Pathophysiologival Changes by Short-term Food Restriction in Obese Diabetic 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: Reduced calorie intake by food restriction leads to extension of life span by exerting beneficial effects on metabolism. Zucker diabetic fatty (ZDF) rat is a type 2 diabetic model with obesity, showing the incidence of diabetes after 7 weeks of age. The present study investigated effects of 2-week food restriction on the pancreas in ZDF rats.

Methods: ZDF rats were pair-fed with Zucker lean (ZL) rats from 7 to 9 weeks of age (The amount of food in ZDF rats was calculated based on the daily food consumption in ZL rats). Body weight and biochemical parameters such as serum glucose, triglyceride, total cholesterol, non-esterified fatty acid and insulin levels, were evaluated. After necropsy, islet size and insulin content in pancreas were measured.

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**Results:** The ZDF rats showed increased blood glucose, insulin and lipid levels at 7 weeks as compared with those in ZL rats. After food restriction for 2 weeks, the blood glucose, insulin, and triglyceride levels in pair-fed ZDF rats were decreased. The islet size in pair-fed ZDF rats decreased as compared with that in ad lib-fed ZDF rats, but the insulin content in pancreas of pair-fed ZDF rats increased remarkably. **Conclusion:** ZDF rats showed improvement of pancreatic disorders by food restriction. Caloric restriction applied at an insulin resistant pre-diabetic stage is an effective means for preventing the pancreatic disorder in diabetes with obesity.

**Keywords:** Food restriction; pancreas; ZDF rat.

**1. INTRODUCTION**

Diabetes has become a global health problem and the incidence of the disease is increasing rapidly in all regions of the world. Type 2 diabetes is brought on by environmental and behavioral factors such as a sedentary life style, overly rich nutrition and obesity [1,2]. In a meta-analysis, lifestyle interventions reduced diabetes mellitus by one-half, and pharmacologic interventions by one-third [3]. Energy intake in excess of energy expenditure induces increase in body weight. Obesity increases the risks of certain diseases, such as hypertension, hypercholesterolemia, hypertriglyceridemia and increased insulin resistance [4-6]. On the other hand, either reduced calorie intake or increased energy expenditure can reduce the body weight, which leads to disappearance of metabolic dysfunction associated with obesity [7]. In rodents, it is also known that food restriction or increased energy expenditure by exercise extends lifespan by exerting beneficial effects on metabolism [8-10]. The Zucker diabetic fatty (ZDF) rats, derived from inbreeding of hyperglycemic Zucker obese rats, has marked hyperglycemia along with insulin resistance, hyperlipidemia and obesity, potentially making it useful as a model of human type 2 diabetes [11,12]. Beneficial effects of food restriction were reported in mice regarding improvement of metabolic health and cellular aging through decreasing hepatic mTOR activity [14]. This information was also considered to be related to ameliorate of diabetes with islet protective action by food restriction. In the present study, we investigated effects of short-term food restriction on pancreas 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 and Niigata University. Ten males of ZDF rats and age-matched male Zucker lean (ZL) rats (Charles River Japan, Yokohama, Japan) were used. ZDF rats at 7 weeks of age were divided into two groups (n=5 in each group): 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 (n=5) from 7 to 9 weeks of age (Fig. 1).

![Fig. 1. Study design, ZDF rats: Male Zucker diabetic fatty rats, at 7 weeks of age were divided into two groups (ad libitum and pair-fed). ZL rats: Male Zucker lean rats](image-url)
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 amount of the food in the restricted group in the ZDF rats was calculated based on the daily food intake in ZL rats. The rats were housed individually in suspended bracket cages in a climate-controlled room with a temperature of 23±3°C, a humidity of 55±15%, and a 12 hours 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 at 7 and 9 weeks of age 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 Pancreatic Islet Cell Size

Measurement of pancreatic islet size was conducted at 9 weeks of age. Animals were euthanized by exsanguination under isoflurane anesthesia. After animals were sacrificed, the pancreas (pancreatic tail) was removed promptly and fixed in 10% neutral buffered formalin. The tissue was paraffin-embedded by standard techniques and cut into thin sections (3 to 5 µm). The sections were treated with hematoxylin and eosin (HE) staining. The islet size on one slide per animal was examined histopathologically and evaluated by another pathologist in a blind manner in accordance with the evaluation criteria. All islets on slides were circled for image analysis and islet size was calculated using the Win ROOF Ver. 5.01 software (Mitani Corporation, Fukui, Japan).

