Chapter 13

Non-Obese Type 2 Diabetes Animals Models

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http://dx.doi.org/10.5772/52712

1. Introduction

Diabetes mellitus has become a global health problem, and the incidence of the disease is increasing rapidly in all regions of the world. Furthermore, the prevalence of diabetes is increasing worldwide at an alarming rate. For example, prevalence of diabetes across the world is forecast to increase from 171 million in 2000 to 366 million in 2030 [1].

Diabetes mellitus is classified into two categories, type 1 and type 2. Type 1 diabetes mellitus (T1D or IDDM; Insulin Dependent Diabetes Mellitus) is characterized by a loss of insulin secretion due to pancreatic β-cell degeneration, leading to autoimmune attack. Type 2 diabetes mellitus (T2D or NIDDM; Non Insulin Dependent Diabetes Mellitus) is metabolic disorder that is caused by insufficient insulin secretion and/or insulin resistance in peripheral and liver tissues. It is known that 90-95% of diabetes is diagnosed as T2D [2]. Development of T2D is usually caused by several factors, which are combined with lifestyle, genetic defects, virus infection, and drugs [3, 4]. Sustained hyperglycemia causes severe diabetic microvascular complications, such as retinopathy, peripheral neuropathy, and nephropathy. In the diabetic states, multiple mechanisms have been implicated in glucose-mediated vascular damage and contribute to diabetic microvascular complications. In addition, postprandial state is also an important factor in the development of macroangiopathy. In diabetes, the postprandial phase is characterized by an exaggerated rise in blood glucose levels. It has recently been shown that postprandial hyperglycemia is relevant to onset of cardiovascular complications. From this evidence, treatment of diabetes has become a part of the strategies for the prevention of diabetic vascular complications.

To help develop new diabetic treatments, it is important to reveal the complex mechanisms of diabetes. In particular, studies using diabetic animal models are essential to aid in clarification of the pathogenesis and progression in human disease course. Types of T2D animal models are classified into three groups, non-obese T2D, obese T2D, and new T2D models. In this chapter, we review these three types of T2D animal models with respect to characteristic features, including impaired glucose tolerance.

2. Non-obese type 2 diabetic animal models

Certain non-obese diabetic models are used in the investigation of T2D in humans. Spontaneous models are well known in this category, e.g., Goto-Kakizaki (GK) rats, Spontaneously Diabetic Torii (SDT) rats, Cohen diabetic rats, Wistar Bonn/Kobori (WBN/Kob) rats, Akita mice, Horino-Niki diabetic (HND) mice, Diabetes Mellitus Saitama (DMS) mice, and Chinese hamsters with spontaneous diabetes (CHAD). Chemically-induced diabetes models such as neonatally streptozotocin-induced (nSTZ) diabetic rats are also used.

2.1. Goto-Kakizaki (GK) rat

2.1.1. Background

Goto-Kakizaki (GK) rat is a non-obese animal with mild T2D [5, 6]. Glucose intolerance is developed early, at 2 weeks of age. Since the GK rat is generally considered as one of the best models of T2D, many researchers have used this animal model to study the physiology of diabetes and its complications, and to evaluate anti-diabetes drugs. In 1973, Goto and Kakizaki of Tohoku University (Japan) started selection of this substrain from Wistar rats by mating pairs with glucose intolerance. Since F8, sister-brother mating has been repeated, and were established as an SPF animal at F29. Today, many colonies of the GK rat exist and the rats are available for purchase from several breeders.

The major quantitative trait locus (QTL) for impaired glucose tolerance is *Niddm1*, identified in chromosome 1. Several loci linked to pathophysiologic characteristics was observed on chromosomes 2, 4, 5, 8, 10, and 17, indicating that the diabetic features in GK rats are inherited as polygenic traits and that GK rats would provide insights into genetics of human T2D [7].

2.1.2. Glucose tolerance and insulin sensitivity

Non-fasting blood insulin levels in GK rats are slightly higher than in age-matched Wistar rats. Impaired glucose-stimulated insulin secretion has been reported in GK rat *in vivo* [8], in the isolated pancreas [9], and in isolated pancreatic islets [10]. Perfusion experiments using isolated pancreas showed that the first phase of insulin secretion by glucose stimulation was impaired in GK rats, although the response to arginine was preserved [9].

"Starfish-shaped" islets are a morphological feature of GK rat. The number of enlarged islets with irregular shape, ill-defined borders, and fibrous strands of endocrine cells is increased in aged GK rats. These islets showed similar or moderately decreased insulin content compared with control rats. Pancreatic glucagon content is at almost the same level as in Wistar rats, and somatostatin content is slightly higher in GK rats [11]. The defective insulin response to glucose in β-cells is due to abnormalities in the function of K^{+}_{ATP} channels and L-type Ca^{2+} channels [12].

The GK rats show mild insulin resistance, mainly considered to be due to increased hepatic glucose production [8]. Decreased glycogen synthesis from glucose, and glucose uptake in skeletal muscle and adipose tissue are also observed in GK rat.

