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Pancreatic Abnormalities at a Young Age in Spontaneously Diabetic Torii (SDT) Rats

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Abstract: The Spontaneously Diabetic Torii (SDT) rat is a non-obese diabetic model showing various metabolic abnormalities in pre-diabetic stage. In this study, researchers investigated the pancreatic function at young ages, 5 and 7 weeks. At 5 weeks, SDT rats showed glucose intolerance and reduction of Homeostasis Model Assessment (HOMA)-β. At 7 weeks, the insulin content in pancreas was also decreased. Insulinogenic index showed no change at 5 and 7 weeks of age. In conclusion, the pancreatic function in SDT rats was decreased from a young age after weaning and the rat is a useful Animal Model to examine the pathophysiological features of Impaired Glucose Tolerance (IGT).

Key words: HOMA, IGT, SDT rat, reduction, insulin content, pancreatic

INTRODUCTION

The presence of both Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG) doubles the risk of progression to diabetes. Insulin secretory defects are seen in IGT and IFG and IGT appears to lead greater Cardiovascular Disease (CVD) risk than does IFG (Bloomgarden, 2008). It is possible to reduce the development of diabetes by intervening in pre-diabetic stage, IGT. In a meta-analysis, lifestyle interventions reduced diabetes by one half and pharmacologic interventions by one third (Gillies *et al*., 2007).

It is essential to investigate metabolic abnormalities in pre-diabetic stage using an animal model of IGT. The Spontaneously Diabetic Torii (SDT) rat is a model of non-obese spontaneous diabetes which was developed by Shinohara *et al*. (2000) and Masuyama *et al*. (2004). SDT rats develop hyperglycemia, hyperlipidemia and hypoinsulinemia from about 16 weeks of age and also ocular complications (cataract and retinopathy) and nephropathy from about 40 weeks of age (Ohta *et al*., 2007; Sasase *et al*., 2006). Also, it is reportedthat SDT rats show various pathophysiological changes in pre-diabetic stage. SDT rats showed a glucose intolerance after 9 weeks of age and decreased the Glucose-Stimulated Insulin Secretion (GSIS) and the β-cell mass in pancreas (Masuyama *et al*., 2004; Matsui *et al*., 2009; Ohta *et al*., 2011). In this study, researchers investigated a pancreatic function at young ages, 5 or 7 weeks of age in 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 weight and blood chemical parameters such as glucose, insulin, Triglyceride (TG) and Total Cholesterol (TC) levels were examined at 5 and 7 weeks of age. Blood samples were collected from the tail vein of non-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 5 and 7 weeks of age. Glucose solution

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(2 g kg^{-1}) was administered to 4 h fasted rats. Blood samples were collected before and 30, 60 and 120 min after glucose loading. Serum glucose and insulin levels were measured as described earlier. To evaluate the insulin response to glucose during OGTT, the insulinogenic index (ainsulin/aglucose) was calculated using incremented serum insulin and glucose levels for 0-30 min after glucose loading. Fasting serum concentrations of both glucose and insulin were used to calculate indices of insulin resistance (Homeostasis Model Assessment (HOMA)-IR) and insulin secretion (HOMA-β) using the following equation:

$$\text{HOMA-IR} = \frac{\text{Fasting insulin } (\mu\text{U mL}^{-1}) \times \text{Fasting glucose (mg mL}^{-1})}{405}$$

$$\text{HOMA-}\beta = \frac{\text{Fasting insulin } (\mu\text{U mL}^{-1}) \times 360}{[\text{Fasting glucose (mg mL}^{-1}) - 63]}$$

Insulin content: Acid/ethanol extraction was performed in accordance with the method of Kenny (1955). Briefly, the pancreas was removed promptly and homogenized in a cold acid/ethanol mixture (75% ethanol, 23.5% distilled water, 1.5% 2N hydrochloric acid) to extract insulin. The level of insulin in the extract was measured with an ELISA kit.

Statistical analysis: Results were expressed as the mean±standard deviation. 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 and serum glucose level in SDT ratswas slightly decreased at 7 weeks of age as compared with those in SD rats. Serum lipid and insulin levels in SDT rats showed no significant change as compared with those in SD rats.

