insulin deficiency, leading to the condition variously referred to as metabolic syndrome or cardiometabolic syndrome (Kendall and Bergenstal, 2001). It is possible to reduce the development of diabetes by intervening before its onset.

It is important to investigate the pathophysiology of not only diabetes mellitus but also pre-diabetes. Animal models of diabetes are very useful for understanding the mechanism of progression of pre-diabetes and onset of diabetes.

At present, Zucker Fatty (ZF) rats (Shibata et al., 1998), Zucker Diabetic Fatty (ZDF) rats (Tokuyama et al., 1995), Goto-Kakizaki (GK) rats (Kimura et al., 1982), Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Kawano et al., 1992), etc., are widely employed as rat models of type 2 diabetes mellitus. The Spontaneously Diabetic Torii (SDT) rat is a model of non-obese spontaneous diabetes which was developed by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) (Masuyama et al., 2004; Shinohara et al., 2000). Male SDT rats develop hyperglycemia, hypoinsulinemia and hyperlipidemia with age and express of urinary glucose from about 20 weeks of age.

SDT rats also develop ocular complications (cataract and retinopathy) and nephropathy from about 40 weeks of age (Shinohara et al., 2000). Sasase et al. (2006) have reported that the lesions in SDT rats are induced by hyperglycemia because an improvement is observed following continuous subcutaneous insulin administration (Ohta et al., 2007; Sasase et al., 2006).

Therefore, the SDT rat is expected to be useful as a new animal model of diabetes-associated complications such as retinopathy and nephropathy. Moreover, it has been reported that SDT rats showed a pancreatic dysfunction before onset of diabetes. An analysis of Glucose-stimulated Insulin Secretion (GSIS) in isolated islets shows a marked reduction in insulin secretion in

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SDT rats at 16 weeks of age (Matsui *et al.*, 2009). In this study, investigated *in vivo* pathophysiological changes in pre-diabetes stage of SDT rats.

MATERIALS AND METHODS

Animals: Male SDT rats were used in this study. Age-matched Sprague Dawley (SD) rats were used as the control animals. SDT and SD 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.

Biophysiological parameters: Body weights and blood chemical parameters such as glucose, insulin, Triglyceride (TG) and Total Cholesterol (TC) levels were examined at 8, 16 and 24 weeks of age. Blood samples were collected from the tail vein of non-fasted or overnight-fasted rats. Serum glucose, TG and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin level was measured with a rat-insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Oral Glucose Tolerance Test (OGTT) was performed at 12 weeks of age. Glucose solution (2 g kg^{-1}) was administered to overnight-fasted rats. Blood samples were collected before and 10, 30, 60 and 120 min after glucose loading. Serum glucose and insulin levels were measured as described earlier. Insulin Tolerance Test (ITT) was performed at 8, 16 and 24 weeks of age. Insulin solution (1 U kg^{-1}) was administered to overnight-fasted rats intravenously. Blood samples were collected 3, 6, 9, 12 and 15 min after insulin injection and the serum glucose levels were measured as described earlier. The rate of glucose disappearance (K value) was calculated from the glucose clearance velocity (t 1/2) with the serum glucose levels between 3 and 15 min after insulin injection.

Pancreatic weakness: Pancreatic weakness was examined by STZ treatment as researchers previously reported (Ohta *et al.*, 2010). Rats at 12 weeks of age received a single intravenous injection of 10 mg kg^{-1} STZ (Sigma, St. Louis, MO, USA) freshly dissolved in saline. Dosage of STZ has been gradually increased from 10-30 mg kg^{-1} every week.

Pancreatic histology: In SDT rats at 15 weeks of age, necropsy was performed. The pancreas was fixed in 10% neutral buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3-5 µm). The sections were stained with Hematoxylin and Eosin (HE). Immunohistochemistry was performed on sections of pancreas with an anti-insulin antibody (Dako, Kyoto, Japan) by indirect staining using peroxidase-conjugated anti-guinea pig immunoglobulin (Dako).

Statistical analysis: Results of biophysiological parameters were expressed as the mean±Standard Deviation (SD). Statistical analysis of differences between mean values was performed using the F test followed by the Student's t test or Aspin-Welch's t test. Differences were defined as significant at p<0.05.

RESULTS AND DISCUSSION

Biological parameters: Body weights and serum parameters in SD rats and SDT rats were shown in Table 1. Body weight in SDT rats was significantly decreased at 24 weeks of age as compared with that in SD rats. Non-fasted serum glucose level in SDT rats at 16 weeks of age showed no significant change as compared with that in SD rats but the glucose level prominently elevated at 24 weeks of age.

