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Effects on Fat Distribution by Food Restriction in Zucker Diabetic Fatty Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TO and MS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors TO, YI, HY and TY managed the analyses of the study, and performed the statistical analyses. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: Zucker diabetic fatty (ZDF) rat is a type 2 diabetic model with hyperphagia, obesity, and the overt fat storage. The present study investigated effects of food restriction for 15 weeks on the fat distribution in ZDF rats.
Methods: ZDF rats were pair-fed with Zucker Lean (ZL) rats from 9 to 24 weeks of age. Body weight and blood chemistry parameters, such as glucose, insulin, triglyceride, total cholesterol and non-esterified fatty acid, were measured every two weeks. The visceral and subcutaneous fat weights were measured at 24 weeks of age by computed tomography (CT) analysis, and the total fat weight and the ratio of visceral fat weight to subcutaneous fat weight (V/S ratio) were determined.
Results: The ZDF rats showed obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia as compared with the ZL rats. Pair-fed ZDF rats showed a temporary decrease in body weight and a suppression of hyperglycemia, but the blood insulin and lipid levels increased. Total fat weight was about 2.4 times higher in the ZDF rats than the ZL rats. The total fat weight in Pair-fed ZDF rats

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was increased by 56%, but the V/S ratio was decreased by 38% at 24 weeks of age.
Conclusion: The change of fat distribution by dietary restriction may be related to the improvement of glucose and lipid metabolic disorders in diabetes mellitus with obesity.

Keywords: Food restriction; fat distribution; visceral fat; Zucker diabetic fatty rat.

1. INTRODUCTION

Obesity plays a key role in the pathophysiology of several metabolic diseases and is a risk factor for diabetes mellitus or dyslipidemia. Type 2 diabetes is a polygenic disorder that is caused by a metabolic and hormonal imbalance between insulin sensitivity in peripheral tissues and insulin secretion from pancreas, both of which might be modified by environmental and genetic factors [1]. Several factors have been identified that cause insulin resistance, and obesity is clearly one. Insulin resistance associated with obesity is more pronounced in patients with centrally localized obesity. 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 [2-4]. Furthermore, visceral fat accumulation leads to atherosclerosis through multiple risk factors, such as hyprglycemia, hyperinsulinemia, dyslipidemia, and hypertension [5,6]. Control of the visceral fat mass plays a key role for treatment of metabolic disorder diseases. Aging in rats is associated with increased fasting and postprandial plasma insulin levels, suggesting an insulin-resistant state. The insulin resistance has correlate with increase of fat weight, especially visceral fat [7,8].

The Zucker diabetic fatty (ZDF) rats, derived from inbreeding of hyperglycemic Zucker obese rats, does have marked hyperglycemia along with insulin resistance, hyperlipidemia, and obesity, potentially making it useful as a model of human type 2 diabetes [9,10]. Moreover, the ZDF rats showed visceral obesity and the insulin action is markedly impaired with visceral fat accumulation [11]. In the present study, we investigated effects of long-term food restriction on the visceral and subcutaneous fat storages and examined the change of fat distribution in ZDF rats.

2. MATERIALS AND METHODS

2.1 Animals

This experiment was conducted in compliance with the Guidelines for Animal Experimentation of Japan Tobacco biological/pharmacological research laboratories, between December 2008 and 2011. Male ZDF rats and age-matched male Zucker Lean (ZL) rats (Charles River Japan, Yokohama, Japan) were used. ZDF rats at 9 weeks of age were divided into two groups: one group was allowed to feed (CRF-1, Charles River Japan, Yokohama, Japan) *ad libitum*, and the other group was pair-fed the amount of food consumed by age-matched ZL rats from 9 to 24 weeks of age. Food consumption of the pair-fed ZDF rats was about 40-60% of that of the *ad libitum*-fed ZDF rats throughout the experimental period. The rats were housed individually in suspended bracket cages in a climate-controlled room with a temperature of 23±3ºC, a humidity of 55±15%, and a 12 h lighting cycle, and had free access to water.

