Comparison between short-term food restriction and exercise on whole body glucose disposal in high-fat fed rats

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Abstract: High-fat diets induce whole-body insulin resistance. The aim of this study was to compare the effect of two interventions : 3-day food restriction (66% of ad libitum fed) and 3-day exercise training (voluntary running wheels), on decreased insulin-mediated whole body glucose uptake in high-fat fed rats (5 mo old) using the hyperinsulinemic euglycemic clamp procedure. The control group was maintained on rat chow alone. After high-fat feeding for 2 wk, insulin-stimulated whole body glucose utilization was significantly decreased by 26%. The exercise training was more effective than food restriction in lowering plasma concentrations of insulin and triacylglycerol and tissue concentrations of triacylglycerol in soleus muscles. Diminished whole-body glucose uptake resulting from high-fat feeding was reversed completely by exercise training, but only partially by food restriction.

The time course of starvation on insulin-stimulated glucose uptake was also observed in high-fat fed rats. Although the extension of starvation time to 48h resulted in decreased plasma glucose, insulin and triacylglycerol concentrations, whole body glucose uptake did not increase further.

These findings suggest that short-term exercise has a higher restorative effect on insulin sensitivity in high-fat fed rats than food restriction, in spite of the same loss in body weight, presumably due in part to improved local lipid availability.

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Key words **:** *hyperinsulinemic euglycemic clamp, insulin resistance, dietary fat, training, food restriction*

INTRODUCTION

Insulin resistance is a characteristic metabolic disorder of obesity and type 2 diabetes. In humans (1) and animals (2, 3), high dietary fat content is considered to be a major cause of obesity. Many studies also have shown that insulin action is impaired in liver, muscle, and adipose tissues of animals with high-fat diets (4-6). Skeletal muscle is quantitatively the most important peripheral tissue for insulin-stimulated glucose clearance, and the degree of insulin resistance is strongly correlated with the local accumulation of triacylglycerol (7, 8).

Clinical trials have shown that energy restriction and/or physical training are important treatments for patients with type 2 diabetes and obesity. Prolonged intervention is characterized by weight loss, decline in blood glucose concentrations and an increase in muscle insulin action (9). We have previously observed that prolonged voluntary exercise training was more effective than food restriction on insulin-stimulated whole body glucose uptake in OLETF rats, animals with some characteristics of type 2 diabetes (10). Recently, Arciero *et al*. also demonstrated that a 10-day exercise training program is more effective than caloric restriction enhancing

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insulin action in patients with impaired glucose tolerance (11). However, many investigations relating the effects on insulin sensitivity of exercise and diet have been performed separately. Consequently, there is little data with which to compare short-term physical activity and dietary interventions in *in vivo* insulin-stimulated glucose disposal.

This study was designed to compare the restorative effects of these interventions, i. e., 3-day food restriction and 3-day exercise program, on insulin resistance in rats fed a high-fat diet. We also examined whether the high-fat induced insulin resistance was improved by prolonged starvation.

MATERIALS AND METHODS

Animal and experimental design

Male Sprague-Dawley rats (5 mo old;Japan SLC, Inc., Shizuoka, Japan) were used in this study. The animals were housed individually in temperature-controlled rooms (23±2℃) under a 12 h light/12 h dark cycle. Before they were assigned to an experimental group, they had free access to a nonpurified diet (Type MF, Oriental Yeast, Tokyo, Japan) and water for 7 d, and then they were randomly divided into four groups of six rats having equivalent mean body weights. One group of rats fed a nonpurified diet following the 2 wk was used as the low-fat group. Three groups (high-fat, exercise, or food restricted) that received the low fat diet for 7 d were followed by 14 d of a high-fat diet consisting of 150 g/kg lard and 50 g/kg sugar in addition to their regular diet (Table 1). Two of the three groups were treated with food restriction or exercise training during the last 3 days of high-fat feeding, respectively. Fundamental changes in body weight of rats reflects energy balance. Therefore, food intake in the food restricted group was adjusted to keep their body weight equal to those of the exercise group, i.e., 66% of the intake of the high-fat group. Each rat in the exercise group was placed in an individual cage with an exercise wheel (Nishin, Tokushima, Japan) and allowed to run at its own pace. The number of revolutions of the wheel per day was recorded with a cyclometer attached to the axis of the wheel to measure the running activity. Food intake and body weight were measured daily. On day 14, the animals underwent euglycemic clamp study at 6 h of fasting.

