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Title: Effects of rapid or slow body weight reduction on glucose tolerance during equivalent weight loss in rats fed a high-fat diet

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Abstract

Excess accumulation of visceral fat induces insulin resistance in skeletal muscle and impairs glucose tolerance. Although weight loss improves glucose tolerance by reducing visceral fat mass, the effects of the speed of weight loss on glucose metabolism are unclear. This study compared the effects of long-term energy restriction–induced weight loss and short-term fasting–induced weight loss on glucose tolerance in rats fed a high-fat diet. After 2 weeks of high-fat diet feeding, male Wistar rats were subjected to either 30% calorie restriction for 2 weeks (CR) or 3-day fasting (FAST). After the intervention period, body weight and intra-abdominal fat mass decreased to a similar extent in both weight-loss groups. The maximum insulin-stimulated glucose uptake and GLUT-4 content in the epitrochlearis muscle were significantly higher in the FAST group than in both control rats fed ad libitum (CON) and the CR group, with no significant differences between the CON and CR groups. Blood glucose levels during the oral glucose tolerance test (OGTT) were similar in the CON and CR groups, but insulin levels were significantly lower in the CR group than in the CON group, indicating improved insulin resistance in the CR group. However, compared with the CON and CR groups, plasma glucose and insulin levels were significantly higher and lower, respectively, in the FAST group during the OGTT. These findings suggest that rapid weight loss through short-term fasting, in contrast to long-term energy restriction, may impair glucose tolerance by reducing insulin secretion capacity.

Key words: weight loss, glucose uptake, glucose tolerance

減量速度の違いが高脂肪食摂取によるインスリン抵抗性改善効果に及ぼす影響

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毎日のエネルギー摂取量を 2〜3 割ほど減らすことで内臓脂肪量が減少し, インスリン抵抗性を改善できることが良く知られている. 一方で, 近年新たな減量方法として, 数日間絶食することで短期間に大きな体重減少効果を得ようとする方法が注目を集めている. しかしながら, この減量法が長期間のエネルギー摂取制限と同様にインスリン抵抗性を改善できるのかは明らかではない. そこで本研究では, 長期間のエネルギー摂取制限と短期間の絶食により同程度に体重を減少させた場合のインスリン抵抗性改善効果について比較検討することを目的とした. 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, 高脂肪食をさらに 2 週間自由摂取させる群（CON 群）, 2 週間にわたり 1 日のエネルギー摂取量を CON 群の 70%に制限することで緩やかに減量させる群（CR 群）, CON 群と同様に高脂肪食を摂取させ, 最後の 3 日間のみ絶食することで CR 群と同程度まで急速に体重を減少させる群（FAST 群）の 3 群に分けた. 飼育期間終了後, 滑車上筋を摘出し糖取り込み速度を測定した. さらに, 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, 飼育期間中の総摂餌量は CR 群に比べて, FAST 群で有意に高い値を示したが, 体重は同程度に減少した. 滑車上筋のインスリン刺激による糖取り込み速度および糖輸送体 GLUT-4 の発現量は, CON 群と CR 群の間に有意な差はなかったが, FAST 群では CON 群および CR 群に比べて有意に高い値を示した. 経口糖負荷試験時の血糖値は, CON 群と CR 群で同程度の値を示したが, インスリン値は CON 群と比較して CR 群で有意に低い値を示したことから, CR 群ではインスリン抵抗性が改善したと考えられる. 一方, FAST 群ではインスリン値が CON 群と比較して顕著に低い値を示し, さらに血糖値は CON 群と比較して有意に高い値を示したことから, 耐糖能が悪化していたと考えられる. 以上の結果から, 短期間の絶食による急速減量は, 脂肪量の減少には効果的であるが, インスリン分泌能力を低下させることで耐糖能を悪化させる可能性が示唆された.

Introduction

Diabetes is a significant public health issue that is rapidly becoming a global epidemic. The incidence of type 2 diabetes is increasing at an alarming rate, and it is expected that the number of people affected by this disease will reach 783.2 million worldwide by the year 2045 [1]. Type 2 diabetes is a metabolic disorder that is marked by reduced insulin action in peripheral tissues, leading to impaired glucose uptake (i.e., insulin resistance), or decreased insulin secretion by the pancreas. The accumulation of excess fat is a well-known contributor to the progression of the disease. Indeed, 60%–90% of individuals diagnosed with type 2 diabetes fall under the categories of overweight (body mass index [BMI] $\geq$ 25 kg/m^2) or obese (BMI $\geq$ 30 kg/m^2) [2]. Studies have demonstrated that overconsumption of energy leading to hypertrophied visceral fat results in the secretion of adipocytokines, including tumor necrosis factor-alpha, resistin, and monocyte migration factor-1. These molecules induce insulin resistance in insulin target organs, particularly skeletal muscle [3, 4]. Therefore, reducing visceral fat mass is crucial in preventing insulin resistance and diabetes.