2.4 Measurements of Insulin Content in Pancreas

Measurement of insulin content in the pancreas was conducted at 9 weeks of age. Pancreatic insulin was extracted by the acid/ethanol extraction method. Briefly, after the animals were sacrificed, the pancreas (pancreatic head) was removed and promptly homogenized in a cold acid / ethanol mixture (75% ethanol, 23.5% distilled water, 1.5% 2N hydrochloric acid) to extract insulin. The levels of insulin in the extract were measured as described above.

2.5 Statistical Analysis

The results of biological parameters are expressed as the mean±standard deviation (SD). Statistical analysis of differences between mean values was performed using an F-test was first performed, when the variance was homogeneous Student’s t-test was applied or when the variance was heterogeneous Aspin-Welch’s t-test was applied (StatLight 2000; Yukms Co., Ltd.). Differences were considered significant at p < 0.05.

3. RESULTS

In ZDF rats at 7 weeks of age, body weight, serum insulin and lipid levels increased as compared with those in ZL rats (Table 1). One ZDF rat showed an increase of serum glucose level. Hyperphagia was also observed in the ZDF rats (data not shown). Serum glucose level in ZDF rats increased significantly as compared with that in ZL rats (Table 1). After food restriction for 2 weeks, in pair-fed ZDF rats, serum glucose, TG and insulin levels decreased significantly as compared with those in ad lib ZDF rats (Table 2). In particular, the elevation of glucose levels was perfectly inhibited. On the other hand, the serum TC level in pair-fed ZDF rats increased as compared with that in ad lib ZDF rats. The NEFA level in pair-fed ZDF rats tended to increase, but not significantly, as compared with that in ad lib ZDF rats.

Islet size in ad lib ZDF rats increased by 2.7-folds at 9 weeks of age, as compared with ZF rats (P value = 0.0021, Figs. 2). The islet size in pair-fed ZDF rats decreased after food restriction for 2 weeks, as compared with that in ad lib ZDF rats (ad lib ZDF rats; 34731±470 µm², pair-fed ZDF rats; 24726±2573 µm², P value = 0.0051). Pancreatic islets in ad lib ZDF rats exhibited multiple irregular projections (Fig. 2B), however, small sized islet and ameliorated multiple irregular projections were observed in pair-fed
ZDF rats (Fig. 2C). Insulin content in ad-lib ZDF rats tended to decrease, but not significantly, as compared with that in ZL rats (Fig. 3). The insulin content in pair-fed ZDF rats was elevated by 2.9-folds after food restriction, as compared with that in ad-lib ZDF rats (ad-lib ZDF rats; 29.2±12.5 µg/g pancreas, pair-fed ZDF rats; 85.1±6.1 µg/g pancreas, P value = 0.00002).

Table 1. Baseline level of body weights and serum parameters in ZDF and ZL rats at 7 weeks of age

|                | Body weight (g) | Glucose (mg/dl) | TG (mg/dl) | TC (mg/dl) | NEFA (µEq/l) | Insulin (ng/ml) |
|----------------|-----------------|-----------------|------------|------------|--------------|-----------------|
| ZDF rat       | 230.9±7.0**     | 180.4±59.3      | 380.5±30.2** | 110.9±5.4** | 402.9±65.3    | 26.3±5.8**      |
| ZL rat        | 167.3±1.8       | 131.2±6.5       | 77.7±25.5  | 88.5±1.4   | 336.7±54.0    | 0.9±0.3         |

Data represent mean ±SD (n=5). *P<0.05, **P<0.01; significantly different from the ZL rats.