A separate complementary study was performed to characterize the relationship between insulin-stimulated whole body glucose uptake and length of starvation time. Twenty-four rats (5 mo old) were divided into four subgroups of six rats each based on body weight. The high-fat diet was provided each day from 0900-1400 h for the 2-wk. Rats were adapted to the meal feeding protocol and food intake was lower by 15% of ad libitum feeding. At the end of the experimental periods, rats were fasted for 6, 12, 24and 48 h, respectively and then underwent euglycemic clamp study.

Table 1. Composition of experimental diets

(g/kg)

	Low-fat diet[1]	High-fat diet[2]
Protein	238	198
Fat	51	201
Carbohydrate	541	492
Energy[3]	3.57	4.27

[1]Oriental yeast, Tokyo, Japan, Type MF.
[2]Lard (150 g/kg) and sugar (50 g/kg) were supplemented to the low-fat diet.
[3]kcal/g.

Measurement of in vivo glucose disposal by euglycemic clamp studies

The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50mg/kg), and catheters were inserted in the left jugular vein and right carotid artery. Before starting the clamp study, blood samples (1 ml) were taken though the catheters from the jugular vein. Rats received a 1-h infusion of insulin (60 pmol/min/kg). A glucose solution (100 g/L) was initiated at t=0. The rate of infusion was adjusted to maintain the plasma concentration of glucose at 110mg/dL. The whole body glucose uptake represents the glucose infusion rate (GIR) during the final 20 minutes.

Plasma glucose levels were determined by the glucose oxidase method (Tido-Tidex, Sankyo, Tokyo, Japan). Insulin levels were determined by an enzyme-linked immunosorbent assay (ELISA) (Levis, rat insulin, Shibayagi, Gunma, Japan) with rat insulin as the standard.

Plasma, liver and muscle lipid assay

Following the completion of the euglycemic clamp test, rats were killed and liver, soleus and the intra-abdominal fat pads (mesenteric, epididymal, and retroperitoneal fat) were surgically removed and weighed. The extraction of lipids from liver and soleus was performed as described by Folch *et al*. (12).

Plasma and tissue triacylglycerol levels were determined by enzymatic methods (Wako, Osaka, Japan). The levels of free fatty acids and cholesterol were determined enzymatically (Wako, Osaka, Japan).

Statistical analysis

Data in the text are expressed as means±SD. Data were analyzed by analysis of variance plus Bonferroni multiple comparison tests. A level of $P < 0.05$ was accepted as significant.

RESULTS

Body weight and food intake

The food intake during the last 3 d was 49.8±6.5 g in the high-fat, 33.1±0.8 g in the food restricted, 35.5±11.4 g in the exercise, and 55.8±11.9 g in the low-fat groups. The calculated energy consumed by high-fat rats was comparable to low-fat rats (616±84 vs. 627±132 kcal/3 d). The mean running distance of the exercise group was 780±431 m/3 d. Animals with food restriction and exercise exhibited significantly higher weight loss (-23.9±8.4 and -22.1±12.5 g/3 d, respectively) than did groups fed high-fat or low-fat diets (-1.5±5.5, and -3.7±12.7 g/3 d, respectively).

Plasma glucose, insulin, and lipids levels

The concentrations of fasting plasma glucose, insulin, and lipids in each group are shown in Table 2. Fasting plasma glucose levels and free fatty acids were similar among the groups. Plasma insulin concentrations tended to be higher in the high-fat group than in the low-fat group. The fasting level of insulin was significantly lowered in the exercise group compared to the high-fat group. Plasma levels of triacylglycerol were significantly increased in the high-fat group compared with the low-fat group. These levels were significantly decreased by exercise compared to those of the high-fat group, but not by food restriction. Plasma levels of cholesterol were significantly increased in the high-fat group, but was not significantly lowered by food restriction or exercise.

Liver and muscle triacylglycerol levels and abdominal fat accumulation

Figure 1 shows the concentrations of triacylglycerol in the liver and soleus, and intra-abdominal fat accumulation for each group. The concentrations of triacylglycerol in the liver and soleus were significantly increased in the high-fat group compared with the low-fat group. These values tended to be decreased by food restriction. Exercise significantly decreased triacylglycerol concentrations in the soleus. The amount of intra-abdominal fat tended to be higher in the high-fat group than in low-fat group. Food restriction and exercise decreased abdominal fat deposition, although not significantly.

In vivo glucose disposal

Figure 2 shows GIR for each group. The GIR was significantly decreased in the high-fat group relative to the low-fat group (11.6±1.0 vs. 15.0±1.2 mg/min/kg BW, $P<0.05$). Food restriction tended to improve GIR (13.7±2.2 mg/min/kg BW, NS vs.

