Research Article

Effect of Food Restriction on Adipose Tissue in Spontaneously Diabetic Torii Fatty Rats

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Spontaneously Diabetic Torii–fa/fa (SDT fatty) rat is a new model of obese type 2 diabetes. SDT fatty rat exhibits obesity associated with hyperphagia. In this study, SDT fatty rats were subjected to pair-feeding with SDT-+/+ (SDT) rats from 6 to 22 weeks of age. The ratio of visceral fat weight to subcutaneous fat weight (V/S) decreased at 12 weeks of age in the pair-feeding rats. The intraperitoneal fat weight such as epididymal and retroperitoneal fat weight decreased, whereas mesenteric fat weight had no change. Cell size of the epididymal fat in the pair-feeding rats tended to decrease. Glucose oxidation level in epididymal fat in the pair-feeding rats at 12 weeks of age was recovered to a similar level with that in SDT rats. These results indicated that SDT fatty rat is a useful model to evaluate the functional or the morphological features in adipose tissue and develop a novel drug for antiobesity.

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1. Introduction

Spontaneously Diabetic Torii (SDT) fatty rat is a new model of obese type 2 diabetes, established by introducing the fa allele of the Zucker fatty rat into the SDT rat genome [1]. SDT rat is a useful model of nonobese type 2 diabetes that spontaneously develops hyperglycemia and glucose intolerance resulting from decreased insulin secretion due to β-cell degeneration [2, 3]. It is worthy of notice that SDT rat shows ocular complications such as venous dilation and meandering vascular networks [4]. SDT fatty rat exhibited hyperphagia and obesity immediately after weaning, and the diabetes and its complications are found at a younger age in SDT fatty rats than in SDT rats [5].

Obesity plays a key role in the pathophysiology of several metabolic diseases and is a risk factor for diabetes mellitus or dyslipidemia. It has been reported that the distribution of fat between subcutaneous and visceral sites affects its metabolic impact. Specifically, increased abdominal adiposity has been identified as a risk factor for diabetes mellitus [6–9]. Aging in rats is associated with increased fasting and postprandial plasma insulin levels, suggesting an insulin-resistant state. The insulin resistance has a correlation with increase of fat weight, especially visceral fat [10, 11]. Food intake in SDT fatty rats at 6 weeks of age showed about twofold increase as compared with that in SDT rats, and body mass index and fat pad weight in SDT fatty rats at 14 weeks of age increased [1]. In SDT fatty rat, which shows hyperphagia and obesity, it is essential that the relationships of the changes between food intake and fat tissue are examined in detail. The present study was conducted to investigate how food restriction in SDT fatty rats affects fat weight, fat mass, or metabolic function of adipose tissue.

2. Materials and Methods

2.1. Animals and Diet. This experiment was conducted in compliance with the Guidelines for Animal Experimentation
of Japan Tobacco biological/pharmacological research laboratories. Male SDT-falaf (SDT fatty) rats and age-matched SDT-+/+ (SDT) rats from our colonies were used. SDT fatty rats at 6 weeks of age were divided into two groups. One group was allowed feed (CRF-1, Charles River Japan, Yokohama, Japan) ad libitum, and the other group was pair-fed the same amount of food consumed by age-matched SDT rats from 6 to 22 weeks of age. Food consumption in the pair-fed SDT fatty rats was about 50%–60% of that in the ad libitum-fed SDT fatty rats during the experimental period. In brief, three groups of rat at 6 weeks of age were prepared: (a) SDT fatty rats fed ad libitum (Fatty Group), (b) SDT fatty rats pair-fed against SDT rats (Fatty-PF Group), and (c) SDT rats fed ad libitum (SDT Group). A satellite group of each of the three groups was established to evaluate additional effects to 12 weeks of age. The rats were housed individually in suspended bracket cages in a climate-controlled room with a temperature of 23 ± 3°C, humidity of 55 ± 15%, and a 12 hours lighting cycle and had free access to water.

2.2. Biological Parameters. Body weights and blood chemical parameters, such as glucose, triglyceride (TG), insulin, leptin, and adiponectin levels, were measured at 6, 12, and 22 weeks of age.