2.2 Biological Parameters

Body weight and biochemical parameters such as serum glucose, triglyceride (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), and insulin levels, were evaluated every two weeks in the non-fasting state. Blood samples were collected from the tail vein of rats. Serum glucose, TG, and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7170S; Hitachi, Tokyo, Japan). Serum insulin levels were measured with a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Serum NEFA levels were measured with a NEFA C test kit (Wako Pure Chem. Ind., Osaka, Japan).

2.3 Measurements of fat Tissue Weight

Visceral and subcutaneous fat weights in each rat were determined at 24 weeks of age by computed tomography (CT) analysis. The fat weights were measured by laboratory X-ray CT device (LATheta, ALOKA Co., LTD., Osaka, Japan). Rats anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital (Tokyo chemical industry, Tokyo, Japan), and about 20 CT photographs in a rat were taken at 5 mm intervals between diaphragm and lumbar

vertebrae. Total fat weight or Visceral/Subcutaneous (V/S) ratio was calculated by visceral and subcutaneous fat weights.

2.4 Statistical Analysis

The results of biological parameters were expressed as the mean ± standard deviation (SD). Statistical analysis of differences between mean values was performed using an F-test, followed by Student's t-test or Aspin-Welch's t-test. Differences were considered significant at $p< 0.05$.

3. RESULTS

In ZDF rats at starting time of an experiment, 9 weeks of age, body weight and serum glucose, insulin, and lipid levels significantly increased as compared with those in ZL rats (Fig. 1). Hyperphagia was also observed in the ZDF rats (data not shown). Body weights in pair-fed ZDF rats decreased from 11 to 15 weeks of age, as compared with those in *ad-lib* ZDF rats, but the body weights were not different from *ad-lib* ZDF rats after 17 weeks of age (Fig. 1A). Hyperglycemia in ZDF rats was almost perfectly inhibited by food restriction during the experimental period (Fig. 1B). The insulin levels in pair-fed ZDF rats increased after 15 weeks of age, as compared with those in *ad-lib* ZDF rats (Fig. 1C). The TG levels in pair-fed ZDF rats increased after 19 weeks of age, as compared with those in *ad-lib* ZDF rats (Fig. 1D). The TC and NEFA levels in pair-fed ZDF rats increased after 11 weeks of age as compared with those in *ad-lib* ZDF rats (Figs. 1E and 1F), and the increase in NEFA levels was sustained during the experimental period.

At 24 weeks of age, total fat weight, which is the sum of visceral fat weight and subcutaneous fat weight, was about 2.4 times higher in the ZDF rats than in the ZL rats (Table 1). Both the visceral and the subcutaneous fat weights in ZDF rats were significantly increased by food restriction (visceral fat weight; 22% increase, subcutaneous fat weight; 95% increase, respectively). The total fat weight in pair-fed ZDF rats was significantly increased by 56%, but the V/S ratio in pair-fed ZDF rats was significantly decreased by 38%.

4. DISCUSSION

It is reported that ZDF rats show hyperglycemia, hyperinsulinemia, and hyperlipidemia about at 9 weeks of age [12], as shown in Fig. 1. Since the remarkable hyperglycemia was sustained in *ad-lib* ZDF rats, the body weight gain was considered to become slow gradually. In other obese diabetic models, such as Otsuka Long Evans Tokushima fatty (OLETF), Spontaneously Diabetic Torii fatty (SDT fatty) rats, and Wistar Bonn/Kobori (WBN/Kob) fatty rats, the hyperglycemia was also inhibited by food restriction [13-18]. On the other hand, the insulin levels in pair-fed rats increased after 15 weeks of age. The reason why is considered that the pancreatic dysfunction in ZDF rats was improved by food restriction. The insulin levels in pair-fed SDT-fatty rats showed a similar change as those in pair-fed ZDF rats [16]. ZDF rats showed increases of lipid levels by food restriction. Since the exogenous intake of lipid, including TG and cholesterol, was decreased by food restriction, the increase of the endogenous production of lipids may be induced. In SDT fatty rats, mRNA expression of HMG-CoA, which is cholesterol-synthetic enzyme, was increased by food restriction [19]. Moreover, in adipose tissue in pair-fed SDT fatty rats, mRNA expression of ACC-1, which is a key enzyme in lipid-synthesis pathway, significantly increased [20].

