Eccentric muscle damage transiently decreases rat skeletal muscle GLUT-4 protein

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Asp, Sven, Søren Kristiansen, and Erik A. Richter. Eccentric muscle damage transiently decreases rat skeletal muscle GLUT-4 protein. J. Appl. Physiol. 79(4): 1338-1345, 1995. — The effects of concentric and muscle-damaging eccentric contractions on muscle glucose transporter GLUT-4 content were studied in rat muscles. Rats were anesthetized, the calf muscles on one side were stimulated electrically for concentric or eccentric contractions, and bilateral calf muscles were obtained in the postexercise period. Inflammatory and phagocytic cells accumulated in the eccentric white and red gastrocnemius muscles, whereas there were only discrete changes in the eccentric soleus. Glycogen was depleted to the same extent in the white and red gastrocnemius muscles after both types of stimulation, and it remained decreased >2 days in eccentric muscles. The total GLUT-4 protein content was decreased in the eccentric white and red gastrocnemius muscles 1 and 2 days after the eccentric stimulation, whereas the maximal activity of glycogen synthase was unaffected at these time points. In conclusion, our one-legged stimulation model caused eccentric muscle damage in the white and red gastrocnemius muscles, whereas only minor damage was observed in the soleus muscle. In damaged muscle, muscle glycogen and GLUT-4 protein content were decreased for >2 days. These findings may suggest (but do not prove) that decreased muscle GLUT-4 protein is involved in the delayed glycogen resynthesis after eccentric exercise.

eccentric exercise; rat glycogen; glucose transporters; GLUT-4; glycogen synthase

ECCENTRIC EXERCISE has been shown to cause muscle damage and impaired postexercise glycogen resynthesis (6, 9, 22, 32), but the mechanisms responsible for this inability to restore muscle glycogen are obscure. There is evidence from transgenic mice overexpressing glucose transporter GLUT-1, suggesting that the rate of glucose transport is the primary factor determining the concentration of glycogen in skeletal muscle at rest (25). In skeletal muscle fibers, GLUT-4 is the predominant glucose transporter (18), and translocation of GLUT-4 from an intracellular pool to the sarcolemma and t-tubules occurs by insulin stimulation (10, 11, 33). The insulin-induced increase in muscle glucose transport and uptake, respectively, correlates with the muscle GLUT-4 protein content in humans (1, 7) and, when combining insulin and exercise, also in rats (13), stressing the importance of this protein for insulin-induced muscle glucose uptake. Recently, Kirwan and co-workers (16) demonstrated that whole body insulin sensitivity was decreased 2 days after eccentric exercise (downhill running), and since the GLUT-4 protein concentration in skeletal muscle can change rapidly (1, 26), a decrease in the GLUT-4 protein concentration could be involved in the impaired insulin sensitivity and muscle glycogen resynthesis after eccentric muscle damage.

The present study was undertaken to develop a rat model for the study of eccentric muscle damage and to elucidate whether eccentric muscle damage in the rat is accompanied by a decrease in the muscle GLUT-4 protein concentration.

MATERIALS AND METHODS

Eccentric stimulation model. Fed Male Wistar rats weighing ~200–240 g were kept on a 12:12-h light-dark cycle. All rats were fed an ad libitum standard chow diet, which was maintained until the end of the experiment. The rats were anesthetized by an intraperitoneal injection of diazepam (a benzodiazepine) and hypnorm (a morphine-like drug), placed on the back, and both hindlimbs were fixed over a plastic block by the use of Velcro strips. The limbs were placed so that the hip joint had an approximate angle of 120°, the knee joint an ~120° angle, and the ankle joint had an ~90° angle. Two groups of rats were studied: concentric and eccentric. Metal hooks were placed under both Achilles tendons and in the eccentric group connected to an air-pressure system, where a standardized air pressure (3 bar) was applied to a piston chamber and used for stretching the active calf muscles. In one hindlimb, the muscles below the knee were stimulated electrically to contraction through a needle electrode placed in the most distal part of the quadriceps femoris muscle and through the hook placed under the Achilles tendon (Fig. 1). The stimulus consisted of four sessions of ten trains, and each train had a duration of 1,000 ms. The frequency of impulses in the train was 100 Hz, and each pulse in the train had a duration of 1 ms. To obtain maximal force output on the strain gauge after the first warm-up session, the stimulus amplitude was increased gradually from 15 to 25 V during the last three sessions. The trains in each session were separated by 4 s, and the sessions were separated by 1 min. The electrical stimulus made the calf muscles contract concentrically. In the eccentric group, the hindlimb was stimulated for concentric contraction as described above, and with a 400-ms delay the active calf muscles were then stretched by a standardized tension (3 bar) transferred from the piston chamber. On stimulus cessation, the calf muscles became inactive instantaneously, and the air pressure was closed, but due to delay in the latter system the nonactive muscles were kept in the stretched position for another 200 ms (Fig. 2). A spring was inserted in series to smoothen the muscle stretch, and a strain-gauge was also inserted to measure the force output and input. All exercise sessions were finished before 12 A.M., and none of the rats was limping in the postexercise period.