Blood samples were collected from the tail vein of rats. Serum glucose and TG levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Serum insulin or leptin levels were measured with a rat insulin- or leptin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Serum adiponectin was measured with a mouse/rat adiponectin ELISA kit (Otsuka pharmaceutical Inc., Tokyo, Japan).

2.3. Fat Tissue Weight.Visceral and subcutaneous fat weights in each rat were determined at 6, 12, and 22 weeks of age by computed tomography (CT) analysis. The fat weights were measured by a laboratory X-ray CT device (LATheta, ALOKA Co., LTD., Osaka, Japan). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital (Tokyo chemical industry, Tokyo, Japan), and about 20 CT photographs per rat were taken at 5 mm intervals between diaphragm and lumbar vertebrae. The ratio of Visceral and Subcutaneous (V/S) were calculated.

Necropsy was performed in satellite groups at 12 weeks of age, and weights of intraperitoneal fat, such as epididymal, retroperitoneal, and mesenteric fat, were determined. Rats were sacrificed by exsanguination under light ether anesthesia, and intraperitoneal fats were collected and weighed. Epididymal fats obtained in this necropsy were used in evaluation of fat cell size and in glucose oxidation and mRNA expression experiments. Since a sufficient fat amount is easily collected, epididymal fats were used in those experiments.

2.4. Fat Cell Size. Cell size of epididymal fat was determined at 12 weeks of age. Cell number of fixed view area was counted for 3 different areas per rat. Cells with incomplete shape on the view frame were not counted. Cells size was estimated as the fixed area divided by the cell number.

2.5. Glucose Oxidation. Glucose oxidation levels on epididymal fat were determined at 12 weeks of age. Adipose tissue samples were incubated in Hanks’ balanced salt solution (pH 7.4, Gibco, Grand Island, NY, USA) containing 4% BSA (bovine serum albumin, Sigma Chemical, St. Louis, MO, USA), 20.72 kBq/mL [U-14C]-glucose (NEN, Boston, MA, USA), and 0–100 mU/mL insulin (from bovine pancreas, Sigma) for 120 minutes at 37°C. Synthesized 14CO2 was trapped with Scintilamine-OH (Dojindo, Tokyo, Japan) on a filter paper (Whatman, Maidstone, UK) and counted using a Liquid Scintillation Analyzer Model 2500 TR (Packard, Meriden, CT, USA). Total lipid synthesis from glucose was estimated by determining the amount of 14C incorporated into total lipids. Total lipids were extracted from the tissues with n-hexane after the addition of Dole’s solution (2-propanol : n-heptane : 1 N H2SO4 = 40 : 10 : 1). Radioactivity in the hexane fractions was measured using a liquid scintillation counter.

2.6. mRNA Quantification with Real-Time Quantitative PCR. Total RNA was extracted from the epididymal fat of the satellite group rats at 12 weeks of age. RNA was transcribed into cDNA using M-MLV reverse transcriptase and random primers (Invitrogen, Carlsbad, CA, USA). The reaction mixture was incubated for 10 minutes at 25°C, 1 hour at 37°C, and 5 minutes at 95°C. Real time PCR quantification was performed in a 50 μL reaction mixture with an automated sequence detector combined with ABI Prism 7700 Sequence Detection System software (Applied Biosystems, Foster City, CA, USA). The reaction mixture contained 50 ng of synthesized cDNA, 3.5 mM MgCl₂, 0.3 μM primers, 0.1 μM probes, and 1.25 units of Ampli Taq Gold. Cycle parameters were 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. The following primers and FAM-conjugated probes were designed using Primer Express software (Applied Biosystems): glucose transporter 4 (GLUT4) (forward, CTACATCGGAACCTTTGAG; reverse, AGTGCGCTGAGCATCACGACGCC; probe, TGC CCG AAA GAGCTCT AAA GCG), tumor necrosis factor α (TNFα) (forward, CCAGGTTCTCTCCAAGGACAA; reverse, CTCCCTGTTATGAAATGGCAAATCT; probe, CCGCGAC-TATGTGTTGCTCCTCACCCACA), lipoprotein lipase (LPL) (forward, TGTCtAATGCACCTTTCACCA; reverse, CATACATCGGCTACGCGT; probe, CAG CAA AAC CTTGTGTGATTCATG), Acetyl CoA carboxylase 1 (ACC-1) (forward, GCGCTATGTTGAGAGACTCC; reverse, CAC CCT CAC AGT TGA CTT GTT TGC; probe, CGG CGA CTT ACG TTC CTA GTT GCA CAA AA) and 18S rRNA (purchased from Applied Biosystems).