2.1.3. Drug treatment and diabetic complications

GK rats have been widely used for evaluating anti-diabetic drugs. Almost all types of such drugs have been tested with GK rats, including sulfonylureas [13], an α-glucosidase inhibitor [14], a thiazolidinedione derivative (troglitazone) [15], a biguanide (metformin) and a gluconeogenesis inhibitor [16], a GLP-1 analog and a dipeptidyl peptidase-4 inhibitor (DPPIV-i) [17], and an SGLT2 inhibitor [18].

In addition to its useful features as a T2D model, GK rat has been used as model of diabetic complications. Reduced motor nerve conduction velocity (MNCV) in the caudal nerve is reported in 2-month-old GK rats [19]. Increase of glomerular basal membrane was observed at 3 months [8]. Electroretinogram (ERG) showed functional abnormalities of photoreceptors in GK rats [20]. Aged GK rats at eight months showed higher endothelial/pericyte ratio than in normal rats, indicating retinopathy [21].

2.2. Neonatally streptozotocin-induced (nSTZ) diabetic rat

2.2.1. Background

Streptozotocin (STZ) is an antibiotic derived from *Streptomyces achromogenes* that has selective toxicity to pancreatic β-cells. STZ induces DNA strand breaks and a consequent excess activation of poly (ADP-ribose) synthetase, an enzyme that repairs DNA, depleting NAD in cells, which leads to energy depletion and finally causes β-cell death [22]. Therefore, STZ is widely used as an agent to induce IDDM (or T1D) experimentally: injection of a single high dose of STZ causes T1D in adult rats.

On the other hand, when the STZ is injected neonatally, rats develop T2D in adulthood. Neonatal rats treated with STZ at birth (nSTZ rat) revealed acute insulin deficient diabetes at 3-5 days after birth [23]. Their pancreatic insulin contents reduced to 7% that of normal rats, and showed hyperglycemia in this period. However, after this period, blood glucose and insulin levels in nSTZ rats were almost the same as in control rats at 3 weeks of age. At eight weeks of age, nSTZ rats showed mild hyperglycemia and impaired glucose tolerance with a 50% decrease in pancreatic insulin content [24].

Recently, Masiello et al. have reported a new method of inducing T2D in rat by administration of STZ combination with nicotinaminde (NA) [25]. Adult (3 months old) Wistar rats treated with NA intraperitoneally 15 min before STZ administration (STZ/NA rat) have shown moderate and stable hyperglycemia with 40% preservation of pancreatic insulin stores. When given a calorie-controlled high fat diet, hyperlipidemia and insulin resistance without obesity were observed [26]. These models of T2D similar to human T2D may provide a particularly advantageous tool for pharmacological investigations of new insulinotropic agents.

2.2.2. Glucose tolerance

The reduction of β-cell number and insulin content in the pancreas leads to defective insulin response *in vivo*. An isolated pancreas perfusion study using adult nSTZ rats showed lack of insulin response to glucose stimulation, indicating loss of β-cell function [27]. Both the first and the second phase of insulin response were severely impaired. Reduction of GLUT2 expression in β-cells may attribute to impaired glucose entry into β-cells and the following insulin secretion [28]. Reduced sensitivity of K_{ATP} channel to extracellular glucose has also been suggested by the patch-clamp technique [29]. Furthermore, an *in vivo* study has indicated that the hepatic glucose production (HGP) in the basal state is higher in adult nSTZ rats than in control animals [30]. From these observations, a lack of insulin response to glucose in pancreas and an increased insulin action upon HGP *in vivo* are major causes of mild basal hyperglycemia and impaired glucose tolerance in nSTZ rats.

2.2.3. Drug treatment

The features of nSTZ rats as a T2D model make this model valuable for evaluation of many hypoglycemic drugs, including a sulfonylurea [31], a thiazolidinedione (pioglitazone) [32], a biguanide (metformin) [33], a glucose sensor enhancer [34], a DPPIV-i [35], and an SGLT2 inhibitor [36]. nSTZ rats are also a useful model for assessment of therapeutic drugs that enhance β-cell regeneration. Tourrel et al. reported improved beneficial effects of GLP-1 and its analog exendin-4 on β-cell mass recovery and glucose homeostasis [37]. Ghrelin, the hunger-stimulating peptide produced in stomach, also promotes regeneration of β-cells in nSTZ rats. Treatment with ghrelin increased pancreatic expression of insulin and Pdx1 mRNA with a consequent improvement of hyperglycemia in nSTZ rats [38].

3. Obese type 2 diabetes animal models

Obesity is a well-established risk factor for many chronic disorders, such as T2D [39]. To understand the complicated features of the disease, spontaneously T2D models provide important knowledge. In particular, the development of diabetic animal models and pathophysiological analyses of the models are very important to aid in clarification of the pathogenesis and the patterns of progression in the human disease course. Genetic models of obesity and diabetes, such as *db*/*db* mice, *ob*/*ob* mice, Zucker diabetic fatty (ZDF) rats, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and Wistar fatty rats are most commonly used in such studies.

