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Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle

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Creer, Andrew, Philip Gallagher, Dustin Slivka, Bozena Jemiolo, William Fink, and Scott Trappe. Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle. J Appl Physiol 99: 950–956, 2005. First published May 5, 2005; doi:10.1152/japplphysiol.00110.2005.—Two pathways that have been implicated for cellular growth and development in response to muscle contraction are the extracellular signal-regulated kinase (ERK1/2) and Akt signaling pathways. Although these pathways are readily stimulated after exercise, little is known about how nutritional status may affect stimulation of these pathways in response to resistance exercise in human skeletal muscle. To investigate this, experienced cyclists performed 30 repetitions of knee extension exercise at 70% of one repetition maximum after a low (2%) or high (77%) carbohydrate (LCHO or HCHO) diet, which resulted in low or high (~174 or ~591 mmol/kg dry wt) preexercise muscle glycogen content. Muscle biopsies were taken from the vastus lateralis before, ~20 s after, and 10 min after exercise. ERK1/2 and p90 ribosomal S6 kinase phosphorylation increased (P ≤ 0.05) 10 min after exercise, regardless of muscle glycogen availability. Akt phosphorylation was elevated (P < 0.05) 10 min after exercise in the HCHO trial but was unaffected after exercise in the LCHO trial. Mammalian target of rapamycin phosphorylation was similar to that of Akt during each trial; however, change or lack of change was not significant. In conclusion, the ERK1/2 pathway appears to be unaffected by muscle glycogen content. However, muscle glycogen availability appears to contribute to regulation of the Akt pathway, which may influence cellular growth and adaptation in response to resistance exercise in a low-glycogen state.

p90 ribosomal S6 kinase; mammalian target of rapamycin

A single bout of resistance exercise (RE) is sufficient to enhance protein synthesis (34, 45), suggesting that a consistent acute stimulation of the protein synthesis pathways in skeletal muscle can partially explain the strength and size improvements with training. Recently, two potential cellular pathways have been implicated for cellular growth and development in response to muscle contraction (25, 36): extracellular signal-regulated kinase (ERK1/2) and Akt intracellular signaling pathways. Once stimulated (phosphorylated), these pathways lead to phosphorylation of the downstream targets responsible for activation of transcriptional and translational factors that serve as the molecular basis for muscle adaptation.

ERK1/2 is phosphorylated rapidly and transiently in response to mechanical stress, with early studies reporting an increase in ERK1/2 phosphorylation after endurance-type exercises, including cycling (1, 22, 37) and running (46). More recently, an increase in ERK1/2 phosphorylation has been shown in response to RE (13, 38). ERK1/2 phosphorylates downstream targets such as p90 ribosomal S6 kinase (p90S6k), MAPK-interacting kinase (Mnk1/2), and mitogen- and stress-activated protein kinase (MSK1/2), which are associated with gene transcription and protein translation (1, 28, 32, 38). Akt is phosphorylated in response to a wide variety of growth factors, including insulin, and, more recently, has been associated with rapid and transient activation in response to exercise in rat and human skeletal muscle (5, 23, 24, 26). Stimulation of the Akt pathway has been shown to mediate skeletal muscle hypertrophy by upregulating proteins involved in mRNA translation, including mammalian target of rapamycin (mTOR), eukaryotic initiation factor 4E-binding protein (4E-BP1), and 70-kDa S6 kinase (p70S6k) (3, 21).

Recent studies have reported a glycogen-related effect in ERK1/2 phosphorylation before and after aerobic exercise, suggesting that ERK1/2 phosphorylation may be influenced by glycogen availability (22, 33). Evidence from cell cultures and rat skeletal muscle also suggest that Akt and mTOR may be regulated by the energy state of the cell (4, 7, 14). Exercise in a glycogen-depleted state presents a unique metabolic challenge to skeletal muscle in which reductions in glycogen content result in a lower rate of glycogen breakdown during exercise (12). To compensate, alternate fuel sources such as lipid and protein are employed to a greater degree in an attempt to maintain cellular energy production, as evidenced by increased induction of metabolic genes during exercise in a glycogen-depleted state (19), as well as during recovery (20).

