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Title: Effects of repetitive or consecutive fasting-induced weight loss on glucose tolerance in rats fed a high-fat diet

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Abstract

Weight loss reduces visceral fat and improves glucose tolerance. Our previous study found that weight loss from 3 consecutive days of fasting led to deteriorated glucose tolerance, but it is not clear whether this was due either to the physiological stress associated with consecutive fasting or that fasting itself. This study aimed to compare the effects of repetitive or consecutive fasting on intra-abdominal fat mass and glucose tolerance in rats fed a high-fat diet. After 2 weeks of high-fat diet feeding, male Wistar rats were divided into three groups matched for body weight: one group continued to receive the high-fat diet ad libitum for 2 weeks (control, CON); the second group fasted for the last 3 days (consecutive fasting, CF); and the third group repeated 1-day fasting three times with a 6-day ad libitum feeding interval (repetitive fasting, RF). Compared with the CON group, the intra-abdominal fat mass was significantly lower in the CF group after the intervention period, and there was a tendency for lower values in the RF group. During the oral glucose tolerance test, plasma glucose level was significantly higher in both fasting groups compared with the CON group, while that in the CF group was significantly higher than that in the RF group. Compared with the CON group, the CF group had significantly lower plasma insulin level, with a tendency for lower levels in the RF group. These findings suggest that even when fasting days are dispersed over multiple occasions, insulin secretion capacity may decrease in a similar manner to consecutive fasting, leading to a deterioration in glucose tolerance.

Keywords; weight loss, fasting, glucose tolerance

分散型および連続型絶食が高脂肪食負荷ラットにおける糖代謝機能に及ぼす影響

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我々はこれまでの研究により，連続3日間の絶食による急速な減量が全身の糖代謝機能を悪化させることを明らかにしてきた．その際，連日にわたり絶食を行うことで，生体に大きな負担がかかり，糖代謝機能が悪化したという可能性が考えられる．そこで本研究では，絶食日を数回に分散し 1 回の絶食に伴う負担を軽減することで，糖代謝機能に対する悪影響をなくすことができるか検討することとした．6 週齢の Wistar 系雄性ラットに高脂肪食を 2 週間摂取させた後，1）引き続き高脂肪食を 2 週間自由摂取させる群（CON 群），2）11 日間は高脂肪食を自由摂取させ，最後の 3 日間連続して絶食させる群（CF 群），3）絶食を週 1 日，合計 3 回に分散して行わせる群（RF 群）の 3 群に分けた．飼育期間終了後に経口糖負荷試験を行い全身の糖代謝機能を評価した．その結果，飼育期間終了後の体重および摂餌量は CON 群と RF 群の間に有意な差は認められなかったが，CF 群における終体重および摂餌量は CON 群および RF 群と比較して有意に低い値を示した．腹腔内脂肪量は CON 群と比較して CF 群では有意に低い値を示し，RF 群においても低値を示す傾向が認められた．経口糖負荷試験時の血漿グルコース濃度は両絶食群で CON 群と比較して有意に高い値を示し，CF 群では RF 群と比べても有意に高い値を示した．一方，血漿インスリン濃度は，CON 群に比べて CF 群では有意に低い値を示し，RF 群においても低値を示す傾向が認められた．以上のことから，絶食日を数回に分散し，1 回の絶食に伴う負担を軽減させたとしても，数日間連続して絶食した場合と同様にインスリンの分泌能力が低下し，全身の糖代謝機能が悪化する可能性が示唆された．

Introduction

The skeletal muscle, which constitutes 40% of human body weight, is the largest metabolic tissue and is responsible for more than 80% of insulin-mediated glucose uptake [1]. It has been reported that diabetic patients exhibit a significant decrease in the glucose disposal of skeletal muscles, and this is considered to be one of the main causes of diabetes [1]. Additionally, the accumulation of excess fat is also a well-known contributor to the progression of the disease, since hypertrophic adipose tissue secretes TNF-α and FFA, which induce insulin resistance in skeletal muscle [2]. Furthermore, it has become evident that excessive accumulation of visceral fat is implicated in the deterioration of glucose uptake in skeletal muscles [3]. Therefore, reducing visceral fat is of paramount importance in the prevention and treatment of diabetes.

