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Effect of Isolation Stress on Glucose/Lipid Metabolism in Spontaneously Diabetic Torii (SDT) Fatty Rats

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Authors' contributions

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

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ABSTRACT

Aim: The Spontaneously Diabetic Torii (SDT) fatty rat is a novel obese type 2 diabetic model, showing hyperphagia, obesity, and diabetes mellitus from a young age. In this study, we investigated the effects of isolation stress on pathophysiology in SDT fatty rats.
Methods: SDT fatty rats (4 weeks old) were housed 3 per cage for 2 weeks and separated as males or females so as each gender will be placed in a separate cage to avoid mating. After acclimatization in 6 weeks of age, the rats were exposed to isolation stress (IS) (one rat per cage, using 5 animals in each sex). In the control group, each sex of experimental rats were housed

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separately continuously 3 per cage (using 6 animals in each sex). Food intake, body weights, and blood chemical parameters, such as glucose, insulin, triglyceride and total cholesterol levels, of the rats from 6 to 15 weeks of age were measured at every 3 weeks. Satellite groups were prepared for pathological analyses. Necropsy of satellite group was performed at 12 weeks of age, and the pathological analyses, such as adrenal, thymus and spleen, were performed.

Results: The blood glucose level in IS group in female SDT fatty rats was significantly increased at 12 weeks of age as compared with that in control group. Female SDT fatty rats showed accelerated diabetic progression, but the male rats did show the effects of IS on the glucose/lipid metabolism. In male SDT fatty rats, an increase of adrenal weight and a decrease of thymus weight were observed in IS group and the female rats in IS group showed a tendency of an increase of adrenal weight and a decrease of thymus weight. In histopathological analyses, adrenal hypertrophy and thymus atrophy were observed in IS group in both male and female rats.

Conclusion: Isolation stress affected the progression of diabetes in female SDT fatty rats. Housing conditions is a factor to care for in evaluation of pathophysiology in diabetic models.

Keywords: Diabetes; isolation stress; SDT fatty rat; male and female rats.

1. INTRODUCTION

Daily exposure to social and psychological stress is associated with lifestyle-related diseases such as insulin resistance, visceral obesity, diabetes, dyslipidemia, cardiovascular diseases and cancer, as well as mental disorders [1-4]. Physiological and psychological stress increase blood pressure and the plasma levels of catecholamines and glucocorticoid by the activation of the sympatho-adrenomedullary system and the hypothalamus-pituitary-adrenal axis [5]. In cynomolgus monkeys, the stress of social subordination has been shown to be associated with increased central fat deposition, which in turn relates to hyperglycemia, dyslipidemia and hypertension [6]. High levels of prolactin and dopamine related to a dysregulated hormone secretion were reported in the isolation stressed ewes [7]. Moreover, in comparison to controls, Sprague-Dawley rats stressed for 28 days had larger adipocytes and a tendency for a heavier fat pad, plus an increased lipoprotein lipase activity in the mesenteric depot [8]. Isolation stress including monotony could induce anxiety and depression like traits in animal models through an IGF-1-serotonin mediated mechanism [9]. Spontaneously Diabetic Torii (SDT) fatty rat, established by introducing the *fa* allele of the Zucker fatty rat into the SDT rat genome, represents a new model of obese type 2 diabetes [10]. SDT fatty rats of both sexes exhibited hyperphagia and obesity after weaning, and the hyperglycemia and the hyperlipidemia were observed from a young age, 4-6 weeks [11,12]. In this study, we investigated the effects of isolation stress (IS) on glucose/lipid metabolism in SDT fatty rats.

2. MATERIALS AND METHODS

2.1 Animals and Isolation Stress

Male and female SDT fatty rats (4 weeks of age; Japan Tobacco Inc., Tokyo, Japan) were housed at three rats per cage. After acclimatization for 2 weeks, the rats were divided into two groups, a control group (three rats per cage, six animals were set in both sexes) and an IS group (one rat per cage, five animals were set in both sexes). Rats were housed in suspended bracket cages and given a standard laboratory diet (CRF-1, Oriental yeast co., ltd. Tokyo, Japan) and water *ad libitum* in a controlled room for temperature, humidity and lightning.

