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High-fat diet impairs the effects of a single bout of endurance exercise on glucose transport and insulin sensitivity in rat skeletal muscle

Satsuki Tanaka[a], Tatsuya Hayashi[a,b,*], Taro Toyoda[c], Taku Hamada[b], Yohei Shimizu[b], Masakazu Hirata[a], Ken Ebihara[a], Hiroaki Masuzaki[a], Kiminori Hosoda[a], Tohru Fushiki[c], Kazuwa Nakao[a]

[a]*Department of Medicine and Clinical Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan*
[b]*Laboratory of Sports and Exercise Medicine, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan*
[c]*Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

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Abstract

A single bout of exercise increases the rate of muscle glucose transport (GT) by both insulin-independent and insulin-dependent mechanisms. The purpose of this study was to determine whether high-fat diet (HFD) feeding interferes with the metabolic activation induced by moderate-intensity endurance exercise. Rats were fed an HFD or control diet (CD) for 4 weeks and then exercised on a treadmill for 1 hour (19 m/min, 15% incline). Insulin-independent GT was markedly higher in soleus muscle dissected immediately after exercise than in muscle dissected from sedentary rats in both dietary groups, but insulin-independent GT was 25% lower in HFD-fed than in CD-fed rats. Insulin-dependent GT in the presence of submaximally effective concentration of insulin (0.9 nmol/L) was also higher in both dietary groups in muscle dissected 2 hours after exercise, but was 25% lower in HFD-fed than in CD-fed rats. Exercise-induced activation of 5′adenosine monophosphate–activated protein kinase, a signaling intermediary leading to insulin-independent GT and regulating insulin sensitivity, was correspondingly blunted in the HFD group. High-fat diet did not affect glucose transporter 4 content or insulin-stimulated Akt phosphorylation. Our findings provide evidence that an HFD impairs the effects of short-term endurance exercise on glucose metabolism and that exercise does not fully compensate for HFD-induced insulin resistance in skeletal muscle. Although the underlying mechanism is unclear, reduced 5′adenosine monophosphate–activated protein kinase activation during exercise may play a role.

1. Introduction

Physical exercise has profound effects on glucose metabolism in contracting skeletal muscle. Exercise activates glucose transport (GT) in skeletal muscle by inducing translocation of glucose transporter 4 (GLUT4) to the cell surface by insulin-independent and insulin-dependent mechanisms (reviewed in Hayashi et al [1]). The activity of insulin-independent GT is markedly enhanced during exercise; and this effect wears off within several hours after exercise, when the postexercise increase in insulin sensitivity that leads to insulin-dependent GT becomes prominent. Wallberg-Henriksson et al [2] showed in isolated rat skeletal muscle that the rate of insulin-independent GT is maximal immediately after exercise, whereas the postexercise increase in insulin sensitivity becomes detectable 3 hours after exercise. Correspondingly, Price et al [3] showed in human muscle that postexercise glycogen repletion occurs in an insulin-independent manner for about 1 hour after exercise, after which insulin-dependent glycogen repletion becomes significant. These exercise-stimulated mechanisms form the basis of practices to prevent individuals from developing glucose intolerance and to improve glycemic control in patients with type 2 diabetes mellitus.

It is of interest to know whether exercise-stimulated GT, including both the insulin-independent and insulin-dependent components, is normal in the state of insulin resistance. Although numerous studies have shown that a

* Corresponding author. Laboratory of Sports and Exercise Medicine, Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-nihonmatsu-cho, Sakyo-ku, Kyoto, 606-8501, Japan. Tel.: +81 75 753 6640; fax: +81 75 753 6640.
E-mail address: tatsuya@kuhp.kyoto-u.ac.jp (T. Hayashi).

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high-fat diet (HFD) causes insulin resistance in muscles at rest, it is unknown whether an HFD interferes with the short-term stimulatory effect of exercise on insulin sensitivity. Only one study has addressed this topic and demonstrated that the postexercise increase in muscle insulin sensitivity is abolished completely in HFD-fed rats [4]. In that study, however, insulin-dependent GT was measured before the insulin-independent glucose uptake wore off (its activity was still 160% higher than the basal uptake), indicating that the net effect of exercise on insulin sensitivity was substantially underestimated because of residual glucose uptake activity. There is considerable controversy over whether an HFD alters the effects of short-term exercise on insulin-independent GT. Most investigators have reported about 50% reduction in the rate of muscle GT stimulated by exercise [5-7] and electrical stimulation [6,8,9] in HFD-fed rodents, although others did not find these effects [4,10]. Moreover, some studies have shown that the reduction in insulin-independent GT was not associated with decreased muscle GLUT4 content [5,6]; but a conflicting result was also reported [8]. Although Hansen et al [9] showed that impairment of the exercise-stimulated GT is associated with decreased GLUT4 translocation to the cell surface, the responsible signaling mechanism remains to be elucidated.