3.1. Zucker diabetic fatty (ZDF) rat

3.1.1. Background

Zucker diabetic fatty (ZDF) rat is an obese animal associated with hyperphagia, hyperglycemia, hyperinsulinemia, and hyperlipidemia. Insulin resistance is caused by age-dependent degeneration in pancreatic β-cells that trigger hyperglycemia. Thus, ZDF rat is a

widely studied model of obesity and insulin resistance and is used for evaluation of anti-diabetic drugs. ZDF rat was discovered in a colony of outbred Zucker fatty (ZF) rat in the laboratory of Dr. Walter Shaw at Eli Lilly Research laboratories during the 1980's. ZF rat, discovered in crosses between Sherman and Merck stock M rats (13M strain) in 1961 [40], was identified as carrying a mutation in *fa* gene, and exhibits hyperphagia/obesity. Dr. Richard Peterson at Indiana University Medical School (IUMS) started selection of this rederivation, and established an inbred line of ZDF rat in 1985. It is well known that sexual differences exist in the incidence and progression of diabetes mellitus in ZDF rat [41]. Diabetes mellitus has developed in more 90% of the males, whereas the blood glucose level remains normal in most females. However, female ZDF rat became diabetic on high-fat diet, and it was shown that the dietary fat content affected development of diabetes in females [41]. Today, ZDF rat is available for purchase from Charles River.

3.1.2. Glucose tolerance and insulin sensitivity

Serum glucose levels in ZDF rat are usually elevated from 7-10 weeks of age. The increase was sustained until about 14 weeks of age. ZDF rats showed hyperinsulinemia from 6 to 12 weeks, but after about 14 weeks of age their insulin levels showed a tendency to decrease. Impaired glucose tolerance has been reported in ZDF rat at 5-7 weeks of age. Glucose intolerance at 12 weeks becomes more severe than that at 5-7 weeks of age [42, 43]. Age-dependent degenerative changes of pancreatic islets showed decreased production and secretion of insulin, and atrophy of islets. Early pathological changes of the pancreatic islets, such as hypertrophy, disarray of islet architecture, and irregular islet boundaries, were observed by 10-12 weeks of age [44, 45]. The specific factor that causes deterioration of pancreatic β-cells has not been identified, but changes in β-cell structure and function have been well studied. It was reported that lipotoxicity based on high plasma free fatty acid could attribute to β-cell dysfunction [46]. Reduction of islet mRNAs in β-cells, such as those for insulin, GLUT2, and glucokinase, contributes to the β-cell deterioration [42]. Furthermore, decrease in GLUT4 expression is also observed in skeletal muscle and adipose tissue of ZDF rat [47].

3.1.3. Drug treatment and diabetic complications

It is well known that ZDF rat is a useful model for evaluating anti-diabetic compounds. Some studies have shown that DPPIV-i improve the diabetic condition in ZDF rat. Other compounds also have been evaluated in ZDF rat, including a sulfonylurea [48], α-glucosidase inhibitors [49], a thiazolidinedione (pioglitazone) [50], a biguanide (metformin) [51], a GLP-1 analog [52], an SGLT2 inhibitor [53], a β3-andrenergic receptor agonist [54], and a variety of other compounds [55-58].

A number of studies demonstrated that ZDF rat can be used as model of diabetic complications. Blood urea nitrogen (BUN) levels and urinary protein excretion in ZDF rat were elevated from about 40-50 weeks of age. Renal morphologic changes were observed at 40 weeks of age [59]. It is shown that ZDF rat exhibits renal hypertrophy. Reduced MNCV

in the sciatic nerve is observed from 12–14 weeks of age in ZDF rats, and endoneurial blood flow (EBF) in the sciatic nerve is also decreased after 24 weeks of age [60]. It is suggested that ZDF rat develops neural dysfunction. The degeneration and swelling of fibrae lentis, formation of Morgagnian globules, and stratification of epithelium lentis cells is observed in ZDF rat at 21 weeks of age [61, 62]. It is shown that diabetic cataract is observed in ZDF rat.

3.2. Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rat

3.2.1. Background

Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rat is a mildly obese animal associated with polydipsia, polyuria, polyphagia, hyperglycemia, and hyperlipidemia. The incidence of diabetes mellitus in this rat might be related to weakness of β-cells. OLETF rat is considered to be a suitable model for understanding the properties of T2D with mild obesity. The spontaneously obese rat with T2D was obtained from a colony of outbred Long-Evans rat, available for purchase from Charles River, in 1984 at laboratory of Otsuka pharmaceuticals, Tokushima [63]. A strain of this rat was established by sister-brother mating with obesity and glucose intolerance. According to the results of a study by Takiguchi [64, 65], a disrupted cholecystokinin-A (CCK-A) receptor gene in peripheral tissues and central nervous system is found in the OLETF rats [64]. The function of CCK-A in central nervous system is to regulate food intake directly [66]. Meanwhile, in peripheral tissues, CCK-A also controls satiety signals through the vagal afferent neurons [67]. Thus, dysfunctional signal of CCK may cause obese T2D, leading to hyperphagia in OLETF rats. Today, OLETF rats are available for purchase from Japan SLC, Inc., but this rat is limited in use to non-profit purposes.