2.2 Biological Parameters

Food intake, body weights and blood chemical parameters, such as glucose, insulin, triglyceride (TG) and total cholesterol (TC) levels, of the rats from 6 to 15 weeks of age were measured at every 3 weeks. Blood samples were collected from the tail vein of non-fasted rats. Serum glucose, TG and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin level was measured with a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan).

2.3 Tissue Sampling and Histopathology

Satellite groups were prepared for pathological analysis. Necropsy of satellite group was performed at 12 weeks of age and organ weights,

such as adrenal, thymus and spleen, were measured. The adrenal and thymus were fixed in 10% neutral buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3 to 5 μm). The sections were stained with hematoxylin and eosin (HE).

2.4 Statistical Analysis

Results of biological parameters and organ weights were expressed as the mean±standard deviation. Statistical analysis of differences between mean values was performed using the F-test, followed by the Student's t-test or Aspin-Welch's t-test (Stat Light 2000; Yukms Co., Ltd.). Differences were defined as significant at $p < 0.05$.

3. RESULTS

Changes in biological parameters are shown in Figs. 1 and 2. Body weights and blood glucose levels in male SDT fatty rats periodically increased after 6 weeks of age, but IS did not significantly affect those levels (Figs. 1A and 1C). The food intakes and the other blood chemical parameters, such as TG, TC, and insulin levels, in the IS group were comparable as compared with those in the control group (Figs. 1B, 1D, 1E and 1F). In female SDT fatty rats, the blood glucose levels gradually increased after 6 weeks of age. The blood glucose level in the IS group was significantly increased at 12 weeks of age as compared with that in the control group (IS group; 690.6±55.8 mg/dl, Control group; 449.0±138.1 mg/dl, 53.8% increase when compared with the control group, Fig. 2C). The other biological parameters in the IS group were not significantly different with those in the control group (Figs. 2A, 2B, 2D, 2E and 2F).

In male SDT fatty rats, an increase of adrenal weight and a decrease of thymus weight were observed in the IS group as compared with those in the control group (18.7% increase in adrenals and -24.5% decrease in thymus when compared with the control group, respectively, (Table 1). Also, female SDT fatty rats in the IS group showed a tendency of an increase of adrenal weight and a decrease of thymus weight (Table 2). Moreover, in histopathological analysis, adrenal hypertrophy and thymus atrophy were observed in the IS group in both male and female rats. In the adrenal cortex in the rats, hypertrophy of zona fasciculata was observed in the IS group (Fig. 3).

4. DISCUSSION

In female SDT fatty rats, the blood glucose levels in the IS group were significantly increased at 12 weeks of age as compared with those in the control group, suggesting that isolation stress accelerated the progression of diabetes in female SDT fatty rats.

It is reported that isolation stress alters hepatic gene expression profiles, especially with reference to lipid metabolism in male BALB/c mice [13]. The gene expression levels involved in the lipid biosynthetic process, such as sterol regulatory element-binding factor (SREBF) 1, fatty acid synthase (FAS), and elongation of very long chain fatty acids protein (ELOVL) 6, were elevated significantly. In this study, however, lipid parameters in the IS group were comparable as compared with those in the control group. In further study, it is necessary to examine the gene expression in liver in SDT fatty rat exposed to social stress. There were no differences in the TG and TC levels between control and IS groups in both sexes.

Table 1. Effect of housing condition on organ weights in male SDT fatty rats at 12 weeks of age

	Adrenal	Thymus	Spleen
Absolute weight (mg)			
Control group	64.8±6.1	560.9±73.0	1210.9±95.2
IS group	76.9±4.3**	423.6±51.3**	1175.9±100.3
Relative weight (mg/g)			
Control group	0.145±0.009	1.263±0.187	2.720±0.198
IS group	0.171±0.014**	0.938±0.116**	2.601±0.192

*Data represents mean±standard deviation (n=5 or 6). **p<0.01; significantly different from Control group*