Protocol. Immediately, and, 1 and 2 days after the bout, bilateral calf muscle samples were obtained from eccentric and concentric rats during anesthesia with pentobarbital sodium. Muscles were also obtained 4 days after eccentric stimulation. The superficial part of the gastrocnemius muscle, which consists mainly of fast-twitch white fibers (2) was cut out. Then the soleus muscle, which consists mainly of slow-twitch red fibers (2), was removed. Finally, a portion of the deep part of the medial head of the gastrocnemius (2), con-
ECCENTRIC MUSCLE DAMAGE AND SKELETAL MUSCLE GLUT-4

sisting mainly of fast-twitch red fibers, was cut out. A well-defined transectional area from the midbelly of each fiber type was dissected, embedded in tissue-tek, and fixed in isopentane, which was cooled to the freezing point in liquid nitrogen. The embedded frozen muscles were stored at −80°C until section. The remaining part of the muscle samples were freeze clamped with tongs cooled in liquid nitrogen and stored at −80°C until analyzed. In separate experiments in which ATP and creatinine phosphate (CP) were measured in the soleus immediately after the eccentric contractions, the muscles were frozen within 20 s after the bout.

Analytic procedures. Muscle samples were freeze-dried and dissected free of blood and connective tissue before analysis. The total water content was calculated as the difference in weight before and after freeze-drying. Glycogen was measured as glucose residues after acid hydrolysis (20). Glycogen synthase was determined essentially by the method of Thomas et al. (31). Activity was measured at 37°C, and the reaction mixture contained uridine-5′-diphosphoglucose at a concentration of 1.5 mM and glucose 6-phosphate at either 0.17 or 8.0 mM, the latter concentration saturating ATP and CP were measured by standard enzymatic methods (20). For histological determination of leukocyte/phagocyte infiltration and focal muscle necrosis, embedded frozen muscle samples were cut in a microtome at a thickness of 10 μm and stained with hematoxylin and eosin.

Total crude muscle membranes (TCM) were obtained as described previously (23). Approximately 30 mg muscle were homogenized two times for 10 s at maximum speed by a Polytron PT-10 (Kinematica, Luzern, Switzerland) in 2 ml of ice-cold buffer (30 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 210 mM sucrose, 2 mM ethylenediamine glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid, 40 mM NaCl, and 0.35 mg/ml polymethylsulfonyl fluoride, pH 7.4). The samples were left on ice for 15 min before TCM were recovered at 4°C by centrifugation at 50,000 rpm for 90 min in a Beckmann 70.1 Ti rotor as described by Plaig et al. (24). The pellet was solubilized in 800 μl 4% sodium dodecyl sulfate

FIG. 1. One-legged stimulation model: experimental setup. Calf muscles on 1 side were stimulated for concentric or eccentric contractions through needle electrode in most distal part of quadriceps muscle and through hook under Achilles tendon. Limb was fixed over plastic block by use of Velcro strips, which are not shown. Force output and input were measured by strain gauge.
FIG. 3. Typical hematoxylin-eosin staining on day 2. A: white gastrocnemius eccentric leg; B: red gastrocnemius eccentric leg; C: soleus eccentric leg. Note presence of phagocytic cells, focal muscle fiber necrosis, and endomysial widening in both eccentric damaged muscles (A and B).
The force-development and stimulus amplitude were re-

TABLE 1. Glycogen concentration in the different fiber types after one-legged concentric or eccentric contractions