Given that the accumulation of visceral fat is a consequence of an imbalance between energy intake and expenditure, a gradual decrease in daily energy intake is recommended in order to reduce visceral fat mass effectively. It has been reported that restricting daily energy intake (calorie restriction: CR method) leads to a reduction in visceral fat mass and an improvement in glucose metabolism both in skeletal muscle and in the body as a whole [5, 6, 7, 8]. However, adherence to the CR method can be challenging for obese individuals who have grown accustomed to consuming an excess amount of energy over an extended period. Indeed, numerous studies have reported that obese people often discontinue weight-loss programs before benefiting from weight loss

[9, 10, 11, 12, 13].

Against this background, short-term fasting-induced weight loss (FAST method) has been proposed in recent years as a more sustainable approach to weight loss [14, 15, 16]. While weight reduction through the FAST method is anticipated to alleviate stress associated with dietary restrictions lasting only a few days, studies involving human subjects have reported that prolonged fasting (72 h) induce insulin resistance [17, 18]. Insulin resistance in skeletal muscle plays a key role in this adaptive response [18, 19]. Meanwhile, several studies in which rats were fasted for 36–48 h (short-term fasting) have reported increased expression of the skeletal muscle glucose transporter GLUT-4, as well as insulin-stimulated glucose uptake capacity [20, 21, 22]. This suggests that weight loss through the FAST method has the potential to improve whole-body glucose metabolism more effectively by enhancing glucose uptake in skeletal muscle. However, previous studies on fasting have focused only on fasting time and have not considered the amount of weight loss or speed of weight loss. Therefore, the effects of the FAST method on glucose metabolism are not conclusively clear because no studies have directly compared the effects of the FAST method with those of the CR method, which is widely recognized for enhancing glucose metabolism in skeletal muscle and the body as a whole. Thus, the purpose of this study was to directly compare the effects of the CR and FAST methods on skeletal muscle glucose metabolism and whole-body glucose tolerance during equivalent weight loss.

Methods

Animal treatment

Five-week-old male Wistar rats (120-140 g body weight) were obtained from CLEA Japan, Inc. and housed individually under a 12:12-h light:dark cycle (lights on 09:00-21:00) in an air-conditioned room (23°C). After 1 week of acclimation, all rats were fed a high-fat diet (Table 1). Previous studies have reported that insulin resistance is induced in rats fed a high-fat diet for 2-8 weeks [23, 24, 25], so after inducing obesity by feeding a high-fat diet for 2 weeks, the animals were divided into three groups, matched for body weight and food efficiency: a control group (CON; n = 8), which continued to receive the high-fat diet ad libitum for the entire 14-day experimental period; a calorie-restriction group (CR; n = 8), which received the standard diet equal to 70% of the average amount of food eaten by the CON group during the 14-day period to decrease their body weight slowly; and a fasting group (FAST; n = 8), which was fed the high-fat diet ad libitum for 11 days and fasted for the last 3 days of the study period to rapidly decrease their body weight to an extent comparable to that of the CR group. Regarding the duration of fasting, we confirmed in our preliminary study that fasting for 3 days reduced body weight to the same extent as restricting energy intake for 2 weeks. We also used the same method in our previous study with non-obese rats [26], and we used the same method in the present study. All rats were permitted ad libitum access to water throughout the 14-day nutritional intervention. Daily monitoring of body mass and dietary consumption was performed throughout the intervention.

Table 1

To eliminate the influence of the most recent diet, on the morning of the experimental day, the food for both the CON and CR groups was removed 6 h before the start of the experiment (the FAST group underwent a 72-h fast). After fasting, the rats were anesthetized with isoflurane, and the epitrochlearis muscle was excised from both forelimbs. One muscle was used to measure the rate of glucose uptake and the other was

used to measure the expression level of the glucose transporter GLUT-4. After muscle dissection was completed, intra-abdominal fat comprising the epididymal, mesenteric, and retroperitoneal fat pads was removed and weighed. The experimental protocol was approved by the Animal Experimental Committee of The University of Tokyo (No. 26-26).

Muscle incubation and measurement of insulin-stimulated 2-deoxyglucose uptake

After dissection, the epitrochlearis muscles were incubated in oxygenated Krebs-Henseleit bicarbonate (KHB) buffer containing 8 mM glucose, 32 mM mannitol, 0.1% bovine serum albumin (BSA; 116 mM NaCl, 4.6 mM KCl, 1.16 mM KH_2PO_4, 25.3 mM $NaHCO_3$, 2.5 mM $CaCl_2$ $2H_2O$, 1.16 mM MgSO4 $7H_2O$) at 35°C for 1 h. The gas phase was 95% O_2 and 5% CO_2.