The purposes of our present study were to determine how HFD affects insulin-independent and insulin-dependent GT activated by a single bout of endurance exercise and to explore the underlying mechanism that leads to the change in exercise-stimulated glucose utilization. We found that both components of exercise-induced GT were impaired by an HFD and that these changes were accompanied by a decrease in 5′adenosine monophosphate (AMP)–activated protein kinase (AMPK) activation in skeletal muscle of rats fed an HFD for 4 weeks.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats at the time of weaning were purchased from Clea Japan (Tokyo, Japan). Animals were fed either control diet (CD) (MF; 3.6 kcal/g, 12% kcal fat, source: soybean; Oriental Yeast, Tokyo, Japan) or HFD (D12493; 5.2 kcal/g, 60% kcal fat, source: soybean/lard; Research Diets, New Brunswick, NJ) for 4 weeks. All animal experiments were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

2.2. Exercise and muscle sampling

The rats were accustomed to a rodent treadmill (Muromachi Kikai, Kyoto, Japan) by running at 14 to 18 m/min on a 15% grade for 5 minutes on the day before the experiment. After an overnight fast, rats performed treadmill running at 19 m/min on a 15% grade for 1 hour or were kept sedentary. To study insulin-independent GT, exercised rats were killed by cervical dislocation immediately after the cessation of running; and the soleus muscles were isolated. Muscles were incubated in 7 mL Krebs-Ringer bicarbonate buffer containing 2 mmol/L pyruvate (KRBP) at 37°C for 20 minutes, and then 3-*O*-methyl-D-glucose (3MG) uptake activity was determined as described previously [11-14]. Muscles dissected from sedentary rats were treated similarly. Some of the muscles were frozen in liquid nitrogen immediately after dissection for analysis of isoform-specific AMPK activity and Western blotting of phosphorylated AMPKα and phosphorylated acetyl–coenzyme A carboxylase (ACC). Muscles from sedentary animals were also analyzed by Western blotting of total AMPKα and GLUT4. For histochemical analysis, soleus muscles were isolated from sedentary animals and frozen in dry ice-cooled 2-methylbutane. Abdominal fat (epididymal, retroperitoneal, and mesenteric fat pads) was collected from sedentary animals and weighed. To study the postexercise effect on insulin-dependent GT, exercised and sedentary rats were placed in separate cages with free access to drinking water but without food for 2 hours, after which the rats were killed by cervical dislocation and the soleus muscles were dissected. Isolated muscles were incubated for 30 minutes in KRBP in the absence or presence of half-maximally effective insulin (0.9 nmol/L) at 37°C, and then 3MG uptake was determined. Some muscles were frozen in liquid nitrogen immediately after incubation for Western blotting of phosphorylated Akt, a signaling intermediary leading to insulin-stimulated GT. To measure glycogen and triglyceride content, muscles were isolated from sedentary rats and from exercised rats immediately and 2 hours after exercise, and frozen in liquid nitrogen. We chose soleus muscle because our preliminary studies showed that soleus muscle provided the most prominent activation of insulin-independent and insulin-dependent GT in response to exercise compared with other muscles including extensor digitorum longus and epitrochlearis.

2.3. 3MG uptake

To assay GT, incubated muscles were transferred to 2 mL Krebs-Ringer bicarbonate buffer containing 1 mmol/L 3-*O*-[methyl-^{3}H]-D-glucose (1.5 μCi/mL) (American Radiolabeled Chemicals, St Louis, MO) and 7 mmol/L D-[^{14}C] mannitol (0.3 μCi/mL) (PerkinElmer Life Science, Boston, MA) at 30°C and incubated for 10 minutes [11-14]. The muscles were weighed and processed by incubating them in 450 μL of 1 mol/L NaOH at 80°C for 10 minutes. Digestates were neutralized with 1 mol/L HCl, and particulates were precipitated by centrifugation at 20 000*g* for 2 minutes. Radioactivity in aliquots of the digested protein was determined by liquid scintillation counting for dual labels.

2.4. Isoform-specific AMPK activity assay

Muscles were treated as described [11-14]. Frozen muscles were homogenized in ice-cold lysis buffer (1:40

wt/vol) containing 20 mmol/L Tris-HCl (pH 7.4), 1% Triton X-100, 50 mmol/L NaCl, 250 mmol/L sucrose, 50 mmol/L NaF, 5 mmol/L sodium pyrophosphate, 2 mmol/L dithiothreitol, 4 mg/L leupeptin, 50 mg/L soybean trypsin inhibitor, 0.1 mmol/L benzamidine, and 0.5 mmol/L phenylmethylsulfonyl fluoride, and centrifuged at 14 000*g* for 30 minutes at 4°C. The supernatants (100 μg of protein) were immunoprecipitated with antibodies directed against the α1 or α2 catalytic subunits of AMPK [11] and protein A–Sepharose CL-4B (Amersham, Buckinghamshire, United Kingdom). Kinase reactions were performed in the presence of SAMS peptide [11], and then ^{32}P incorporation was quantitated with a scintillation counter.

2.5. Western blotting

For analysis of phosphorylated AMPKα, total AMPKα, phosphorylated ACC, and phosphorylated Akt, muscles were homogenized in lysis buffer used for isoform-specific AMPK activity. Lysates were solubilized in Laemmli sample buffer containing mercaptoethanol and boiled. For analysis of GLUT4, muscles were homogenized in ice-cold buffer containing 250 mmol/L sucrose, 20 mmol/L 2-[4-(2-hydroxyethyl)-1-piperadinyl] ethonsulforic acid (HEPES) (pH 7.4), and 1 mmol/L EDTA, and centrifuged at 1200*g* for 5 minutes. The supernatant was centrifuged at 200 000*g* for 60 minutes at 4°C. The resulting pellet was solubilized in Laemmli sample buffer containing dithiothreitol. Samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membranes (PolyScreen; PerkinElmer, Wellesley, MA). Blocked membranes were incubated with phosphospecific AMPKα Thr^{172} (Cell Signaling Technology, Beverly, MA), total AMPKα (Cell Signaling Technology), phosphospecific ACC Ser^{79} (Upstate Biotechnology, Lake Placid, NY), phosphospecific Akt (Ser^{473}) (Cell Signaling Technology), and GLUT4 (Biogenesis; South Coast, United Kingdom) antibodies. Proteins were visualized with enhanced chemiluminescence reagents (Amersham). The signal was quantified with a Lumino-Image Analyzer LAS-1000 System (Fuji Photo Film, Tokyo, Japan).