Table 1: Body weights and serum parameters in male SD rats and SDT rats

Characteristics	Age (weeks of age)		
	8	16	24
Body weight (g)			
SD rat	290.0±15.4	525.3±26.3	622.5±58.5
SDT rat	289.6±14.3	500.6±11.5	520.4±40.6**
Glucose (mg dL^{-1})			
Non-fasted			
SD rat	156.5±6.7	136.2±5.2	128.7±9.3
SDT rat	170.8±5.0**	182.8±65.0	611.5±150.0**
Fasted			
SD rat	883.7±10.5	116.0±9.6	104.5±6.5
SDT rat	65.0±7.3**	115.7±13.7	186.4±87.8
Insulin (ng mL^{-1})			
Non-fasted			
SD rat	2.8±0.9	4.3±1.4	3.2±0.8
SDT rat	1.8±0.4*	5.0±1.1	1.4±1.0**
Fasted			
SD rat	0.3±0.1	1.4±0.8	1.4±0.3
SDT rat	0.3±0.1	0.9±0.4	0.5±0.2**
Triglyceride (mg dL^{-1})			
Non-fasted			
SD rat	175.0±43.2	270.1±31.3	253.2±62.3
SDT rat	112.8±13.2*	262.6±90.0	446.1±251.7
Fasted			
SD rat	137.8±27.4	94.1±31.4	160.8±54.3
SDT rat	104.4±12.9*	91.1±19.5	80.3±24.8*
Total cholesterol (mg dL^{-1})			
Non-fasted			
SD rat	92.7±11.8	79.0±17.2	107.4±16.9
SDT rat	74.5±3.4*	83.5±6.1	87.2±14.9
Fasted			
SD rat	83.6±10.5	75.8±10.6	86.1±9.7
SDT rat	84.8±2.7	69.2±4.9	64.2±5.8**

Data represents means±sd (n = 6); *p<0.05, **p<0.01; significantly different from age-matched SD rats
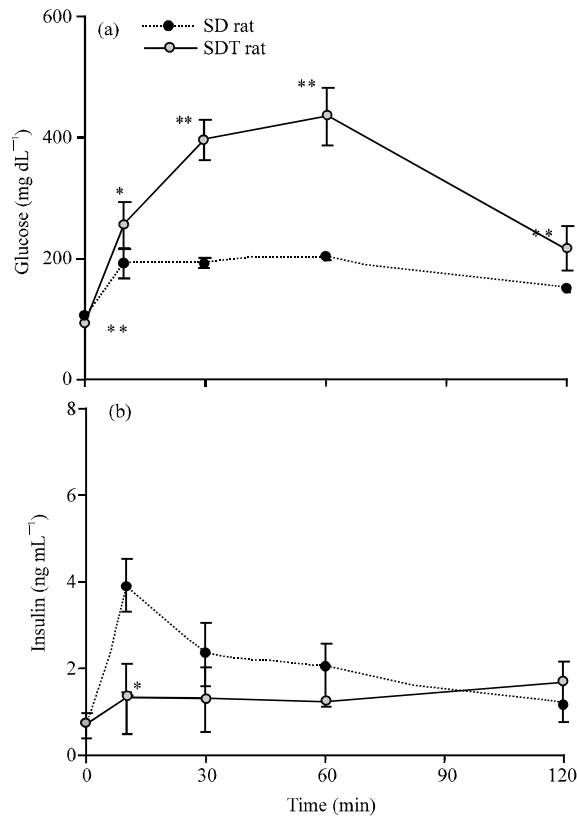
Fig. 1: Changes of serum glucose and insulin levels after glucose loading in SD and SDT rats at 12 weeks of age; blood samples were taken via the tail vein before and 10, 30, 60 and 120 min after glucose loading; data represent means±SD (n = 5); *p<0.05, ** p<0.01; significantly different from the SD rat

Both non-fasted and fasted serum insulin levels were significantly decreased in SDT rats at 24 weeks of age. A significant hyperlipidemia was not observed until 24 weeks of age. Serum glucose levels after glucose loading in SDT rats at 12 weeks of age were significantly increased as compared with those in SD rats (Fig. 1a). In SDT rats, a serum insulin level at 10 min after glucose loading significantly decreased (Fig. 1b). In OGTT, SDT rats at 12 weeks of age showed glucose intolerance and reduction of GSIS. K values in ITT were shown in Fig. 2. A K value in SDT rats at 16 weeks of age was significantly increased as compared with that in SD rats. The values at 8 and 24 weeks of age were not different between SD rats and SDT rats. In ITT, SDT rats at 16 weeks of age showed an increase of insulin sensitivity.