We used 2-deoxyglucose (2-DG) to measure the rate of muscle glucose uptake, based on the method described by Higashida et al. (2013) [27]. After incubation, to remove glucose from the extracellular space, the muscles were incubated in KHB buffer containing 2 mM sodium pyruvate, 36 mM mannitol, 0.1% BSA, and 10 mU/mL insulin at 30°C for 30 min. Glucose uptake was measured after incubation at 30°C for 30 min in KHB buffer containing 8 mM 2-Deoxyglucose (2DG), 32 mM mannitol, 0.1% BSA, and 10 mU/mL insulin. Previous studies on fasting have reported altered GLUT-4 content [20, 27]. Therefore, the present study used an insulin concentration of 10 mU/mL, the maximum insulin stimulus, to examine insulin responsiveness as defined by GLUT-4 content [28]. The muscles were blotted and frozen in liquid nitrogen, and then weighed, homogenized in 0.3 M perchloric acid, and centrifuged at 1000 × g. After centrifugation, the supernatant was collected and neutralized by the addition of 2 N

KOH, followed by fluorometric measurement of 2-deoxyglucose-6-phosphate. The intracellular accumulation of 2-deoxyglucose-6-phosphate has been shown to reflect muscle glucose uptake activity.

Tissue homogenization

Frozen epitrochlearis muscle was homogenized in ice-cold radio-immuno precipitation assay lysis buffer (EMD Millipore, Temecula, CA) containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM ethylenediaminetetraacetic acid, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The homogenates were frozen and thawed three times to disrupt the intracellular organelles and then rotated end-over-end at 4°C for 60 min to solubilize the protein. The homogenized samples were centrifuged at 700 × g for 5 min at 4°C and the supernatants were harvested.

Western blotting

The protein concentrations of the abovementioned supernatants were also used for the measurement of GLUT-4 content. Samples were prepared in Laemmli sample buffer (Wako Pure Chemical Industries, Osaka, Japan). Equal amounts of sample protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10% resolving gel and transferred to polyvinylidene difluoride membranes at 200 mA for 90 min. After transfer, the membranes were blocked for 1 h at room temperature in Tris-buffered saline with 0.1% Tween 20 (TBS-T; 20 mM Tris base, 137 mM NaCl; pH 7.6) supplemented with 5% (w/v) non-fat powdered milk. Membranes were incubated overnight at 4°C with the primary antibody diluted 1:5000 in TBS-T containing 5%

BSA. The primary antibody used was anti-GLUT-4 (a gift from the laboratory of Dr. John O. Holloszy, Washington University, St. Louis, MO). After incubation with the primary antibody, membranes were incubated for 1 h at room temperature with the secondary antibody (goat anti-rabbit IgG) diluted 1:10,000 in TBS-T containing 1% non-fat powdered milk. Bands were visualized by enhanced chemiluminescence reagent (GE Healthcare Life Sciences, Piscataway, NJ) and quantified by Image Studio (LI-COR, Lincoln, NE). The membranes were stained with Ponceau S (Sigma-Aldrich) to verify equal loading of protein across lanes.

Oral glucose tolerance test

In a separate subsequent experiment intended to further evaluate the effects of weight loss on glucose tolerance, an oral glucose tolerance test (OGTT) was performed. On the day of the experiment, the CON and CR groups were allowed free access to their respective diets until 7:00 AM (food was removed at this time). After fasting (CON and CR groups, 6 h; FAST group, 72 h), glucose (2 g/kg of body weight) was orally administered using a stainless-steel gavage needle. Blood was drawn from the tail vein into capillary tubes before and at 30, 60, and 120 min after glucose administration. The capillary tubes were then centrifuged at 10,000 × g for 10 min, and the resulting supernatants (i.e., plasma samples) were stored at −80°C. The trapezoidal rule was used to calculate the total areas under the curve for plasma glucose, insulin, and C-peptide.

Plasma glucose, insulin, and C-peptide concentrations

Plasma glucose was determined using the Glucose C2 Test (Wako Pure Chemical Industries). The concentrations of plasma insulin and C-peptide (Mercodia

AB, Uppsala, Sweden) were measured using enzyme-linked immunospecific assay kits according to the manufacturer's instructions.

Statistics

The data are presented as means ± SEM. In the experiment, statistical analysis was performed using either one-way analysis of variance or two-way repeated analysis of variance followed by Tukey's post hoc test. Correlations between two variables were examined using Pearson's correlation analysis. All statistical analyses were performed using BellCurve for Excel ver.2.2 (Social Survey Research Information, Tokyo, Japan). 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. Although the body weight of the CON group continued to increase, the CR group, which underwent a 30% reduction in daily energy intake compared with the CON group for 2 weeks, exhibited a moderate increase in body weight. The FAST group exhibited a rapid decrease in body weight during the final 3 days of treatment, resulting in a final body weight at the end of the intervention period that was equivalent to that of the CR group. The body weight in the CR group became significantly different from the CON and FAST groups at day 4. The body weight in the FAST group became significantly different from the CON group at day 12 (1 day after the onset of fasting). Final body weight was significantly lower in both the CR and

Figure 1

FAST groups than in the CON group ($p < 0.001$; Table 2). Total intra-abdominal fat weight was also significantly lower in both weight-loss groups than in the CON group ($p < 0.001$), with no significant differences between the CR and FAST groups (Table 2).

