Exercise training increases ERK2 activity in skeletal muscle of obese Zucker rats

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Osman, Abdullah A., Joe Hancock, Desmond G. Hunt, John L. Ivy, and Lawrence J. Mandarino. Exercise training increases ERK2 activity in skeletal muscle of obese Zucker rats. J Appl Physiol 90: 454–460, 2001.—Acute exercise and training increase insulin action in skeletal muscle, but the mechanism responsible for this effect is unknown. Activation of the insulin receptor initiates signaling through both the phosphatidylinositol (PI) 3-kinase and the mitogen-activated protein kinase [MAPK, also referred to as extracellular signal-regulated kinases (ERK1/2)] pathways. Acute exercise has no effect on the PI3-kinase pathway signaling elements but does activate the MAPK pathway, which may play a role in the adaptation of muscle to exercise. It is unknown whether training produces a chronic effect on basal activity or insulin response of the MAPK pathway. The present study was undertaken to determine whether exercise training improves the activity of the MAPK pathway or its response to insulin in obese Zucker rats, a well-characterized model of insulin resistance. To accomplish this, obese Zucker rats were studied by using the hindlimb perfusion method with or without 7 wk of treadmill training. Activation of the MAPK pathway was determined in gastrocnemius muscles exposed in situ to insulin. Compared with lean Zucker rats, untrained obese Zucker rats had reduced basal and insulin-stimulated activities of ERK2 and its downstream target p90 ribosomal S6 kinase (RSK2). Seven weeks of training significantly increased basal and insulin-stimulated ERK2 and RSK2 activities, as well as insulin stimulation of MAPK kinase activity. This effect was maintained for at least 96 h in the case of ERK2. The training-induced increase in basal ERK2 activity was correlated with the increase in citrate synthase activity. Therefore, 7 wk of training increases basal and insulin-stimulated ERK2 activity. The increase in citrate synthase activity may be related to the response of muscle to training.

mitogen-activated protein kinase; extracellular signal-regulated kinase; exercise; insulin; Zucker fatty rats

INSULIN EXERTS ITS EFFECTS by binding to the insulin receptor and starting a series of events that begins with activation of its intrinsic tyrosine kinase activity, which first phosphorylates the beta subunit of the insulin receptor itself. The stimulation of receptor kinase activity induces the phosphorylation of nonreceptor proteins, including insulin receptor substrate (IRS)-1 and the tyrosine kinase Shc (27, 29). Phosphorylation of IRS-1 by insulin receptor tyrosine kinase, for example, creates binding sites for several Src (a tyrosine kinase family) homology-2 domain proteins, including the regulatory subunits of phosphatidylinositol 3-kinase (PI3-kinase) and Grb/Sos complex (12, 27). PI3-kinase appears to be particularly important for mediating most of the metabolic effects of insulin and its downstream signaling events (18, 26). On the other hand, Shc and the Grb2/Sos complex link insulin receptor signaling to activation of the mitogen-activated protein kinase [MAPK or extracellular signal-regulated kinase (ERK1/2)] cascade, which is not required for insulin’s metabolic effects but mediates mitogenic signaling (22). MAPK activity is increased when Grb2/Sos complex is recruited to the plasma membrane, in which Sos increases the rate of exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on Ras, the protein product of the ras gene, which, in turn, activates MAPK kinase (MEK1), a threonine-tyrosine kinase that phosphorylates and activates ERK1 and ERK2 (32). ERK2 is the most abundant MAPK isoform in skeletal muscle. One of the downstream elements phosphorylated by ERKs is p90 ribosomal S6 kinase (RSK2).