Serum glucose levels at 30 and 60 min after glucose loading in SDT rats at 5 and 7 weeks of age significantly increased as compared with those in SD rats (Fig. 1a and c). Serum insulin levels at 30 and 60 min after glucose loading and the insulinogenic index in SDT rats showed no significant change as compared with those in SD rats (Fig. 1b and d, Table 2). Masuyama *et al*. (2004) showed glucose intolerance at 8 weeks of age in SDT rats and in the present study, the glucose intolerance was shown at the younger age, 5 weeks of age. GSIS at 12 or 16 weeks of age was reduced in pre-diabetic stage of SDT rats (Matsui *et al*., 2009; Ohta *et al*., 2011) but the GSIS did not decrease in SDT rats at 5 and 7 weeks of age (Fig. 1). Fasted insulin levels (before glucose loading) in SDT rats were reduced as compared with those in SD rats (Fig. 1b and d).

At 5 and 7 weeks of age, HOMA-β in SDT rats was decreased as compared with that in SD rats (Table 1). Moreover, HOMA-IR in SDT rats was reduced at 7 weeks of age as compared with that in SD rats. HOMA-IR was used as an index of insulin resistance and HOMA-β as an index of insulin secretory function derived from fasting blood glucose and insulin concentrations (Matthews *et al*., 1985; Wallace *et al*., 2004). The predictability of these markers for future development of type 2 diabetes was suggested in previous studies (UK Prospective Diabetes Study Group, 1995; Haffner *et al*., 1997; Matsumoto *et al*., 1997). Increased

Table 1: Body weights and serum parameters in non-fasted SD rats and SDT rats

	Age (weeks of age)	
Parameters	5	7
Body weight (g)		
SD rat	189.0±9.900	309.0±09.00
SDT rat	192.0±7.400	287.3±15.90*
Glucose (mg dL^{-1})		
SD rat	156.8±7.100	149.7±7.400
SDT rat	138.8±16.50	136.2±9.500*
Insulin (ng mL^{-1})		
SD rat	1.01±0.25	2.68±0.69
SDT rat	1.49±0.81	1.99±1.17
Triglyceride (mg dL^{-1})		
SD rat	98.4±36.20	145.5±39.30
SDT rat	82.5±31.80	172.7±32.20
Total cholesterol (mg dL^{-1})		
SD rat	90.8±4.70	78.3±9.700
SDT rat	86.3±6.20	80.8±2.600

Data represents means±standard deviation (n = 4 or 6). *p<0.05; significantly different from age-matched SD rats

Table 2: Insulinogenic index and HOMA indices in SD rats and SDT rats

	Age (weeks of age)	
Indices	5	7
Insulinogrnic index		
SD rat	0.029±0.011	0.033±0.008
SDT rat	0.036±0.013	0.036±0.008
HOMA-IR		
SD rat	13.6±4.3	22.3±6.5
SDT rat	7.8±1.1	11.1±3.8*
HOMA-β		
SD rat	309.1±49.9	531.1±97.6
SDT rat	155.2±22.7**	314.9±103.5**

Data represents means±standard deviation (n = 5). *p<0.0, **p<0.01; significantly different from age-matched SD rats