3.2.2. Glucose tolerance and insulin sensitivity

Non-fasting plasma glucose levels in OLETF rats were elevated from 18 weeks of age, and the increase was sustained until 40 weeks of age. Diabetes mellitus developed in about 90% of OLETF rats at 30 weeks of age, whereas the plasma glucose level remained normal in most females at 24 weeks of age [63, 68]. Sexual differences exit in the incidence and progression of diabetes mellitus in OLETF rats [69]. In glucose tolerance test, marked elevation of plasma glucose and insulin level responses to glucose are observed at 24 weeks of age [63]. Impaired glucose tolerance becomes more severe after 24 weeks of age. Age-dependent degenerative changes of pancreatic islets are observed from 16 weeks of age [70]. The pathological changes of the pancreatic islets, such as hypertrophy, atrophy of insulin positive-β-cells, fibrosis, and indistinct, irregular islet boundaries, were observed by 30 weeks of age [71]. These dysfunctions of β-cells seem to cause the development of glucose intolerance in OLETF rats. Insulin resistance has been reported in OLETF rats at 16 weeks of age, as measured by hyperinsulinemic euglycemic clamp technique [70]. In adipocytes, the GLUT4 protein expression considerably decreased in OLETF rats at 30 weeks of age. The decrease in GLUT4 protein in muscles is also observed in OLETF rats at 30 weeks of age [72]. These abnormalities of GLUT4 protein expression lead to insulin resistance in the peripheral tissues of OLETF rats.

3.2.3. Drug treatment and diabetic complications

OLETF rats have been widely used for pharmacological evaluation while testing for many anti-diabetic drugs, including a Ca^{2+} antagonist [73], sulfonylureas [74], an α-glucosidase inhibitor [75], a thiazolidinedione [76], a biguanide (metformin) and a gluconeogenesis inhibitor [77], and a GLP-1 analog [78].

OLETF rats are also used as a model for assessment of diabetic complications. It was reported that histopathological changes in the kidney were observed after 23 weeks of age. OLETF rats at 55 weeks of age showed an expansion of the mesangial matrix and aneurismal dilatation of intraglomerular vessels [63]. Development of diabetic nephropathy was also observed in OLETF rats. It is known that lenticular sorbitol level increases in OLETF rats from 40 weeks of age [79]. OLETF rats show swelling and liquefaction of lens fibers in the subcapsular and supranuclear region at 60 weeks of age. It is suggested that diabetic cataract is observed in OLETF rats.

3.3. Wistar fatty rat

3.3.1. Background

Wistar fatty rat develops obesity with hyperphagia, hyperglycemia, hyperinsulinemia, hyperlipidemia, and glucose intolerance. Wistar fatty rat is a good model for studying obesity and insulin resistance, and for evaluation of anti-diabetic drugs. Wistar fatty rat was established as a congenic line of the insulin resistance of the Wistar Kyoto strain (WKY) rat by introducing the *fa* allele of the ZF rat for obesity into the WKY rat genome in the laboratory of Dr. Hitoshi Ikeda at Takeda Chemical Industries [80]. At 5th generation of backcrossing, male obese animals exhibit hyperglycemia, and were established as Wistar fatty rat at 10th generation. Sex differences in diabetes have been reported in Wistar fatty rat. The female Wistar fatty rat sustained the euglycemia until 22 weeks of age [81]. The molecular basis for this sex difference has not been identified.

3.3.2. Glucose tolerance and insulin sensitivity

Nonfasting plasma glucose levels in Wistar fatty rats were elevated until 8 weeks of age, and this level was sustained until 24 weeks of age. Wistar fatty rats also exhibited hyperinsulinemia and hypertriglyceridemia. Wistar fatty rat is a widely studied model used to investigate the pathogenesis of obesity and insulin resistance, and for evaluation of anti-diabetic drugs. In glucose tolerance test conducted at 12 weeks of age, Wistar fatty rat showed higher serum glucose and insulin levels after glucose loading compared with WKY rat, and glucose intolerance became more severe age-dependently. Pronounced glucose intolerance was observed in Wistar fatty rat. Hypertrophied pancreatic islets in Wistar fatty rat were increased in pancreas compared with WKY rat [80]. Insulin resistance has been reported in Wistar fatty rats, confirmed by glucose clamp technique [82]. Decreased insulin-stimulated glycogen synthesis and glycolysis in the isolated soleus muscles, and insulin-stimulated glucose oxidation and lipogenesis in adipocytes were observed in Wistar fatty

rats [83]. It is considered that these abnormalities in the peripheral tissues lead to insulin resistance in Wistar fatty rats.