2.7. Statistical Analyses. Results are expressed as the mean ± standard deviation (SD). Statistical analysis of differences between mean values was performed using the F-test,
3. Results

3.1. Biological Parameters. Body weight in the SDT fatty rats was already elevated as compared with that in the SDT rats at the starting point of this experiment, 6 weeks of age (mean value: SDT fatty rats, 207 g; SDT rats, 174 g). Body weight in the Fatty Group showed a significant increase at 12 weeks of age as compared with that in the SDT Group, whereas body weight in the Fatty-PF Group showed similar changes at 12 and 24 weeks of age to those in the SDT Group (Figure 1(a)). Serum glucose level in the SDT fatty rats was already elevated as compared with that in the SDT rats at 6 weeks of age (mean value: SDT fatty rats, 350 mg/dL; SDT rats, 200 mg/dL). Glucose levels in both the Fatty and Fatty-PF rats showed similar changes to those in the SDT rats (Figure 1(b)). Serum triglyceride levels were elevated in the SDT fatty rats compared with those in the SDT rats at 6 weeks of age (mean value: SDT fatty rats, 200 mg/dL; SDT rats, 100 mg/dL). Hypertriglyceridemia in Fatty rats was temporally improved by food restriction, and the hyperinsulinemia was maintained to 12 weeks of age. Serum leptin and adiponectin levels were elevated in the Fatty-PF rats. Data represent means ± SD (n = 5-6). *P < .05, **P < .01: significantly different from SDT rat.

followed by the Student’s t-test or Aspin-Welch’s t-test. Differences were defined as significant at P < .05.
of age (mean value: SDT fatty rats, 313 mg/dL; SDT rats, 158 mg/dL). Serum glucose level in the Fatty Group was elevated to 600–700 mg/dL at 12 and 22 weeks of age. Serum glucose level in the Fatty-PF Group was suppressed to a similar level as in the SDT Group at 12 weeks of age, whereas glucose level in the Fatty Group was elevated to 600–700 mg/dL at 12 and 22 weeks of age. Serum adiponectin level in the Fatty Group was significantly increased at 12 and 22 weeks of age compared with those in the Fatty Group, whereas the adiponectin level in the Fatty-PF Group was sustained during the experimental period. Serum leptin level decreased to a level similar to that in the SDT Group (mean value: SDT fatty rats, 313 mg/dL; SDT rats, 2.2 ng/mL). The elevation in visceral fat weight or V/S ratio: Fatty Group, 47.9 g or 0.75; see Table 1, Figure 2). Moreover, subcutaneous fat weight in the Fatty-PF Group was increased as compared with that in the Fatty Group. Similar changes were observed at 22 weeks of age, but the changes were not significant (Table 1).

In examination of intraperitoneal fat weight at 12 weeks of age, epididymal and retroperitoneal fat weights in the Fatty-PF Group were decreased at 12 weeks of age as compared with those in the Fatty Group, whereas the mesenteric fat weights between two groups were not changed (Table 2).

Food restriction in SDT fatty rats induced decreases of visceral fat weight and V/S ratio.

3.3. Fat Cell Size. Fat cell size at 12 weeks of age in the Fatty Group was about 2.3 times higher than that in the SDT Group (mean ± SD: Fatty Group, 8059 ± 1967 μm²; SDT Group, 3553 ± 341 μm²). The fat cell size in the Fatty-PF Group (mean ± SD: 6080 ± 355 μm²) tended to be decreased by about 25% as compared with that in the SDT Group (Figure 3).