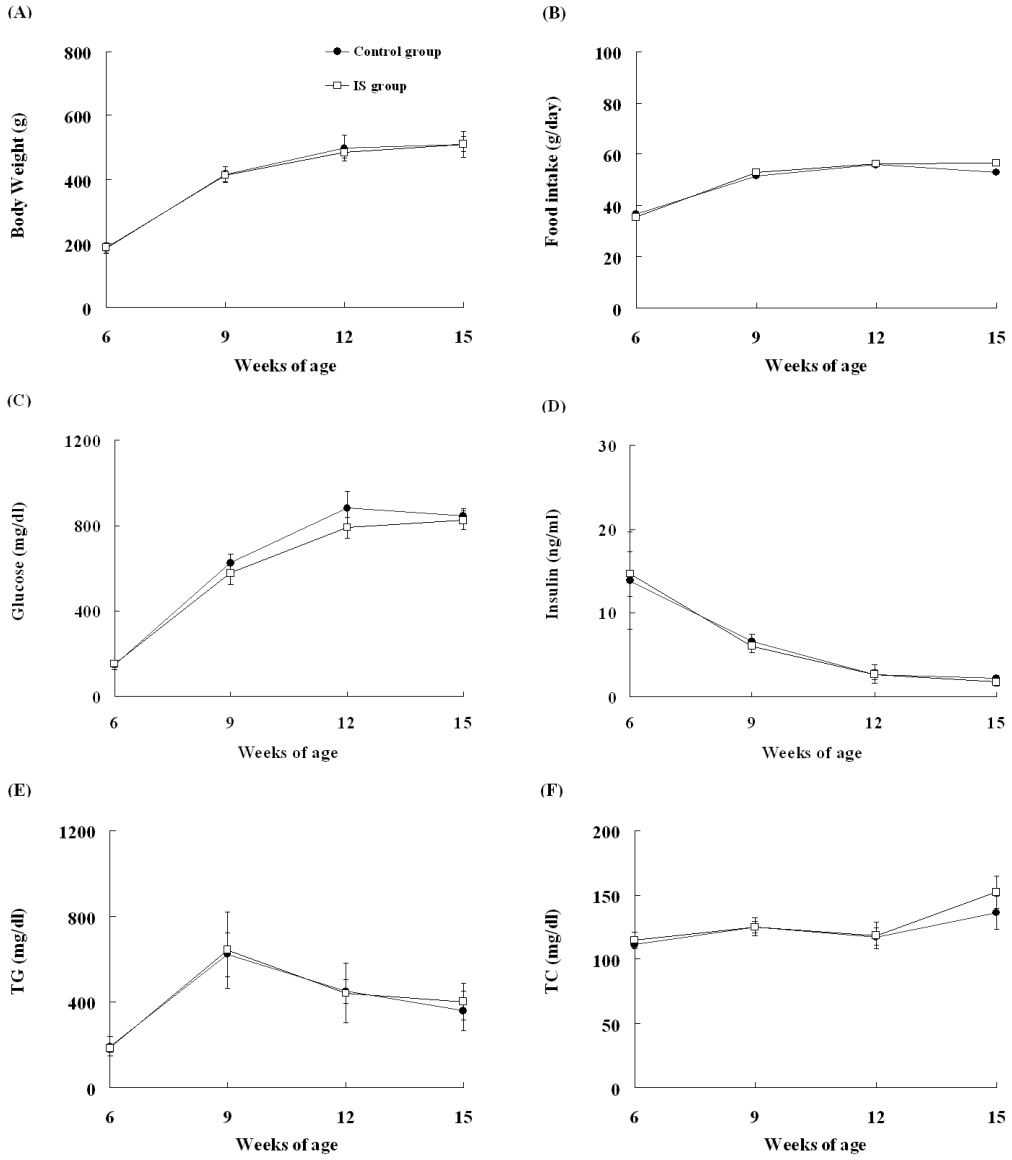

Fig. 1. Changes in biological parameters (A, Body weight; B, Food intake; C, Glucose; D, Insulin; E, TG; F, TC) in control group and isolation stress (IS) group of male SDT fatty rats

Data (except for food intake) represent mean ± standard deviation (n=5 or 6)

Table 2. Effect of housing condition on organ weights in female SDT fatty rats at 12 weeks of age

	Adrenal	Thymus	Spleen
Absolute weight (mg)			
Control group	56.1±6.5	640.9±60.7	1055.8±119.2
IS group	62.6±5.6	595.7±72.6	987.0±107.4
Relative weight (mg/g)			
Control group	0.149±0.016	1.702±0.060	2.803±0.175
IS group	0.166±0.015	1.582±0.207	2.610±0.163

Data represents mean±standard deviation (n=5 or 6)