3.3.3. Drug treatment and diabetic complications

Wistar fatty rats have been used as a good model for evaluation of a number of anti-diabetic drugs, including a biguanide [84], an α-glucosidase inhibitor [75], a thiazolidinedione [85], and an DPPIV-i [86].

Wistar fatty rats are also used as a model of diabetic complications. It was reported that age-related increases in urinary NAG (N-acetyl-beta-D-glucosaminidase) and urinary protein and albumin excretion in Wistar fatty rat were elevated from 5-11 weeks of age. Wistar fatty rats at 26 weeks of age showed an expansion of the glomerular mesangial matrix and local formation of a nodular-like lesion. The development of diabetic nephropathy was observed in Wistar fatty rats [87]. Reduced MNCV in the fibula nerve and histopathological changes, such as demyelination and axonal degeneration, were observed in Wistar fatty rats [88]. It is suggested that Wistar fatty rat develops neural dysfunction.

4. New type 2 diabetes animal models

4.1. Spontaneously Diabetic Torii (SDT) rat

4.1.1. Background

The Spontaneously Diabetic Torii (SDT) rat is a new inbred strain of Sprague-Dawley (SD) rat established as a non-obese model of type 2 diabetes mellitus. Glucosuria appeared at approximately 20 weeks in male SDT rats. The cumulative incidence of diabetes was 100% by 32 weeks in male SDT rats, while it was only 33% in females even at 65 weeks. A clear sex difference is observed in the onset of diabetes in SDT rats. Male SDT rats showed high plasma glucose levels (over 700 mg/dL) by 20 weeks [89]. As a result of chronic severe hyperglycemia, the SDT rats developed severe complications in eyes, peripheral nerves, and kidneys. Especially, ocular complications including the diabetic retinopathy in SDT rats is noteworthy [90]. Of many diabetic ocular complications, cataract, retinopathy, and neovascular glaucoma (hemorrhagic glaucoma) are the most important clinically. SDT rats are the first diabetic model with all of these complications [89, 90].

4.1.2. Glucose tolerance and insulin sensitivity

In SDT rats, development of hyperglycemia may be more dependent on decreased insulin secretion than insulin resistance, as shown by the fact that the blood insulin concentration tended to be lower than in normal SD rats even before the onset of diabetes, and marked hypoinsulinemia developed after the onset of hyperglycemia [91-93], indicating that this strain of rat is a model of non-obese T2D associated with impaired insulin secretion. It is clinically known that glucose tolerance decreases before the onset of T2D. In oral glucose tolerance test in SDT rats, glucose tolerance markedly decreased at least 3 months before

manifestation of hyperglycemia (around 16 weeks old), and the rate of rise in blood glucose level after glucose-loading increased with age. We examined the glucose tolerance periodically at 5, 9, and 13 weeks of age in SDT rats (Figure 1.).

