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Insulin resistance and low sympathetic nerve activity in the Tsumura Suzuki obese diabetic mouse: a new model of spontaneous type 2 diabetes mellitus and obesity

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Abstract

Tsumura Suzuki obese diabetic (TSOD) mouse is a new model of type 2 diabetes mellitus and obesity. The TSOD mice had hypoadiponectinemia. To assess the glucose utilization and insulin sensitivity, we examined the effect of insulin (1U/kg) on peripheral tissue glucose uptake in vivo in the TSOD and Tsumura Suzuki nonobese mouse using 2-deoxy-D-[^{3}H]glucose. The rates constant of glucose uptake in basal condition without insulin were similar in the peripheral tissues in both strains. Insulin-stimulated glucose uptake by skeletal muscles and adipose tissues in vivo was diminished in the TSOD mice. In addition, we assessed norepinephrine turnover in brown adipose tissue and adrenal epinephrine (E) content and E turnover because disturbances in the sympathetic activities relate to many features in obese and diabetic syndrome. In these mice, the rate of norepinephrine turnover was decreased, and adrenal E content was at most one half of the Tsumura Suzuki nonobese mice and E turnover had extremely low rates. The TSOD mice showed hypercorticosteronemia. These results suggest that TSOD mice have insulin resistance and both low sympathetic nervous activities and low adrenomedullary activity, and have high adrenocortical activity, which are significant features of the TSOD mouse.

1. Introduction

Tsumura Suzuki obese diabetic (TSOD) mouse strain, a new polygenic model of spontaneous type 2 diabetes mellitus, develops moderate degrees of obesity and diabetes [1], which are apparent in animals older than 12 weeks. Phenotypic characterization shows hyperphagia, polydipsia, polyuria, hyperglycemia, and hyperinsulinemia [2]. Obesity and diabetes progress for about 6 months further. Although obesity is evident in both sexes of the TSOD mice, diabetes is overt only in males [2]. Through a whole genome scan of quantitative trait loci affecting body weight, and blood glucose and insulin concentrations, 3 major loci meeting the criteria for linkage have been identified [1]. Each locus has distinct effects on different diabetic trait and indicates that diabetes and obesity are controlled by distinct combination of genetic loci in the TSOD mouse [1].

Several characteristics in this mouse resemble human obese type 2 diabetes mellitus. In this study, to assess the glucose utilization and insulin sensitivity in TSOD mouse, we examined the effect of insulin on the rate of glucose uptake in skeletal muscles, adipose tissue, and brown adipose tissue (BAT) in vivo using 2-deoxy-D-[^{3}H]glucose (2-[^{3}H]DG) [3,4]. It is well known that impaired glucose metabolism in peripheral tissues such as skeletal muscle plays a critical role in the development of insulin resistance. Insulin resistance and decreased insulin stimulated glucose use are hallmarks of type 2 diabetes mellitus. Tissue glucose uptake is a major contributory factor to systemic glucose homeostasis. The results in TSOD mice are compared with those in a control Tsumura Suzuki nonobese (TSNO) mice to clear features of the TSOD mouse as a type 2 diabetic obese animal model.

Many features in the syndrome of some obese and diabetic model animals may be attributable to disturbances in the sympathetic nerve activities and adrenomedullary system. It has been proposed that disturbances of the

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sympathetic nerves impair catabolic processes of metabolism [5-7] and energy expenditure [8], and result in obesity [9]. The sympathetic nervous system also affects the rate of glucose uptake in peripheral tissues. Activation of sympathetic nerves enhanced the rate constant of glucose uptake in certain sympathetically innervated tissues [4,10]. In rodent, the sympathetic regulation of BAT function is well known, and BAT plays an important role in regulation of energy balance. Much of that increase in energy expenditure has been attributed to accelerated thermogenesis by BAT [11]. Obese (*ob/ob*) mice and rats (*fa/fa*) have high efficiencies of energy retention and this has been linked to low sympathetic activity in their BAT [12,13]. In the TSOD mouse, it is conceivable that sympathetic tone and the sympathetic activity in BAT are decreased. In the present study, we investigate norepinephrine (NE) turnover in BAT to clarify whether low sympathetic activity is found in the TSOD mouse. In addition, we measured adrenal epinephrine (E) content and E turnover rate as an index of adrenomedullary activity. We also measured plasma concentration of the adrenocortical hormone, corticosterone.