2.6. Histochemical analysis

Serial sections (10 μm thick) were used for muscle fiber typing and intramyocellular lipid (IMCL) measurement. To determine muscle fiber type (type I, IIa), myosin adenosine triphosphatase (ATPase) staining was performed as described [15,16]. Sections were incubated in acidic (30 mmol/L sodium barbital and 50 mmol/L sodium acetate, adjusted to pH 4.3 with HCl) or alkaline buffer (50 mmol/L $CaCl_2$ and 75 mmol/L NaCl, adjusted to pH 10.6 with NaOH) and then incubated in staining buffer (2.8 mmol/L adenosine triphosphate, 50 mmol/L $CaCl_2$, 75 mmol/L NaCl, adjusted to pH 9.4 with NaOH), followed by immersion in 1% $CaCl_2$, 2% $CoCl_2$, and 1% $(NH_4)_2S$. The sections were treated in ethanol and xylol, dried in the air, and then mounted with Aquatex (Merk, Darmstadt, Germany). Fiber type distribution was determined by counting the number of each fiber type in 100 contiguous fibers in a muscle section. To determine IMCL content, the oil red O (ORO) staining procedure and stained area measurement were performed as described [17]. Sections were incubated with formaldehyde-methanol (1:1 vol/vol) and then incubated with ORO solution followed by extensive wash with distilled water. The sections were dried in the air and then mounted with Aquatex. Images from each section were saved as gray-scale images, and the digitized data were then analyzed using the freeware ImageJ software (http://rsb.info.nih.gov/). The amount of IMCL in each fiber was quantified as the percentage of the area occupied by ORO-stained droplets (total area occupied by lipid droplets of a muscle fiber) × 100/total cross-sectional area of the fiber. Lipid area was calculated for each of 3 different fields within the section, and a mean percentage was then calculated for each muscle [17].

2.7. Muscle glycogen and triglyceride content measurement

Glycogen content was assayed as described [12,14]. Frozen muscles were weighed and digested in 1 mol/L NaOH (1:9 wt/vol) at 80°C for 10 minutes. The digestates were neutralized with 1 mol/L HCl, and then 6 mol/L HCl was added to obtain a final concentration of 2 mol/L HCl. The digestates were incubated at 85°C for 2 hours and then neutralized with 5 mol/L NaOH. The concentration of hydrolyzed glucose residues was measured enzymatically using the hexokinase glucose assay reagent (Glucose CII Test; Wako, Osaka, Japan). Triglyceride content was measured as described [18]. Total lipids were extracted from muscles with isopropyl alcohol–heptane (1:1 vol/vol) and saponified in ethanolic KOH (0.5 mol/L). Free glycerol concentration was then determined using a commercial kit (Triglyceride E Test; Wako).

2.8. Blood sample analysis

Blood samples were collected from the tail vein using heparinized glass tube 3 days before the experimental day after an overnight fast. Plasma levels for glucose (Glutest-Ace; Sanwa Kagaku Kenkyusyo, Nagoya, Japan), insulin (rat insulin ELISA kit; Morinaga, Yokohama, Japan), leptin (rat leptin radioimmunoassay kit; LINCO, St Charles, MO), triglycerides (Triglyceride E Test; Wako), and lactate (Lactate Pro; Arkray, Kyoto, Japan) were measured. Lactate concentration was also measured on the experimental day immediately after exercise.

2.9. Statistical analysis

Results are presented as means ± SE. The significance of difference between 2 groups was evaluated using Student *t* test. Multiple means were compared by analysis of variance followed by post hoc analysis using Dunn's procedure. $P < .05$ was considered statistically significant.

3. Results

3.1. Metabolic parameters in rats fed the CD and HFD

Table 1 summarizes the basic characteristics of the CD- and HFD-fed rats (Table 1). Rats fed the HFD for 4 weeks were slightly heavier and had higher plasma concentrations of glucose, insulin, triglycerides, and leptin than did CD-fed rats.

3.2. HFD increases IMCL in soleus

We analyzed the influence of HFD on IMCL concentration by ORO staining (Fig. 1A-B). Muscle fiber type was determined by myofibrillar ATPase histochemical staining (Fig. 1C-F). The fiber type proportions did not differ significantly between CD-fed and HFD-fed rats (CD, 80.7% ± 1.2% type I and 19.3% ± 1.2% type IIa fibers; HFD, 79.2% ± 2.5% type I and 20.8% ± 2.5% type IIa fibers). In both muscle fiber types, the IMCL content in HFD-fed rat muscle was twice as high as that in muscles from CD-fed rats. In CD-fed rats, the IMCL content was 2.3% ± 0.5% in type I fibers and 5.9% ± 1.3% in type IIa fibers. In HFD-fed rats, the respective values were 4.5% ± 0.9% and 12.8% ± 2.0% ($P < .05$ vs CD) (Fig. 1G). The IMCL content in the total soleus muscle was also twice as high in the HFD group than in the CD group (3.0% ± 0.7% vs 6.2% ± 1.0%, $P < .05$) (Fig. 1G).

3.3. HFD attenuates activation of insulin-independent GT induced by one bout of endurance exercise

To analyze insulin-independent GT stimulated by one bout of exercise, soleus muscles were dissected and 3MG uptake was determined ex vivo in the absence of insulin immediately after exercise (Fig. 2). Exercise elicited significant activation of insulin-independent 3MG uptake by 3.4 times in muscles from CD-fed rats and by 2.9 times in muscles from HFD-fed rats. However, the rate of insulin-independent 3MG uptake stimulated by exercise was 25% lower in muscles from HFD-fed rats (0.15 ± 0.02 μmol/[g h]) than in muscles from CD-fed rats (0.11 ± 0.01 μmol/[g h]) ($P < .05$). Basal glucose uptake was not affected by dietary manipulation.