Both the visceral and the subcutaneous fat weights in pair-fed ZDF rats increased, but the V/S ratio decreased. The increases of fat weights in pair-fed ZDF rats may be related with increases of the serum insulin levels. It is reported that total fat weight including visceral fat and subcutaneous fat was decreased by food restriction in normal rat [21-23], OLETF rats [15], and Zucker fatty rats [24]. In the pair-fed SDT fatty rats, the visceral fat or the V/S ratio significantly decreased, whereas the subcutaneous fat tended to increase [20]. The change of fat tissue weights by food restriction in our study was inconsistent with the previous reports. In the study using OLETF rats (experimental period, 12 weeks), the food restriction was subjected to dietary restriction of about 70% of the food intake of the control group [15]. As a result, the weights of abdominal subcutaneous fat pads and the sum of abdominal fat pad weights were markedly lower in food restriction group than in the control group. On the other hand, in the study using SDT fatty rats (experimental period, 16 weeks), food consumption in the pair-fed SDT fatty rats was about 50-60% of that in the control SDT fatty rats [20]. As a result, the food restriction in SDT fatty rats induced decreases of visceral fat weight and V/S ratio. In this study, food consumption in the

pair-fed ZDF rats was about 40-60% of that in the *ad libitum*-fed ZDF rats, and the inhibition percentage in food restriction was comparable to that in the SDT fatty rats. In both SDT fatty rats and ZDF rats, the decrease of V/S ratio was induced by food restriction. Inhibition ratio in food restriction may be related with changes of fat tissue weights. Moreover, the food restriction period is a considerable factor. In this study, the decrease of V/S ratio in pair-fed ZDF rats is an important perspective to argue development of diabetes mellitus. In clinical studies, it is also reported that the shift of energy accumulation from visceral to subcutaneous fat may contribute to the improvement of insulin resistance [25,26]. Evaluation for the pattern of that fat distribution in obese diabetic rats is considered to be very important to elucidate pathophysiological features in human obesity.