Several studies have shown that activation of the PI3-kinase signaling pathway in response to insulin is reduced in muscles of obese and Type 2 diabetic subjects and in animal models of insulin resistance (1, 5, 25). One such animal model is the obese Zucker rat (fa/fa). This rat exhibits severe skeletal muscle insulin resistance that is characterized by pronounced hyperinsulinemia, a decrease in insulin-stimulated glucose uptake (9), and marked obesity. These symptoms are similar to those observed in the early stage of human
Type 2 diabetes. Thus the obese Zucker rat (fa/фа) may be a useful model for obesity and Type 2 diabetes. Recent evidence shows that insulin-induced PI3-kinase activity associated with IRS-1 and IRS-2 is reduced in skeletal muscle of fat Zucker rats (1, 17). In contrast, activation of the MAPK pathway by insulin was not impaired in vasculature tissue, and this was accompanied by increased basal MAPK activity (17). Moreover, recent studies in obese and Type 2 diabetic humans indicate that, although the PI3-kinase pathway is markedly insulin resistant in muscle, activation of ERK2, the predominant MAPK isoform in skeletal muscle, remains intact (10, 20). Currently, there is no information available regarding insulin’s ability to activate the MAPK pathway in skeletal muscle of the obese Zucker rat. The first purpose of this study, therefore, was to determine whether insulin activation of the MAPK pathway in skeletal muscle from overweight Zucker rats is normal or insulin resistant. Another purpose was to determine whether exercise training increased basal or insulin-stimulated MAPK pathway activity.

In rodents and humans, an acute bout of exercise activates both the MAPK and c-Jun NH2-terminal kinase (JNK) pathways (2, 14). These observations led to the hypothesis that the stress of exercise alters skeletal muscle growth and metabolism through activation of the MAPK or related pathways (14). Exercise training increases muscle oxidative capacity, capillary density, and fiber size in obese Zucker rats (31) and limits muscle insulin resistance as well (6, 16). It is possible that some of these adaptations are initiated via activation and possible adaptation of the MAPK system to repeated bouts of exercise. However, the adaptations of this pathway to exercise training have not been evaluated. A third purpose of this study, therefore, was to determine whether exercise training-induced changes in MAPK pathway activity are related to adaptation of skeletal muscle to training.

METHODS

Experimental Animals

Eight obese female Zucker rats (fa/фа) and eight of their lean littersmates (fa?) (all animals were 14 wk old) were studied basally (no insulin) or under insulin stimulation. Three other groups of Zucker rats (fa/фа; n = 7 each) were trained for 5 days/wk for 7 wk on a motorized treadmill. Training began when the rats were 7 wk old and finished at 14 wk; thus all groups of rats were studied at the same age. Training began with rats running at 15 m/min for 10 min on an 8% grade. The work rate was gradually increased during the next 6 wk until the rats were continuously running for 90 min at 22 m/min on an 8% grade. Thereafter, the rats continued the running protocol until 2 days before hindlimb perfusion, at which time they were also required to run for 10 min at 26 m/min after a 5-min rest period. All rats were housed three animals to a cage and were provided laboratory chow and water ad libitum. Temperature was maintained at 21°C, and an artificial 12:12-h light-dark cycle was set. Food was withdrawn 12–14 h before the experiments.

Surgical Preparation and Hindlimb Perfusion

After an overnight fast that ended between 10:00 and 15:00, rats were anesthetized with a 6.5-mg/100 g body wt intraperitoneal injection of pentobarbital sodium. The surgical procedure for hindlim perfusion of the rats and the perfusion apparatus were similar to those previously described (16). Blood samples were collected under basal conditions for assays of plasma glucose (glucose oxidase method) and insulin (radioimmunoassay). After completion of the surgical preparations, the gastrocnemius muscle was removed from the left leg immediately before cannulation, split into two sections over ice, and clamped frozen. Cannulas were then inserted into the abdominal aorta and vena cava of the rats, and the right hindlimbs were washed out with 35 ml of Krebs-Henseleit buffer. Immediately thereafter, the cannulas were placed in line with the perfusion system, and the hindlimbs were allowed to stabilize during a 10-min nonrecirculating washout period. The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing 4.5% dialyzed BSA, 20% washed, time-expired human red blood cells, 1 mM glucose, 10 μU/ml Humulin (Lilly, Indianapolis, IN), and 0.2 mM pyruvate. Perfusion flow rate during the washout period was 5 ml/min. After 10 min (washout period), the arterial line was switched to a perfusate with 10 μU/ml Humulin, 6 mM glucose, 0.2 mM pyruvate, 2 mM mannitol, 0.2 μCi/ml 2-3H(deoxyglucose, 0.15 μCi/ml 14C[mannitol, and the same concentrations of human red blood cells and BSA as used during the washout period. Perfusion was performed at 37°C and continued for a total of 22 min, at which time the right gastrocnemius muscle was excised, split, and clamped frozen. Both left and right gastrocnemii were stored at −80°C until analysis.

