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Effects of alternate-day fasting on high-fat diet-induced insulin resistance in rat skeletal muscle

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ABSTRACT

Aims: The purpose of this study was to investigate the effects of alternate-day fasting (ADF) on insulin-stimulated glucose transport activity in skeletal muscle in rats fed a high-fat diet.
Main methods: Male Wistar rats were placed on a high-fat diet (n = 24) or standard chow diet (Chow, n = 12) for 10 weeks. Rats fed the high-fat diet were separated into two groups after 4 weeks. One group was subjected to ADF for the subsequent 6 weeks (HF-ADF, n = 12), and the other group was maintained on an ad libitum diet (HF-AL, n = 12). After the 10-week dietary intervention, measurements of insulin-stimulated glucose uptake and insulin tolerance test (ITT) were performed.
Key findings: Whereas the total intra-abdominal fat mass in the HF-AL group was significantly higher than in the Chow and HF-ADF groups, there was no significant difference between the Chow and HF-ADF groups. However, insulin-stimulated glucose uptake in skeletal muscles was significantly lower in both high-fat fed groups than in the Chow group. Muscle GLUT-4 protein content in HF-AL is significantly lower (~30%) than in Chow, and further reduction (~42%) was observed in the HF-ADF group rats. The HF-ADF and HF-AL group rats had less reduction in glycemia than did the Chow group rats during ITT.
Significance: ADF was unable to eliminate high-fat diet-induced muscle insulin resistance, despite a substantial decrease in total intra-abdominal fat mass. This might have resulted from a reduction in GLUT-4 protein in both HF-AL and HF-ADF rats compared to the Chow group.

Introduction

Over the past three decades, there has been an explosive increase in the prevalence of type 2 diabetes (Wild et al., 2004). Insulin resistance is an early and key defect associated with type 2 diabetes (Martin et al., 1992). Insulin resistance in skeletal muscle seems to play an important role in the development of type 2 diabetes since most glucose disposal occurs in skeletal muscle after a meal. During the hyperinsulinemic euglycemic clamp procedure, about 80–90% of the glucose was taken up in skeletal muscle, and the insulin-mediated glucose disposal in the skeletal muscle of type 2 diabetes subjects was reduced to about 50% of the value shown in control subjects (DeFronzo et al., 1981).

Since a strong negative correlation between insulin-stimulated glucose transport activity in skeletal muscle and visceral fat mass was observed (Kim et al., 2000), skeletal muscle insulin resistance is postulated to be caused by an excessive accumulation of visceral fat. The typical Western diet, which is very high in fat and sucrose, is considered a major factor in the visceral fat accumulation and the development of muscle insulin resistance. In contrast, dietary strategies that are designed to prevent or reverse increased visceral fat mass may help to prevent the development of diet-induced muscle insulin resistance and diabetes.

Dietary interventions that reduce daily energy intake by 20 to 40% of baseline requirements, also known as calorie restriction (CR), have been shown to cause numerous physiological benefits in both human and animals (Fontana and Klein, 2007). With respect to muscle insulin resistance, Kim et al. (2000) reported that restricting the daily intake of a high-fat diet to 75% of ad libitum completely prevented the high-fat diet-induced increase in visceral fat and muscle insulin resistance in rats, suggesting that CR is a useful tool to prevent or ameliorate diet-induced muscle insulin resistance.

Recently, reducing the energy intake by alternate-day fasting (ADF, food provided on every second day) has been proposed as an alternative to CR. Recent studies have demonstrated that ADF lowers body weight, waist circumference and visceral fat mass in both animals and humans (Varady and Hellerstein, 2007, 2008), suggesting that ADF can reproduce many of the effects of CR on the risk factors

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for diseases. These findings led us to hypothesize that ADF could also be a useful dietary treatment to ameliorate diet-induced muscle insulin resistance, which is associated with visceral fat accumulation. Since, to date, only one study was performed to examine the effects of reducing energy intake by ADF on glucose metabolism, and non-obese subjects were recruited for participation in that study (Heilbronn et al., 2005), it is still unclear whether ADF can improve obesity-induced insulin resistance in skeletal muscle. In this context, the present study evaluated the effects of ADF on high-fat diet-induced obesity and muscle insulin resistance in rats.

Materials and methods

Materials

The reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad (Hercules, CA, USA). Monoclonal β-actin antibody and horseradish peroxidase (HRP)-conjugated secondary antibodies were from Sigma (St. Louis, MO, USA) and Jackson ImmunoResearch Laboratories (West Grove, PA, USA), respectively. Polyclonal antiserum specific for the GLUT-4 was a generous gift from Mike Mueckler (Washington University, St. Louis, MO, USA). Enhanced chemiluminescence (ECL) reagent was purchased from Amersham (Arlington Heights, IL, USA). All other chemicals were obtained from Sigma.