Total food intake for 2 weeks was significantly lower in both weight-loss groups than in the CON group ($p < 0.001$). Furthermore, total food intake in the FAST group was significantly higher than that in the CR group ($p < 0.05$; Table 2).

There was no significant difference in muscle wet weight of the epitrochlearis muscle among the three groups (Table 2).

Table 2

Insulin-stimulated 2-DG uptake in rat epitrochlearis muscle

Uptake of 2-DG in the epitrochlearis muscle when stimulated by the maximally effective insulin dose (10 mU/mL) was about 100% higher in the FAST group than in the CON and CR groups, with no significant differences between the CON and CR groups ($p < 0.001$; Fig. 2).

Figure 2

GLUT-4 protein content in rat epitrochlearis muscle

Although the GLUT-4 protein content of the epitrochlearis muscle did not differ between the CON and CR groups, that in the epitrochlearis muscle was significantly higher in the FAST group than in the CON and CR groups ($p < 0.001$; Fig. 3).

Figure 3

Relationship of skeletal muscle glucose uptake rate with intra-abdominal fat mass and GLUT-4 content

Intra-abdominal fat mass was significantly and negatively associated with glucose uptake ($p < 0.05$; Fig. 4A), while GLUT-4 protein content was significantly and

Figure 4

positive correlated with glucose uptake ($r = 0.626$, $p < 0.01$; Fig. 4B).

Oral glucose tolerance test

Plasma glucose area under the curve (AUC) values did not significantly differ between the CON and CR groups but were significantly higher in the FAST group than in the CON and CR groups (CON vs FAST, $p < 0.001$; CR vs FAST, $p < 0.001$; Fig. 5A, B).

Plasma insulin AUC values were significantly lower in the CR group than in the CON group ($p < 0.01$). Moreover, the FAST group showed significantly lower values compared with the CON and CR groups (CON vs FAST, $p < 0.001$; CR vs FAST, $p < 0.01$; Fig. 5C, D).

The plasma concentration of C-peptide can be used as an index of insulin secretion. Plasma C-peptide AUC values were significantly lower in the FAST group compared with the CON group ($p < 0.05$), but there was no significant difference between the CR and FAST groups (Fig. 5E, F).

Figure 5

Discussion

In this study, we compared the effects of the speed of different weight-loss methods on glucose metabolism in skeletal muscle and the body as a whole. We found that while total food intake was significantly higher in the FAST group compared with the CR group, both weight-loss methods resulted in a comparable reduction in body weight and intra-abdominal fat content. These results suggest the possibility that weight loss through the FAST method is a more convenient approach in terms of being able to consume a larger quantity of food during the weight-loss period while still achieving

297 reductions in body weight. However, while weight loss achieved through the FAST
298 method enhances skeletal muscle glucose uptake, it also impairs pancreatic insulin
299 secretion capability, leading to a reduction in glucose tolerance.

300 More than 80% of glucose uptake occurs in the skeletal muscles, and the glucose
301 uptake capacity of skeletal muscles is reported to be reduced in patients with type 2
302 diabetes [29]. It has also been reported that there is a negative correlation between
303 visceral fat mass and glucose uptake capacity in skeletal muscle [30], suggesting that it is
304 important to decrease visceral fat mass in order to increase the glucose uptake capacity
305 of skeletal muscle. As shown in Fig. 2, the maximal insulin-stimulated glucose uptake
306 in skeletal muscle was significantly higher in the FAST group than in the other two
307 groups. In addition, despite fasting for 3 days, the FAST group showed a reduction in
308 intra-abdominal fat comparable to that of the CR group, which had undergone energy
309 restriction for 2 weeks, and a negative correlation was observed between visceral fat
310 mass and glucose uptake in the skeletal muscles (Fig. 4A). The rate-limiting step of
311 glucose metabolism in skeletal muscle is the transport of glucose into the cells, and a
312 strong correlation has been observed between maximum insulin-stimulated glucose
313 uptake capacity and GLUT-4 content [31]. We found that the protein content of GLUT-4
314 was significantly higher in the FAST group compared with the other two groups, and a
315 strong correlation was observed between glucose uptake and the GLUT-4 content in the
316 skeletal muscle (Figs. 3, 4B). These findings suggest that in the FAST group, reducing
317 intra-abdominal fat mass and increasing GLUT-4 content might enhance glucose
318 uptake in skeletal muscle.