Table 1
Metabolic parameters in rats under HFD and CD feeding

	CD	HFD
Body weight (g)	193 ± 2	203 ± 3 *
Food intake (kcal/d)	54 ± 1	58 ± 1 *
Plasma glucose (mg/dL)	78 ± 2	88 ± 2 *
Plasma insulin (mg/dL)	1.3 ± 0.1	2.5 ± 0.2 *
Plasma triglycerides (mg/dL)	98 ± 4	122 ± 8 *
Plasma leptin (ng/mL)	1.6 ± 0.2	7.6 ± 0.4 *
Abdominal fat (g)	5.3 ± 0.5	9.7 ± 0.4 *

Male Wistar rats at the time of weaning were fed CD or HFD for 4 weeks. Body weight, abdominal fat weight, and plasma parameters were measured at the end of week 4. Blood samples were obtained after an overnight fast at 9:00 to 11:00 AM. Data are means ± SE; n = 6 to 19 per group.

* $P < .05$ vs CD-fed group.

3.4. One bout of endurance exercise activates insulin-dependent GT after exercise, but does not fully compensate for insulin resistance in muscle from HFD-fed rats

The effect of the HFD on insulin-dependent GT is shown in Fig. 3. The rate of insulin-dependent 3MG uptake was 59% lower in muscles from sedentary HFD-fed rats than in sedentary CD-fed rats (0.12 ± 0.02 vs 0.05 ± 0.01 μmol/[g h], $P < .05$), indicating marked insulin resistance in the HFD-fed animals (Fig. 3; insulin+, sedentary). Two hours after exercise, when insulin-independent 3MG uptake stimulated by exercise had declined significantly (Fig. 3; insulin−, 2 hours postexercise), insulin-dependent 3MG uptake was markedly higher in muscles from exercised animals than in muscles from sedentary rats in both dietary groups (Fig. 3; insulin+, 2 hours postexercise). The net increase in the rate of insulin-stimulated 3MG uptake was similar in both dietary groups (CD, 0.33 ± 0.03 μmol/[g h] vs HFD, 0.27 ± 0.02 μmol/[g h]). However, the rate of insulin-stimulated 3MG uptake was still 25% lower in HFD-fed rats than in CD-fed rats (0.36 ± 0.03 vs 0.27 ± 0.03 μmol/[g h], $P < .05$).

3.5. HFD attenuates muscle AMPKα2 activation by one bout of endurance exercise

We evaluated whether the HFD affects muscle AMPK activity, a signaling intermediary leading to insulin-independent GT [12,19-21] and regulation of insulin sensitivity [22-24]. Neither diet nor one bout of exercise had an effect on AMPKα1 activity (Fig. 4A). In contrast, exercise increased AMPKα2 activity by 1.6 times in muscle from CD-fed rats ($P < .05$), whereas in muscle from HFD-fed rats, AMPKα2 activation did not change significantly (Fig. 4B). Interestingly, the basal AMPKα2 activity was 1.3 times higher in muscle from HFD-fed rats than from CD-fed rats ($P < .05$), whereas the AMPKα2 activity immediately after exercise was similar in both dietary groups (Fig. 4B). Therefore, the exercise-mediated response of AMPKα2 activity was significantly lower in HFD-fed than in CD-fed rats. This is consistent with the findings of AMPKα2 activity: exercise increased phosphorylation of the Thr172 residue of AMPKα, an essential site for full kinase activation (Fig. 4C), and the Ser79 residue of ACC, a known substrate of muscle AMPK (Fig. 4D), in muscles from CD-fed animals, but not in HFD-fed animals. The protein level of AMPKα did not differ between muscles from both dietary groups (Fig. 4E).

3.6. One bout of endurance exercise dose not affect nsulin-stimulated phosphorylation of muscle Akt in CD- and HFD-fed rats

To determine whether HFD impairs the downstream of phosphatidylinositol 3-kinase (PI-3 kinase), we measured the