Excessive accumulation of visceral fat arises from an imbalance wherein energy intake exceeds energy expenditure, and thus a weight loss strategy that decreases the amount of food intake is effective in reducing visceral fat mass. Weight loss methods involve either a slow approach that involves restricting daily energy intake (i.e., calorie restriction; CR) or a rapid approach that involves fasting for several days [4]. Although many studies have reported that CR has positive effects on skeletal muscle and glucose tolerance [5, 6, 7, 8], it was not clear whether fasting was as effective a weight-loss method as CR. Therefore, in our previous study, we compared skeletal muscle and whole-body glucose metabolism in obese rats under conditions where body weight was equivalently reduced by following two distinct weight loss methods: a 30% daily energy restriction over a 2-week period (CR method) and a 3-day fasting (FAST method) [9]. The results revealed that with the FAST method, weight and visceral fat reduction were achieved within a few days and to a similar extent as the CR method. Moreover, while the FAST method increased the GLUT-4 content in skeletal muscles, thereby enhancing skeletal muscle glucose uptake capacity, the FAST method appeared to decrease insulin secretion, leading to a decline in whole-body glucose tolerance [9]. However, it remains unclear whether the observed 3-day fasting-induced deterioration in glucose tolerance was a consequence primarily of prolonged periods of fasting (i.e., consecutive days of fasting) or if it is inherently attributable to the fasting itself. Therefore, this study aimed to investigate whether repetitive fasting mitigates the adverse effects on glucose metabolism associated with consecutive days of fasting in rats fed a high-fat diet.

Methods

Animals

Five-week-old male Wistar rats were obtained from CLEA Japan (Tokyo, Japan) and individually housed in stainless steel cages in a controlled environment with a temperature of 22°C ± 2°C and a 12-h light–dark cycle (lights on from 9 AM to 9 PM). The rats were fed powdered rodent diet ad libitum (CE-2, CLEA Japan) and water. After 1 week of acclimation, all rats were fed a high-fat diet (Table 1). Previous research has indicated that rats developed insulin resistance after being fed a high-fat diet for 2-4 weeks [10, 11]. Therefore, following a 2-week high-fat diet to induce obesity, the animals were divided into three groups, matched for body weight and food efficiency: a control group (CON; n = 5), a consecutive fasting group (CF; n = 5), and a repetitive fasting group (RF; n = 5). The CON group continued to have *ad libitum* access to the high-fat diet for 2 weeks. The CF group had ad libitum access to the high-fat diet for 11 days, followed by 3 days of fasting. The RF groups were made to fast for 1 day in a given week, for a total of 3 times (1 day fasting on the day of grouping, day 7, and day 14). All rats

Table 1

were permitted *ad libitum* access to water throughout the 14-day intervention. Body mass and food intake were recorded during the dietary intervention. The experimental protocol was approved by the Animal Experimental Committee of The University of Tokyo (No. 26-26).

Oral glucose tolerance test (OGTT)

After the 2-week experimental period, the oral glucose tolerance test (OGTT) was conducted. To eliminate the influence of recent food intake, food was removed from the CON group 6 h before the experiment. Glucose was administered orally at a dose of 2 g per kg of body weight, and blood samples were collected from the tail vein immediately before administration and at 30, 60, 90, and 120 min after administration. The capillary tubes were then centrifuged at 10,000 × g for 10 min. Plasma glucose and insulin concentrations were measured using the Glucose CII test kit (Wako Pure Chemical Industries, Osaka, Japan) and an ELISA kit (Mercodia AB, Uppsala, Sweden), respectively. The trapezoidal rule was used to calculate the total areas under the curve for plasma glucose and insulin.

Statistical analysis

Data are presented as the mean ± standard error. Welch's analysis of variance (ANOVA) was used for between-group comparisons, followed by post hoc multiple comparisons using the Games–Howell test. In the experiment that involved OGTT, two-way analysis of variance was performed, followed by Tukey's post hoc test. All statistical analyses were performed using GraphPad Prism version 10.1.0 Software (GraphPad, San Diego, CA, USA). Statistical significance was defined as $p < 0.05$.

Results

Body weight, total intra-abdominal fat weight, and total food intake

Changes in body weight throughout the intervention period are shown in Figure 1. Final body weight was significantly lower in the CF group compared with both the CON group and the RF group (CON vs CF, $p < 0.01$; CF vs RF, $p < 0.05$; Table 2). Although body weight decreased with each fasting day in the RF group, there was no significant difference compared with the CON group due to the substantial food intake after each fasting period. Intra-abdominal fat mass was significantly lower in the CF group compared with the CON group ($p < 0.01$; Table 2), and the RF group tended to have lower intra-abdominal fat mass compared with the CON group ($p = 0.08$), with no significant differences between the CF and RF groups. Although total food intake did not significantly differ between the CON and RF groups, total food intake in the CF group was significantly lower than that in the CON and RF groups (CON vs CF, $p < 0.05$; CF vs RF, $p < 0.05$; Table 2).