With consideration of the priority given to the maintenance of muscle glycogen, it is possible that acute adaptations to RE, including protein synthesis, may be influenced by muscle glycogen content. Therefore, the purpose of this study was to investigate the immediate effect of ERK1/2 and Akt, as well as their downstream targets p90S6k and mTOR, in response to a bout of RE performed with high compared with low muscle glycogen content. We hypothesized that phosphorylation of the ERK1/2 and Akt pathways would be elevated to a greater degree in response to RE with high muscle glycogen availability than in response to RE with low muscle glycogen availability.

METHODS

Subjects. Eight healthy, experienced (>2 yr) male cyclists were recruited to participate in the study. Age, height, weight, percent body fat, and maximal O2 consumption (VO2 max) were as follows: 24 ± 1 yr, 72 ± 4 kg, 8 ± 2%, and 63 ± 4 ml·kg⁻¹·min⁻¹, respectively. Single-muscle-fiber myosin heavy chain composition is shown in...
Aerobic testing. In the week before testing, subjects performed a graded exercise test to exhaustion on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands) to determine aerobic capacity (V\(\text{O}_2\) max). Expired air was measured at 30-s intervals using gas analyzers calibrated to gases of known O\(_2\) and CO\(_2\) concentrations. At 2 min of two-arm cycling exercise to further reduce whole body glycogen levels, V\(\text{O}_2\) max, as described previously (6).

Strength testing. Bilateral isotonic quadriceps strength was determined using a unilateral knee extensor device (Cybex Eagle) in a manner utilized previously in this laboratory (30, 31, 38–40). Briefly, assessment of the one repetition maximum (1 RM) was determined by increasing weight until full knee extension could not be achieved. Each attempt was separated by a 2-min recovery period. The weight lifted during the actual experimental trial (3 sets of 10 repetitions) was determined as 70% of 1 RM.

Experimental trials. Subjects underwent two trials, a low-carbohydrate (LCHO) and a high-carbohydrate trial (HCHO), based on dietary manipulation throughout the trial. Each experimental trial was separated by 1 wk and took place over a 3-day period. Trials involved two glycogen-depletion protocols in combination with dietary manipulation on days 1 and 2 and an RE trial on day 3. Early in the evening of day 1, subjects performed 60 min of cycling exercise at ~68% of V\(\text{O}_2\) max to reduce muscle glycogen levels. This was followed by 30 min of two-arm cycling exercise to further reduce whole body glycogen stores (19, 42). In the morning of day 2, subjects performed an additional 75 min of cycling at ~68% of V\(\text{O}_2\) max, followed by six 1-min maximal sprints separated by 1-min rest intervals. Subjects then performed 30 min of arm-cycling exercise. Early in the morning of day 3, subjects arrived at the laboratory after a 12-h fast and performed three sets of 10 repetitions of bilateral knee extension exercise at 70% of 1 RM separated by a 2-min recovery period.

Experimental feedings. During the 2-day glycogen-depletion period leading to the experimental trial, subjects were fed an isocaloric (5,206 ± 119 kcal LCHO or 5,211 ± 117 kcal HCHO) diet containing 2% carbohydrate, 80% fat, and 18% protein or 80% carbohydrate, 7% fat, and 13% protein, respectively. A total of four meals were provided, including dinner on day 1 and breakfast, lunch, and dinner on day 2. Subjects were free to drink water ad libitum throughout each trial. A normal meal (55% carbohydrate, 30% fat, and 15% protein) was provided after completion of the exercise trial on day 3. Total caloric intake was determined as the sum of the total energy expenditure during the depletion protocols and an estimation of the subject’s metabolic rate (29).

Muscle biopsy. A total of three percutaneous needle biopsies (2) with suction were taken from the vastus lateralis muscle on day 3 of each trial. Samples were immediately separated into longitudinal sections and frozen in liquid nitrogen for future analysis. On arrival at the laboratory on day 3, subjects were required to rest for 30 min in a supine position. A resting muscle biopsy was then taken, and the RE protocol was performed. A second biopsy was obtained immediately (~20 s) after exercise from the same leg (different incision). A third biopsy was taken from the opposite leg 10 min after exercise. It should be noted that the muscle biopsy procedure in humans typically yields 75–100 mg of tissue. As a result, the number of nutritional and signaling analyses that could be performed as part of this investigation was limited to those described below.