Food restriction in SDT fatty rats induced a miniaturization of the fat cell size.

3.4. Glucose Oxidation. Both basal glucose oxidation levels and insulin-stimulated glucose oxidation levels in adipose tissue of SDT fatty rats tended to be decreased as compared

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Table 1: Changes of fat tissue weights in Fatty Group, Fatty-PF Group, and SDT Group. Visceral and subcutaneous fat tissue weights were determined by computed tomography analysis. Data represent mean ± SD (n = 5-6).

|                  | 6 weeks of age | 12 weeks of age | 22 weeks of age |
|------------------|----------------|-----------------|-----------------|
| **Fatty Group**  |                |                 |                 |
| Visceral fat (g) | 9.8 ± 1.0      | 47.9 ± 1.9      | 85.9 ± 15.8     |
| Subcutaneous fat (g) | 16.3 ± 1.7 | 39.2 ± 10.9      | 51.6 ± 37.3     |
| Total fat (g)    | 26.1 ± 2.4     | 87.1 ± 11.0     | 137.5 ± 52.5    |
| V/S ratio        | 0.60 ± 0.07    | 1.29 ± 0.31     | 2.04 ± 0.66     |
| **Fatty-PF Group** |              |                 |                 |
| Visceral fat (g) | 10.5 ± 1.0     | 37.0 ± 1.4##    | 75.5 ± 2.5      |
| Subcutaneous fat (g) | 16.5 ± 1.9 | 50.8 ± 9.0       | 59.8 ± 8.8      |
| Total fat (g)    | 27.1 ± 2.8     | 87.8 ± 9.2      | 135.2 ± 10.4    |
| V/S ratio        | 0.64 ± 0.04    | 0.75 ± 0.15##   | 1.28 ± 0.19     |
| **SDT Group**    |                |                 |                 |
| Visceral fat (g) | 2.9 ± 0.8      | 17.8 ± 2.8      | 27.1 ± 10.6     |
| Subcutaneous fat (g) | 2.2 ± 0.6 | 9.1 ± 2.3       | 13.5 ± 6.2      |
| Total fat (g)    | 5.1 ± 1.4      | 26.9 ± 5.0      | 40.6 ± 16.7     |
| V/S ratio        | 1.31 ± 0.07    | 2.01 ± 0.22     | 2.30 ± 0.89     |

## P < .01: significantly different from Fatty Group.
with those in SDT rats (Figure 4), and especially in 100 nM insulin, the decrease of glucose oxidation in the Fatty Group was significant. Glucose oxidation levels in the Fatty-PF Group were recovered to similar levels as in the SDT Group.

Food restriction in the SDT fatty rats induced improvement of the metabolic function of adipose tissue.

3.5. mRNA Expression in Adipose Tissue. GLUT4 and ACC-1 mRNA levels between the Fatty Group and the SDT Group were not changed, whereas those levels in the Fatty-PF Group were elevated as compared with that in the Fatty Group (Figures 5(a) and 5(b)). LPL mRNA level in the Fatty Group increased as compared with that in the SDT Group, and the levels in the Fatty-PF Group were, furthermore, elevated (Figure 5(c)). TNFα mRNA level in the Fatty Group tended to increase as compared with that in the SDT Group, whereas the levels between the Fatty Group and the Fatty-PF Group were not changed (Figure 5(d)).
4. Discussion

SDT rat, which is a model of nonobese type 2 diabetes, was established in 1997 by Shinohara et al. [2]. The rat spontaneously develops hyperglycemia and glucose intolerance resulting from decreased insulin secretion due to β-cell degeneration [12]. Interestingly, SDT rat shows ocular complications, such as venous dilation and meandering vascular networks, which are caused by hyperglycemia [4]. It is also reported that SDT rat is useful as a model of diabetic nephropathy [13]. More recently, Masuyama et al. [1] produced an SDT fatty rat by introducing the fa allele of the Zucker fatty rat into the SDT rat genome. SDT fatty rats manifest hyperphagia, obesity, hyperglycemia, and hyperlipidemia. The fatty rats develop diabetes from 5 weeks of age, a development time that is quite earlier than for SDT rats [5]. This early incidence or progression of diabetes is considered to be caused by hyperphagia, which is associated with a leptin signal abnormality induced by introduction of the fa allele. In this study, we investigated the effects of food restriction on bloodchemical parameters and adipose tissue in SDT fatty rats.