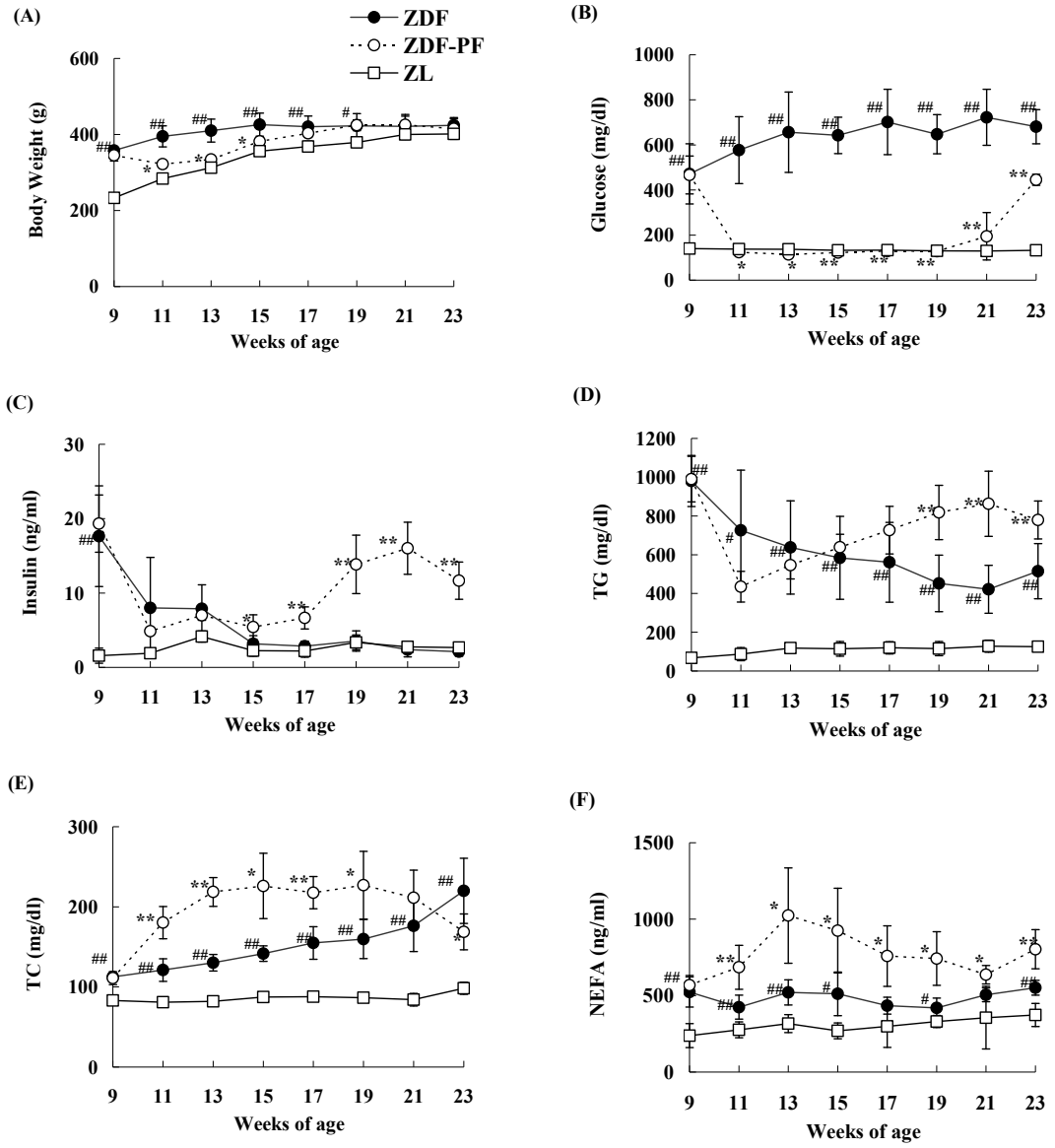

Fig. 1. Changes in body weight (A) and serum parameters ((B) Glucose; (C) Insulin; (D) Triglyceride (TG); (E) Total cholesterol (TC); (F) Non-esterified fatty acid (NEFA)) in ZDF, pair-fed ZDF (ZDF-PF), and ZL rats.Data represent means ± SD (n=5)

**p<0.05, ** p<0.01: significantly different from the ZDF rat. #p<0.05, ##p<0.01: significantly different from the ZL rat*

Table 1. Fat weights in ZDF, pair-fed ZDF, and ZL rats. Visceral and subcutaneous fat weights were determined by computed tomography analysis

	Visceral fat (g)	Subcutaneous fat (g)	Total fat (g)	V/S ratio
ZDF rat	43.0±5.4##	36.8±5.8##	79.8±11.1##	1.17±0.05
Pair-fed ZDF rat	52.4±3.9*	71.8±5.4**	124.1±7.3**	0.73±0.07**
ZL rat	18.2±3.0	14.8±2.5	32.9±5.2	1.24±0.12

*Data represent means ± SD (n=5). ##p<0.01: significantly from the ZL rat, *P<0.05, **P<0.01: significantly from the ZDF rat*

5. CONCLUSION

The change of fat distribution by dietary restriction may be related to the improvement of glucose and lipid metabolic disorders in diabetes mellitus with obesity.

6. LIMITATIONS

Some limitations exist in this study. It is difficult to establish the appropriate inhibition rate in food restriction and the food restriction period individually.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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