Treatment of animals

Male Wistar rats (30–50 g body weight) were obtained from CLEA Japan (Tokyo, Japan) and placed on either a high-fat diet (n = 24) or a standard chow diet (Chow group, n = 12) for 10 weeks. The high-fat diet was prepared using lard, corn oil, sucrose, and casein (32, 18, 27 and 23% of total calories, respectively), supplemented with vitamins (22 g/kg, AIN93 vitamin mix, CLEA Japan, Tokyo, Japan), minerals (51 g/kg, AIN93G mineral mix, CLEA Japan, Tokyo, Japan), and methionine (4.4 g/kg, Wako Pure Chemical, Osaka, Japan). The standard rat chow, CE-2, was obtained from CLEA Japan (Tokyo, Japan). It contained 59% carbohydrate, 12% fat, and 29% protein as percentages of calories. The energy content of the high-fat diet was 5.1 kcal/g, whereas that of the rat chow was 3.4 kcal/g. This diet intervention protocol using the standard chow and high-fat diet has been well-established and used frequently in the nutrition research field (Kim et al., 2000; Hancock et al., 2008; Terada et al., 2012). The rats were provided the diets and water ad libitum. Feeding of this high-fat diet for 4–8 weeks has been shown to cause severe muscle and whole-body insulin resistance in rats (Kim et al., 2000; Terada et al., 2012).

Rats fed the high-fat diet were separated into two groups matched for body weight after 4 weeks on the diet. The animals in one group (HF-ADF group, n = 12) were subjected to alternate-day fasting (food provided every second day) for the subsequent 6 weeks. The other group (HF-AL group, n = 12) was maintained on an ad libitum diet.

On the day before the glucose uptake experiment, HF-ADF as well as HF-AL and Chow groups were allowed free access to diets until 7:00 PM (food was removed after 7:00 PM). After overnight fasting (17–19 h) rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight), and the epitrochlearis and triceps muscles were excised. The order of anesthetization and excision was randomized among the three groups. After the muscle dissection was completed, blood samples were collected via cardiac puncture, and then the epididymal, mesenteric, and retroperitoneal fat pads were removed and weighed. This research was approved by the Committee for Animal Experimentation in the School of Sport Sciences at Waseda University.

Muscle incubations and measurements of insulin-stimulated 2-deoxyglucose uptake

After dissection, the epitrochlearis muscles were incubated with shaking at 35 °C in flasks containing 4 ml of oxygenated Krebs–Henseleit bicarbonate buffer (KHB) containing 8 mM glucose, 32 mM mannitol, and 0.1% radioimmunoassay-grade bovine serum albumin (BSA) for 1 h. The gas phase was 95% O_2–5% CO_2.

We used 2-deoxyglucose (2-DG) to measure the rate of muscle glucose uptake based on the method described by Koshinaka et al. (2008). To remove glucose from the extracellular space and induce stimulation by insulin, we incubated the muscles for 30 min at 30 °C in 4 ml of oxygenated KHB containing 2 mM sodium pyruvate, 36 mM mannitol, 0.1% BSA, and 10 mU/ml insulin. After the rinse step, the muscles were incubated for 30 min at 30 °C in 4 ml of KHB containing 8 mM 2-DG, 32 mM mannitol, 0.1% BSA, and 10 mU/ml insulin with a gas phase of 95% O_2–5% CO_2 in a shaking incubator. The muscles were then blotted and frozen in liquid nitrogen. The muscles were 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 (2DG6P). The intracellular accumulation of 2DG6P has been shown to reflect muscle glucose transport activity (Koshinaka et al., 2008).

Insulin-tolerance test

In a separate subsequent experiment intended to further evaluate the effects of ADF on whole-body insulin sensitivity in the rats fed the high-fat diet, an insulin-tolerance test was performed. For the insulin-tolerance test, another set of male Wistar rats (n = 6 in each group) was treated for 10 weeks as described above, and these rats received an intraperitoneal injection of human regular insulin (Lilly, Indianapolis, IN, USA) at a dose of 0.75 U/kg body weight after overnight fasting. Blood was drawn from the tail at 0, 30, 60, and 120 min and was assayed for blood glucose with a glucose analyzer (ONE TOUCH Assist, LifeScan, Milpitas, CA, USA).

Western blot analysis

Frozen epitrochlearis muscles were homogenized in ice-cold RIPA buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM ethylenediaminetetraacetic acid (EDTA), and protease inhibitor cocktail. Protein concentrations were measured with a BCA protein assay kit (Pierce, Rockford, IL, USA). Samples were prepared in Laemmli sample buffer (Wako Pure Chemical, Osaka, Japan). Equal amounts of sample protein were subjected to SDS-PAGE (10% resolving gels) and then transferred to polyvinylidene difluoride (PVDF) membranes at 220 mA for 1 h. After transfer, the membranes were blocked for 1 h at room temperature in Tris-buffered saline with 0.1% Tween 20 (TBST; 20 mM Tris base, 137 mM NaCl, pH 7.6) supplemented with 10% nonfat powdered milk. Membranes were incubated overnight with antibodies specific for GLUT-4 at concentrations of 1:10000 at 4 °C. The HRP-conjugated secondary antibody (goat anti-rabbit IgG) was used at a concentration of 1:10000. Bands were visualized by ECL and quantified using densitometry. The expression of β-actin was also measured as an internal control.

Analytical procedure

Serum glucose, free fatty acid (FFA), and triglyceride concentrations were determined using kits (Glucose C2 Test Wako, NEFA-C Test Wako, and Triglyceride E Test Wako, respectively) obtained from Wako Pure Chemical (Osaka, Japan). Serum concentrations of insulin, adiponectin, tumor necrosis factor-α (TNFα), and monocyte