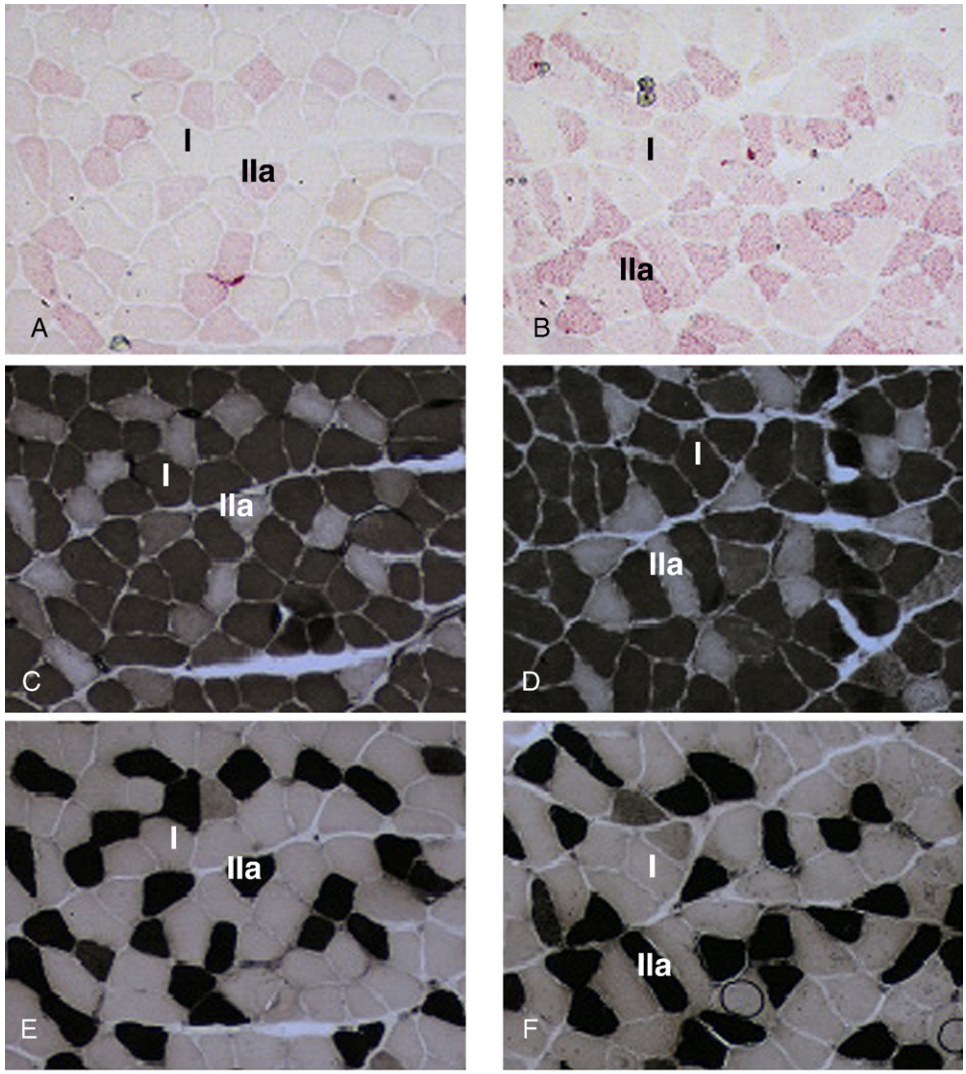

Fig. 1. High-fat diet increases IMCL content in soleus muscle. Representative transverse sections of soleus muscle dissected from CD-fed (A, C, and E) and HFD-fed (B, D, and F) rats (80× magnification). A and B, Oil red O staining of IMCL. Oil red O stains neutral lipid (mainly triglycerides) with an orange-red tint, and lipid droplets are seen as distinct spots of stain (A, B). C and D, Myosin ATPase staining (pH 4.3). Light and dark fibers are type IIa and I, respectively (C, D). E and F, Myosin ATPase staining (pH 10.6). Light and dark fibers are type I and IIa, respectively (E, F). G, Fiber type–specific IMCL content, expressed as a percentage of the area of lipid stained. Data are means ± SE; n = 7 per group. *$P < .05$ vs CD-fed group.

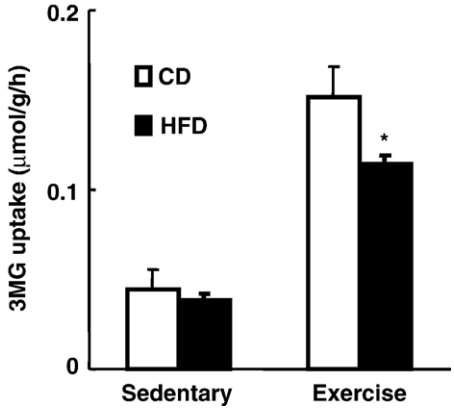

Fig. 2. High-fat diet attenuates activation of insulin-independent GT induced by one bout of moderate-intensity endurance exercise. Rats ran on a treadmill for 1 hour at 19 m/min at a 15% incline. Soleus muscles were isolated from exercised rats immediately after the cessation of running and from rats kept sedentary. Isolated muscles were incubated for 20 minutes in the absence of insulin, and 3MG uptake was determined. Data are means ± SE; n = 5 to 9 per group. *$P < .05$ vs CD-fed group.

phosphorylation of the Ser473 residue of Akt in muscles dissected 2 hours after exercise and stimulated with insulin in vitro (Fig. 5). Despite the significant increase in insulin-stimulated 3MG uptake in muscles from exercised rats (Fig. 3), Akt phosphorylation in muscle was not affected by exercise in CD- or in HFD-fed rats. The level of basal and insulin-stimulated Akt phosphorylation was not affected by the dietary manipulation.

3.7. HFD dose not change muscle GLUT4 protein level

To test the possibility that the decrease in insulin-independent and insulin-dependent GT activity caused by HFD is mediated by a reduction in the GLUT4 content of skeletal muscle, we measured soleus muscle GLUT4 protein content (Fig. 6). The GLUT4 protein content did not differ between soleus muscles from CD- and HFD-fed rats.

3.8. HFD does not change muscle glycogen concentration, but increases muscle triglyceride content

Basal and postexercise glycogen concentrations were similar in soleus muscle in CD- and HFD-fed rats (Table 2). In contrast, muscle triglyceride content was higher in muscle from HFD-fed rats than from CD-fed rats (Table 2) ($P < .05$).

3.9. Effect of one bout of endurance exercise on blood lactate concentration

To evaluate the intensity of exercise, we measured blood lactate concentration at rest and immediately after exercise. Compared with basal values, exercise increased blood lactate by 1.8 times in CD-fed rats (from 1.7 ± 0.2 to 3.1 ± 0.1 mmol/L, $P < .05$) and by 2.1 times in HFD-fed rats (from 1.6 ± 0.1 to 3.4 ± 0.2 mmol/L, $P < .05$). Blood lactate concentration did not differ between dietary groups.