Materials

An anti-ERK2 monoclonal antibody, a murine ERK2 antibody, produced in rabbit immunized with a fast performance liquid chromatography-purified recombinant murine MAPK (p42ERK), a glutathione S-transferase (GST)-MAPK (inactive), (anti-RSK2 antibody, and 3R S6 RSK substrate peptide were purchased from Upstate Biotechnology (Lake Placid, NY). An anti-MEK1 monoclonal antibody was obtained from Transduction Laboratories (Lexington, KY). [γ-32P]ATP was purchased from NEN Life Science Products (Boston, MA). Protein A-agarose, protein G-agarose, myelin basic protein (MBP), and all other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Muscle Processing

Muscle samples were homogenized in ice-cold lysis buffer containing in mM 50 HEPES (pH 7.6), 150 NaCl, 2 EDTA, 20 β-glycerophosphate, 20 sodium pyrophosphate, 10 NaF, 2 mM Na3VO4, 1 CaCl2, 1 MgCl2, 1 phenylmethylsulfonyl fluoride (PMSF), and 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10% glycerol, and 1% NP-40. Homogenates were centrifuged at 15,000 g for 1 h at 4°C, and muscle debris was removed. Protein concentrations in crude homogenates were estimated by the Lowry method (23). The supernatant was stored at −80°C until used.

Western Blotting

Muscle protein (250 μg) was solubilized in SDS sample buffer, boiled for 5 min, loaded onto a 10% SDS-polyacrylamide gel, subjected to electrophoresis, and transferred to nitrocellulose membranes. The membranes were then blocked in TBST (20 mmol/l Tris·HCl, pH 7.5, 150 mmol/l
NaCl, 0.05% Tween 20) containing nonfat dried milk for 1 h at room temperature. The membranes were incubated at 4°C overnight with mouse monoclonal ERK2 or MEK1 antibodies at a 1:1,000 dilution in blocking buffer for each. After they were washed three times (5 min each) in TBST, the membranes were incubated with secondary antibody (goat anti-mouse coupled to horseradish peroxidase; Amersham) in TBST in a dilution of 1:2,000 and incubated for an additional 1 h at room temperature. The membranes were then washed three times in TBST and developed using the enhanced chemiluminescence detection system according to the manufacturer’s protocol (Amersham). The autoradiographs were subjected to scanning densitometry, and the densities of products were quantified using an imaging densitometer fitted with Molecular Analyst Software.

**ERK2 Activity Assay**

The ERK2 activity assay was performed as described previously (24). An aliquot of rat muscle protein (300 μg) was incubated with 10 μl of anti-murine ERK2 polyclonal antibody at 4°C overnight and subsequently adsorbed to 80 μl of 50% slurry of protein A-agarose beads for an additional 2 h. The immune complexes were washed three times with ice-cold lysis buffer and twice with kinase reaction buffer [100 mM Tris·HCl (pH 7.5), 40 mM magnesium acetate, 0.4 mM EGTA, 0.4 mM orthovanadate, 2 mM DTT]. After the washes, the immunoprecipitates were suspended in 40 μl of kinase reaction buffer containing 20 μM ATP, 10 μCi/sample of [γ-32P]ATP (6,000 Ci/mmol) and 0.25 mg/ml myelin basic protein (MBP) as substrate. The suspension was incubated with agitation at 30°C for 45 min. The reaction was terminated by transferring 25-μl aliquots onto P-81 phosphocellulose paper disks in duplicate and washed four times (5 min each, 300 ml wash) in 0.75% H3PO4. The disks were washed once with acetone and air-dried, and the 32P incorporated into the MBP was measured by liquid scintillation counting. “Specific” kinase activity was determined by subtracting the radioactivity detected in the absence of substrate from that detected in the presence of substrate, and the radioactivity count was normalized for ERK2 protein content.