Table 2

Figure 1

Oral glucose tolerance test

In the two fasting groups, the pre-administration blood glucose levels were significantly lower compared to the CON group (CON vs CF, $p < 0.01$; CON vs RF, $p < 0.01$; Fig. 2A), and then rose to a level similar to that of the CON group 60 minutes after glucose administration. Furthermore, in the CF group, plasma glucose concentrations at 90 and 120 minutes after glucose administration were significantly higher compared to the CON and RF groups (Fig. 2A). Plasma glucose area under the curve (AUC) values were significantly higher in both fasting groups compared with the CON group. (CON vs CF, $p < 0.01$; CON vs RF, $p < 0.05$; Fig. 2B). Additionally, the plasma glucose AUC values in the CF group were significantly higher than those in the RF group (CF vs RF, $p < 0.05$; Fig. 2B).

The plasma insulin concentrations after glucose administration were lower in the two fasting groups compared with the CON group (Fig. 2C). Insulin AUC value in the CF group was significantly lower than that in the CON and RF groups (CF vs CON, $p < 0.05$; CF vs RF, $p < 0.05$; Fig. 2D). The insulin AUC value in the RF group showed a tendency toward lower values compared with the CON group ($p = 0.06$).

Figure 2

Discussion

Our previous study revealed that fasting for 3 days deteriorates glucose tolerance. We investigated whether the negative effects of fasting on glucose metabolic capacity could be eliminated by repetitive fasting on 3 separate days. In the RF group, intra-abdominal fat mass was reduced and glucose tolerance was impaired via lower insulin secretion, as in the CF group. Thus, it was suggested that even if the duration of each fasting period was shortened, repetitive fasting may worsen whole-body glucose metabolism.

It has been reported that more than 80% of carbohydrates ingested through diet and other means are processed by skeletal muscle, and that skeletal muscle glucose uptake capacity is reduced in patients with type 2 diabetes [1)]. Regarding the glucose uptake capacity of skeletal muscle, it has also been reported that there is a negative correlation between visceral fat mass and glucose uptake capacity in skeletal muscle [3)], suggesting the importance of reducing visceral fat mass in order to improve the whole-body glucose metabolism. As shown in Table 2, in the CF group, which underwent three consecutive 3 days of fasting, a significant reduction in intra-abdominal fat mass was observed compared to the CON group. Additionally, although not statistically significant compared to the CON group, there was a trend towards lower intra-abdominal fat mass in the RF group, which repeated one day of fasting three times (p=0.08). While the total food intake was significantly lower in the CF group compared to the CON group, no significant difference was observed in the RF group. As shown in Fig. 1, the body weight in the RF group substantially increased due to refeeding after fasting, suggesting that the final body weight and intra-abdominal fat mass in the RF group reflect the outcome of the last fasting period. However, Izumida et al. (2013) have reported that subjecting mice to a 24-hour fasting period activates the liver–brain–adipose neural axis due to the hepatic sensing glycogen depletion, resulting in a shift in the energy source from glucose to fat [12]. Therefore, even a 1-day fasting may lead to effective reduction in intra-abdominal fat mass.

Given the indication that weight reduction through repeated fasting may not lead to weight loss as significant as that achieved through consecutive fasting, it nonetheless suggested the potential for reducing intra-abdominal fat mass. Therefore, glucose tolerance was examined by the OGTT. The results showed that glucose AUC values were significantly higher in the CF group than in the CON group. In addition, in the RF group, although glucose AUC values during the OGTT were lower than those in the CF group, the glucose AUC value was higher compared with the CON group. Therefore, it is suggested that weight reduction involving fasting may potentially impair whole-body glucose metabolism, regardless of changes in the method of fasting. To clarify the cause of these results, we examined insulin concentration during OGTT and found that insulin secretion was decreased in the two fasting groups (Fig. 2C, D). Therefore, it has been suggested that, similar to consecutive fasting, repetitive fasting also impairs glucose tolerance by reducing insulin secretion.