Pancreatic weakness: Both SD and SDT rats did not develop diabetes at 10 mg kg^{-1} STZ. All SDT rats showed
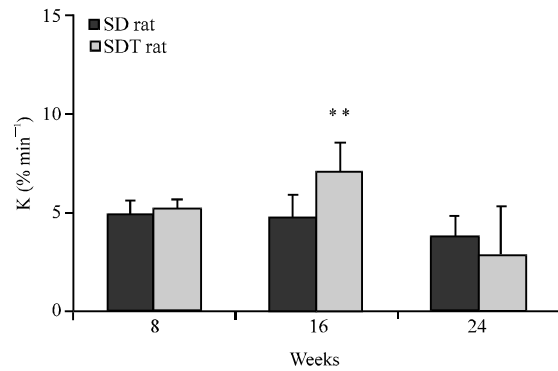

Fig. 2: The rate of glucose disappearance (K) in SD and SDT rats at 8, 16 and 24 weeks of age. Serum glucose levels were measured at 3, 6, 9, 12 and 15 min after insulin injection and the K value was calculated. Data represent means±SD (n = 5 or 6); **p<0.01; significantly different from the SD rat

hyperglycemia (749.6 mg dL^{-1} in average) after 20 mg kg^{-1} STZ injection (Fig. 3a). Whereas in SD rats, 20 mg kg^{-1} of STZ did not significantly increase the blood glucose levels. All SD rats showed hyperglycemia (706.0 mg dL^{-1} in average) after 30 mg kg^{-1} STZ injection. After 20 mg kg^{-1} STZ injection, insulin levels in SDT rats decreased rapidly (0.2 ng mL^{-1} in average). On the other hand, STZ treated SD rats showed hypoinsulinemia (0.4 ng mL^{-1} in average) after 30 mg kg^{-1} STZ injection.

Pancreatic histopathology: Histopathological changes in the pancreas including atrophy of islets, multiple irregular projection of islets and islet fibrosis were observed in SDT rats at 15 weeks of age (Fig. 4a). Furthermore, β-cells in islets of the SDT rats were segmented (Fig. 4b).

The SDT rat which develops hypoinsulinemia and hyperglycemia with age is a spontaneous model of non-obese type 2 diabetes mellitus (Masuyama *et al.*, 2004; Shinohara *et al.*, 2000). The mechanism of onset of diabetes mellitus in SDT rats has been unclear.

Shinohara *et al.* (2000) however have reported that lesions, including bleeding and inflammatory cell infiltration were observed in the pancreatic islets of pre-diabetic SDT rats (Shinohara *et al.*, 2000). Moreover, a reduction in insulin secretion in islet of SDT rats is reported by Matsui *et al.* (2009).

In this study, researchers investigated *in vivo* pathophysiological changes in pre-diabetes stage of SDT rats. At 16 weeks of age (before the onset of diabetes), hyperglycemia, hypoinsulinemia and hyperlipidemia were not observed in SDT rats whereas the weight loss, the elevated serum glucose level and the decreased serum insulin level were observed at 24 weeks of age (Table 1). These changes coincided with those reported by Masuyama *et al.* (2004).
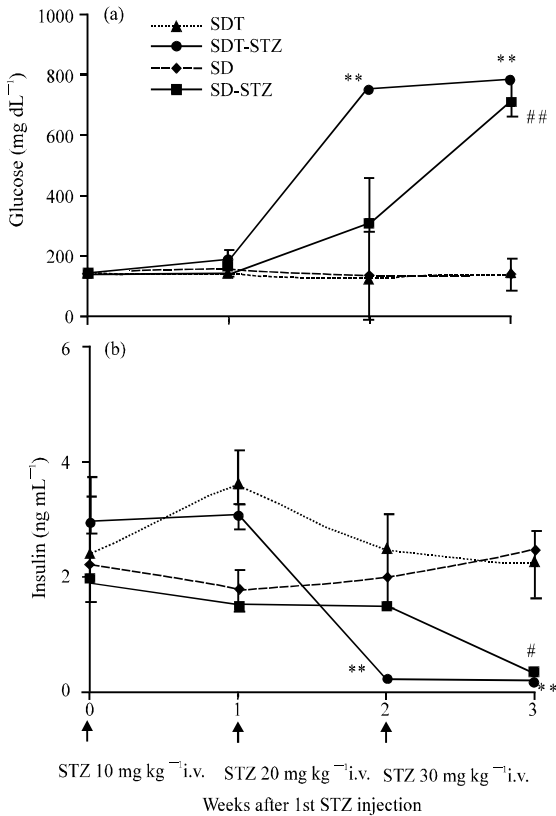
Fig. 3: Changes of serum; a) glucose and b) insulin levels in SD or SDT rats with injection of streptozotocin; data represent means±SD (n = 4 or 5); ** p<0.01; significantly different from the control SDT rats; #p<0.05, ##p<0.01; significantly different from the control SD rats
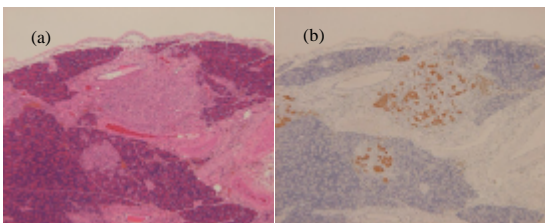