**MEK1 Activity Assay**

MEK1 activity was assayed as previously described, with some modifications (2). Muscle protein (250 μg) was incubated with 1.25 μg/tube of anti-MEK1 monoclonal antibody at 4°C for 3 h followed by incubation with 50 μl of protein G-agarose beads for an additional 2 h. Immunoprecipitates were washed twice with lysis buffer and twice with MEK1 kinase buffer [25 mM HEPES (pH 7.5) 10 mM MgCl2, 2 mM DTT]. After the washes, the immune complexes were resuspended in 80 μl of MEK1 kinase buffer containing 50 μM ATP, 10 μCi/sample of [γ-32P]ATP (6,000 Ci/mmol) and, a recombinant kinase inactive mouse GST-MAPK/ERK2 (1.4 μg/tube) as substrate. The suspension was incubated with agitation for 30 min at 30°C and was terminated by adding 40 μl of SDS sample buffer. Products were boiled for 5 min and resolved on 10% SDS-PAGE. Gel was dried, and the phosphorylated GST-ERK2 (62 kDa) were quantified by PhosphorImager and ImageQuant Software (Molecular Dynamics).

**RSK Activity Assay**

The RSK activity assay was performed as previously described, with some modifications (2). Aliquots of muscle protein (400 μg) were precleared with protein A-agarose beads for 30 min at 4°C, incubated with 4 μg of polyclonal anti-RSK2 antibody for 2 h at 4°C, and then incubated with 80 μl of 50% slurry of protein A-agarose beads for an additional 2 h. The immune complexes were washed twice with lysis buffer, twice with LiCl buffer [500 mM LiCl, 100 mM Tris·HCl (pH 7.6), 0.1% Triton X-100, 1 mM DTT], and once with RSK kinase buffer (30 mM Tris, pH 7.4, 10 mM MgCl2, 0.1 mM EGTA, 1 mM DTT). The immune complexes were resuspended in 50 μl of RSK kinase buffer containing 50 μg of 3R S6 peptide as substrate (final concentration 0.2 μg/μl), 50 μM ATP, and 10 μCi/sample of [γ-32P]ATP (6,000 Ci/mmoll). Reactions were incubated with agitation at 30°C for 15 min and terminated by adding 10 μl of stopping solution containing 0.6% HCl, 1 mM ATP, and 1% BSA. The suspensions were then centrifuged at 15,000 g for 5 min at 4°C. After centrifugation, 20-μl aliquots of the supernatant were spotted onto P-81 phosphocellulose paper disks in duplicate and washed four times in 0.75% H3PO4 for at least 10 min each. The paper disks were washed once with acetone, air-dried, and counted for 32P.

**Citrate Synthase Activity**

To verify that there was a training response of the gastrocnemius muscle, citrate synthase activity was assayed as previously described (28).

**Statistical Analysis**

All data are expressed as means ± SE. A one-tailed paired Student’s t-test was used to test for differences between basal and insulin stimulation points, as appropriate, with P < 0.05 considered statistically significant. Statistical significance of differences among lean, obese, and trained rats was determined using analysis of variance.

**RESULTS**

**Animal Characteristics**

Untrained obese Zucker rats were significantly heavier than their lean littermates (373.1 ± 4.8 vs. 200.1 ± 4.5 g, P < 0.001). Exercise-trained obese Zucker rats weighed 356.1 ± 3.2 g (24-h group), 353.5 ± 2.8 g (96-h group), and 348.5 ± 8.1 g (7-day group). None of the groups of trained Zucker rats had weights that were significantly lower than those of the untrained obese rats. Citrate synthase activity in untrained obese rats was 49.0 ± 2.5 μmol·min⁻¹·g⁻¹. Training increased citrate synthase activity to 78.8 ± 4.0 and 73.9 ± 4.4 μmol·min⁻¹·g⁻¹ 24 and 96 h after the last exercise bout, respectively (P < 0.001). Seven days after the last exercise bout, citrate synthase activity (59.7 ± 2.6 μmol·min⁻¹·g⁻¹) was still increased compared with the untrained value (P < 0.05). Fasting plasma glucose and insulin concentrations are given in Table 1. Plasma glucose concentrations were slightly, but not significantly, increased in the untrained obese rats compared with the lean controls. Exercise training did not significantly decreased plasma glucose concentrations. In contrast, plasma insulin concentrations were dramatically increased in the untrained obese rats compared with the lean controls (P < 0.001) and were not significantly decreased by exercise training.
Table 1. Plasma glucose and insulin values