<table>
<thead>
<tr>
<th></th>
<th>Concentric, mmol/kg dry wt</th>
<th>Eccentric, mmol/kg dry wt</th>
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<tr>
<td></td>
<td>Post</td>
<td>Day 1</td>
</tr>
<tr>
<td>WG-C</td>
<td>194.6±14.0</td>
<td>186.7±5.6</td>
</tr>
<tr>
<td>WG-E</td>
<td>80.1±6.9*</td>
<td>181.0±4.8</td>
</tr>
<tr>
<td>RG-C</td>
<td>188.9±20.1</td>
<td>200.1±14.7</td>
</tr>
<tr>
<td>RG-E</td>
<td>103.7±15.3*</td>
<td>193.2±10.5</td>
</tr>
<tr>
<td>S-C</td>
<td>151.4±10.5</td>
<td>137.7±10.6</td>
</tr>
<tr>
<td>S-E</td>
<td>193.1±13.6*</td>
<td>133.4±7.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6–8 observations in each group. WG-C, white gastrocnemius control leg; WG-E, white gastrocnemius exercise leg; RG-C, red gastrocnemius control leg; RG-E, red gastrocnemius exercise leg; S-C, soleus control leg; S-E, soleus exercise leg. * Significantly different (P < 0.05) from control value.

(SDS), 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4, and stored in aliquots at −20°C for subsequent determination of protein concentration and Western blotting. Protein concentration was determined by the bichinchoninic acid method (30). Samples were loaded on a discontinuous gradient (8–18%) SDS gel, electrophoresed on a horizontal Multisphor system (Pharmacia, Uppsala, Sweden), and then transferred to an Immobilon membrane (Millipore, Bedford, MA) by electrotransfer. Total protein was stained with 0.2% Ponceau S and again destained with water. Subsequently, the Immobilon P membrane was blocked in 10 mM Tris, 0.9% NaCl (TS buffer, pH 7.4) containing 2.5% bovine serum albumin (BSA) and 2.5% defatted milk powder at 37°C for 90 min followed by 90-min incubation with primary antibody (1.25 μg immunoglobulin G/ml) in 5% BSA in TS buffer at room temperature. We used a mouse monoclonal antibody produced against a synthetic peptide corresponding to the C-terminal amino acids of GLUT-4. After incubation with the primary antibody, the Immobilon membrane was washed four times for 3 min in TS buffer and then incubated for 1 h with horseradish peroxidase-labeled goat anti-mouse antibody diluted in 5% BSA in TS buffer (1:2,000). Membranes were finally washed three times for 3 min in 0.5% Triton X-100 in TS buffer, followed by a 3-min wash in TS buffer alone. Antibody-antigen complexes were visualized by enhanced chemiluminescence (Amersham, Buckinghamshire, UK) on Hyperfilm. Densiometric scanning was performed within the linear response range (CREAM software; Kern-En-Tee Software Systems, Copenhagen, Denmark).

The force-development and stimulus amplitude were re-

Statistics. Mean values were compared by the Student’s paired t-test. The Bonferroni correction factor was used in the case of glycogen, GLUT-4, water content, and the activity of glycogen synthase in the two legs and in each fiber type where multiple comparisons were performed. The level of significance was set at P < 0.05.

RESULTS

Phagocytic/inflammatory cells had accumulated, and there was focal muscle fiber necrosis and endomysial widening in eccentrically exercised red and white gastrocnemius muscles 1, 2, and 4 days after the exercise. Only discrete changes were found in the eccentric soleus, and no accumulation of inflammatory cells was found in any of the concentric muscles. Representative photomicrographs of muscles sampled on day 2 after eccentric contractions are shown in Fig. 3.

The mean force output in the eccentric rats during the concentric part of the contractions that lasted 400 ms was 3.86 ± 0.27, 2.74 ± 0.24, 2.30 ± 0.23, and 1.79 ± 0.21 N, respectively, during the four sessions. During the eccentric part, which lasted 600 ms, the force input was 29.92 ± 0.80, 27.99 ± 0.54, 27.04 ± 0.50, and 25.96 ± 0.55 N, respectively. The force input that kept the nonactive muscles in the stretched position for another 200 ms did not change over the sessions and was on average 23.62 ± 0.24 N. The mean force generated in concentric rats where the contractions lasted 1,000 ms was 3.25 ± 0.26, 4.73 ± 0.38, 4.71 ± 0.35, and 4.41 ± 0.36 N in the different sessions, respectively.

