Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats

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Hernandez, Jazmir M., Mark J. Fedele, and Peter A. Farrell. Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. J. Appl. Physiol. 88: 1142–1149, 2000.—The temporal pattern for changes in rates of protein synthesis and glucose uptake after resistance exercise, especially relative to each other, is not known. Male Sprague-Dawley rats performed acute resistance exercise (n = 7) or remained sedentary (n = 7 per group), and the following were assessed in vivo 1, 3, 6, 12 and 24 h later: rates of protein synthesis, rates of glucose uptake, phosphatidylinositol 3-kinase (PI3-kinase) activity, and p70S6k activity. Rates of protein synthesis in mixed gastrocnemius muscle did not increase until 12 h after exercise (e.g., at 12 h, sedentary = 138 ± 4 vs. exercised = 178 ± 6 nmol phenylalanine incorporated·g muscle·h⁻¹, mean ± SE, P < 0.05), whereas at 6 h after exercise rates of glucose uptake were significantly elevated (sedentary = 0.18 ± 0.020 vs. exercised = 0.38 ± 0.024 μmol glucose 6-phosphate incorporated·kg muscle·h⁻¹·min⁻¹, P < 0.05). At 24 h after exercise, rates of protein synthesis were still elevated, whereas glucose uptake had returned to basal levels. Arterial insulin concentrations were not different between groups at any time. Non-insulin-stimulated activities of PI3-kinase and p70S6k were higher at 6, 12, and 24 h after exercise (P < 0.05), and, generally, these occurred when rates of protein synthesis (12 and 24 h) and glucose uptake were elevated (6 and 12 but not 24 h) by exercise. These data suggest that regulators of protein synthesis and glucose uptake may respond to the same contraction-generated signals with different kinetics or that they respond to different intra- or extracellular signals that are generated by exercise contractions; insulin.

Endurance exercise stimulates higher rates of glucose uptake immediately and for prolonged periods after the stress (36, 48). In contrast, resistance exercise, which includes eccentric contractions, results in reduced glucose uptake during the recovery period (1–4), and such a reduction could negatively impact the energy-requiring process of protein synthesis. One approach to studying the coordination of these two processes, glucoregulation and protein synthesis, is to determine whether they change with a similar time course after exercise. Resistance exercise may be a good model for elucidating such a time course. Lowering a weight with the legs requires eccentric contractions as the person or animal lowers itself from a standing position. Therefore, it is possible that glucose uptake is impaired after resistance exercise, which contains eccentric contractions; however, the time course over the first day of this impairment is not known.

In a like manner, exercise changes intracellular cell signaling pathways involved in glucose uptake (19); however, most of these observations are limited to only a few hours after exercise (25–27, 46, 48, 49). A recent study by Baar and Esser (6) demonstrated that 6 h after acute in situ resistance exercise the activity of p70S6k is elevated and that this elevation persists for 36 h after acute exercise. Neither rates of protein synthesis nor rates of glucose uptake were measured in that study; however, the suggestion that p70S6k activation could be critical for exercise-induced muscle growth requires further investigation.

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Our goals for this study were to measure rates of protein synthesis, glucose uptake, and selected intracellular cell signaling pathways that might control both processes at various times after resistance exercise.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Male Sprague-Dawley rats were used in all experiments and were housed in temperature- and humidity-controlled holding facilities with lights on at 0700 and off at 1900. Rats were fed ad libitum a standard rodent diet (PMI Feeds 5001), which contained 24% protein, 12% fat, 50% carbohydrate, 7% ash, 6% fiber, and vitamins. Each exercise or sedentary group had seven rats.

Design

Seventy nondiabetic rats (238 ± 4 g body wt at death) were studied at specific time intervals after acute resistance exercise (described below). Rats were randomly assigned to groups and were studied 1, 3, 6, 12, or 24 h after exercise. Sedentary rats were studied simultaneously. The feeding schedule for this study was somewhat complicated in that all rats were fasted for 5 h before the determination of rates of protein synthesis and glucose uptake (described below). To ensure that the rats were similarly fed, we taught the rats to eat within a 1-h period by making food available during a short period of time for several days before an experiment. The rats learned to eat when food was available. They were then returned to a normal eating schedule for 2 days before the experimental day. A schedule of feeding, exercise, and tissue procurement for this study is provided in Fig. 1.