2. Methods

2.1. Animals

Male TSOD and TSNO mice (Imamichi Institute for Animal Reproduction, Ibaraki, Japan) aged 16 to 18 weeks were used. They were maintained individually in a temperature-controlled (23°C ± 1°C) room with a 12-hour light-dark cycle (light on 7:00 AM–7:00 PM) and given free access to laboratory chow and water. Urine glucose was confirmed with a test stick (Wako, Osaka, Japan). All the TSOD mice showed an apparent glucosuria.

All experimental procedures in these studies were approved by the laboratory animal committee of Tsumura & Co (Ibaraki, Japan) and met the guidelines of the Japanese Association for Laboratory Animal Science.

2.2. 2-Deoxy-D-glucose uptake

One week before the experiment, a heart catheter (a silicon tube with an outer diameter of 1.0 mm and inner diameter of 0.5 mm) was inserted through the right jugular vein to facilitate the administration of 2-[^{3}H]DG and [^{14}C]sucrose, and blood sampling [14]. The residual segment of the silicone tubing was passed under the skin and pulled out from the back of the neck. The body weight recovered within a week. The uptake of 2-deoxy-D-glucose (2-DG) in skeletal muscles and 2 types of adipose tissue, white adipose tissue and BAT, was examined according to the procedure described previously [4] with slight modifications as follows. Mice were anesthetized with pentobarbital sodium (60 mg/kg IP). After a 40-minute period of sedation, each mouse was injected with 3.7×10^5 Bq of 2-[^{3}H]DG and 7.4×10^4 Bq of [^{14}C]sucrose (ICN Radiochemicals, Irvine, CA) dissolved in 50 μL saline solution through the cardiac catheter. The catheter was immediately flushed with 50 μL saline. Insulin (1 U/kg) was injected into the right atrium through the cardiac catheter 10 minutes before the injection of the 2-[^{3}H]DG. Blood samples (50 μL) were taken −10, 0, 10, and 20 minutes before and after injection of the tracers, and replaced with an equivalent volume of saline. After the last blood sample was obtained, pentobarbital sodium (100 mg/kg) was injected through the cardiac catheter. The following tissues and organs were carefully dissected and freed of all extraneous materials as soon as possible: heart (left ventricle), retroperitoneal and epididymal white adipose tissue, interscapular BAT, and skeletal muscles (gastrocnemius, soleus, and extensor digitorum longus [EDL]). All tissues were rapidly frozen on dry ice, weighted, and solubilized in 0.5 mL of 0.5 N NaOH at 80°C for 1 hour. The solution was added with 1 mL of 3 % perchloric acid and centrifuged. The radioactivities of ^{3}H and ^{14}C in 1 mL of the supernatant were measured in a liquid scintillation fluid (Ultima Gold; Packard Bioscience, Wellesley, MA) using Tri-Carb scintillation analyzer (Packard; model 2000CA). Plasma samples were also analyzed for radioactivities of 2-[^{3}H]DG and [^{14}C]sucrose. The rate constant (K_i) of net tissue uptake of 2-[^{3}H]DG was calculated using the following equation [3]:

$$K_i = CK_p/C_{p0}\left(1 - e^{-K_p t}\right)$$

where C is the intracellular concentration of 2-[^{3}H]DG (disintegration per minute/mg tissue) at death, K_p is the rate constant of plasma disappearance of 2-[^{3}H]DG. C_{p0} is the extrapolated plasma 2-[^{3}H]DG concentration at time 0, and t is the duration of the test, that is, 20 minutes. C was calculated from the radioactivities of ^{3}H and ^{14}C in the plasma and tissue. K_p was evaluated from a best-fit line provided by the linear regression analysis.