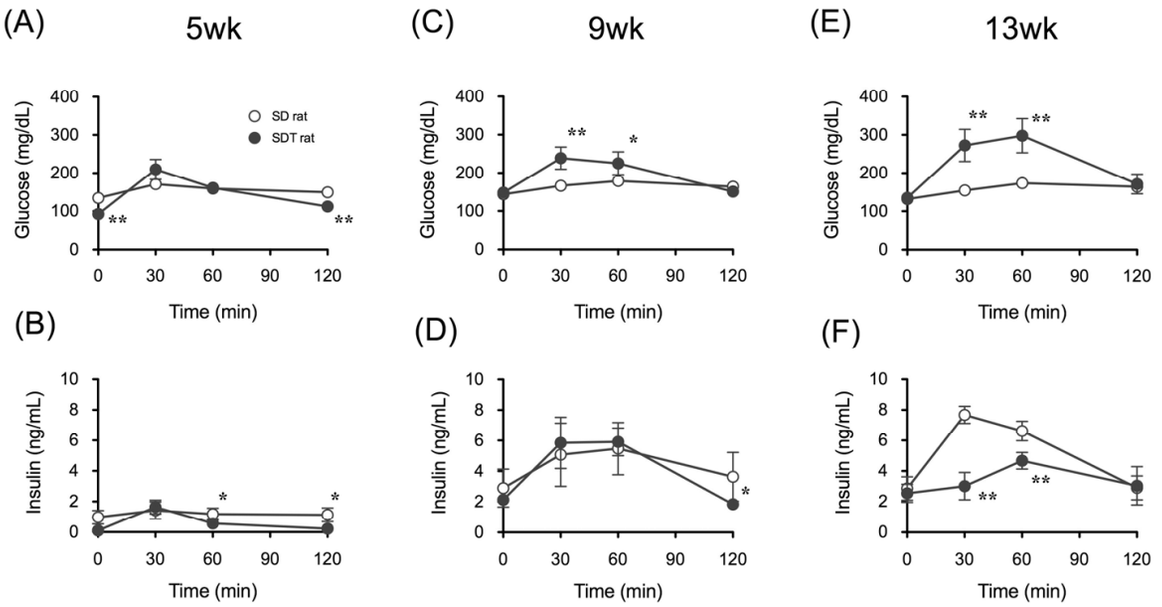

Figure 1. Glucose tolerance test (GTT) at 5, 9, and 13 weeks of age in SD rats and SDT rats. Glucose solution (2g glucose/kg) were administered to SD rats and SDT rats. The glucose and the insulin levels were examined at immediately before glucose-loading, 30, 60, and 120 min after glucose-loading. (A), (B) 5 weeks of age, (C), (D) 9 weeks of age, (E), (F) 13 weeks of age. The data are shown as the mean ± standard deviation (n=4-6). * P<0.05, ** P<0.01 significantly different from SD rats.

At 5 weeks of age, blood glucose level at 30 min after glucose-loading in SDT rats tended to increase as compared with that in SD rats (Figure 1A.). The blood glucose level before glucose-loading and the level at 120 min after glucose-loading in SDT rats significantly decreased as compared with those in SD rats. The blood insulin level at 30 min after glucose-loading was not different from that in SD rats, but the insulin levels at the other points significantly decreased as compared with those in SD rats (Figure 1B.). Also, the insulinogenic index at 5 weeks of age in SDT rats was comparable with that in SD rats (Figure 2.).

At 9 weeks of age, the blood glucose levels after glucose-loading in SDT rats were more elevated as compared with those in the rats at 5 weeks of age, suggesting that the glucose tolerance was deteriorated with age (Figure 1C.). The insulin levels at points except for 120 min after glucose-loading in SDT rats was comparable with those in SD rats (Figure 1D.), but the insulinogenic index showed a lower level than SD rats (Figure. 2). At 13 weeks of age, the impaired glucose tolerance (IGT) in SDT rats was accelerated and a peak value in the blood glucose level reached to about 300 mg/dl (Figure 1E.). The insulin level at 30 min after glucose-loading did not increase in SDT rats (Figure 1F.), suggesting that the glucose stimulated insulin secretion (GSIS) was almost deleted in the rats at 13 weeks of age (Figure 2.). In male rats, the severity of impaired glucose tolerance before the onset of diabetes was closely correlated with the age. Impaired glucose tolerance was related to decreased insulin

secretory response after glucose-loading, and decrease in the fasting plasma insulin concentration (lower than 1 ng/ml) and loss of insulin secretory response after glucose-loading were also observed after the onset of diabetes (Figure 3.).

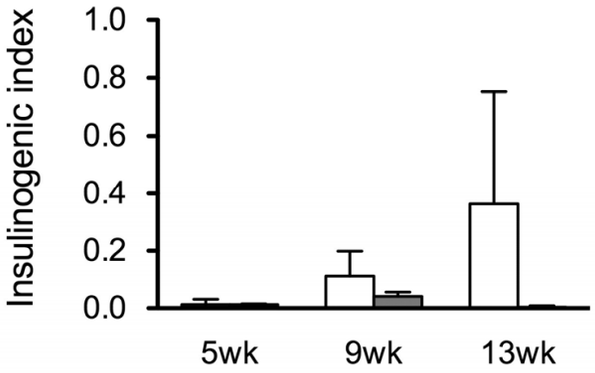

Figure 2. Insulinogenic index at 5, 9, and 13 weeks of age in SD rats and SDT rats. Insulinogenic index (ΔInsulin/ΔGlucose) was calculated using incremental blood insulin and glucose levels for 0 to 30 min after glucose-loading. The data are shown as the mean ± standard deviation (n=4-6).