Procedures

Resistance exercise. Rats performed resistance exercise that has been previously described (17). Briefly, rats were operantly conditioned to touch an illuminated bar low on a Plexiglas exercise cage and then were taught to stand and touch the high bar 50 times during one acute exercise session. Once the learning process was completed (2–3 sessions), weighted vests were strapped over the scapulae and the rats were required to touch the high bar 50 times during one acute exercise session. We defined “acute” resistance exercise as four separate sessions with 1 day of rest between sessions. Rats performed 50 repetitions each day with 0.2 (day 1), 0.4 (days 2 and 3), and 0.6 (day 4) g weighted vests/g body wt. Previous work showed that rats naive to the lifting procedure would not lift the 0.6 g body wt on the first day weights were applied to the vest. This protocol can be considered as “acute” because it does not result in changes in body or muscle weight (14, 18). Exercise sessions occurred in the dark (red light) at appropriate times during the day. Sedentary rats were placed in the lifting cages at least three times during the week of acute exercise and were given four electric shocks to simulate some of the stress experienced by the exercised groups. One of these shock control sessions occurred at the same time exercising rats were lifting in the time course study.

Muscle glucose uptake. Measurements of muscle glucose uptake and rates of protein synthesis occurred at various time intervals after the last bout of acute resistance exercise based on the time course design. Rats were anesthetized with methoxyflurane and placed on a heating pad, and then the left carotid artery and right jugular vein were cannulated. One milliliter of arterial blood was taken to determine plasma concentrations of insulin and glucose. Rats remained unconscious after the placement of catheters and during the measurement of rates of protein synthesis and glucose uptake. Total time between the onset of anesthesia and completion of surgery was 10–15 min.

An index of glucose uptake was determined according to Kraegen et al. (32) in vivo 30 min after infusion of 14C-labeled 2-deoxyglucose ([1-14C]DOG; 30 µCi). Glucose uptake was estimated from the content of [1-14C]DOG 6-phosphate in muscle, the plasma concentration of the [1-14C]DOG, and the plasma concentration of nonradioactive glucose 30 min after the infusion of the tracer.

Rates of protein synthesis. Twenty minutes after the infusion of [1-14C]DOG, a flooding dose (21) of L-[2,3,4,5,6-3H]-phenylalanine (1 mCi/rat; Amersham Life Science, Arlington Heights, IL) in unlabeled phenylalanine (150 mM; 1 ml/100 g body wt, total volume) was injected into the venous catheter over a 15-s period. Arterial blood (1 ml) was taken at 6 and 10 min after infusion of the flooding dose, and then the left gastrocnemius and soleus muscles were excised and immediately dropped into liquid nitrogen and used for the determination of rates of protein synthesis. The right leg was used for the determination of glucose uptake. Because of limitations in the amount of muscle tissue, glucose uptake index, and phosphatidylinositol 3-kinase (P13-kinase) and p70S6k activities were not measured in the soleus muscle.

Assays

Materials. Affinity-purified rabbit polyclonal p70S6k antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein A-Sepharose CL-4B and protein G-Sepharose were purchased from Pharmacia (Piscataway, NJ). [1-14C]DOG was purchased from DuPont NEN (Boston, MA). Phosphatidylinositol was purchased from Avanti Polar Lipids. All other biochemicals were from Sigma Chemical (St. Louis, MO), and radiochemicals were from DuPont NEN.

Protein synthesis. Frozen muscles were stored at −70°C until phenylalanine incorporation into TCA-precipitable protein was analyzed using dabsylation of the amino acid and measurement on an HPLC (12). Radioactivity in the phenylalanine peak was measured by liquid scintillation counting.