2.3. Concentration of plasma insulin and adiponectin

Plasma samples were obtained from cardiac catheter under anesthesia as described in the measurement of 2-DG uptake. Concentration of plasma insulin, adiponectin, and glucose were measured in samples obtained before administration of 2-DG and insulin. Plasma insulin and adiponectin were assayed by enzyme-linked immunosorbent assay (Shibayagi, Gunma, and Otsuka, Tokyo). Plasma glucose was assayed using glucose oxidase kit (Wako).

2.4. Norepinephrine turnover

Norepinephrine turnover was assessed from a decline of tissue NE contents after inhibition of catecholamine biosynthesis with α-methyl-*p*-tyrosine (α-MT, 200 mg/kg as a free base, IP, Sigma Chemical, St Louis, MO) [15-17]. Tissue NE disappeared at a constant rate for at least 8 hours after administration of the drug [15]. Before (zero time) and 4 hours after injection of a methyl ester of α-MT, the animals were decapitated. Brown adipose tissue and

adrenal gland were removed and frozen on dry ice and stored at −80°C until assay of catecholamine content (within 2 weeks). Tissues were homogenized in 0.2 N perchloric acid containing 0.1 mmol/L EDTA and internal standard, isoproterenol (50 μg in the adrenal gland, 500 ng in the BAT). The homogenate was centrifuged at 10 000*g* for 15 minutes and the supernatant was filtered with a 0.45-μm membrane. Norepinephrine and E contents in the filtrate were assayed directly using high-performance liquid chromatography with electrochemical detector system [17]. Fractional turnover rates of NE and E were calculated by linear regression of the ln NE and E content vs time (0 and 4 hours). Norepinephrine and E turnover rates were then calculated as fractional turnover rate multiplied by endogenous NE or E content at zero time.

2.5. Concentration of plasma corticosterone

Blood samples were taken from the cavernous sinus with a capillary syringe between 2:00 and 3:00 PM. Plasma corticosterone was assayed by enzyme-linked immunosorbent assay (Assay Designs, Ann Arbor, MI).

2.6. Data analysis

All values are expressed as means ± SE. The statistical comparisons between the 2 groups were made using the Student *t* test. The other data were evaluated by analysis of variance, with a post hoc analysis of Duncan multiple-range test.

3. Results

3.1. Hypoadiponectinemia, hyperglycemia, and hyperinsulinemia in TSOD mice

Plasma glucose, insulin and adiponectin levels, and body weights in control TSNO and TSOD mice are presented in Table 1. The TSOD mice showed a significant increase in plasma glucose and insulin levels, and body weight compared with that in the TSNO mice. The TSOD mice had obesity and diabetes. Hypoadiponectinemia in addition to hyperglycemia and hyperinsulinemia was observed in the TSOD mice ($P < .01$). Plasma adiponectin level in the TSOD mouse was about one fourth of that in the control TSNO mouse.

Table 1
Plasma glucose, insulin, and adiponectin concentration, and body weight and food intake in the TSNO and TSOD mice

	TSNO	TSOD
Plasma glucose (mg/dL)	221.6 ± 7.4	461.2 ± 19.1*
Plasma insulin (ng/mL)	2.7 ± 0.4	27.0 ± 1.9*
Plasma adiponectin (μg/mL)	18.1 ± 0.8	4.9 ± 0.2*
Body weight (g)	38.7 ± 0.4	59.5 ± 0.7*
Food intake (g/d)	4.1 ± 0.1	5.6 ± 0.2*

Values are expressed as means ± SE for 8 to 9 mice.
* $P < .01$ compared with the values of TSNO mice.