In this study, differences in glucose tolerance occurred between the CF and RF groups, even though the number of fasting days was the same. The mechanism for these differences is not clear, but the following possibilities may account for this phenomenon. Insulin secretion during the OGTT were significantly higher in the RF group than in the CF group (Fig. 2C, D), suggesting that insulin secretion capacity may have been preserved in the RF group. Insulin secretion is regulated by several events starting with the influx of glucose through glucose transporters. GLUT-2 is the major glucose transporter isoform in rodent pancreatic β [13] cells, and GLUT-2 in the pancreas is thought to play an important role in insulin secretion [14, 15,16]. In addition, it has been reported that with an increasing duration of fasting (i.e., in hypoglycemic experiments where rats were infused with either normal saline or insulin), there is a corresponding decrease in GLUT-2 gene expression, reaching an 85% reduction on the fourth day of fasting [17]. On the other hand, this study also reports that by increasing glucose concentration (i.e., continuous infusion of 50% glucose), the gene expression level of GLUT-2 is increased by 46% after 5 days [17]. Based on the above, although the RF group underwent a total of 3 days of fasting, it is believed that the effects of fasting on GLUT-2 were eliminated by 6 days of refeeding after fasting. Therefore, it is highly probable that the final day of fasting in the RF group has an impact on the whole-body glucose metabolism. While the present study did not investigate the expression levels of GLUT-2 in the pancreatic β-cells, it is possible that in the group subjected to one day of fasting (RF group), the gene expression of GLUT-2 remained higher than that in the group subjected to three days of fasting (CF group). The differences in gene expression due to variations in the fasting period could potentially alter insulin secretion capacity, leading to differences in glucose tolerance. To explore this possibility, future research should compare the effects of variations in fasting durations on insulin secretion capacity using isolated β-cells.

Conclusion

Even when the fasting days are spread out over several days, the ability to secrete insulin may be reduced and glucose tolerance may deteriorate as when fasting for several consecutive days.

Conflict of Interests

All authors declare no conflict of interests.

Author contributions

Y.N., M.I., S.N., and S.U. performed the experiments. Y.N. and S.T. contributed to the conception and experimental design, data analyses and interpretation of the findings, and the preparation of the manuscript. All authors approved the final version of the manuscript.

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Figure legends

Fig. 1. Changes in body weight during the intervention period. CON, ad libitum-fed control group; CF, consecutive fasting–induced weight-loss group; RF, repetitive fasting–induced weight-loss group. Values are means ± SEM, n = 5.

Fig. 2. Effects of weight loss induced by consecutive fasting versus repetitive fasting on glucose tolerance in rat fed a high-fat diet. Plasma glucose (A) and insulin responses (C) after oral glucose administration. AUCs for plasma glucose (B) and insulin (D) during the 120-min period after oral glucose administration. Values are means ± SEM, n = 5. * and ** indicate significant differences from the values obtained in the CON group at $p < 0.05$ and $p < 0.01$, respectively. § and §§ indicate significant differences from the values obtained in the CF group at $p < 0.05$ and $p < 0.01$, respectively.

Table 1 Composition of the experimental high-fat diet

Ingredients	(g/kg)
Sucrose	347.286
Casein	293.400
Lard	180.000
Canola oil	100.000
Methionine	5.000
Vitamin mix (AIN-93-VX)	22.000
Mineral mix (AIN-93G-MX)	51.000
Choline bitartrate	1.300
tert-Butylhydroquinone	0.014

Table 2 Body weight, intra-abdominal fat weight and total food intake in rats

	CON	CF	RF
Initial body weight (g)	228 ± 3	228 ± 3	228 ± 5
Final body weight (g)	316 ± 8	264 ± 5**	292 ± 7§
Intra-abdominal fat weight (g)	20.4 ± 0.7	13.8 ± 1.2**	16.8 ± 1.2
Total food intake (g)	239 ± 11	194 ± 8*	222 ± 6§

CON, ad libitum-fed control group; CF, consecutive fasting–induced weight-loss group; RF, repetitive fasting–induced weight-loss group. Values are means ± SEM, n = 5. * and ** indicate significant differences from the values obtained in the CON group at $p < 0.05$ and $p < 0.01$, respectively. § indicates a significant difference from the values obtained in the CF group at $p < 0.05$.