The glycogen concentration decreased to the same extent in the white and red gastrocnemius after both types of exercise, and the concentration had returned to control values <1 day after the concentric stimulation, whereas it remained decreased for >2 days after the eccentric bout (Table 1). The glycogen concentration was on average decreased by 21% (P < 0.05) in the eccentric and concentric soleus muscle immediately after the bout (Table 1), and the high-energy phosphates had also decreased in the eccentric soleus (ATP 28.96 ± 0.91 vs. 30.64 ± 0.96 mmol/kg dry wt, P < 0.05 and CP 28.87 ± 0.73 vs. 51.00 ± 4.09 mmol/kg dry wt, P < 0.05). Two days after eccentric contractions, glycogen in the soleus was decreased slightly compared with control muscles (Table 1).

The total GLUT-4 content was decreased in the eccentric white and red gastrocnemius muscles 1 and 2 days after contractions (Table 2). It was not significantly lower than the control value immediately after the bout or on day 4 (Table 2 and Fig. 4). The GLUT-4 protein concentration was not different from the control value in the eccentric soleus at any stage or in any of the concentric muscles on day 2 (Table 2).

The maximal activity of glycogen synthase was unaffected 1 and 2 days after the exercise in all eccentric muscles (Table 3), whereas there was a slightly higher maximal activity in the control red gastrocnemius on day 1 compared with day 2. The fractional velocity of glycogen synthase was slightly lower in the eccentric red gastrocnemius compared with control on day 1 (Table 3).
Table 2. GLUT-4 protein content in the different muscles fiber types after one-legged concentric or eccentric contractions

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Concentric</th>
<th>Eccentric</th>
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<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Post</td>
</tr>
<tr>
<td>WG-C</td>
<td>4.6 ± 2.2</td>
<td>4.5 ± 3.7</td>
</tr>
<tr>
<td>WG-E</td>
<td>4.6 ± 2.8</td>
<td>3.8 ± 2.9</td>
</tr>
<tr>
<td>RG-C</td>
<td>8.7 ± 2.5</td>
<td>7.8 ± 2.0</td>
</tr>
<tr>
<td>RG-E</td>
<td>9.5 ± 2.0</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td>S-C</td>
<td>9.0 ± 1.2</td>
<td>10.4 ± 1.5</td>
</tr>
<tr>
<td>S-E</td>
<td>9.5 ± 2.2</td>
<td>9.9 ± 1.1</td>
</tr>
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Values are means ± SE of 6–8 observations in each group. GLUT-4 values are given in arbitrary units, expressed as % of a rat heart standard per milligram sample protein. * Significantly different (P < 0.05) from control value.

The water content was increased in the eccentric damaged muscles during the total experimental period. In the eccentric soleus and in all the concentric muscles, the content was increased immediately after the bout but had returned to control values <1 day after the bout. There was a slight increase on day 4 in the eccentric soleus (Table 2).

**Discussion**

Our one-legged stimulation model caused eccentric muscle damage in the white and red gastrocnemius, whereas only minor damage was observed in the soleus muscle. The principal findings in this study are that eccentric muscle damage causes a transient decrease in muscle GLUT-4 protein content. This change did not simply reflect unspecific muscle damage, since the maximal enzyme activity (which presumably reflects enzyme concentration) of another of the insulin-regulatable proteins, namely glycogen synthase, was unchanged by eccentric muscle activity.

Muscle-damaging exercise impairs the ability to resynthesize glycogen (6, 9, 22, 32), and eccentric exercise induces more muscle damage than exercise with a major concentric component (5). The mechanisms behind the sustained low glycogen after eccentric exercise are obscure, but a recent study in humans (16) demonstrated that whole body insulin action is impaired after eccentric exercise, indicating that reduced net glucose uptake into the muscle cell could be of importance. Accumulation of phagocytic/inflammatory cells can be seen after muscle-damaging eccentric exercise (6, 32), and is especially pronounced in rats (3) (Fig. 3); it has been suggested that competition between these cells and muscle fibers for available plasma glucose actually causes the impaired glycogen resynthesis (32). Furthermore, phagocytic cells have been shown to produce a factor stimulating glycolytic flux in muscle (12, 28), thus possibly diverting glucose away from muscle glycogen synthesis. However, these hypotheses remain speculative, and if they were true one might expect to find an increase in the insulin-stimulated glucose uptake after eccentric muscle damage, rather than a decrease, as found in the study by Kirwan et al. (16).