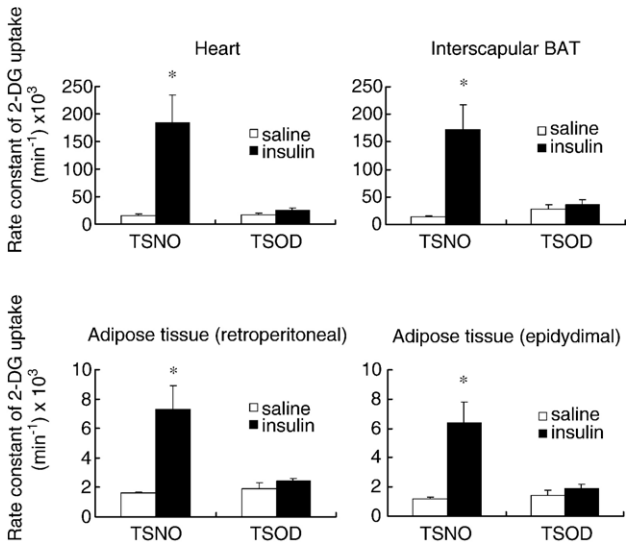

Fig. 1. Effects of insulin on the rate constant of 2-[^{3}H]deoxyglucose uptake in heart, interscapular BAT, and adipose tissues in TSNO and TSOD mice. The rate constant of 2-[^{3}H]deoxyglucose uptake was calculated as described in the Methods section. Results are expressed as means ± SE for 4 mice. *$P < .05$ compared with the TSNO mice.

3.2. Effect of insulin on glucose uptake by peripheral tissues in TSNO and TSOD mice

The K_i of net tissue glucose uptake was obtained under pentobarbital sodium anesthesia for heart muscle, adipose tissues, BAT, and skeletal muscles. The mass of adipose tissues in TSOD mice seems to be several times of that in the TSNO mice. The EDL and soleus muscles have been known to be mainly composed of fast-twitch glycolytic fibers and slow-twitch oxidative fibers, respectively. The

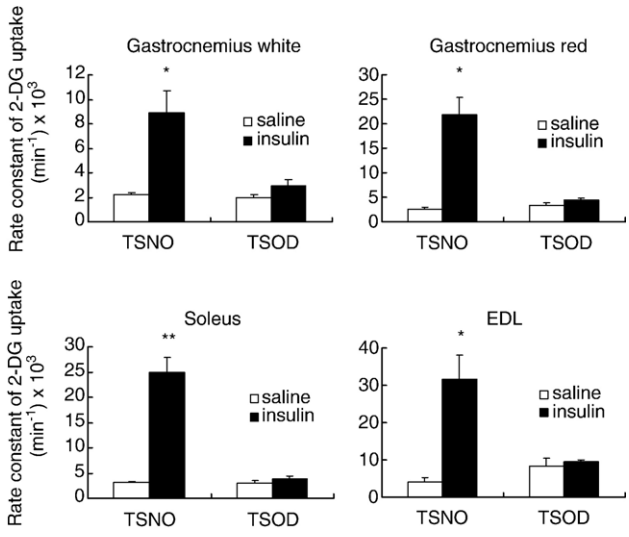

Fig. 2. Effects of insulin on the rate constant of 2-[^{3}H]deoxyglucose uptake in 4 different hindlimb skeletal muscles, gastrocnemius white, gastrocnemius red, soleus, and extensor digitorum longus (EDL), in TSNO and TSOD mice. Results are expressed as means ± SE for 4 mice. *$P < .05$, **$P < .01$ compared with the TSNO mice.

Table 2
Norepinephrine turnover rate in the heart and BAT in TSNO and TSOD mice

	TSNO	TSOD
Heart		
Tissue wet weight (mg)	149.9 ± 2.1	160.6 ± 2.3*
NE content (ng/g tissue)	893.2 ± 27.5	933.8 ± 44.9
NE, 4 h after α-MT (ng/g tissue)	541.1 ± 48.7	790.8 ± 20.6**
Fractional turnover rate (%/h)	12.1 ± 2.2	4.1 ± 0.6**
NE turnover rate (ng hour^{-1} g tissue^{-1})	110.6 ± 19.5	38.5 ± 5.6**
BAT		
Tissue wet weight (mg)	175.2 ± 12.7	232.6 ± 16.1*
NE content (ng/g tissue)	506.5 ± 62.9	315.2 ± 22.7*
NE, 4 h after α-MT (ng/g tissue)	171.6 ± 17.4	185.1 ± 10.8
Fractional turnover rate (%/h)	24.2 ± 1.8	12.6 ± 1.2**
NE turnover rate (ng hour^{-1} g tissue^{-1})	122.7 ± 9.3	39.8 ± 3.8**