Table 2. Effect of short-term food restriction and exercise on plasma levels of glucose, insulin, and lipids in rats fed high-fat diet

	Low-fat	High-fat	Food restricted	Exercise
Glucose (mg/dL)	104±9	110±15	101±8	117±18
Insulin (ng/mL)	4.9±3.4	7.9±3.1	5.4±2.4	3.5±2.3[2]
Free fatty acids (mEq/L)	0.55±0.29	0.48±0.19	0.54±0.36	0.46±0.13
Triacylglycerol (mg/dL)	74±23	128±51[1]	82±32	65±36[2]
Total cholesterol (mg/dL)	71±12	91±18[1]	75±15	82±20

Results are expressed as means±SD for six rats.
[1]Significantly different from low-fat group, $P<0.05$.
[2]Significantly different from high-fat group, $P<0.05$.

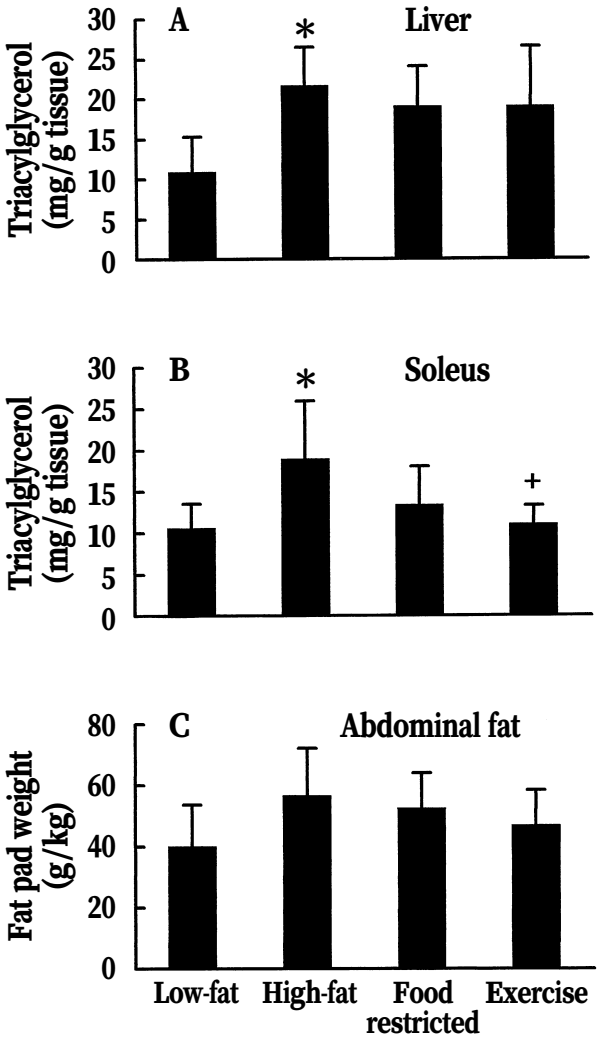

Fig. 1. Effects of food restriction and exercise training for 3 days on triacylglycerol levels of liver (A) and soleus muscle (B) and accumulation of abdominal fat (C) in rats fed a high-fat diet. Results are expressed as means±SD (n=6). *Significantly different from low-fat group, P<0.05. +Significantly different from high-fat group, P<0.05.

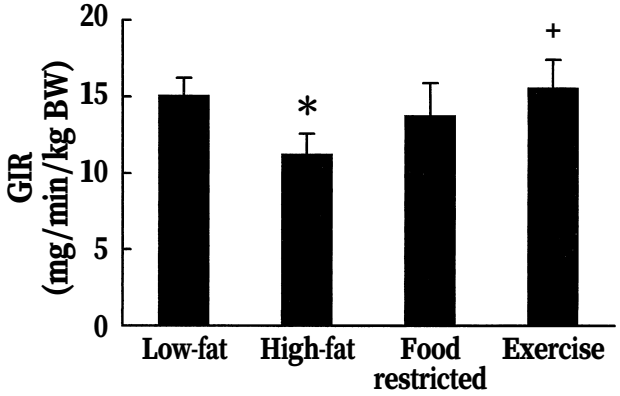

Fig. 2. Effects of food restriction and exercise for 3 days on glucose infusion rates (GIR) in rats fed a high-fat diet. Results are expressed as means±SD (n=6). *Significantly different from low-fat group, P<0.05. +Significantly different from high-fat group, P<0.05.

high-fat group) and exercise training significantly increased it (15.5±1.9 mg/min/kg BW, P<0.01 vs. high-fat group).