4. Discussion

Endurance exercise has long been advocated as beneficial for patients with insulin resistance associated with type 2 diabetes mellitus and obesity. This is based partly on the observation that, even in people with insulin resistance, endurance exercise stimulates muscle glucose uptake in skeletal muscle by 2 distinct mechanisms: one insulin independent and one insulin dependent (reviewed in Hayashi et al [1]). Reversal of the short-term increase in GT after cessation of contractile activity is followed by a marked increase in the sensitivity of muscle to insulin. We found a significant increase in insulin-independent GT followed by insulin-dependent GT in rat soleus muscle after a 1-hour bout of treadmill running in both the CD-fed and HFD-fed rats. In both dietary groups, the mild increase in blood lactate concentration (<4 mmol/L) (Results) and significant reduction in muscle glycogen content (Table 2) after exercise suggest that rats performed moderate-intensity endurance exercise and that muscle glucose utilization was substantially activated by exercise. Similar lactate concentration and glycogen content also indicate that the exercise intensity did not differ between the dietary groups.

Although we found that exercise activated GT in skeletal muscle from both CD- and HFD-fed rats, the absolute rates of insulin-independent and insulin-dependent GT were lower in muscles from HFD-fed rats than in muscles from CD-fed rats; in contrast, muscle GLUT4 levels were similar between the groups (Figs. 2 and 3). Our results are consistent with previous studies demonstrating that exercise-stimulated insulin-independent GT is impaired in muscles from HFD-fed rats in the absence of a reduction in muscle GLUT4 content [5,6]. Liu et al [4] measured insulin-dependent GT in

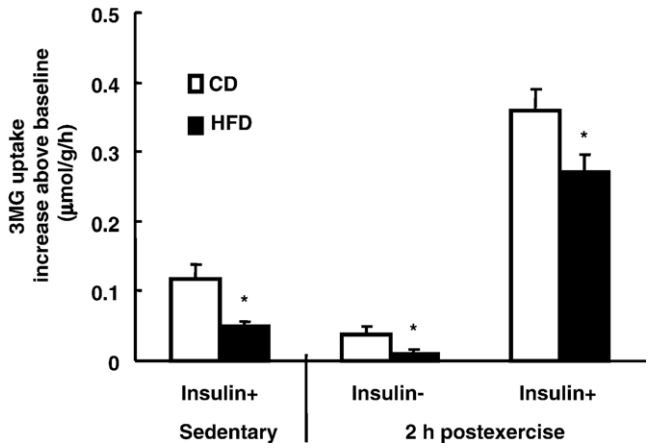

Fig. 3. One bout of endurance exercise activates insulin-dependent GT after exercise, but does not fully compensate for reduced insulin sensitivity in muscle from HFD-fed rats. Soleus muscles were isolated from exercised rats 2 hours after exercise and from sedentary rats. Isolated muscles were incubated for 30 minutes in the absence (insulin−) or presence of 0.9 nmol/L insulin (insulin+), and 3MG uptake was determined. Baseline (insulin-independent) activity was subtracted in each diet group. Data are means ± SE; n = 10 per group. *$P < .05$ vs CD-fed group.