Values are expressed as means ± SE. Tissue weights of heart and BAT (n = 15), and NE contents before (0 time, n = 6) and 4 hours after (n = 9) administration of α-MT were represented. Fractional turnover rate (%/h) was calculated by linear regression of the ln NE and E content vs time (0 and 4 hours). Norepinephrine turnover rate (ng hour^{-1} g tissue^{-1}) was estimated as product of fractional turnover rate times NE content at time 0.

* $P < .05$ compared with the TSNO mice.

** $P < .01$ compared with the TSNO mice.

white part of gastrocnemius is fast-twitch glycolytic fibers and the red part is slow-twitch oxidative fibers. In the basal condition, the K_i value of glucose uptake was similar in peripheral tissues in both strains, TSNO and TSOD mice. The values varied considerable among different tissues, being high in the heart and BAT, and low in the adipose tissue. The K_i values of glucose uptake were not significantly different in both types of muscle fibers. Administration of insulin (1 U/kg) increased glucose uptake in all tissues examined in the TSNO mice (Figs. 1 and 2). The K_i values of heart muscle and BAT were increased about 12 times that of in the basal controls. The K_i values of skeletal muscles (gastrocnemius white muscle, gastrocnemius red muscle, soleus, and EDL), and adipose tissues (retroperitoneal and epididymal), were increased about 4 to 8 times that of in the basal controls. On the other hand, no significant increment of glucose uptake was observed in the TSOD mice (Figs. 1 and 2). These results indicate that TSOD mice have insulin resistance.

3.3. Norepinephrine turnover in BAT and E in adrenal gland

We assessed NE turnover in sympathetically well-innervated peripheral organs, heart and BAT. Table 2 shows tissue weights, NE contents before (zero time) and 4 hours after injection of α-MT, and the calculated fractional turnover rate (%/h) and the NE turnover rate (ng hour^{-1} g tissue^{-1}). Norepinephrine content in the BAT of TSOD mice was significantly lower than that of in the TSNO mice ($P < .05$). Norepinephrine content roughly reflects the density of sympathetic innervations of each organ. Injection of α-MT caused a decrease in NE content. The degree of decrease in NE contents during 4 hours was significantly lower in the TSOD tissues. Accordingly, fractional turnover rates in these tissues of TSOD mice were significantly lower than that in the TSNO mice ($P < .01$). Calculated NE turnover rate of the heart and BAT in TSOD mice were 35% and 32% of corresponding tissues in the control TSNO mice ($P < .01$).

To assess adrenomedullary activity, we measured catecholamine content and turnover in the adrenal gland. Table 3 shows tissue weight, content, and turnover of NE and E. In the TSOD mice, hypertrophy of the adrenal gland was observed. The weight of adrenal gland in TSOD mice was about 1.3 times as heavy as in the TSNO mice ($P < .05$). However, NE and E content in the TSOD adrenal gland was 83% ($P < .05$) and 46% ($P < .01$) of the TSNO mice. Compared with the control TSNO mice, calculated E turnover rate of the adrenal gland in TSOD mice was extremely low ($P < .05$). These data suggest that TSOD mice have low adrenomedullary activity.

3.4. Plasma corticosterone

Hypercorticosteronemia was observed in the TSOD mice ($P < .01$). Plasma corticosterone concentrations in the control TSNO and TSOD mice were 18.5 ± 3.8 ng/mL (SE, n = 6) and 145.0 ± 17.2 ng/mL (SE, n = 7), respectively.