Fig. 4: Histopathological analysis of pancreas; SDT rats at 15 weeks of age; original magnification x 100; a) HE stain; b) immunostained with anti-insulin

In diabetic animal models, fasted plasma lipid levels are elevated (Dolphin *et al.*, 1987; Hennes *et al.*, 1988; Michaelis *et al.*, 1988). But fasted serum triglyceride and total cholesterol levels were decreased in SDT rats at 24 weeks of age. The reason is unknown. In OGTT, a marked decrease in GSIS was observed in SDT rats at 12 weeks of age (Fig. 1) with abnormalities in insulin

secretion noted prior to the onset of diabetes mellitus. Regarding the etiology of type 2 diabetes mellitus in humans and the question of whether impaired insulin secretion or insulin resistance is the initial symptom, most epidemiological studies conducted to date have reported that decreased insulin secretion precedes the onset of type 2 diabetes (Kosaka, 2002; Kosaka *et al.*, 1977). The present study also confirmed a decrease in GSIS prior to the onset of diabetes mellitus in SDT rats. Therefore, the SDT rat is a useful animal model for the analysis of the etiology of diabetes mellitus in humans. Type 2 diabetes mellitus is a heterogenous syndrome of polygenic origin which involves both defective insulin secretion and peripheral insulin resistance (Unger, 1995; Unger and Grundy, 1985). ITT was performed in order to evaluate the insulin sensitivity in SDT rats.

Interestingly before the onset of diabetes, SDT rats at 16 weeks of age showed an increase of insulin sensitivity (Fig. 2). Since the insulin-secretion deficiency exists in SDT rats, the enhancement of insulin sensitivity might be caused by any compensatory mechanism. It is reported that the amount of β-cell increased to compensate for insulin resistance (Kubota *et al.*, 2000; Terauchi *et al.*, 2007). In further study, it is necessary to examine the insulin resistance of SDT rats in pre-diabetic stage. In this study, STZ was used as diabetogenic agent to examine the pancreas weakness in SDT rats. STZ induced selective destruction of pancreatic β-cells and it is used to induce both type 1 and 2 diabetes (Szkudelski, 2001). The dosage of 10-30 mg kg^{-1} STZ was performed one by one. Both SD and SDT rats did not develop diabetes at 10 mg kg^{-1} STZ. All SDT rats showed hyperglycemia after 20 mg kg^{-1} STZ injection (Fig. 3a). Whereas in SD rats, 20 mg kg^{-1} of STZ failed to elevate blood glucose level.

All SD rats showed hyperglycemia after 30 mg kg^{-1} STZ injection (Fig. 3a). After 20 mg kg^{-1} STZ injection, insulin levels in SDT rats decreased rapidly with the diabetes onset (Fig. 3b). Since SDT rats with STZ at a dose level of 20 mg kg^{-1} showed a significant hyperglycemia, the rats did not receive 30 mg kg^{-1} STZ. A lower dosage of STZ (20 mg kg^{-1}) made SDT rats diabetic. It is considered that a pancreatic weakness exists in pre-diabetic stage of SDT rats. Also, ZF rat which is a genetic obese model, shows a pancreatic weakness (Ohta *et al.*, 2010).

Histopathological sever changes such as atrophy of islets, multiple irregular projection of islets and islet fibrosis were observed in SDT rats at 15 weeks of age (Fig. 4a). Similar pancreatic changes are observed in ZF rats and GK rats (Ohta *et al.*, 2003, 2010). In pre-diabetic stage of SDT rats, not only pancreatic dysfunction but also morphological abnormalities in pancreas exist obviously.

CONCLUSION

In pre-diabetic SDT rats, a reduced GSIS, a pancreatic weakness, pancreatic histological abnormalities and an increased insulin sensitivity were observed. SDT rat is a useful model for the analysis of the progression of pre-diabetes and the onset of type 2 diabetes.

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