<table>
<thead>
<tr>
<th></th>
<th>Lean Zucker Rats</th>
<th>Obese Zucker</th>
<th>24 h</th>
<th>96 h</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>66 ± 3</td>
<td>87 ± 12</td>
<td>72 ± 4</td>
<td>78 ± 7</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>19 ± 4</td>
<td>243 ± 24*</td>
<td>197 ± 21*</td>
<td>248 ± 20*</td>
<td>224 ± 20*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.001 vs. lean Zucker rats.

Effect of Insulin Resistance on MAPK Signaling

Protein expression of ERK2 and MEK1 in muscle of obese Zucker rats. To examine whether the expression of ERK2 or MEK1 protein is changed in obese Zucker rats, homogenates of basal and insulin-stimulated gastrocnemius muscle from lean and obese rats were separated by SDS-PAGE and immunoblotted with anti-ERK2 or anti-MEK1 antibody. The ERK2 and MEK1 protein bands were visualized by the enhanced chemiluminescence system, and the immunoblots were quantified using scanning densitometry. The results are given in Table 2. Under basal conditions, there were no significant differences in ERK2 protein content between lean and obese rats. Insulin perfusion did not change the expression level of ERK2 in either group of rats (data not shown), so basal and insulin values were averaged. Similarly, there was no difference between MEK1 protein in lean and untrained obese rats.

Stimulation of MAPK signaling components by insulin in Zucker rat muscle. To assess the regulation of the MAPK pathway by insulin in obese rats, ERK kinase activity assays were performed by using anti-ERK2 immunoprecipitates of muscle samples and MBP as a substrate. The effect of insulin on ERK2 activity is shown in Fig. 1. Both basal and insulin-stimulated ERK2 activity were decreased in untrained obese Zucker rats compared with lean controls (P < 0.01). Exercise training significantly increased basal and insulin-stimulated ERK2 activity in rats studied 24 h after the last training session, and basal ERK2 activity remained increased for at least 96 h after training (P < 0.01 vs. untrained obese rats). By 7 days, the effect had waned, although basal ERK2 activity was still increased over the nontrained animals (P < 0.05). Basal ERK2 activity in obese rats studied 24 or 96 h after the last training session was significantly greater than that in lean Zucker rats (P < 0.01). Basal ERK2 activity was significantly correlated with citrate synthase activity in muscle of untrained and trained obese Zucker rats (Fig. 2).

MEK1 and MEK2, the upstream activators of ERKs, are dual-specificity enzymes that have been shown to increase ERK1/2 activity by phosphorylating both Thr-183 and Tyr-185 residues (32). Therefore, we determined whether exercise training increases MEK1 activity or alters its response to insulin in rat hindlimb muscle. MEK1 activity was assayed in muscle protein by measuring the ability of MEK1 immune complexes to phosphorylate a recombinant kinase-inactive MAPK (p42MAPK). The results are shown in Fig. 3. Although ERK2 activity was significantly reduced in the obese rats, MEK1 activity was not reduced significantly, either basally or after insulin stimulation. Exercise training significantly increased insulin-stimulated, but not basal, MEK1 activity. Twenty-four hours after the last training session, insulin-stimulated MEK1 1 activity was increased significantly over that of obese (P < 0.01) and lean rats (P < 0.05). This effect waned rapidly, disappearing by 96 h after the last training bout.