Under food restriction, body weights in the Fatty Group were regulated to similar levels as those in the SDT Group (Figure 1(a)). The rats in the Fatty-PF Group showed an improvement of hyperglycemia and a tendency of TG levels to be decreased at 12 weeks of age (Figures 1(b) and 1(c)). These improvements in glucose and TG levels were also reported under food-regulated conditions of Zucker diabetic fatty rats [14] and by food restriction in OLETF rats [15]. Moreover, food restriction in normal rats (Wistar rats) did not show a lowering effect on fasting plasma glucose level, whereas the fasting plasma insulin level decreased [16]. The improvement in the Fatty-PF Group was not continuously observed, and the elevation of glucose and TG levels was observed at 22 weeks of age (Figures 1(b) and 1(c)). Since SDT fatty rats might have weakness in pancreatic function [1], it is considered that the insulin secretion is deteriorated, and the incidence of diabetes in the rats cannot be suppressed by food restriction alone. Serum insulin level in the Fatty-PF Group was decreased to a similar level in the SDT fatty Group at 22 weeks of age (Figure 1(d)). Also, elevation of TG levels at 22 weeks of age in the Fatty-PF Group is considered to be caused by the insulin deficiency. Both serum leptin level and serum adiponectin level in SDT fatty rats
were increased by food restriction (Figures 1(e) and 1(f)). It is reported that plasma leptin level was decreased, and plasma adiponectin level was increased by food restriction in Wistar rats and OLETF rats [15, 16]. The change in adiponectin level was consistent with the result in our study, but the change in leptin level was inconsistent with our result. Since leptin resistance exists in SDT fatty rats, the serum leptin level may not be decreased by food restriction. Furthermore, the increase in serum leptin levels in food-restricted SDT fatty rat is considered to be caused by the increase of subcutaneous fat weight (Table 1). On the other hand, the increase of serum adiponectin levels in food-restricted SDT fatty rat is considered to be related to the decrease of visceral fat weight. In the investigation on the effects of food restriction on biochemical parameters in SDT fatty rats, a temporal improvement of hyperglycemia and an increase of adipokine level were observed. Especially, the improvement of hyperglycemia was observed, and the reason is considered to be related with the background factor in SDT rats.

In analysis of fat tissue weights in the pair-feeding SDT fatty rat, the visceral fat or the V/S ratio significantly decreased, whereas the subcutaneous fat tended to increase as compared with the SDT fatty rat (Table 1). It is reported that total fat weight, including visceral fat and subcutaneous fat, was decreased by food restriction in normal rats [16–18], OLETF rats [15], and Zucker fatty rats [19]. The change of visceral fat in our study was consistent with results in some reports, but the change of subcutaneous fat mass in our study was inconsistent with the some results. Those changes of fat tissue weight in this study were similar with changes after treatment with thiazolidinediones [20, 21]. After pioglitazone treatment, subcutaneous fat area increased, whereas visceral fat area and the ratio of visceral to subcutaneous fat decreased [20, 21]. In human obesity, the contribution of abdominal visceral and subcutaneous fat is widely noticed [22, 23]. There is a strong association between visceral adiposity and insulin resistance patterns of glucose homeostasis, including in type 2 diabetes mellitus [24, 25]. Furthermore, visceral fat is related to essential hypertension, dyslipidemia, and cardiovascular disease [26, 27]. Evaluation for the pattern of that fat distribution in SDT fatty rat is considered to be very important to elucidate pathophysiological features in human obesity.