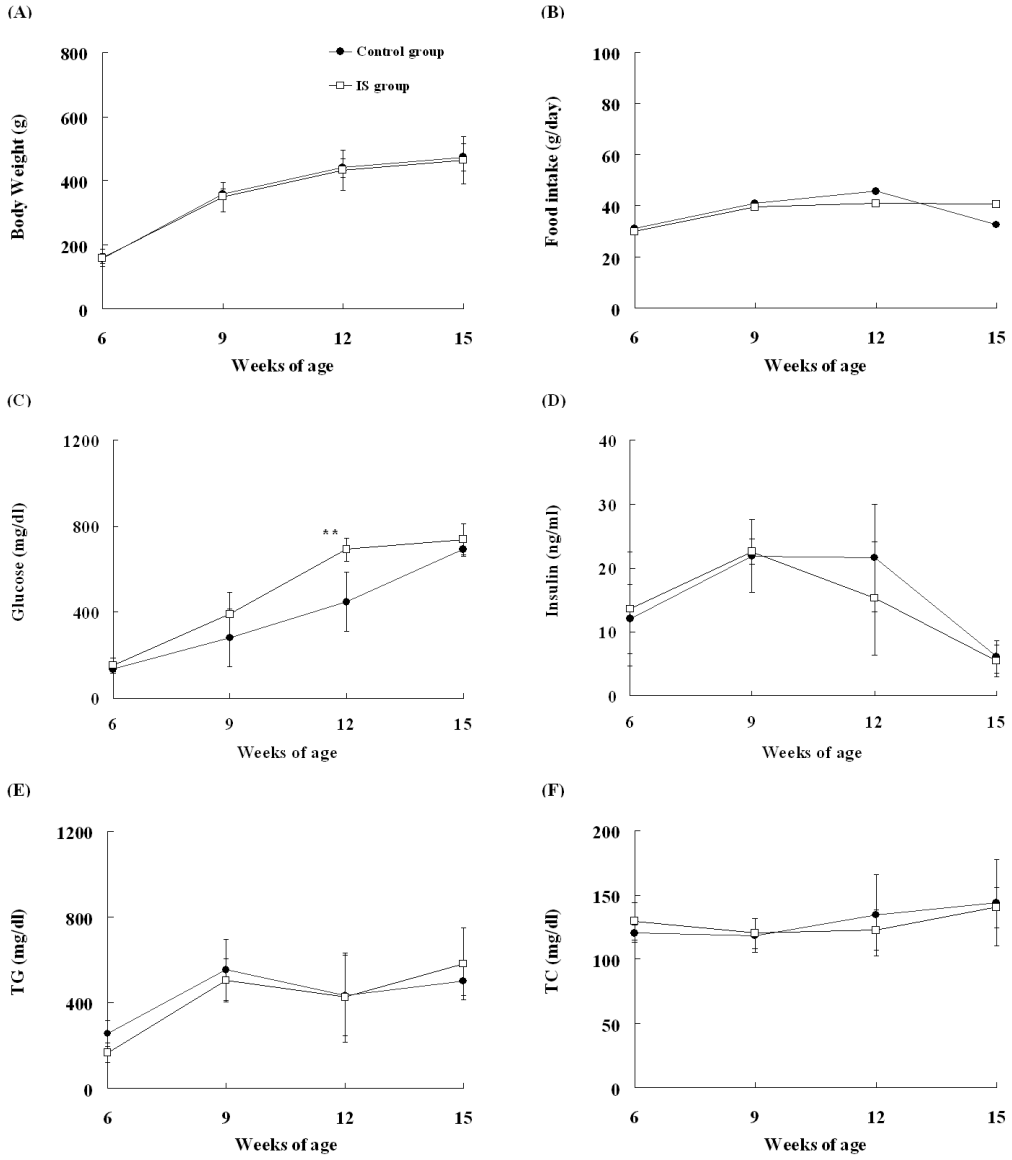

Fig. 2. Changes in biological parameters (A, Body weight; B, Food intake; C, Glucose; D, Insulin; E, TG; F, TC) in control group and isolation stress (IS) group of female SDT fatty rats

Data (except for food intake) represent mean±standard deviation (n=5 or 6)
*** p<0.01; significantly different from the control*