Table 2. Expression of ERK2 and MEK1 in Zucker rat gastrocnemius muscle

<table>
<thead>
<tr>
<th></th>
<th>Lean Zucker Rats</th>
<th>Obese Zucker Rats</th>
<th>24 h</th>
<th>96 h</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERK2</td>
<td>1.31 ± 0.02</td>
<td>1.35 ± 0.03</td>
<td>1.28 ± 0.02</td>
<td>1.27 ± 0.03</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>MEK1</td>
<td>6.82 ± 0.17</td>
<td>6.99 ± 0.14</td>
<td>6.85 ± 0.15</td>
<td>6.98 ± 0.05</td>
<td>6.97 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in arbitrary density units; n, no. of rats. ERK2, extracellular signal-regulated kinase; MEK1, mitogen-activated protein kinase kinase.
Acute Exercise and Training Increase Insulin Action

Muscle contraction or exercise does not appear to directly influence insulin receptor signaling at the level of the insulin receptor, nor does it appear to influence IRS-1 tyrosine phosphorylation or the association of PI3-kinase with IRS-1 (13, 15, 19). The insulin receptor, like many other tyrosine kinase receptors, also activates the MAPK pathway. This action of insulin is not directly related to its metabolic effects (7, 11) but is likely to be responsible for the effects of insulin on cell growth and differentiation. Although it is known that a single exercise bout or acute muscle contraction activates ERK2, it is not known whether chronic exercise training increases basal ERK2 activity or its response to insulin. The present study was undertaken to determine 1) the extent of insulin resistance in the MAPK pathway in obese Zucker rats, a rodent model of insulin resistance and 2) whether 7 wk of exercise training increases MAPK pathway activity or its response to insulin. To assess activity of the MAPK pathway, insulin stimulation of ERK2, MEK1, and RSK2 was determined in gastrocnemius muscle taken from rats before and after hindlimb perfusion. Untrained fatty rats (n = 8) and groups of fatty rats that had been trained for 7 wk and then detrained for 24 or 96 h or 7 days (n = 7 each) were compared. Values are means ± SE. Gastrocnemius from the control insulin-stimulated leg were homogenized and immunoprecipitated with an anti-ERK2 antibody, and activity assays were performed as described in the text. ERK2 catalytic activity (dpm \( \times 10^{-5} \) per unit protein per 20 min) is shown. a, P < 0.05 vs. lean Zucker rats; b, P < 0.01 vs. lean Zucker rats; c, P < 0.05 vs. untrained obese Zucker rats; d, P < 0.01 vs. untrained obese Zucker rats.

Fig. 3. Effects of 7 wk of training on basal (open bars) and insulin-stimulated (solid bars) mitogen-activated protein kinase kinase (MEK1) activity (dpm \( \times 10^{-5} \) per unit protein per 20 min) in gastrocnemius muscle from Zucker rats who underwent hindlimb perfusion. Untrained lean Zucker rats (n = 8) served as controls. Untrained fatty rats (n = 8) and groups of fatty rats that had been trained for 7 wk and then detrained for 24 or 96 h or 7 days (n = 7 each) were compared. Values are means ± SE. Gastrocnemius from the control insulin-stimulated leg were homogenized and immunoprecipitated with an anti-MEK1 antibody, and activity assays were performed as described in the text. a, P < 0.05 vs. lean Zucker rats; d, P < 0.01 vs. untrained fatty rats.

Fig. 4. Effects of 7 wk of training on basal (open bars) and insulin-stimulated (solid bars) p90 ribosomal S6 kinase (RSK2) activity (dpm \( \times 10^{-5} \) per 20 min) in gastrocnemius muscle from Zucker rats who underwent hindlimb perfusion. Untrained lean Zucker rats (n = 8) served as controls. Untrained obese Zucker rats (n = 8) and groups of fatty rats that had been trained for 7 wk and then detrained for 24 or 96 h or 7 days (n = 7 each) were compared. Values are means ± SE. Gastrocnemius from the control insulin-stimulated leg were homogenized and immunoprecipitated with an anti-RSK2 antibody, and activity assays were performed as described in the text. b, P < 0.01 vs. lean Zucker rats; c, P < 0.05 vs. untrained fatty rats.