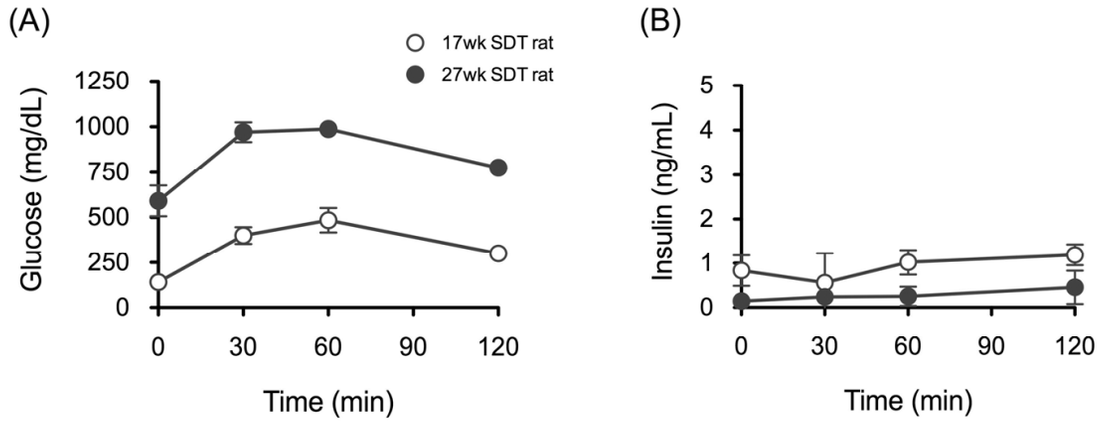

Figure 3. Glucose tolerance test (GTT) at 17 and 27 weeks of age in SDT rats. Glucose solution (2g glucose/kg) were administered to SDT rats. The glucose (A) and the insulin (B) levels were examined at immediately before glucose-loading, 30, 60, and 120 min after glucose-loading. The data are shown as the mean ± standard deviation (n=5).

In addition, the insulin secretion level in pancreatic islets of Langerhans from SDT rats after glucose treatment markedly decreased at 12 weeks of age and thereafter compared with normal SD rats. Likewise, the mRNA expression levels for GLUT2 and glucokinase (GK) in the isolated pancreatic islets of Langerhans markedly decreased at 12 weeks and thereafter in SDT rats [94]. In female rats, glucose tolerance also decreased, at 25 weeks and thereafter, but insulin was secreted after glucose-loading, indicating that some factors cause insulin resistance or insulin requirement in the females, unlike in the males [95].

It is reported that the pancreatic insulin content in SDT rats at 7 weeks of age decreased as compared with that in SD rats [96]. In human, β cell mass in impaired fasting glucose (IFG) subjects significantly decreased as compared with that in nondiabetic subjects [97]. In further study, the change of β-cell mass in pre-diabetic SDT rats should be elucidated.

Other non-obese type 2 diabetic models, such as GK rats and the nSTZ rats, did not show a pre-diabetic state. Since the SDT rat shows a pre-diabetic state for a long term, the rat is valuable as IGT model as well as a type 2 diabetic model.

4.1.3. Drug treatment

In previous study, α-glucosidase inhibitor voglibose was administered to male SDT rats in a pre-diabetic stage, and the effects of voglibose on the glucose intolerance and the development of diabetes were investigated [98]. In SDT rats at 10 weeks of age, a single dose of voglibose (0.03, 0.1, 0.3 mg/kg) improved the glucose tolerance dose-dependently. Moreover, voglibose was administered as a dietary mixture to SDT rats from 10 to 20 weeks of age. As a result, voglibose suppressed the incidence of diabetes in SDT rats. In clinical study, α-glucosidase inhibitor, such as voglibose and acarbose, showed a prevention of type 2 diabetes mellitus [99, 100]. The pharmacological intervention delayed progression of IGT to diabetes. The results showed that pharmacological intervention with voglibose in SDT rats with IGT can delay progression to T2D. SDT rat is considered to be useful for development of a preventive drug on T2D. SDT rat is available for purchace from CLEA Japan.

4.2. Spontaneously Diabetic Torii *Leprfa* (SDT fatty) rat

4.2.1. Background

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 [101]. 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 [102]. Obesity plays key roles in the pathophysiology of several metabolic diseases and is a risk factor for diabetes mellitus and dyslipidemia. Based on the above concept, a novel model of obesity-related diabetes was established by Masuyama *et al.* [103]. They established a congenic line of the Spontaneously Diabetic Torii (SDT) rat by introducing the *fa* allele of the ZF 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 inter-crossing between *fa*-heterozygous littermates.

4.2.2. Glucose tolerance, insulin sensitivity and drug treatment

Metabolic disorder in SDT fatty rats was obviously promoted as compared with SDT rats [104, 105]. 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. With early incidence of diabetes mellitus, diabetes-associated complications in SDT fatty rats were seen at younger ages than those in the SDT rats. SDT fatty rats did not almost show a pre-diabetic state, since the rats showed a hyperglycemia

from a young age. However, the glucose intolerance in SDT fatty rats is considered to exist with the progression of diabetes mellitus.

We evaluated the pharmacological effects of an anti-diabetic drug, DPPIV-i 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 DPPIV-i. When DPPIV-i was administered at doses of 1 and 10 mg/kg, the impaired glucose tolerance was improved dose dependently and insulin secretion was enhanced (Figure 4.).