Body weight decreased with increasing duration of starvation, i.e., starvation of 6, 12, 24, and 48 h groups decreased body weight by -0.1±11.6, -2.5±4.2, -20.9±5.0, and -33.1±10.9 g, respectively. The relationships between duration of starvation and biochemical findings are shown in Table 3. With prolongation of starvation time, plasma glucose levels decreased and plasma free fatty acids increased. The concentrations of plasma insulin in rats were slightly increased up to 12 h of starvation and then decreased slightly up to 24 and 48 h of starvation. Table 3 shows the changes in plasma lipids in the starved rats. Plasma triacylglycerol and cholesterol concentrations reached a maximum value after 12 h,

Table 3. Effect of food deprivation on plasma glucose, insulin, and lipids levels in rats fed high-fat diet with time

	Food deprivation time			
	6 h	12 h	24 h	48 h
Glucose (mg/dL)	104±5	98±10	92±11	76±9[1]
Insulin (ng/mL)	5.7±2.5	6.1±2.1	5.2±1.0	2.5±0.9[1]
Free fatty acids (mEq/L)	0.32±0.09	0.43±0.10	0.50±0.24	0.55±0.22
Triacylglycerol (mg/dL)	136±92	220±126	55±21[1]	39±8[1]
Total cholesterol (mg/dL)	83±7	91±12	55±12[1]	57±10[1]

Results are expressed as means±SD for six rats.
[1]Significantly different from 6 h group, P<0.05.

then decreased at 24 and 48 h of starvation. Figure 3 shows the changes in triacylglycerol levels of the liver and soleus muscle in the starved rats. The concentrations of liver triacylglycerol showed a progressive decrease during the starvation period. Soleus triacylglycerol concentrations in rats showed little change over starvation time. Figure 4 shows the change in GIR in the rats under starvation. The GIR reached a maximum value after 24 h, and then decreased at 48 h of starvation.

DISCUSSION

The purpose of this study was to compare short-term food restriction with exercise in improving insulin resistance induced by a high-fat diet. Negative caloric balance rapidly improves insulin action in insulin resistant individuals with obesity (13, 14). Food restriction and exercise training induces negative caloric balance by energy restriction or increased energy consumption. Body weight gain in rats reflects the general energy balance. Therefore, food intake in the restricted group was restricted to keep their body weight the same as those of the exercise group.

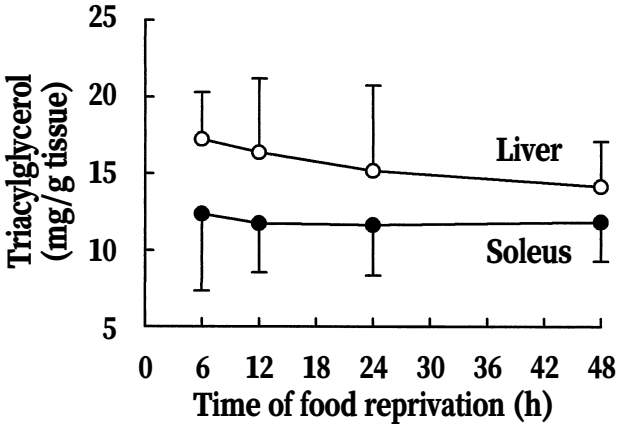

Fig. 3. Effects of duration of fasting time on concentrations of triacylglycerol in liver and soleus. The animals were fed once a day between 0900 to 1400 h. Results are means±SD for n=6 rats at each time point.

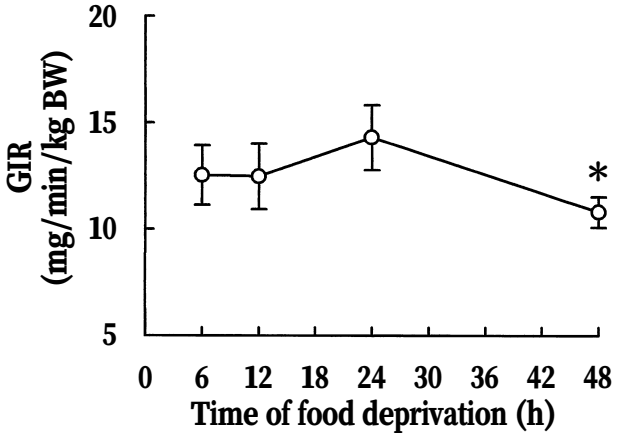

Fig. 4. Effects of duration of starvation on glucose infusion rates (GIR) in rats. The animals were fed once a day between 0900 to 1400 h. Results are means±SD for n=6 rats at each time point. *Significantly different from 6 h group, P<0.05.