**DISCUSSION**

**Acute Exercise and Training Increase Insulin Action**

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With regard to insulin resistance in the obese Zucker rat, the present results show that both basal and insulin-stimulated ERK2 activity were markedly (>50%) reduced in untrained obese rats compared with lean control rats. These results are consistent with other studies showing that insulin receptor signaling through the PI3-kinase pathway is reduced in this rodent model of insulin resistance (1). In this sense, the Zucker rat differs from human obesity or Type 2 diabetes, which are characterized by insulin resistance in the PI3-kinase, but not the MAPK, pathway (10, 20). These results from skeletal muscle also differ from those in microvascular cells from obese Zucker rats, in which insulin stimulation of the MAPK pathway was normal (17). Thus insulin resistance in the obese Zucker rat appears to be tissue specific. We also determined the insulin response of other signaling elements related to ERKs, namely the upstream activator MEK1 and the downstream serine kinase RSK2. Unlike ERK2 activity, MEK1 activity was not reduced significantly in the untrained obese rats compared with lean controls. The decrease in ERK2 activity without a concomitant decrease in MEK1 suggests that ERK2 is a specific site of insulin resistance or that other upstream kinases are responsible for activating ERK2 in the muscles of Zucker rats. On the other hand, RSK2 activity in the obese rats paralleled ERK2 activity, with a reduction in absolute activity but a retention of the ability of insulin to increase RSK2 activity relative to its basal value. This pattern is consistent with RSK2 being activated by ERK2.

With regard to the effect of exercise conditioning, training increased basal ERK2 and RSK2 activity, although the effect on RSK2 activity had a shorter duration. However, training did not increase the basal activity of MEK1. Thus, although MEK1, ERK2, and RSK2 are known to be consecutive elements in a MAPK signaling pathway, the exercise training-induced changes in enzymatic activity of these proteins were not always coordinated. There are at least two possible explanations for this result. First, other kinases at the level of MEK1 that were not assayed might have been increased by training and could have contributed to phosphorylation and activation of ERK2. Second, it is possible that the increase in ERK2 phosphorylation and activity was brought about not by an increase in the activity of a kinase but rather by a decrease in the activity of an ERK2 phosphatase. A recently identified mammalian ERK1/2 protein tyrosine phosphatase, VHR, may be responsible for inactivation of ERK1/2 (30). The potential importance of coordinate kinase-phosphatase activity in regulation of ERK activity has been demonstrated by evidence suggesting that inhibition of tyrosine phosphatase activity increased ERK activity and transcription of the insulin gene (4).

The present results show that exercise training normalized activity of ERK2 under conditions of maximal insulin stimulation. At least two possible mechanisms could explain this result. First, it is possible that training merely increased the basal activity of ERK2, and the response (increment over basal activity) to insulin was about the same in the trained and untrained obese rats. This interpretation would lead to the conclusion that training had no effect on insulin action. Second, it is possible that the training did not increase basal ERK2 activity, but, rather, it markedly improved the sensitivity of ERK2 to insulin stimulation. In this case, the basal hyperinsulinemia that was still present after training might have stimulated ERK2 activity, and then maximal ERK2 activation was attained during perfusion with maximal insulin (10 mU/ml). Because acute exercise increases ERK activity and because exercise in other conditions has so far failed to influence insulin signaling (13, 15), the first explanation may be more likely. However, the latter explanation cannot be ruled out. Regardless of which is true, exercise training resulted in normal ERK2 activity during maximal insulin.

This study was also undertaken to determine whether training-induced changes in MAPK activity are related to the response of skeletal muscle to training. The activity of ERK2 was significantly correlated with that of citrate synthase in trained and untrained obese Zucker rats, providing evidence that is consistent with the notion that activation of ERK2 is responsible for at least some of the muscle-specific responses to training. Several mechanisms have been proposed to account for this activation of ERK2. Stimulation of the MAPK pathway via RAS activation can occur in response to an increased rate of guanidine nucleotide exchange stimulated by reactive oxygen species (21). Because protein kinase C also can activate the MAPK pathway at the level of MEKK (Raf), it is also possible that activation of Ca2+-dependent protein kinase C isoforms during exercise might chronically activate ERKs (3). Likewise, stretch has also been shown to activate protein kinase C and various MAPK isoforms (3).

In summary, the results of this study show that MAPK pathway activity is reduced in the skeletal muscle of insulin-resistant obese Zucker rats. Exercise training increased both basal and insulin-stimulated ERK2 and RSK2 activity. The change in ERK2 activity was related to the increase in citrate synthase activity, suggesting that at least some of the training response of skeletal muscle may be mediated by ERK activation.

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