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Fig. 1. Description of the fasting, feeding, and exercise schedule for each group on the day of the infusion. Feeding time = 1-h feeding period when animals received rat chow ad libitum. Boxes = 5-h fasting period before muscle extraction. Circles = time exercise was performed. Sedentary rats were subjected to the same schedule as their exercised counterparts.
GLUCOSE AND PROTEIN METABOLISM AFTER RESISTANCE EXERCISE

Glucose uptake. Gastrocnemius muscle concentration of [1-14C]DOG 6-phosphate was determined by column chromatography separation of free [1-14C]DOG from [1-14C]DOG 6-phosphate (32). Briefly, 0.2 g of powdered gastrocnemius muscle was homogenized in 1.5 ml of double distilled water, boiled for 3 min, and then centrifuged at 10,000 rpm for 10 min. The supernatant was deproteinized with 2 mM ZnSO4 and 5 mM Ba(OH)2; 0.5 ml of the supernatant was applied to a chromatography column containing 1 ml of ion-exchange resin (Dowex 2-x-8; 100–200 mesh, Sigma). Five milliliters of double distilled water were applied to the column to elute free [1-14C]DOG. A subsequent elution with 10 ml of 0.2 M formic acid/0.5 M ammonium acetate, pH 4.9, was performed to yield [1-14C]DOG 6-phosphate. A consistent fraction of the eluates was placed in 10 ml of scintillation cocktail (Ready Flow III from Beckman) and counted in a Beckman scintillation counter. Plasma samples were treated according to Oshima et al. (39) for the determination of [1-14C]DOG concentration. Thirty microliters of plasma were deproteinized with 120 µl of 2 mM ZnSO4 and 120 µl of 5 mM Ba(OH)2 and then centrifuged at 10,000 rpm, 4°C, for 10 min; 250 µl of the supernatant were placed into 10 ml of scintillation cocktail and counted in a Beckman LS 6500 scintillation counter. Appropriate corrections for quench were applied. Glucose uptake was calculated as micromoles of [1-14C]DOG 6-phosphate incorporated per kilogram of muscle per minute.

p70S6k activity. Approximately 100 mg of the mixed gastrocnemius were homogenized in ice-cold buffer according to Foll et al. (19). The solubilization buffer contained 50 mM HEPES (pH 7.5), 137 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 2 mM NaN3, 10 mM sodium pyrophosphate, 10 mM NaF, 2 mM EDTA, 1% NP-40, 10% glycerol, 2 µg/ml aprotinin, 10 µg/ml anti-pan p70S6k polyclonal antibody. The immunocomplexes were washed and incubated with a lipid substrate (phosphatidylinositol 3-monophosphate, phosphatidylinositol 4-monophosphate, and phosphatidylinositol 3,4,5-trisphosphate) were extracted with chloroform-methanol (1:1, by vol), and the lower organic phase was subjected to TLC for separation and identification by their comigration with a phosphatidylinositol 4-phosphate standard. Radioactivity of the phosphorylated products was determined by counting 25 µl of the mixture to phosphocellulose P-81 paper (Whatman). The filters were washed three times with 0.85% phosphoric acid and once with acetone, placed in 10 ml of scintillation cocktail (Ready Flow III from Beckman), and counted in a liquid scintillation counter with correction for several types of quench. Samples in the absence of peptide substrate were processed identically for determination of nonspecific binding to the phosphocellulose paper. p70S6k activity was calculated as picomoles of phosphate incorporated into the S6 substrate per milligram of protein per minute (33).

Plasma glucose was measured in duplicate using a Beckman model 2 autoanalyzer. Plasma insulin was measured in duplicate using a double-antibody RIA (37) as modified in our laboratory (13), which could detect 10 pmol of insulin per tube and has an interassay coefficient of variation of 3%.