Table 2. Effects of the food restriction on the body weights and serum parameters in ZDF, pair-fed ZDF, and ZL rats at 9 weeks of age

|                | Body weight (g) | Glucose (mg/dl) | TG (mg/dl) | TC (mg/dl) | NEFA (µEq/l) | Insulin (ng/ml) |
|----------------|-----------------|-----------------|------------|------------|--------------|-----------------|
| ZDF rat       | 395.1±27.6##    | 430.2±45.2##    | 927.4±103.3## | 105.0±7.5## | 454.2±70.4    | 17.8±5.9##      |
| Pair-fed ZDF  | 321.8±9.6**     | 112.2±4.7**     | 388.0±131.1** | 133.8±7.7** | 795.8±332.9   | 4.7±1.1**       |
| ZL rat        | 283.9±9.3       | 132.6±10.0      | 100.8±44.5 | 82.0±6.6   | 373.6±163.9   | 2.0±0.5         |

Data represent mean ±SD (n=5). ##P<0.01; significantly different from the ZL rats, **P<0.01; significantly different from the ZDF rats.

Fig. 2. Islet size in ZDF, pair-fed ZDF (ZDF-PF) and ZL rats (A). The islet size were measured at 9 weeks of age. Data shown as mean ±SD (n=5). ** p<0.01; significantly different from the ZDF rat. ##p<0.01; significantly different from the ZL rat. Pancreas in (B) ZDF, (C) pair-fed ZDF and (D) ZL rats at 9 weeks of age. HE stain. Bar = 100 µm. Pancreatic islets in ZDF rats exhibited multiple irregular projections (B), small sized and ameliorated islet figure in pair-fed ZDF rats (C).
Fig. 3. Insulin content in ZDF, pair-fed ZDF (ZDF-PF) and ZL rats. The insulin content were measured at 9 weeks of age. Data shown as mean ±SD (n=5). ** p<0.01; significantly different from the ZDF rat

4. DISCUSSION

In this study, the ZDF rats showed hyperlipidemia and hyperinsulinemia at 7 weeks of age and hyperglycemia at 9 weeks of age. Moreover, the insulin levels in ZDF rats decreased from 7 to 9 weeks of age. It was also reported that ZDF rats showed hyperglycemia and hyperlipidemia after 8 or 9 weeks of age. Blood insulin levels in ZDF rats accompanied by insulin resistance reached a peak at 10 weeks of age and decreased gradually after 10 weeks of age [13]. In these obese diabetic rats accompanied by insulin resistance, large sized islet was observed in ZDF rats, on the one hand, small sized islet was noted in Spontaneously Diabetic Torii fatty (SDT fatty) rats. Serum glucose, TG and insulin levels in ZDF rats decreased after food restriction for 2 weeks. In other obese diabetic models, such as Zucker fatty (ZF), Otsuka Long Evans Tokushima fatty (OLETF) and SDT fatty rats, the hyperglycemia and the hypertriglyceridemia were also inhibited by food restriction [15-18]. In ZF and OLETF rats, restriction of food or calorie intake resulted in sustained or stable suppression of hyperinsulinemia [15,17]. Moreover, SDT fatty rats showed suppression of hyperinsulinemia temporarily [18,19]. In pair-fed ZDF rats, hyperglycemia and hypertriglyceridemia were ameliorated, but the hypercholesterolemia was not improved by food restriction. The hypercholesterolemia may be caused by the leptin signal abnormality in liver and an increase in cholesterol absorption, as shown in pair-fed SDT fatty rats [19].

Islet size in ZDF rats decreased after food restriction for 2 weeks, and the insulin content increased significantly. It is considered that a decrease of calorie intake prevents the exhaustion of pancreas in ZDF rats, resulting in the increase of insulin content. In SDT fatty rats, food restriction induced an increase of islet size [18]. The result of islet size in pair-fed ZDF rats was significantly larger than that in pair-fed SDT fatty rats. Since the blood insulin level in pair-fed ZDF rats decreased after food restriction (Table 2), the decrease of islet size is considered to be related with a decreased of the blood insulin level in the rats. Increases of insulin content after food restriction were also observed in other diabetic models. In SDT fatty rats, the insulin content after food restriction increased by 4.2-folds [18]. These islet findings were also considered to be related pancreas-protective action by food restriction in diabetic patients with obesity.
5. CONCLUSION

The islet size in pair-fed ZDF rats decreased, but the insulin content in the pancreas increased. ZDF rats showed improvement of pancreatic disorders by food restriction. Caloric restriction applied at an insulin resistant pre-diabetic stage is an effective means for preventing the pancreatic disorder in diabetes with obesity.