Western blotting. Muscle samples for ERK1/2, p90\(\text{rSk}\), Akt, and mTOR were homogenized in a commercial extraction buffer (BioSource International, Camarillo, CA) containing 1% SDS. Homogenates were centrifuged at 13,000 rpm for 10 min at 4°C, and the supernatant was extracted and stored at −80°C. Protein concentrations of the homogenates were determined by a bicinchoninic acid protein assay kit (Sigma, St. Louis, MO) in a 96-well plate and reader (Wallac 1420 Victor-2 plate reader, EG & G Wallac, Turku, Finland).

For Western blotting, homogenates were resolved by SDS-PAGE (10% for ERK1/2 and Akt, 8% for p90\(\text{rSk}\), and 6% for mTOR) and electrophoretically transferred (Idea Genie, Idea Scientific, Minneapolis, MN) to a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was blocked with 5% BSA (ERK1/2 and Akt) or 5% milk (p90\(\text{rSk}\) and mTOR) in Tris-buffered saline (pH 7.6) and 0.1% Tween 20 for 60 min and then incubated with the appropriate primary antibody overnight at 4°C (with the exception of Akt on Ser\(^{473}\), which was blocked overnight and incubated for 2 h in primary antibody at room temperature on the following day). Blots were rinsed in Tris-buffered saline (4 times for 10 min each), incubated in a horseradish peroxidase-conjugated secondary antibody, and then exposed to an enhanced chemiluminescent substrate (Chemiglow, Alpha Innotech, San Leandro, CA). Visualization and quantification of phosphorylated sites were performed using a chemiluminescent imaging system (Chemilnager 4000, Alpha Innotech). Equal protein loading was verified by Ponceau S staining.

Sizes of the immunodetected proteins were confirmed by molecular weight marker proteins (Amersham Biosciences; Cell Signaling Technologies, Beverly, MA).

Primary antibodies for total and phospespecific ERK1/2 (Thr\(^{183}\) and Tyr\(^{197}\)) and phosphospecific Akt (Ser\(^{473}\)) were acquired from Biosource International. Phosphospecific p90\(\text{rSk}\) (Ser\(^{95}\)), total p90\(\text{rSk}\), mTOR, and Akt primary antibodies, as well as anti-rabbit horseradish peroxidase-linked secondary antibodies were acquired from Cell Signaling Technologies.

Muscle glycogen and triglyceride. Total muscle glycogen and triglyceride concentrations were determined from freeze-dried muscle samples. For muscle glycogen, samples were hydrolyzed in HCl, and the resultant glucose residues were determined using a standard enzymatic technique with fluorometric detection (18). A modified version of the chloroform-methanol method was used to determine total muscle triglyceride (9).

Blood samples. Blood samples were taken from an antecubital vein in conjunction with each biopsy. Commercially available kits were used to determine plasma glucose (ThermoTrace, Noble Park, Victoria, Australia) and free fatty acid (Wako Chemicals, Richmond, VA) concentrations. Plasma insulin values were analyzed by an enzyme-linked immunosorbent assay (DRG International).

Single-fiber analysis. Individual muscle fibers were dissected into 80 µl of sample buffer [10% SDS, 6 mg/ml EDTA, 0.06 M Tris (pH 6.8), 2 mg/ml bromphenol blue, 15% glycerol, and 5% β-mercaptoethanol] and stored at −20°C until they were assayed. To determine myosin heavy chain (MHC) composition, single fibers were subjected to SDS-PAGE as described previously (40). Briefly, samples were run overnight at 4°C on a Hoefer SE 600 gel electrophoresis unit utilizing a 3.5% (wt/vol) acrylamide stacking gel with a 5% separating gel. After electrophoresis, the gels were silver stained as described by

<p>| Table 1. Single-fiber MHC composition |
|-------------------------------|----------------|</p>
<table>
<thead>
<tr>
<th>MHC, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>I/II</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>I/IIax</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>I/IIIax</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>I/III</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>II</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>II/IIax</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of fibers studied for each subject. MHC, myosin heavy chain. |
Giulian et al. (10). MHC isoforms (I, I/IIa, I/IIax, IIa, IIax, and IIX) were identified according to migration rate and compared with molecular weight standards.

Statistical analysis. Values are means ± SE. A repeated-measures ANOVA was used to compare differences in phosphorylation of ERK1/2, p90S6K, Akt, and mTOR, as well as the blood parameters [glucose, free fatty acids (FFA), and lactate]. Bonferroni’s post hoc test was used when necessary to determine pairwise differences. A paired t-test was also used where applicable to determine differences in values. Significance was set at P ≤ 0.05.