319 Kim et al. demonstrated that even when rats were subjected to a high-fat diet for 4
320 weeks, restricting daily food intake to 70% of freely fed rats prevented the accumulation

of intra-abdominal fat and improved the insulin-stimulated skeletal muscle glucose uptake to a level comparable to rats consuming a normal diet [30]. In this study, similar to Kim et al., the daily food intake of the CR group was limited to 70% of the CON group for 2 weeks. As a result, the intra-abdominal fat content in the CR group was significantly lower compared to the CON group (Table 2). However, in contrast to Kim et al.'s findings, the insulin-stimulated glucose uptake rate in the epitrochlearis muscle did not differ between the CON and CR groups. While there are differences in the duration of interventions, the reason for this discrepancy in results is not clear. One potential contributing factor could be the difference in insulin concentrations used for glucose uptake measurements between this study and Kim et al. (10 mU/ml vs. 2 mU/ml). It is known that the glucose uptake stimulated by high concentrations of insulin is defined as "insulin responsiveness," and the expression level of GLUT-4 is a determining factor in this response [28]. Previous studies have not observed an increase in GLUT-4 expression in skeletal muscles following weight reduction through the CR method [32, 33]. Consequently, it is plausible that no difference was observed in the insulin-stimulated skeletal muscle glucose uptake between the CON and CR groups in this study. On the other hand, various studies have indicated that weight reduction through the CR method improves insulin sensitivity, specifically in the context of lower insulin stimulation [33, 34]. Therefore, while this study did not observe an improvement in insulin responsiveness of skeletal muscle glucose uptake following maximal insulin stimulation through the CR method, it is conceivable that an improvement in insulin sensitivity might have been detected if measurements were conducted at lower insulin concentrations.

We performed the OGTT to evaluate glucose tolerance in the FAST group, which

showed a substantial increase in skeletal muscle glucose uptake even when weight loss was comparable to that of the CR group. Although the plasma glucose concentration before glucose administration was lower in the FAST group due to 3-day fasting, it increased to the same level as the other two groups and remained high in the latter part of the OGTT. The plasma glucose AUC in the FAST group was significantly higher compared with the other two groups (Fig. 5B). Given that the glucose uptake capacity of the skeletal muscle was increased in the FAST group, factors other than peripheral tissue glucose disposal capacity were considered to be involved. Therefore, we measured the insulin concentration, which plays a role in promoting glucose uptake in peripheral tissues such as skeletal muscle. The plasma insulin AUC during the OGTT in the FAST group was significantly lower compared with the CON and CR groups (Fig. 5D), suggesting that the observed increase in plasma glucose AUC in the FAST group may be attributable to lower insulin concentrations. C-peptide is released in equivalent amounts with insulin and is not cleared by the liver or other peripheral tissues, in contrast to insulin, making it an indicator of insulin secretion [35]. We measured the C-peptide concentration and found that it was significantly lower in the FAST group than in the CON group (Fig. 5E, F). In a previous study, Fink et al. reported a reduction in insulin secretion from the pancreas of mice subjected to 48-h fasting [36]. Similarly, in humans, where insulin resistance is induced by prolonged fasting, 60-h fasting has also been reported to decrease insulin secretion [19]. Our results are consistent with those of Fink et al., and although short-term fasting improves skeletal muscle metabolic function, insulin secretion capacity was significantly reduced, which led to impaired glucose tolerance. The mechanism underlying the remarkable decrease in insulin secretion in the FAST group was not elucidated in this

study. However, previous studies have reported that short-term fasting suppresses autophagy in pancreatic beta cells, leading to a decrease in insulin secretion [37]. Therefore, it is plausible that this mechanism might have played a role in the present study. To evaluate this, it will be necessary to isolate and analyze pancreatic islets, using specialized techniques.

In the CR group, the AUC of plasma insulin concentration during the OGTT was significantly lower compared with the CON group, while the AUC of plasma glucose concentration was similar to that of the CON group. Many studies examining the CR method have reported a significant decrease in insulin concentration, although no significant changes in blood glucose levels during the OGTT were observed [38, 39]. Therefore, the present results suggest that blood glucose was lowered with a smaller amount of insulin, indicating an improvement in insulin resistance. The mechanism underlying the decrease in insulin secretion is not clear. However, a previous study using isolated islets reported an increase in insulin secretion capacity with the CR method [40]. It is plausible that the reduced insulin levels are attributable to enhanced insulin sensitivity in insulin target organs. Described above, the CR method has been reported to improve skeletal muscle insulin sensitivity, which is characterized by enhanced glucose uptake in response to low-dose insulin stimulation [33, 34]. Therefore, although the high-concentration insulin stimulation used in our study did not lead to an improvement in skeletal muscle glucose uptake, as shown in the OGTT, the glucose uptake capacity under low and physiological insulin conditions appeared to be enhanced in the CR group. Consequently, the enhancement in glucose uptake capacity might have contributed to the amelioration of glucose tolerance during the OGTT.