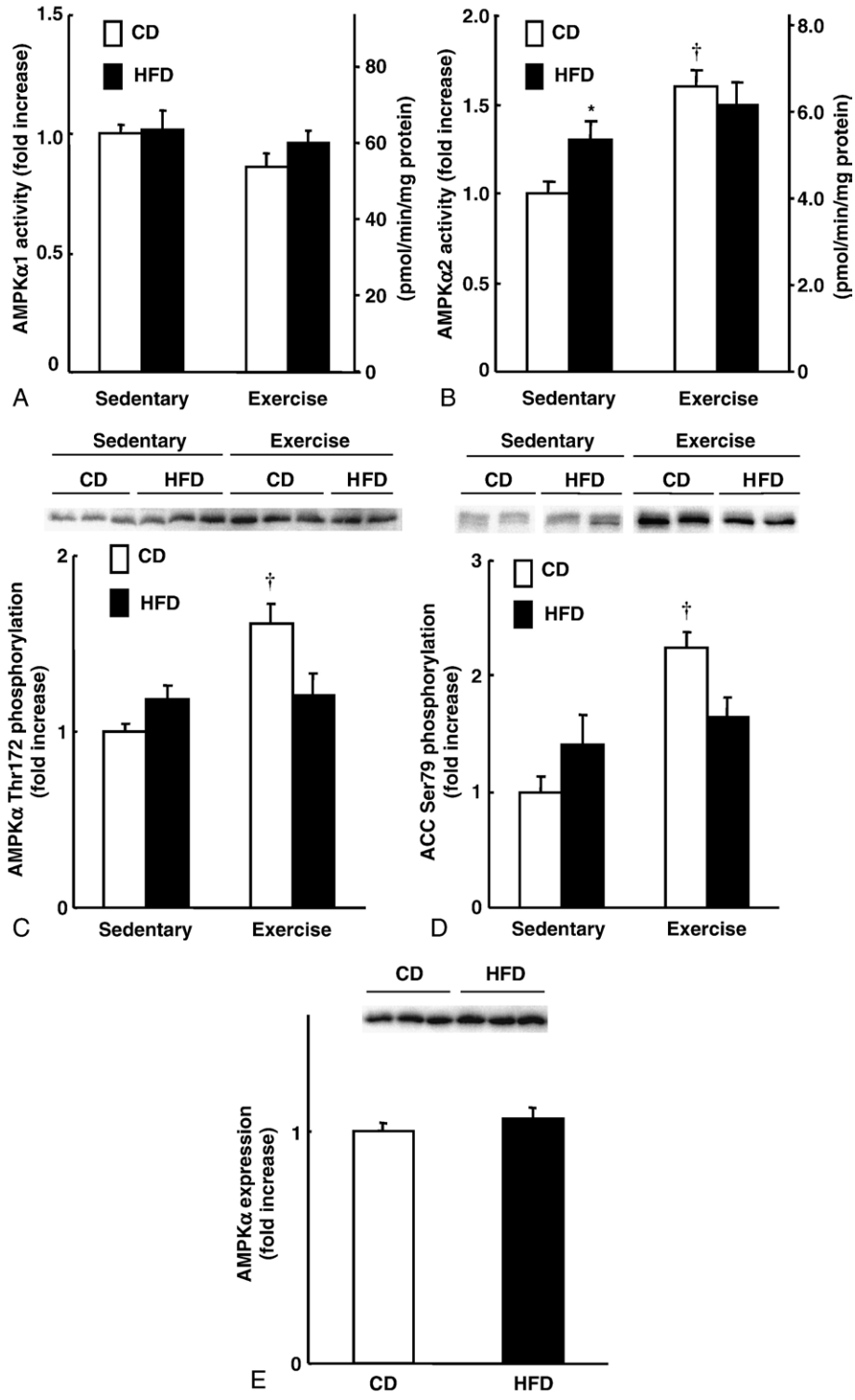

Fig. 4. High-fat diet attenuates muscle AMPKα2 activation induced by one bout of endurance exercise. Soleus muscles were isolated from exercised rats immediately after exercise and from sedentary rats. Isoform-specific AMPK activity was determined in anti-AMPKα1 (A) and α2 (B) immunoprecipitates. Muscles were also subjected to Western blot analysis using antiphosphorylated AMPK (C), antiphosphorylated ACC (D), and anti-AMPKα (E) antibodies. Fold increases are expressed relative to the activity of muscles from the sedentary CD-fed group. Data are means ± SE; n = 13 to 16 per group. *$P < .05$ vs CD-fed group, †$P < .05$ vs corresponding sedentary group.

epitrochlearis muscle in the presence of half-maximally effective concentration of insulin (0.8 nmol/L) after rats swam for 2 hours. They found no increase after exercise in insulin-dependent GT in muscles from HFD-fed rats because of the maintenance of a high level of insulin-independent GT in skeletal muscle even at 3.5 hours after exercise. In

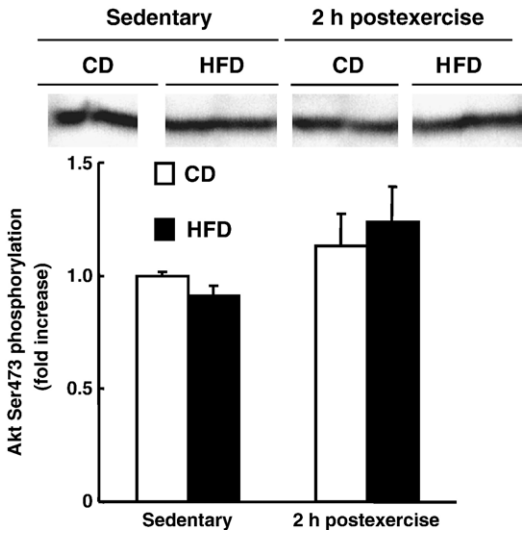

Fig. 5. One bout of endurance exercise does not affect insulin-stimulated phosphorylation of Akt in CD- and HFD-fed rats. Soleus muscles were isolated from exercised rats 2 hours after exercise and from sedentary rats, and then incubated for 30 minutes in the presence of insulin (0.9 nmol/L). Muscles were subjected to Western blot analysis using antiphosphorylated Akt antibody. Fold increases are expressed relative to the level in muscles from the sedentary CD-fed group. Data are means ± SE; n = 9 per group.

contrast, insulin-independent GT was substantially lower 2 hours after exercise in our study; and insulin-dependent GT was lower in muscles from HFD-fed animals (Fig. 3). Thus, it seems reasonable to speculate that prolonged HFD feeding evokes "exercise resistance" as well as insulin resistance; that is, HFD does not allow physiological exercise to activate GT or insulin sensitivity to the level achieved by similar exercise in skeletal muscle of CD-fed animals.

The blunted AMPK activation during exercise may be part of the mechanism leading to impaired exercise-

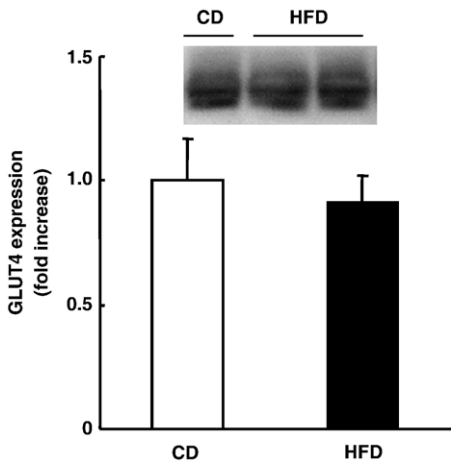

Fig. 6. High-fat diet feeding does not change muscle GLUT4 protein level. The GLUT4 protein level was determined in soleus muscle using Western blot analysis. Fold increases are expressed relative to the level in the CD-fed group. Data are means ± SE; n = 6 to 7 per group.

Table 2
Muscle glycogen and triglyceride concentrations in rats under HFD and CD feeding

	Sedentary	Immediate postexercise	2 h postexercise
Muscle glycogen (μmol/g wet weight)			
CD	23.6 ± 0.8	7.6 ± 0.6†	18.1 ± 1.0‡
HFD	22.4 ± 0.6	8.9 ± 0.4†	15.9 ± 0.5‡
Muscle triglycerides (μmol/g wet weight)			
CD	6.1 ± 0.5	4.9 ± 0.1†	5.0 ± 0.3†
HFD	7.8 ± 0.2*	7.1 ± 0.5*	6.5 ± 0.5†*

Soleus muscles were isolated from sedentary and exercised rats immediately and 2 hours after exercise, and glycogen and triglycerides concentrations were determined. Data are means ± SE; n = 6 to 14 per group.