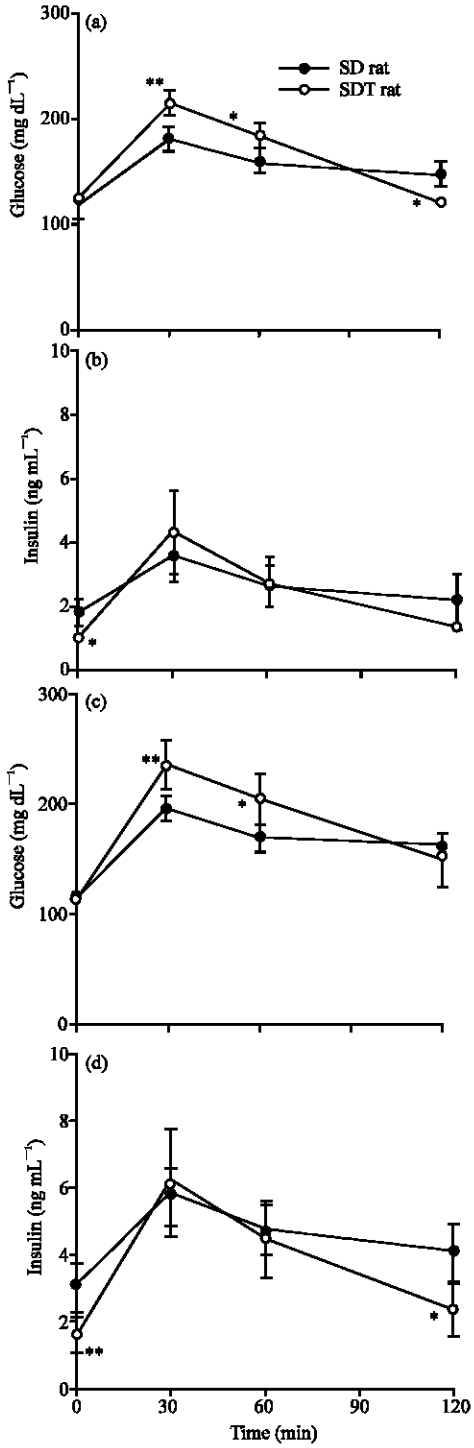

Fig. 1: a, b) Changes of serum glucose and insulin levels after glucose loading in SD and SDT rats at 5 weeks of age; c, d) 7 weeks of age. Blood samples were taken via the tail vein before and 30, 60 and 120 min after glucose loading. Data represent means±standard deviation (n = 5). *p<0.05, **p<0.01; significantly different from the SD rat

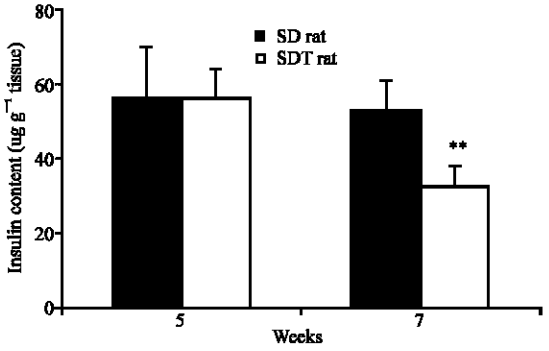

Fig. 2: Pancreatic insulin content in SD and SDT rats at 5 and 7 weeks of age. Data represent means±standard deviation (n = 4 or 6). **p<0.01; significantly different from the SD

change in HOMA-IR was associated with glycemic progression in human (Rhee *et al*., 2011; Li *et al*., 2007) and the HOMA-IR was improved by drug therapy (Li *et al*., 2007). HOMA-β was reduced in type 2 diabetic patients and the HOMA-β was improved in nateglinide or repaglinide treatment (Li *et al*., 2007) and in thiazolidinediones-treatment (Campbell and Mariz, 2007). Also, the improvement of HOMA-IR or HOMA-β was observed in diabetic model with anti-diabetic drug treatment (Pickavance and Wilding, 2007; Zhang *et al*., 2008). Since, HOMA-IR in SDT rats was not increased as compared with that in SD rats (Table 2), the SDT rats at 5 or 7 weeks of age are not considered to be insulin resistance. In SDT rats, the fasted insulin level and the HOMA-β was significantly reduced from 5 weeks of age. The reduced insulin secretion indicated by HOMA-β analysis suggests that glucose intolerance at a young age may be caused by endogenous insulin secretory dysfunction.

Insulin content: The insulin content of the pancreas of 5 weeks of age in SDT rats was similar to that of SD rats (SD rat: 55.9±14.3 μg g^{-1} tissue vs. SDT rat: 56.2±7.8 μg g^{-1} tissue) (Fig. 2). At 7 weeks of age, the insulin content of the pancreas of SDT rats decreased approximately 40% that of SD rats (SD rat: 52.7±8.1 μg g^{-1} tissue vs. SDT rat: 32.2±5.6 μg g^{-1} tissue) (Fig. 2). It is reported that a quantitative decrease of pancreas was observed in human with metabolic abnormalities. Obese humans with IFG and type 2 diabetes had a 40 and 63% deficit and lean cases of type 2 diabetes had a 41% deficit in relative β-cell volume compared with non-diabetic obese and lean cases, respectively (Butler *et al*., 2003). On the other hand, Meier *et al*. (2009) reported that pancreatic β-cell area was not different from Normal Glucose Tolerance (NGT) and IGT/IFG (Meier *et al*., 2009). There

is controversy whether β-cell mass is decreased in pre-diabetic stage of human. Zucker Fatty (ZF) rat is a disease model of obesity and metabolic syndrome resulting from hyperphagia owing to the loss of function of the leptin receptor but it does not exhibit hyperglycemia (Iida *et al*., 1996; Liu *et al*., 2002). In ZF rats, the insulin content per beta cell mass was decreased as compared with that in Zucker Lean (ZL) rats (Delghingaro-Augusto *et al*., 2009).

In SDT rats, the reduction of insulin content in pancreas was observed at 7 weeks of age but the GSIS has not changed until 7 weeks of age (Fig. 1 and 2). It is considered that a quantitative alternation occurs before the quality change in pancreas of SDT rats. The reduction of insulin content may be related with a decrease of endogenous insulin secretion.

CONCLUSION

Pancreatic dysfunction and glucose intolerance were observed from a young age in SDT rats. The SDT rat is a useful animal model to examine the pathophysiological features of IGT.

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