RESULTS

Depletion protocols and work output. Each depletion protocol was carried out to completion regardless of carbohydrate condition. The relative exercise intensity and respiratory exchange ratio during the depletion protocols (combined days 1 and 2) remained similar between trials: 68 ± 1% and 0.85 ± 1, respectively, for LCHO trials and 68 ± 1% and 0.86 ± 1, respectively, for HCHO trials. Each subject completed all 30 repetitions during the RE bout, regardless of dietary condition.

Blood parameters. Plasma glucose, FFA, and insulin levels are shown in Table 1. Preexercise plasma glucose was lower (P < 0.05) during the LCHO than during the HCHO trial. Measurements immediately and 10 min after exercise were not different between trials. The LCHO condition resulted in elevated (P < 0.05) FFA values at all time points compared with the HCHO condition. Insulin levels were not different between groups at any time point.

Muscle glycogen. Preexercise glycogen was higher (P < 0.05) in the HCHO than in the LCHO trial (Table 2). In response to RE, glycogen levels decreased (P < 0.05) in both groups; however, 43% more (P < 0.05) glycogen was used during the HCHO trial. The 10-min recovery period had no effect on muscle glycogen in the LCHO trial; however, in the HCHO trial, muscle glycogen increased by 14% (P < 0.05) from the postexercise measurement.

Intramuscular triglycerides. Intramuscular triglyceride (IMTG) concentration was 40% higher (P < 0.05) in the LCHO than in the HCHO trial before exercise (Table 2). A 21% decrease (P < 0.05) in IMTG was shown immediately after RE in the LCHO trial only.

Intracellular signaling. Densitometric analysis of the combined ERK bands (ERK1/2) showed that, at rest, ERK1/2 phosphorylation was unaffected by muscle glycogen content (Fig. 1). Immediately after RE, ERK1/2 phosphorylation remained unchanged, and no differences were noted between the exercise trials. After 10 min of recovery, ERK1/2 phosphorylation increased 1.2-fold (P < 0.05) in the LCHO and HCHO trials. There were no differences in preexercise phosphorylation of p90S6K between groups (Fig. 2). RE did not change p90S6K phosphorylation immediately after exercise in either condition. p90S6K significantly increased 10 min after exercise, with phosphorylation increasing 5.5-fold (P < 0.05) and 2.9-fold (P = 0.05) in the LCHO and HCHO trials, respectively.

Akt phosphorylation was similar in both groups before and immediately after exercise (Fig. 3). After 10 min of recovery in the HCHO trial, Akt phosphorylation increased 1.5-fold (P < 0.05). During the LCHO trial, Akt phosphorylation remained unchanged throughout all time points. The mTOR response was similar to that of Akt after RE during both trials (Fig. 4); however, the mean changes were not significant.

DISCUSSION

The intent of this investigation was to determine the interactive effects of preexercise muscle glycogen content and RE on the phosphorylation state of the ERK1/2 and Akt signaling pathways. The dietary intervention employed in combination with the glycogen-depletion protocols performed during this investigation resulted in a 3.4-fold increase in HCHO compared with LCHO preexercise muscle glycogen availability.

Table 2. Plasma metabolite and hormone concentrations before, after, and 10 min after resistance exercise after an LCHO or HCHO diet

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Post 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCHO</td>
<td>HCHO</td>
<td>LCHO</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.9±0.1*</td>
<td>5.3±0.1</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.7±0.1*</td>
<td>0.5±0.1</td>
<td>0.8±0.1*</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>13.4±7.7</td>
<td>16.8±7.9</td>
<td>16.0±7.6</td>
</tr>
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</table>

Values are means ± SE. LCHO and HCHO, low and high carbohydrate; FFA, free fatty acid; Pre, before; Post, after; Post 10, 10 min after. *P < 0.05 vs. HCHO.
(Table 3). Muscle glycogen levels declined after RE in both trials, although 43% more glycogen was utilized during the HCHO trial. IMTG concentrations were greater before RE in the LCHO trial and utilized to a greater degree during RE (Table 3), consistent with results obtained by Essen-Gustavsson and Tesch (8), who reported a similar relation between preexercise IMTG content and IMTG utilization during RE.