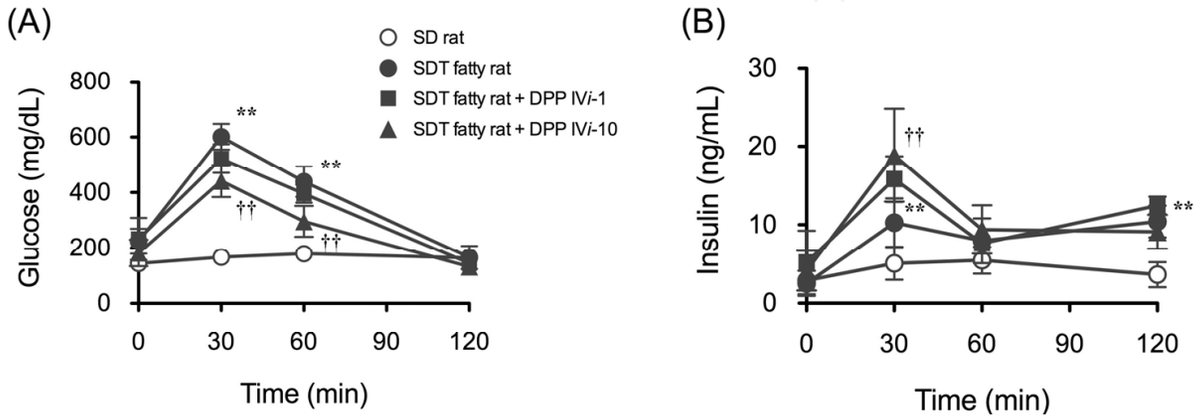

Figure 4. Effect of DPPIV-i on blood glucose (A) and insulin (B) levels in glucose-loaded SDT fatty rats. DPPIV-i was administered orally 30 min before glucose-loading (1g glucose/kg). The data are shown as the mean ± standard deviation (n=5). * P<0.05, ** P<0.01 significantly different from the control. #P<0.05, ## P<0.01 significantly different from SD rat.

Furthermore, we investigated the chronic effect of DPPIV-i in male SDT fatty rats. DPPIV-i (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 DPPIV-i, and the hemoglobin A1c (HbA1c) levels at 4 weeks after the administration tended to decrease (HbA1c level, control: 7.31 ± 0.22%, DPPIV-i 1 mg/kg: 6.99 ± 0.24%, DPPIV-i 10 mg/kg: 7.03 ± 0.17%). There was no change in body weights during the experimental period. DPPIV-i is expected to control postprandial hyperglycemia in patients with type 2 diabetes mellitus without increasing body weight. SDT fatty rats at 9 weeks of age showed a prominent hyperglycemia after glucose-loading (Figure 4A.). The glucose levels at 30 and 60 min after glucose-loading in the SDT fatty rats significantly increased as compared with those in SD rats. Moreover, the insulin levels at 30 and 120 min after glucose-loading in the SDT fatty rats increased as compared with those in SD rats (Figure 4B.). The GSIS in SDT fatty rats was accelerated as compared with SD rats, suggesting that hyperinsulinemia (insulin resistance) exists in the SDT fatty rats at 9 weeks of age. In the other hand, the insulinogenic index in SDT fatty rats was lower than that in SD rats (SDT fatty rat, 0.021 ± 0.010, and SD rat, 0.112 ± 0.087, respectively). Glucose intolerance in SDT fatty rats is considered to be related with both the insulin resistance and the impaired insulin secretion. SDT fatty rat is available for purchace from CLEA Japan.

Table 1. Characteristic of type 2 diabetes models.

	Non-obesity			Obesity			
	GK	nSTZ	SDT	ZDF	OLETF	Wistar fatty	SDT fatty
Sexual dimorphism	No	No	Yes (Male>Female)	Yes	Yes (Male)	Yes	No
Obesity	No	No	No	Yes	Yes	Yes	Yes
Hyperphagia	No	No	No	Yes	Yes	Yes	Yes
Pancreatic islet	Degeneration	Degeneration	Degeneration	Hypertrophy → Degeneration	Hypertrophy	Hypertrophy	Hypertrophy → Degeneration
β cell number	Decrease	Decrease	Decrease	Increase → Decrease	Increase → Decrease	Increase	Increase → Decrease?
Impaired insulin secretion	Yes	Yes	Yes	Yes (Hypersecretion)	Yes	Yes? (Hypersecretion)	Yes (Hypersecretion)
Onset of diabetes	8wk	8wk	20wk	7wk	18wk	8wk	5wk
Insulin resistance	Yes	–	?	Yes	Yes	Yes	Yes
Diabetic complications	Nephropathy (Mild)		Nephropathy	Nephropathy	Nephropathy	Nephropathy	Nephropathy
	Retinopathy		Retinopathy				Retinopathy /Uveitis
	Neuropathy			Neuropathy		Neuropathy	Neuropathy
			Cataract	Cataract	Cataract		Cataract
							Osteoporosis

5. Conclusion

There are many type 2 diabetic animal models, and they can be classified into the following three groups: non-obese T2D, obese T2D, and new type 2 diabetic models.

Each of these models has different features as described above (Table 1.), and each model acts as an important tool for revealing the complex mechanisms of diabetes and developing new anti-diabetic drugs. Studies using diabetic animal models are especially essential to aid in clarification of the pathogenetic development in human T2D. On the other hand, these animal models do not exhibit the human characteristics perfectly. New T2D animal models that more closely resemble the human conditions are required for further investigation of the disease mechanisms in the future.

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