In male SDT fatty rats, an increase of adrenal weight and a decrease of thymus weight were observed in the IS group and the female rats in the IS group showed a tendency of an increase of adrenal weight and a decrease of thymus weight. Those changes of organ weights were considered to be caused by isolation stress. Similar responses in mice have been reported [13,14]. Moreover, in histopathological analysis, hypertrophy of the adrenal cortex was observed in the IS group. Adrenal hypertrophy was observed in mice exposed to isolation stress for 7 or 30 days. The thymus and spleen weights in the mice exposed to isolation stress indicated a lower tendency than those in the control mice. Isolation stress is considered to induce the activation of the sympatho-adrenomedullary system or the hypothalamus-pituitary-adrenal (HPA) axis in both male and female SDT fatty rats, but the deterioration of glucose metabolism

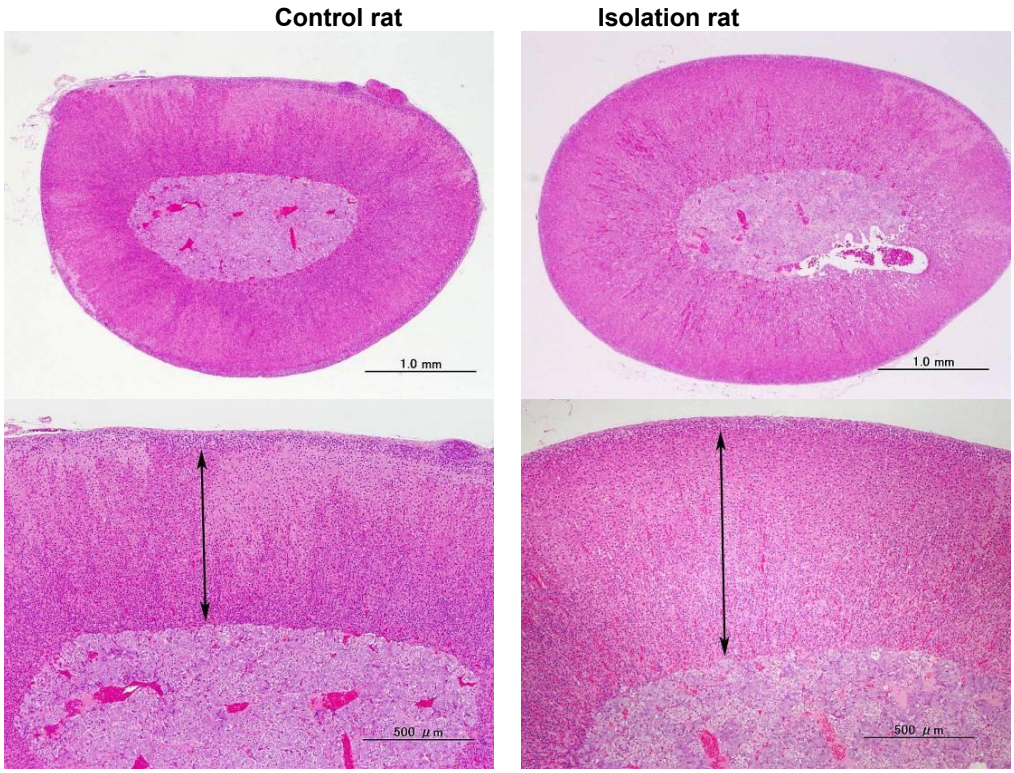

Fig. 3. Histopathological analysis of adrenal; Control rat and IS rat at 12 weeks of age. HE stain. Arrows: zona fasciculata in the adrenal cortex

was observed only in the female SDT fatty rats. Since the progression of diabetes in the male rats was more severe than that in the female rats, it is considered that it was difficult to observe the effects of isolation stress on glucose/lipid metabolism in male SDT fatty rats. Blood corticosterone levels in the IS group were not significantly different from those in the control group (data not shown). Other studies showed that feeding under stress is induced insulin secretion for remodeling energy stores from muscle to fat, especially in the abdominal region [15,16] and fasting/starvation activates the HPA axis to increase circulating glucocorticoid levels [17]. Therefore, the corticosterone levels follow a circadian rhythm, it was considered necessary to examine the fluctuation of the levels within a day.

5. CONCLUSION

Isolation stress affected the progression of diabetes in female SDT fatty rats. Housing conditions constitute a factor to take care for in evaluation of pathophysiology in diabetic models.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal protocols used in this study were strict compliance with our own Laboratory Guidelines for Animal Experimentation. This study was conducted from May 2011 to April 2012.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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