Statistical Analysis

Statistical differences between sedentary and exercised groups were analyzed using repeated-measures ANOVA. The design was a two (exercise status) by five (hours postexercise) factorial ANOVA for repeated measures. This analysis provided F ratios pertinent to whether the groups differed according to exercise status or whether the groups within each group based on exercise status (e.g., sedentary or exercised) differed across time. When significant F ratios were calculated, a Student-Newman-Keuls post hoc test was used to identify means that differed significantly at P < 0.05.

RESULTS

The four sessions of acute exercise did not adversely affect the rat's health, since body weights were stable and hematoctit and Hb were similar between groups (data not shown). Plasma glucose and insulin concentrations were determined at the start of the tracer infusion and just before tissue procurement. During this 30-min period under anesthesia, there was no change in arterial glucose; however, plasma insulin increased about 1.5-fold. The increase in insulin as well as the absolute concentrations of insulin and glucose were similar (P > 0.05) for exercised and sedentary groups. Therefore, the concentrations shown in Table 1 are the averages of those two time points, since this provides a better indicator of circulating glucose and insulin concentrations during the period of amino acid and glucose incorporation into muscle.

Table 1 provides arterial plasma concentrations for insulin and glucose for each group.

Figure 2 shows that there is a delay of at least 6 h after exercise before elevations in protein synthesis occurred in gastrocnemius muscle; the delay was even longer in soleus muscle. The percent increases in gastrocnemius muscle (data not shown) for synthesis at 12 h (Fig. 2) postexercise were similar to those reported in prior studies (14, 15). The increases at 24 h, however,
exceed (increase of 42%) those that we normally observe at 16 h postexercise (~20–30%). Figure 3 shows that a similar time course occurred in soleus muscle with the exception that the elevation at 12 h was not statistically significant ($P = 0.09$).

Figure 4 provides data for glucose uptake. Glucose uptake was significantly lower at 3 h postexercise and significantly higher in exercised rats at 6 and 12 but not 24 h postexercise. Figure 5 provides data for the time course in activation of p70$^{	ext{S6K}}$. The activity of this enzyme was significantly higher in exercised animals at 12 and 24 h postexercise. Figure 6 provides data for the time course in activation of PI3-kinase. The activity of this enzyme was significantly higher in exercised animals at 6, 12, and 24 h postexercise.

**DISCUSSION**

The present data provide new insights into some aspects of the regulation of protein synthesis and glucose uptake after resistance exercise. The time courses for the effects of prior exercise on rates of protein synthesis and glucose uptake were not similar. Protein synthesis in gastrocnemius muscle did not change for at least 6 h after exercise, whereas glucose uptake initially decreased (3 h) and then markedly increased at 6 h, a time when rates of protein synthesis were not different between exercised and sedentary rats. At 12 h, both protein synthesis and glucose uptake were elevated, whereas at 24 h postexercise only rates of synthesis were elevated. A differential time course between rates of protein synthesis and glucose uptake to the same stress may be due to activation of different intracellular pathways (40, 45), some of which are regulated by insulin or muscle contractions per se (46), as well as many circulating factors or intracellular pathways that were not evaluated in this study.

It is reasonable to investigate an interaction between glucose uptake/availability and the effects of resistance exercise on protein synthesis because Roy et al. (44) have shown that glucose supplementation in humans after such exercise can augment elevations in rates of protein synthesis when the supplementation is given within 1 h after exercise. All rats were fasted for 5 h before tissue procurement. This length of fast was chosen because a prolonged fast will reduce rates of protein synthesis, whereas studying rats in the fed state could result in markedly different insulinemia. The 5-h fast must be considered because some exer-

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Table 1. Arterial plasma glucose and insulin concentrations at the time of tissue procurement and measurement of rates of protein synthesis and glucose uptake