Cherel et al. reported that in non-obese rats, by the 3-day fasting, there was a

decrease in skeletal muscle weight compared to non-fasting rats, while in obese rats, there was no decrease in skeletal muscle weight by the 3-day fasting [41]. In addition, Goodman et al. reported that in obese rats, the breakdown of skeletal muscle protein was suppressed during fasting, but this adaptation was lost when body fat stores were nearing exhaustion [42]. In our previous study with non-obese rats, we observed a decrease in muscle weight and muscle protein content in the plantaris muscle that underwent weight loss using the FAST method [26], whereas there was no decrease in the weight of the epitrochlearis muscle in this study (Table 2). Therefore, based on the results of the previous studies and the present study, it is possible that weight loss in obese rats using the FAST method may be less likely to reduce skeletal muscle mass than in non-obese rats. However, this study only examined the epitrochlearis muscle and did not compare skeletal muscle used in our previous study. It will be necessary to conduct future studies on other skeletal muscles, including plantaris and soleus muscles, to determine whether weight loss using the FAST method is a weight loss method that is less likely to cause muscle atrophy in obese rats.

Conclusion

During equivalent weight loss, short-term fasting–induced weight loss enhances skeletal muscle glucose uptake capacity compared with weight loss induced by energy restriction. However, it also impairs glucose tolerance by reducing insulin secretion from the pancreas.

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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590 **Figure legends**

591 Fig. 1. Changes in body weights during the intervention period. CON, ad libitum-fed
592 control group; CR, daily energy restriction–induced weight-loss group; FAST, fasting-
593 induced weight-loss group. Values are means ± SEM, n = 8. * and ** indicate
594 significant differences from the values obtained in the CON group at $p < 0.05$ and $p <$
595 0.01, respectively. † and ‡ significant differences from the values obtained in the FAST
596 group at $p < 0.05$ and $p < 0.01$, respectively.
597
598 Fig. 2. Effects of weight loss on insulin-stimulated glucose transport activity. CON, ad
599 libitum-fed control group; CR, daily energy restriction–induced weight-loss group;
600 FAST, fasting-induced weight-loss group. Values are means ± SEM, n = 8. *** indicates
601 a significant difference from the values obtained in the CON group at $p < 0.001$. §§§
602 indicates a significant difference from the values obtained in the CR group at $p < 0.001$.
603
604 Fig. 3. GLUT-4 content in skeletal muscle. CON, ad libitum-fed control group; CR,
605 daily energy restriction–induced weight-loss group; FAST, fasting-induced weight-loss
606 group. Values are means ± SEM, n = 8. *** indicates a significant difference from the
607 values obtained in the CON group at $p < 0.001$. §§§ indicates a significant difference
608 from the values obtained in the CR group at $p < 0.001$.
609
610 Fig. 4. Relationship of glucose transport with intra-abdominal fat mass (A) and GLUT-4
611 content in rat skeletal muscle (B). CON, ad libitum-fed control group; CR, daily energy
612 restriction–induced weight-loss group; FAST, fasting-induced weight-loss group. Values
613 are means ± SEM, n = 8.
614

615 Fig. 5. Effects of weight loss on plasma glucose, insulin, and C-peptide levels in rats fed
616 a high-fat diet. Plasma glucose (A), plasma insulin (C), and plasma C-peptide (E)
617 concentration after oral glucose administration (2.0 g/kg of body weight) in rats. The
618 area under the curves (AUCs) for plasma glucose (B), plasma insulin (D), and plasma
619 C-peptide (F) during the 120-min period after oral glucose administration were
620 calculated in accordance with the trapezoidal rule. CON, ad libitum-fed control group;
621 CR, daily energy restriction–induced weight-loss group; FAST, fasting-induced weight-
622 loss group. Values are means ± SEM, n = 8. *, ** and *** indicate significant
623 differences from the values obtained in the CON group at $p < 0.05$, $p < 0.01$ and $p <$
624 0.001, respectively. §§ and §§§ significant differences from the values obtained in the CR
625 group at $p < 0.01$ and $p < 0.001$, respectively.
626
627

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

633 Table 2 Body weight, intra-abdominal fat weight, total food intake, and muscle wet
634 weight in rats

	CON	CR	FAST
Initial body weight (g)	276 ± 6	276 ± 5	276 ± 5
Final body weight (g)	362 ± 10	304 ± 4***	305 ± 6***
Intra-abdominal fat weight (g)	27.6 ± 1.6	18.4 ± 1.0***	16.3 ± 1.0***
Total food intake (g)	242 ± 6	166 ± 1***	187 ± 4***,§§
Epitrochlearis muscle weight (mg)	57.8 ± 2.7	55.3 ± 2.3	57.2 ± 4.5

635 CON, ad libitum-fed control group; CR, daily energy restriction–induced weight-loss group; FAST, fasting-
636 induced weight-loss group. Values are means ± SEM, n = 8.
637 *** indicates a significant difference from the values obtained in the Con group at $p < 0.001$.
638 §§ indicates a significant difference from the values obtained in the CR group at $p < 0.01$.
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Figure 1