With respect to the ERK1/2 and Akt signaling pathways, the main findings of this investigation are as follows: 1) The ERK1/2 pathway was unaffected by muscle glycogen availability in response to RE. 2) Akt phosphorylation was influenced by muscle glycogen availability. 3) Akt phosphorylation was not associated with an increase in mTOR phosphorylation.

As anticipated, ERK1/2 phosphorylation was increased under high muscle glycogen conditions following RE. These results were expected on the basis of our previous RE study (38) and others (1, 12, 29, 30). Surprisingly, however, ERK1/2 phosphorylation was also increased after RE in a low muscle glycogen state, providing evidence that ERK1/2 phosphorylation is primarily controlled by contraction-mediated stress as opposed to metabolic processes. Recent studies measuring ERK1/2 in response to aerobic exercise with low, normal, or high muscle glycogen content have provided mixed results (22, 33). Watt et al. (33) reported an increase in ERK1/2 phosphorylation at rest and during aerobic exercise with normal glycogen availability compared with exercise with low muscle glycogen. In contrast, aerobic exercise with low muscle glycogen availability has been shown to increase ERK1/2 phosphorylation to a greater extent than exercise in a supercompensated state (22).

Discrepancies between the present results and those derived from previous investigations are difficult to compare directly because of different modes of exercise (RE vs. aerobic). Nevertheless, in each case, ERK1/2 phosphorylation increased after exercise, suggesting that ERK1/2 activation may be independent of muscle glycogen content. Interestingly, ERK1/2 phosphorylation did not occur immediately after RE, as has been previously reported (13, 38). This may be due to differences in study populations utilized. In the present study, a homogeneous population of experienced cyclists and triathletes was used, as opposed to an untrained population. The significance of the present population compared with untrained
Muscle glycogen and IMTG concentrations in vastus lateralis muscle before, after, and 10 min after resistance exercise after an LCHO or HCHO diet

<table>
<thead>
<tr>
<th></th>
<th>LCHO</th>
<th>HCHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG, mmol/kg dry wt</td>
<td>174±24*</td>
<td>591±35</td>
</tr>
<tr>
<td>Δ MG</td>
<td>115±15†</td>
<td>488±28†</td>
</tr>
<tr>
<td>IMTG, mmol/kg dry wt</td>
<td>47±7*</td>
<td>28±2</td>
</tr>
<tr>
<td>Δ IMTG</td>
<td>-59±16</td>
<td>-103±25</td>
</tr>
<tr>
<td></td>
<td>37±6†</td>
<td>27±3</td>
</tr>
<tr>
<td></td>
<td>-10±2</td>
<td>-4</td>
</tr>
</tbody>
</table>

Values are means ± SE. IMTG, intramuscular triglyceride, MG, muscle glycogen. *P < 0.05 vs. LCHO. †P < 0.05 vs. Pre. ‡P < 0.05 vs. Post.

populations employed in other studies may be the result of a delayed ERK1/2 signaling response in conditioned muscle. Indeed, the magnitude of exercise-induced signaling responses has been shown to be greater in untrained than in trained subjects working at the same relative intensity (47). Downstream of ERK1/2, p90rSk was activated in a manner similar to ERK1/2 in both trials, as shown previously (1, 15, 38, 46).

Bodine et al. (3) were the first to demonstrate the importance of chronic Akt pathway stimulation in skeletal muscle hypertrophy utilizing a compensatory hypertrophy model in rats. Since that time, a number of investigations have reported an increase in Akt phosphorylation in human and rat skeletal muscle after endurance exercise (23, 24, 26) and in rat skeletal muscle in response to RE (5). To our knowledge, this is the first study to show an increase in Akt phosphorylation after an acute bout of RE in human skeletal muscle. Akt phosphorylation increased 1.5-fold 10 min after exercise in the HCHO trial. Bolster et al. (5) established a similar time course of Akt phosphorylation after RE in rat skeletal muscle, reporting increased activation 5 min after exercise, with maximal activation 10 min after exercise. Plasma insulin levels measured from blood samples taken before, immediately after, and 10 min after exercise in the present study revealed no differences in insulin concentration between trials or during the exercise bout. Because insulin has been shown to be a potent stimulator of Akt, these data suggest that the elevation in Akt phosphorylation 10 min after exercise was most likely an exercise-induced response.