According to CT analysis at 6, 12, and 22 weeks of age, we showed significant difference in the ratio of visceral fat to subcutaneous fat at 12 weeks of age (Table 1). So we investigated fat weight, fat cell size, glucose oxidation, and quantification of mRNA at this age. In examination of intraperitoneal fat weight, epididymal and retroperitoneal fat weights in the SDT fatty rats were decreased by food restriction for 6 weeks, whereas the mesenteric fat weight was not changed (Table 2). Epididymal and retroperitoneal fat weights in Wistar rats and OLETF rats had been decreased by food restriction [15, 16]. Furthermore, the mesenteric fat weight in OLETF rats was lowered by the food restriction for 12 weeks. A decrease of mesenteric fat weight may not be caused by food restriction for 6 weeks, which was a condition in our study.

Fat cell size in the SDT fatty rat tended to decrease by food restriction (Figure 3). It is reported that a decrease of energy intake, that is, a restriction of food intake, induces miniaturization of fat cells in Sprague-Dawley rats [28, 29]. In general, food restriction is considered to induce an increase in the number of small adipocytes in rats. Furthermore, it is reported that a miniaturization of fat cell size induces increase of plasma adiponectin levels [30]. It is possible that the increase of serum adiponectin levels in the pair-feeding rat (Figure 1(f)) was induced by the miniaturization of fat cell size.

Insulin stimulated-glucose oxidation in the pair-feeding SDT fatty rats was elevated as compared with that in the SDT fatty rats (Figure 4), and it is considered that the glucose metabolic function in the SDT fatty rat was promoted by food restriction. Glucose utilization index and glucose metabolic index of retroperitoneal and epididymal adipose tissues has been also increased in food-restricted Wistar rats [16, 17]. Since it is reported that insulin stimulated-glucose oxidation was elevated by a reinforcement of insulin sensitivity [31], the recovery of glucose oxidation in the food-restricted SDT fatty rats may be caused by the improvement of glicolipid abnormalities, such as the decrease of serum glucose level and TG level (Figures 1(b) and 1(c)). Also, increases of blood leptin level and adiponectin level are considered to induce good effects on the metabolic function of adipose tissue [16, 32–35]. Furthermore, GLUT4, LPL, and ACC mRNA expressions in the pair-feeding SDT fatty rats increased (Figure 5). It is reported that these mRNA expressions in adipose tissue is increased by insulin treatment [36, 37]. Since the serum insulin level in the pair-feeding SDT fatty rats at 12 weeks of age increased (Figure 1(d)), the elevation of these mRNA expressions is considered to be induced by the increase of the insulin levels. Furthermore, another reason for the elevation of these mRNA expressions is considered to be reinforcement of insulin sensitivity by food restriction (Figure 4). Decrease of TNFα mRNA expression and increase of GLUT4 mRNA expression were shown in treatment with thiazolidinediones [38, 39], but the TNFα mRNA expression did not change in this study (Figure 5(b)). Although this evaluation of adipose tissue was

| Table 2: Intraperitoneal fat tissue weights at 12 weeks of age. Data represent mean ± SD (n = 6). |
|----|----|----|
| Epididymal fat (g) | Retroperitoneal fat (g) | Mesenteric fat (g) |
| Fatty Group | 14.6 ± 0.7 | 16.4 ± 1.4 | 10.9 ± 0.6 |
| Fatty-PF Group | 12.0 ± 1.8## | 12.8 ± 1.7## | 10.7 ± 0.9 |
| SDT Group | 6.8 ± 0.7 | 8.7 ± 1.0 | 5.5 ± 0.6 |

##P < .01: significantly different from Fatty Group.
performed by using epididymal fats, the further examination by using other adipose tissue might be essential. In the investigation on the effects of food restriction on adipose tissue in SDT fatty rats, a decrease of visceral fat weight and a miniaturization of the fat cell size were observed. Moreover, the food restriction induced an improvement of the metabolic function on adipose tissue.

Food restriction in SDT fatty rats partially improved the glycolipid parameters and induced a decrease of the visceral fat weight. Furthermore, the food restriction affected the fat cell size and the metabolic function in the adipose tissue. In conclusion, SDT fatty rat is a useful model to evaluate the functional or the morphological features in adipose tissue and develop a novel drug for antiobesity.

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