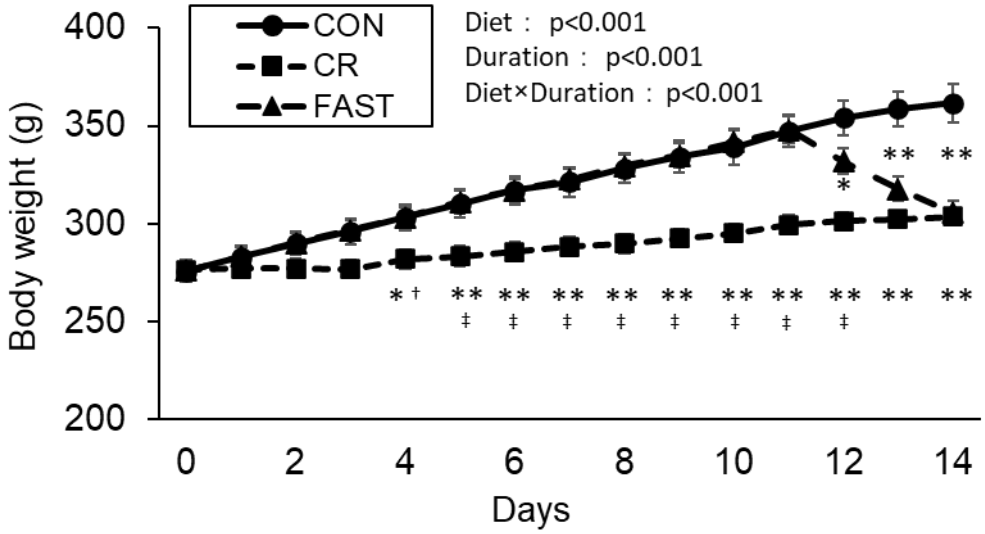

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Figure 2

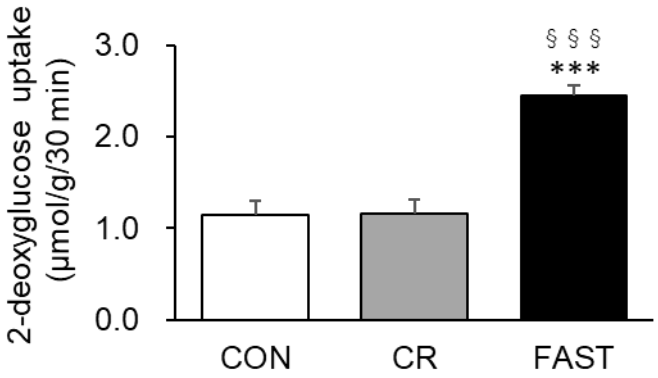

Figure 3

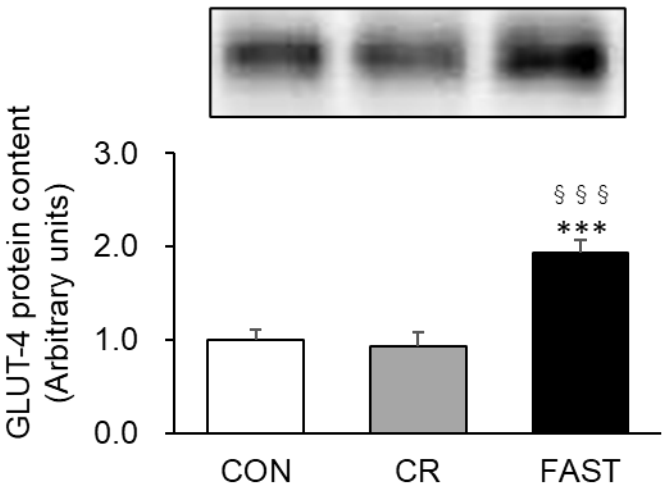
GLUT-4 protein content
(Arbitrary units)
3.0
2.0
1.0
0.0
§ § §
***
CON
CR
FAST