Although the present findings, together with recent findings in rat and human skeletal muscle (5, 23), support enhanced phosphorylation of Akt in response to exercise, this remains a controversial topic. Previous studies have failed to show enhanced Akt phosphorylation in response to endurance exercise in human skeletal muscle (35, 44) and electrical stimulation in rats (17). Variations in the phosphorylation of Akt after exercise may be the result of different modes, intensity, and duration of exercise and/or study populations. The time point at which the postexercise muscle sample is taken may also play a role, as evidenced by the present data. The mechanism preventing an increase in Akt phosphorylation after RE in the LCHO trial is not completely understood; however, AMP-activated protein kinase (AMPK) may play a role. AMPK is considered an energy-sensing protein kinase responding to changes in the cellular ATP-to-AMP ratio in response to muscle contraction (41). In rat skeletal muscle, pharmacological stimulation of AMPK has been shown to suppress phosphorylation of Akt, mTOR, and p70S6k (4). These data suggest that when skeletal muscle is under energetic stress, phosphorylation of the Akt pathway is suppressed, likely in an attempt to maintain cellular energy levels. In human skeletal muscle, previous studies showed that AMPK activation is elevated at rest and during exercise in a glycogen-depleted state (22, 42, 43).

Akt has previously been shown to phosphorylate mTOR on Ser2448 (16). Although mTOR phosphorylation on Ser2448 followed a phosphorylation pattern similar to Akt after the HCHO trial, mTOR phosphorylation was not significantly affected by RE with low or high muscle glycogen content. A similar disassociation between Akt and mTOR phosphorylation at Ser2448 has been observed in HEK 293 cells and rat skeletal muscle in response to stimulation, although phosphorylation of the downstream intermediates 4E-BP1 and p70S6k was elevated (5, 27). On the basis of these data, it appears that phosphorylation of Akt may enhance protein synthesis through the activation of 4E-BP1 and p70S6k independent of mTOR.

In contrast to the aforementioned findings, electrical stimulation enhances mTOR phosphorylation on Ser2448 in rat skeletal muscle (17). However, elevation was observed only in muscles containing a predominant percentage of fast-twitch fibers. Although these findings suggest that changes in mTOR2448 phosphorylation resulting from electrical stimulation may be fiber type specific, fiber type-specific variation in mTOR2448 phosphorylation may be a distinct result of the stimulus or stimulation patterns utilized to contract the muscle. In the present study, muscle samples were collected from the vastus lateralis, revealing a mixed distribution of MHC I, IIa, and IIx proteins. On the basis of the predominant type II fiber profiles expressed in the rat skeletal muscle and the mixed MHC content of the muscle samples in the present study, any fiber type-specific mTOR2448 interactions may have prevented a clear representation of mTOR2448 phosphorylation in response to RE in human skeletal muscle.

Recently, it has been suggested that aerobic exercise in a low muscle glycogen state is advantageous, leading to increases in muscle glycogen content, time to exhaustion, and metabolic enzyme activity compared with 10 wk of normal training (11). These data are consistent with studies investigating metabolic gene expression during recovery from exercise (20) and after exercise in a glycogen-depleted state (19), providing evidence that depressed muscle glycogen levels enhance metabolic adaptations. Although these data would suggest a possible benefit from exercise in a low glycogen state, the present data suggest that exercise in a depleted state may attenuate growth by preventing the stimulation of proposed signaling pathways. Although care must be taken in making direct comparisons between data sets because of the different modes of exercise, there appears to be an interesting interaction between glycogen concentration and training adaptations, including growth and metabolic regulation, that warrants further investigation.
In conclusion, on the basis of the present findings, it appears that mechanical stress associated with exercise is a powerful stimulator of ERK1/2 and p90RSK phosphorylation, independent of glycogen concentration. However, muscle glycogen availability appears to play a role in regulation of the Akt pathway, inasmuch as Akt phosphorylation was elevated only after RE in the HCHO trial. Thus the present findings suggest that although the ERK1/2 pathway may be unaffected by muscle glycogen, exercising in a glycogen-depleted or malnourished state may disrupt mechanisms involved with protein translation through the Akt pathway. In this manner, adaptations to an acute bout of exercise may be blunted.

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GRANTS

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