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 which is based on the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aguiar EJ, Morgan PJ, Collins CE, Plotnikoff RC, Callister R. Efficacy of interventions that include diet, aerobic and resistance training components for type 2 diabetes prevention: A systematic review with meta-analysis. Int J Behav Nutr Phys Act. 2014;11:2.
2. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001;414:782-787.
3. Gillies CL, Abrams KR, Lambert PC, Cooper NJ, Sutton AJ, Hsu RT, Khunti K. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. BMJ. 2007;334:299.
4. Taskinen MR, Borén J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis. 2015;239:483-495.
5. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: A dose-response study. Metabolism. 1990;39:452-459.
6. Rexrode KM, Manson JE, Hennekens CH. Obesity and cardiovascular disease. Curr Opin Cardiol. 1996;11:490-495.
7. Brown T, Avenell A, Edmunds LD, Moore H, Whittaker V, Avery L, Summerbell C. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. Obes Rev. 2009;10:627-638.
8. Masoro EJ. Caloric restriction and aging: An update. Exp Gerontol. 2000;35:299-305.
9. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. J Nutr Biochem. 2005;16:129-137.
10. Parizkova J. Interaction between physical activity and nutrition early in life and their impact on later development. Nutr Res Rev. 1998;11:71-90.
11. Peterson RG, Shaw WN, Neel MA, Little LA, Eichberg J. Zucker diabetic fatty rat as a model for non-insulin-dependent diabetes mellitus. ILAR News. 1990;32:16-19.
12. Clark JB, Palmer CJ, Shaw WN. The diabetic Zucker fatty rat. Proc Soc Exp Biol Med. 1983;173:68-75.
13. Bates HE, Kiraly MA, Yue JTY, Montes DG, Elliott ME, Riddell MC, et al. Recurrent intermittent restrain delays fed and fasting hyperglycemia and improves glucose return to baseline levels during glucose tolerance tests in the Zucker diabetic fatty rat-role of food intake and corticosterone. Metabolism. 2007;56:1065-1075.
14. Schloesser A, Campbell G, Glüer CC, Rimbach G, Huebbe P. Restriction on an energy-dense diet improves markers of metabolic health and cellular aging in mice through decreasing hepatic mTOR activity. Rejuvenation Res. 2015;18:30-39.
15. Maddox DA, Alavi FK, Santella RN, Zawada ET. Prevention of obesity-linked renal disease: Age-dependent effects of dietary food restriction. Kidney Int. 2002;62:208-219.
16. Koide N, Oyama T, Miyashita Y, Shirai K. Effects of calorie-restricted low-carbohydrate diet on glucose and lipid metabolism in Otsuka Long Evans Tokushima fatty rats. J Atheroscler Thromb. 2007;14:253-260.
17. Kimura M, Shinozaki T, Tateishi N, Yoda E, Yamauchi H, Suzuki M, et al. Adiponectin is regulated differently by chronic exercise than by weight-matched food restriction in...
hyperphagic and obese OLETF rats. Life Sci. 2006;79:2105-2111.
18. Ishii Y, Ohta T, Sasase T, Morinaga H, Miyajima K, Kakutani M. Effect of food restriction on pancreatic islets in Spontaneously Diabetic Torii fatty rats. J Vet Med Sci. 2011;73:169-175.
19. Matsui K, Ohta T, Morinaga H, Sasase T, Fukuda S, Ito M, et al. Effects of preventing hyperphagia on glycolipid metabolic abnormalities in Spontaneously Diabetic Torii fatty rats. Anim Sci J. 2008;79:605-613.

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