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Figure 4

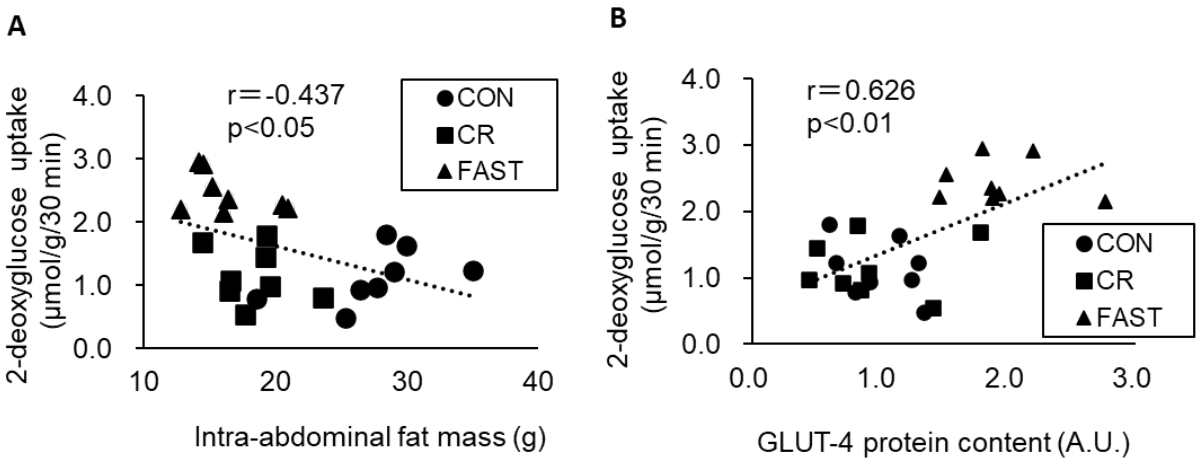

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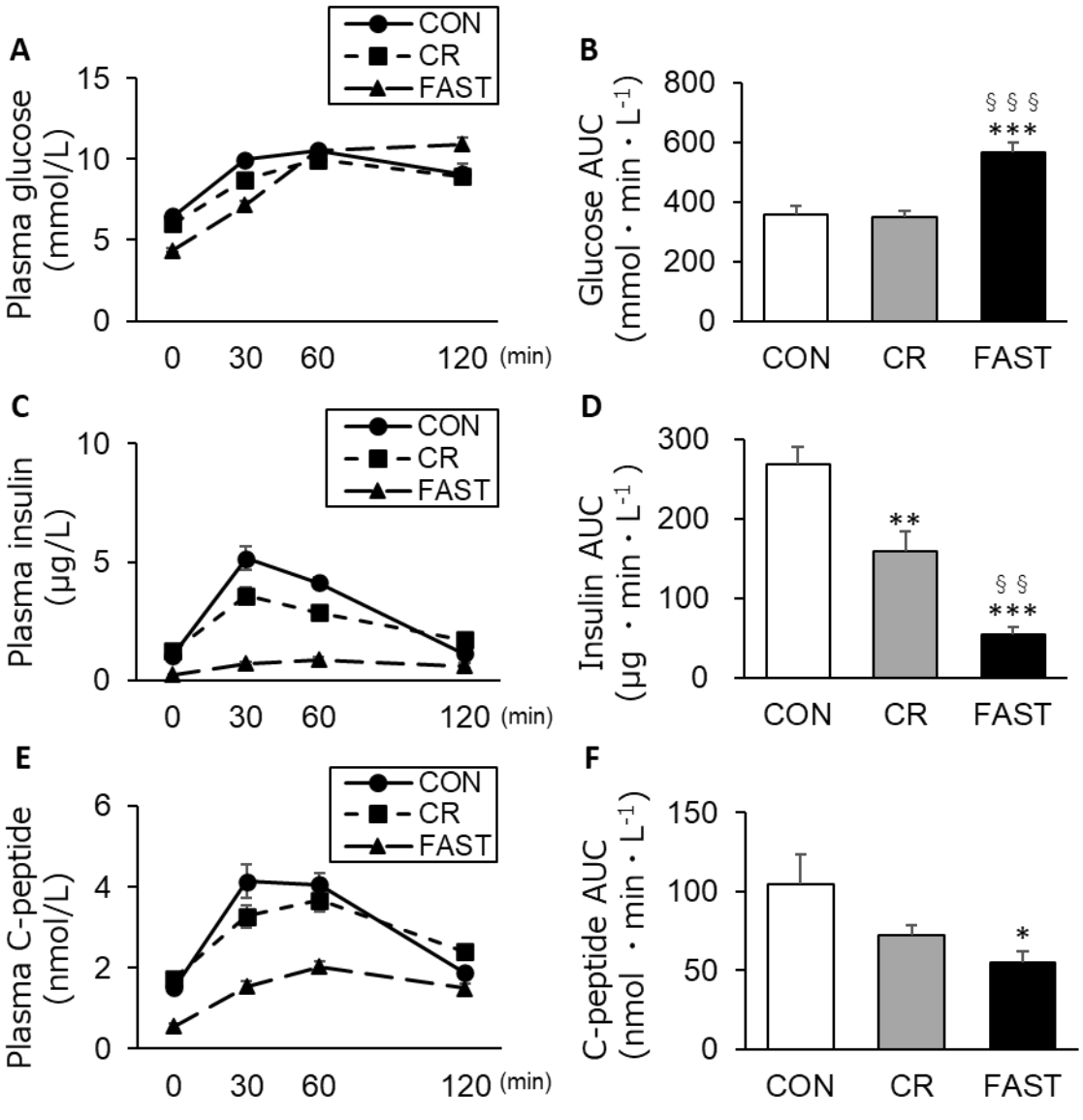
Figure 5



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