<table>
<thead>
<tr>
<th>Group</th>
<th>Time Postexercise</th>
<th>Glucose, mM</th>
<th>Insulin, pM</th>
<th>Glucose, mM</th>
<th>Insulin, pM</th>
<th>Glucose, mM</th>
<th>Insulin, pM</th>
<th>Glucose, mM</th>
<th>Insulin, pM</th>
<th>Glucose, mM</th>
<th>Insulin, pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>1 h</td>
<td>10.1 ± 0.2</td>
<td>418 ± 52</td>
<td>9.8 ± 0.15</td>
<td>347 ± 90</td>
<td>10.2 ± 0.2</td>
<td>396 ± 82</td>
<td>10.3 ± 0.3</td>
<td>412 ± 48</td>
<td>10.5 ± 0.14</td>
<td>400 ± 52</td>
</tr>
<tr>
<td>Exercised</td>
<td>3 h</td>
<td>10.9 ± 0.1</td>
<td>451 ± 62</td>
<td>10.4 ± 0.17</td>
<td>458 ± 66</td>
<td>10.6 ± 0.2</td>
<td>378 ± 5.6</td>
<td>10.8 ± 0.19</td>
<td>292 ± 44</td>
<td>10.6 ± 0.13</td>
<td>444 ± 35</td>
</tr>
</tbody>
</table>

Values are means ± SE.
cised groups were fed after exercise, whereas others were not. Rats in the 1- and 3-h groups did not eat after exercise, whereas rats in the 6-, 12-, and 24-h groups did. The feeding schedule, however, was successful since insulinemia and circulating glucose were similar for all groups. Although this was effective for negating large differences in circulating insulin, careful evaluation of the impact of food intact on our results is required. In particular, the 6-h postexercise group had food available for 1 h after exercise, and this was the time when rates of glucose uptake were first elevated after exercise. It is doubtful, however, that the food per se was responsible for elevated glucose uptake because the sedentary rats received food in a manner identical to the exercised rats and glucose uptake was similar for sedentary rats that did not eat after shock exposure (1- and 3-h groups) and the 24-h group that had food for 19 h after exercise and was then fasted for 5 h. Rates of glucose uptake affect glycogen formation, and muscle glycogen status can alter glucose uptake (see review in Ref. 23). Very little is known, however, about the effects of acute resistance exercise on glycogen status. After chronic resistance exercise (training), the muscle glycogen concentration is significantly higher when assessed 96 h after the last exercise session in humans (29).

Fig. 4. Glucose metabolic index as an estimation of glucose uptake was measured in mixed gastrocnemius in sedentary (open bars) and exercised (solid bars) rats; n = 7 for each group per time. *Significantly higher in exercised groups and #significantly lower in exercised group, P < 0.05. Bars represent means ± SE.

No prior studies have documented the time course of rates of protein synthesis and glucose uptake using frequent assessments over the first 24-h postexercise. Booth and Watson (10) reviewed the available data up to 1985 and suggested that rates of protein synthesis are consistently elevated when studied more than 1 h postexercise, but it must be noted that the data included in Table 3 of that review are not specific to resistance exercise. Later studies by Wong and Booth (50–52) suggested that such elevations are apparent between 12–17 and 36–41 h after in situ nonvoluntary contractions depending on the number of repetitions and load per repetition required in the protocol. The time course of elevations in rates of protein synthesis is probably model and species specific because a longer delay after exercise is noted in our in vivo model using rats than that reported using traditional weight lifting in humans, in which significant elevations in rates of protein synthesis (and proteolysis) are noted 4 h after exercise (8, 41) and are probably maintained for at least 1 day (47).

Arterial plasma insulin concentrations were not different between exercised and sedentary groups when measured during the isotope infusion. This was an
expected finding, since the rats were fed identically before exercise and this type of exercise does not alter circulating insulin concentrations (14). These data agree with our previous work showing that elevations in protein synthesis after similar exercise are independent of insulin concentrations as long as a low but critical amount of insulin is available in vivo (15). Such a concept is compatible with many studies (16, 31) that show that severe diabetes or the absence of insulin in an in situ hindlimb perfusion model (30) markedly reduces basal rates of protein synthesis.