In this study, the high-fat fed rats developed hyperinsulinemia, hypertriacylglycerolemia, and showed increased muscle and liver triacylglycerol concentrations and impaired insulin sensitivity. The impairment of glucose utilization caused by a high-fat diet was improved by both interventions : a 3-day period of volunteer exercise or moderate restriction of food intake. However, exercise training had a greater improvement than food restriction. Additionally, whole body glucose use did not increase further during prolonged starvation. The marked increase in glucose disposal induced by exercise training, despite lower plasma insulin levels, suggested that exercise is more effective than food restriction in high-fat fed rats when compared in the short term.

Studies in animals have shown that high-fat diets can induce profound and widespread tissue insulin-resistance (5, 15-17). Our present study, as well as studies by others (5, 17) showed that even when the diets were fed in isocaloric amounts, insulin action throughout the whole body deteriorated markedly within a short (2-3 wk) period of the high-fat diet. Anai *et al*. reported that insulin-stimulated whole body glucose uptake in the rats fed a high-fat diet for 2 wk was reduced by 58% compared with that in rats fed a low-fat diet (17). Our findings showed that insulin-mediated glucose uptake in rats fed high-fat for 2 wk was reduced by 26% compared to those of the low-fat fed control. One of the differences between the study by Anai *et al*. and ours is dietary fat content ; that is, their study used 60% fat content vs. 20%. The insulin responsiveness during the hindlimb perfusion technique has been reported to be negatively correlated to an increasing percentage of dietary fat in rats fed different diets (18).

On the other hand, it was shown that in the high-fat fed rats, there was a strong negative correlation between muscle triacylglycerol content and *in vivo* insulin-mediated glucose uptake (8, 19). Our findings also showed a build-up of muscle triacylglycerol with high-fat feeding (1.8-fold increase) after 2 wk.

This study showed that exercise and food restriction can reverse diet-induced insulin resistance.

Although there were no differences in plasma glucose and free fatty acid concentrations between exercise rats and food restricted rats, a marked decrease in plasma insulin was noted in trained rats, but not in food restricted rats. The triacylglycerol concentrations were lower in soleus muscles isolated from exercise and food restricted rats than in those of the rats fed a high-fat diet. Exercising also resulted in a greater improvement in glucose disposal than food restriction. The findings in this study showed that exercise is more effective in this regard than food restriction, since exercise rats had lower intramusclar triacylglycerol levels. In muscle and adipose tissue, phosphatidylinositol (PI) 3-kinase, is considered to be important for insulin-induced glucose uptake via the translocation of GLUT4 from the intracellular membrane to the plasma membrane (20-22). In fact, previous studies have shown the insulin-induced activation of PI3-kinase to be decreased in muscle and abdominal fat in high-fat fed rats (17) and diabetic rodent models (23, 24). Recently, Dean *et al*. have shown that calorie restriction does not alter the timing or amount of insulin-stimulated increase in insulin receptor substrate-1-PI 3-kinase activity in muscle (25). Our findings are consistent with the evidence that exercise induces larger increases in the sensitivity and responsiveness of skeletal muscle to insulin than food restriction (9, 11, 26). Large increases in muscle GLUT-4 content have been observed in skeletal muscle of humans and rats after a few days of exercise (27), although we did not address this topic in our study.

Recently, Etgen *et al*. reported that nitric oxide stimulates muscle glucose transport though a calcium/contraction- and PI3-kinase independent pathway (28). Our previous study showed that the urinary NO_2 excretion rate was significantly greater in exercise rats than in food restricted rats (10). These factors may also explain improved glucose uptake and an increase in muscle GLUT-4.

Although early studies reported an enhancement of *in vitro* insulin-stimulated glucose uptake in muscles of starved rats, this *in vivo* study showed a decrease in glucose uptake with duration of starvation. Similar findings were observed by Charron *et al*. (29). A marked decrease in the intrinsic activity of transporters from skeletal muscle has also been reported during starvation in rats (30). The insulin resistance associated with prolonged fasting may be one of the metabolic adaptations that is known to spare glucose during starvation (31, 32). Insulin resistance effectively decreases glucose metabolism in insulin-responsive tissues, and spares limited dietary carbohydrates for use by insulin-independent cells such as cerebral tissue and erythrocytes.

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