* $P < .05$ vs CD-fed group.

† $P < .05$ vs sedentary group.

‡ $P < .05$ vs immediate postexercise group.

stimulated glucose metabolism in skeletal muscle. In the present study, short-term exercise increased the activity of the α2 isoform of muscle AMPK from baseline in CD-fed rats, but not in HFD-fed rats. Correspondingly, exercise stimulated the phosphorylation of AMPKα Thr172 and ACC Ser79 only in muscles from CD-fed animals. AMPK is a heterotrimeric serine-threonine protein kinase comprising a catalytic α subunit and regulatory β and γ subunits. Two distinct α isoforms, α1 and α2, are expressed in skeletal muscle; and both isoforms can be activated in response to muscle contraction by AMP-independent or AMP-dependent mechanisms [13]. AMPK has been implicated in a number of exercise-stimulated metabolic events in skeletal muscle, including insulin-independent GT and GLUT4 translocation [12,19-21], insulin sensitivity [22-24], fatty acid oxidation by the inactivation of ACC [12,25,26], GLUT4 expression [12,27-31], and glycogen utilization [14,32-34]. The mechanism underlying the significantly higher basal α2 activity in muscle from HFD-fed rats than in CD-fed rats (Fig. 4B) is unknown; but increased serum leptin concentration might play a role because, in vivo, both short- [35] and long-term [36] administrations of leptin activate AMPKα2 activity.

Although the predominant activation of AMPKα2 by exercise is consistent with most previous studies [12,37,38], we have recently reported that AMPKα1 activity is more sensitive to physical or physiological stress than AMPKα2 is and that AMPKα1 activity increases markedly during dissection, whereas AMPKα2 activity does not change [13]. Thus, it may be difficult to measure the α1 activity because it is disturbed by additional activation during dissection; only after high-intensity exercise, when the activation by muscle contraction exceeds that of the isolating stimuli, would AMPKα1 activity be detectable. In our 2006 study [13], we stabilized isolated muscle in KRBP for 60 minutes, which decreased α1 activity to a constant level and allowed us to observe the activation of AMPKα1 by electrical stimulation. This α1 activation was associated with corresponding increases in AMPKα phosphorylation, insulin-independent GT, and ACC phosphorylation.

Moreover, α1 was activated even by low-intensity contraction, which was characterized by the absence of an increase in AMP concentration or in the ratio of AMP to adenosine triphosphate. These observations suggest that the α1 isoform is the predominant form activated by low-intensity contractions and lead us to believe that activities of both α2 and α1 isoforms increase in response to moderate-intensity treadmill exercise used in our current study.

The mechanisms underlying the postexercise increase in insulin sensitivity, which probably relates to GLUT4 translocation in exercised muscle [39], are presumed to be mediated by multiple factors, including AMPK [22-24], muscle glycogen concentration, humoral factors, and autocrine-paracrine mechanisms (reviewed in Hayashi et al [1]). In the present study, Akt phosphorylation in response to insulin stimulus in muscle of HFD-fed rats did not decrease at rest or after exercise despite the blunted insulin-dependent GT. This result is consistent with a previous study showing that muscle insulin resistance induced by an HFD is not accompanied by impairment of Akt activation [40] and with studies demonstrating that the postexercise increase in insulin sensitivity does not accompany an enhancement of the insulin signal [22,39,41,42]. We conclude that some functional changes in the GLUT4 translocation system or signal transduction mechanism distal to Akt phosphorylation play a role in HFD-induced insulin resistance and impaired postexercise increase in insulin sensitivity.

People with type 2 diabetes mellitus exhibit remarkable muscle insulin resistance and abnormal lipid metabolism, which are also common among HFD-fed individuals. In contrast, previous studies have shown that the insulin-independent GT system activated by exercise is intact in diabetic people, unlike in the case of HFD-fed experimental animals. Kennedy et al [43] showed that muscles from people with type 2 diabetes mellitus retain the capacity to translocate GLUT4 to the sarcolemma in response to short-term exercise. Correspondingly, Musi et al [38] demonstrated that exercise normally activates muscle AMPK in people with type 2 diabetes mellitus. We note that individuals with type 2 diabetes mellitus generally have normal muscle GLUT4 protein levels [44,45]. Similarly, the genetically insulin-resistant obese Zucker rat has severe defects in insulin-stimulated glucose uptake [46] and GLUT4 translocation [47] despite normal levels of total muscle GLUT4 protein [46]. In contrast, these animals have normal increases in contraction-stimulated glucose uptake [48] and GLUT4 translocation [49,50]. To our knowledge, no study has addressed whether insulin sensitivity increases normally after a single bout of endurance exercise in people with type 2 diabetes mellitus or obese Zucker rats. Our finding that the HFD attenuated contraction-induced GT in muscle raises the possibility that the pathophysiological condition induced by the HFD differs from the condition exhibited by humans with type 2 diabetes mellitus and genetically insulin-resistant animals.

In summary, our study provides new evidence to suggest that moderate-intensity endurance exercise activates both insulin-independent and insulin-dependent components of muscle GT even when combined with HFD-induced insulin resistance. However, these metabolic effects are significantly reduced by an HFD; and consequently, exercise cannot compensate totally for muscle insulin resistance in HFD-fed rats. Although the precise mechanism is not clear, an HFD may evoke these metabolic impairments by reducing AMPK activation in contracting skeletal muscle. Appropriate fat intake might be important for efficient activation of glucose metabolism by exercise.

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