Figure 1

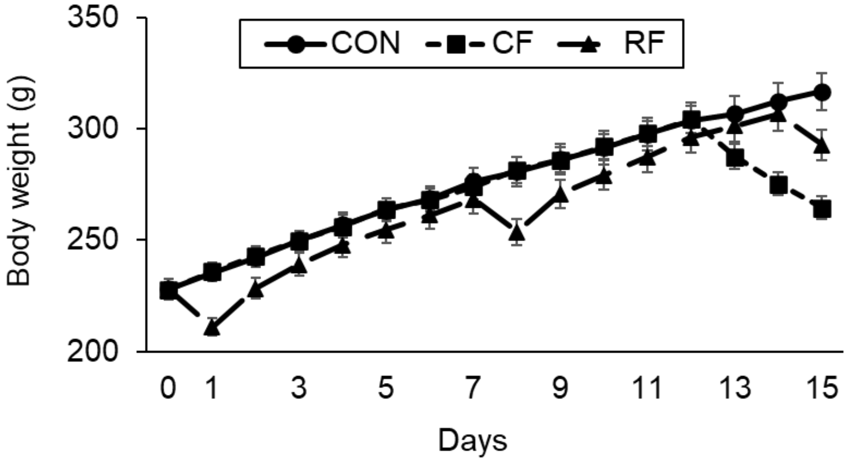
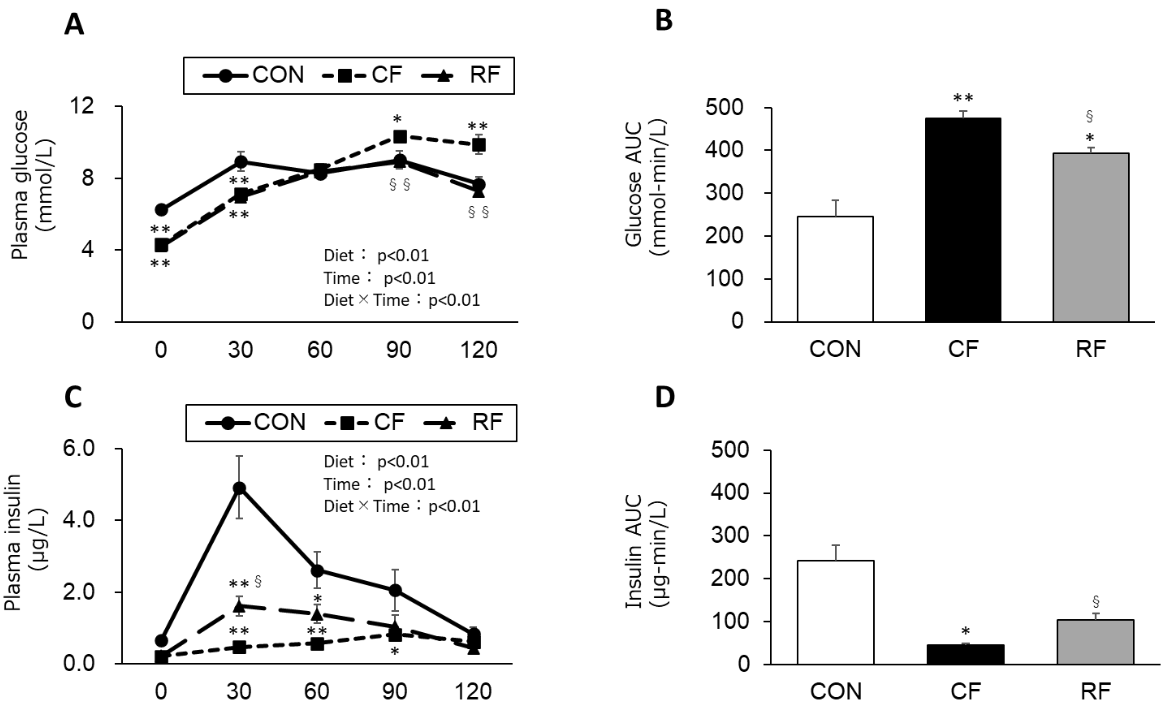


Figure 2


A
CON
CF
RF
Plasma glucose (mmol/L)
Diet： p<0.01
Time： p<0.01
Diet×Time：p<0.01
B
Glucose AUC (mmol·min/L)
CON
CF
RF
C
Plasma insulin (µg/L)
Diet： p<0.01
Time： p<0.01
Diet×Time：p<0.01
D
Insulin AUC (µg·min/L)