4. Discussion

In the first part of this study, we demonstrated that TSOD mice have insulin resistance. Tsumura Suzuki obese diabetic mice show decreased insulin-mediated glucose uptake in skeletal muscles and adipose tissues (Figs. 1 and 2). Decreased insulin-stimulated glucose uptake is a hallmark of type 2 diabetes mellitus. Hypoadiponectinemia in addition to hyperglycemia and hyperinsulinemia was observed in the TSOD mice (Table 1). Next, we indicated that TSOD mice have low sympathetic nervous activity and low adrenomedullary activity. Both NE turnover rates in heart and BAT and E turnover rate in adrenal gland decrease

Table 3
Catecholamine content and turnover rate in the adrenal gland

Adrenal	TSNO	TSOD
Tissue wet weight (mg)	4.47 ± 1.1	5.87 ± 0.21*
NE content (μg per organ)	4.42 ± 0.13	3.69 ± 0.24*
NE fractional turnover rate (%/h)	1.62 ± 0.48	1.31 ± 0.71
NE turnover rate (ng/h per organ)	71.6 ± 21.2	48.3 ± 26.2
E content (μg per organ)	16.99 ± 0.16	7.83 ± 0.23**
E fractional turnover rate (%/h)	1.51 ± 0.44	0.29 ± 0.64
E turnover rate (ng/h per organ)	256.5 ± 74.8	22.5 ± 50.5*

Values are expressed as means ± SE. Tissue weights (n = 15), NE and E contents (0 time, n = 6), and calculated NE and E turnover rates (n = 9) were presented. Norepinephrine and E turnover rates were calculated a product of fractional turnover rate times NE and E contents at time 0.

* $P < .05$ compared with the TSNO mice.

** $P < .01$ compared with the TSNO mice.

in the TSOD mice compared with that of in the control TSNO mice (Tables 2 and 3).

Administration of insulin (1 U/kg) increased the rate constant (K_i) of glucose uptake in all tissues examined in TSNO mice (Figs. 1 and 2). On the other hand, no significant increment of glucose uptake was observed in the TSOD skeletal muscles and adipose tissues (Figs. 1 and 2). Thus, TSOD mice have insulin resistance. It seems that insulin resistance in the TSOD mice associated with a defect in key molecules of both glucose transporter (GLUT) 4 trafficking and insulin signaling pathways, as reported in other animal models [18]. Miura et al [19] reported that translocation of GLUT-4 from intracellular compartment to plasma membrane in response to insulin was reduced in the TSOD mice. Concentrations of GLUT-4 in the plasma membrane in the basal condition were not significantly different in the TSOD mice compared with the controls [19]. Consistent with the report, the basal K_i value of glucose uptake was similar in respective tissues in both strains, TSNO and TSOD mice (Figs. 1 and 2). The mass of adipose tissues in TSOD mice is several times that of the TSNO mice. The perirenal fat weight in TSOD mice was about 3 times of the controls [2]. It seems probable that the gross amount of basal glucose utilization of adipose tissues in TSOD mice increases several times that of in the TSNO mice. The maintenance and/or increase of fat amount in TSOD mice may be mainly sustained by the basal glucose utilization. Hypoadiponectinemia in the TSOD mice may have reflected on the insulin resistance. Recent studies have indicated a critical role for adiponectin in controlling energy substrate metabolism by enhancing insulin sensitivity in skeletal muscles [20,21]. Plasma adiponectin levels are reduced in a variety of obese and insulin-resistant states in mice [22] and humans [23].