Muscle contractions during the exercise used in this study required concentric contractions during the lifting and eccentric contractions during the lowering movement (verified using electromyography, data not shown). Exercise requiring purely eccentric contractions of red gastrocnemius muscle in rats caused reduced maximal but not submaximal insulin-stimulated glucose uptake (2, 4) when assessed 2 days after exercise. Our data at 3 h postexercise are somewhat consistent with this work, since glucose uptake was reduced at 3 h postexercise and plasma insulin was not changed by prior exercise. The decline in glucose uptake was transient, however, since significant increases occurred at 6 and 12 h postexercise. This increase may have been due to the concentric component of muscle activation, since this type of contraction results in elevated basal and insulin-stimulated glucose uptake (25, 27, 36, 43). Together, these data in rats show that resistance exercise, which requires both eccentric and concentric contractions, results in a transient decline in basal glucose uptake but is followed by a prolonged period of enhanced glucose uptake.

Enhancement of glucose uptake and protein synthesis after exercise is the result of a complex activation of intracellular signaling pathways (23, 48). Cheatham et al. (11) have provided evidence using 3T3-L1 fibroblasts that PI3-kinase may be a point of divergence for several pathways regulated by insulin, including potential pathways activated by insulin are known to be unresponsive to muscle contractions (24). The present study is the first to provide a detailed time course showing prior resistance exercise activation of glucose uptake, protein synthesis, PI3-kinase, and p70S6 kinases at times when insulin concentrations are not different between exercised and sedentary groups. Such data are compatible with dual but separate regulation of specific intracellular pathways by muscle contractions and insulin. The complexity of these intracellular pathways will require extensive work before there is a complete understanding of which pathways are most important when the organism transitions from a catabolic to an anabolic state as it must do after resistance exercise.

Intracellular cell signaling molecules such as mitogen-activated protein kinase (MAPK) may also be important to the coordinated changes in glucose uptake and transcriptional or translational regulation after exercise, as well as being a potential signal transducer for contractile (mechanical?) activity per se. Sherwood et al. (46) demonstrated that phosphorylation of p42MAPK and phosphorylation of p44MAPK are elevated immediately after in situ muscle contractions. Such elevations, if sustained for more than 6 h after exercise, may promote enhanced glucose uptake but may not be related to elevations in rates of protein synthesis. From a glucose uptake perspective, however, recent evidence (26) suggests that MAPK signaling is not related to changes in glucose transport immediately after soleus muscle contractions in vitro. From a protein synthesis perspective, Azpiazu et al. (5) have demonstrated, using rat diaphragm muscle studied in vitro, that the phosphorylation of eukaryotic initiation factor 4E binding protein (4E BP-1) is MAPK independent but p70S6k dependent. Phosphorylation of 4E BP-1 leads to dissociation of 4E BP-1 from eukaryotic initiation factor 4E, and this is consistent with elevations in rates of protein synthesis of specific proteins. The dissimilarity of our model of in vivo resistance exercise to in situ-stimulated muscle contractions and the dissimilarity of the time after exercise when observations were made limit
our speculation about the role of MAPK in the glucoregulatory and anabolic processes we measured.

In summary, the time course for changes in protein synthesis and glucose uptake were not similar during the first 24 h after resistance exercise. Glucose uptake increased sooner after exercise than protein synthesis but returned to values found in sedentary animals faster than protein synthesis. The activity of two important kinases, PI3-kinase and p70S6K, was not increased in this model for 6 h after exercise but then remained elevated for a prolonged period. These elevations occur when glucose uptake and protein synthesis were also elevated; however, such a relationship does not seem to be mandatory. These data support the general conclusion that regulators of protein synthesis and glucose uptake in skeletal muscle either respond to the same exercise-generated signals with different time kinetics or respond to different intracellular signals generated by the same stress.

We thank Marlin Druckenmiller, J Josh Ryan, and J Jaycee Kostyak for expert technical skills.

This study was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-43127. Address for reprint requests and other correspondence: P. A. Farrell, Noll Physiological Research Center, University Park, PA 16802 (E-mail: paf4@psu.edu).

Received 7 October 1999; accepted in final form 8 December 1999.

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