Our data also suggest that both low sympathetic nervous activities and low adrenomedullary activity are significant features of the TSOD mouse. These systems are indispensable regulatory mechanisms in the body for adequate adaptation in response to various situations. Disturbance of both systems must affect intermediate energy substrates metabolism, glucose utilization, and various hormone secretion, which can contribute to the development of hyperglycemia, hyperinsulinemia, and obesity and diabetic syndrome. The sympathetic regulation of BAT function is well established. Brown adipose tissue is a major site for thermogenesis in rodents, which is under direct control of the sympathetic nervous system. On this basis, reduced turnover of NE in BAT of obese *fa/fa* rats and *ob/ob* mice has been interpreted as showing a lack of sympathetic stimulation [12,13]. Thus, reduced NE turnover in BAT of TSOD mouse may reflect on energy expenditure and subsequent ability to retain dietary energy with a higher efficiency. It is probable that mobilization of fat, lipolysis, produced by the sympathetic nerve activity [14], is low in the TSOD mice. A state of low sympathetic activities, low NE turnover, seems to be responsible for the increase of BAT mass (Table 2).

Many researchers have reported low NE turnover values in heart as well as BAT in obese animal models such as *ob/ob* mouse, *db/db* mouse, and monosodium glutamate–treated mouse. Knehans and Romsos [24] have reported that rates of NE turnover in heart in preobese (2 weeks old) were about half of the lean littermates, but the difference diminished at 8 weeks of age. Increase in the amount of food intake in the *ob/ob* mice has the possibility to raise NE turnover in heart [24]. In BAT, rates of NE turnover were slower in the preobese *ob/ob* mice than in lean littermates and remained slow at 8 weeks of age [24].

Lowering of NE turnover in sympathetically innervated organs developed in this TSOD mouse, which may be the result from abnormalities in the central nervous system especially in the hypothalamus. Of the hypothalamic structures, the ventromedial hypothalamus (VMH) is intimately involved in sympathetic facilitation [7,25] and believed to be one of the most effective regions in the energy substrate metabolism [6,7]. Stimulation of the VMH increases metabolic activity of BAT [25,26]. Hypothalamic obesity after VMH lesion is well known. The VMH sympathetic nervous system also affects glucose utilization in the peripheral tissues. Electrical and chemical stimulation of the VMH preferentially increases glucose uptake by the heart, skeletal muscles, and BAT through mediation of the sympathetic nerves innervating the tissues [4,27]. Several lines of evidence have been interpreted as indicating that the mechanism by which VMH stimulation increases glucose uptake is different from that of insulin [10,27]. Different mechanisms are thought to operate in the effects of insulin and NE; whereas insulin stimulates translocation of GLUT-4 to the plasma membrane, NE causes activation of GLUT-1 present in the plasma membrane [28]. The VMH stimulation increased apparent functional activity of glucose transporters in the plasma membrane [10]. It is likely that glucose uptake mechanism via sympathetic nervous system is also reduced in the TSOD mouse.

The adrenal gland of TSOD mouse exhibit hypertrophy (Table 3). Therefore, it is surprising that E content was at most one half of the TSNO control, and the E turnover rate was extremely low (Table 3). The data suggest that adrenomedullary system in the TSOD mouse is dysfunctional. Because E functions in mobilization of energy stores, reduced E release may have contributed to the enhanced storage of body energy observed in TSOD mouse. Adrenomedullary E content and excretion were reduced in the VMH-lesioned obese rats [29]. On the other hand, it is possible that production of corticosterone in adrenal cortex increases. In fact, hyperadrenocorticosteronemia was observed in the TSOD mouse in our data. It has been proposed that obese animal models have an overactive hypothalamo-pituitary-adrenal axis with resulting hypercorticosteronemia [30]. Hypercortisolism has been proposed to contribute to obesity and diabetes [31]. Insulin-stimulated muscle glucose utilization was markedly decreased by corticosterone administration, indicating insulin resistance [32]. In

addition, it has been reported that corticosterone reduces NE turnover in BAT and inhibits BAT function [33]. Hypercorticosteronemia seems to also influence the development of obesity and insulin resistance in the TSOD mouse.

In this study, we showed that TSOD mouse has insulin resistance, hypoadiponectinemia, and hypercorticosteronemia, and that the TSOD mouse is an interesting type 2 diabetic animal model that accompanies an impairment of both sympathetic nerve activity and adrenomedullary activity. Tsumura Suzuki obese diabetic mouse seems to be very valuable for studying the development of type 2 diabetes mellitus.

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