From studies in transgenic mice overexpressing GLUT-1 protein, there is evidence that the primary factor determining the steady-state concentration of glycogen in skeletal muscle is the rate of glucose transport (25). In the present study, the GLUT-4 protein concentration was decreased in the eccentric white and red gastrocnemius muscles 1 and 2 days after the bout, and the glycogen concentration was decreased at similar time points in eccentric damaged muscles. Therefore, these findings are in accordance with the hypothesis that the decrease in GLUT-4 content might be responsible for the decreased rate of net glycogen deposition after eccentric exercise. Arguing against such a mechanism is the fact that GLUT-4 was decreased to very low levels in the FTW fibers on day 1 and 2, whereas the values in FTR fibers were not decreased to nearly the same extent, and yet net glycogen resynthesis was equally impaired after eccentric contractions in FTW and FTR fibers (Table 1 and 2). This could suggest that the simultaneous decrease in glycogen and GLUT-4 content is coincidental and not causally related. Another possibility for the rate-limiting step in glycogen synthesis would be the activity of glycogen synthase. However, neither fractional velocity nor total activity was adversely affected by eccentric exercise on day 1, and only a small decrease in fractional velocity was found in FTR fibers on day 2 (Table 3). Thus changes in glycogen synthase activity are not likely to be responsible for the persistent low muscle glycogen concentration after eccentric contractions.
As judged by leukocyte infiltration, increase in water content, and decrease in GLUT-4 protein content, the FTW fibers were more afflicted by eccentric contractions than the FTR fibers, whereas the STR fibers of the soleus were very little affected if at all. This variation could be due to differences in fiber depolarization, mechanical strain, or inherent differences in susceptibility to muscle damage by glycolytic compared with oxidative fibers. Even though the needle and hook we used for stimulation were inserted through tissue (through the most distal part of the quadriceps muscle and tissue under the Achilles tendon), it could be argued that the direct method of stimulation did not depolarize the deeper red fibers of the gastrocnemius and the soleus to the same extent as the superficial FTW fibers. However, similar glycogen depletion of the FTW and FTR fibers immediately after either concentric or eccentric contractions argues against a difference in recruitment within the gastrocnemius. As regards the soleus, this muscle was definitely electrically stimulated as judged by the significant breakdown of glycogen, ATP, and CP. The small breakdown of glycogen in the soleus muscle is seen during electrical stimulation, even when executed through the motor nerve (14), unless stimulation is very intense (27). The much less muscle damage in the soleus compared with the gastrocnemius could be caused by less fiber strain during eccentric contractions. However, without individual measurements of tension in the soleus and gastrocnemius muscles, such a possibility is hard to evaluate. Finally, the observed graded degree of muscle damage could be due to inherent increased susceptibility of glycolytic vs. oxidative fibers for eccentric muscle damage, as suggested (19).

Eccentric exercise is known to cause muscle damage and leakiness of the muscle membranes as judged by large increases in the plasma concentration of intramuscular enzymes such as creatine kinase (21). It is interesting that the GLUT-4 content is markedly decreased by eccentric muscle damage, whereas a cytosolic protein like glycogen synthase apparently is unaffected. The mechanism behind the GLUT-4 susceptibility to eccentric muscle damage is not known but may be related to its cycling from an intracellular storage site to the plasma membrane and back (8, 15, 17). If the plasma membrane is damaged, GLUT-4 may not be translocated properly and may be susceptible to premature degradation. Interestingly, we found no effect on the GLUT-4 content immediately after eccentric contractions (Table 2), which might suggest that immediately after the bout glycogen resynthesis is unpained by eccentric contractions. This cannot be determined from the present study, since the earliest sampling time in recovery from contractions was day 1. However, such a contention is in accordance with earlier findings in humans, where the ability to restore glycogen after glycogen-depleting cycling followed by one-legged eccentric exercise was normal in the eccentric thigh for the first 6 h after the bout and impaired thereafter (32).

It has recently been shown that both GLUT-4 mRNA and protein content are increased 24 h after a 2-h bout of swimming (concentric exercise) in rats (26). In contrast, in the present study, GLUT-4 protein was not increased 2 days after concentric stimulation. This might indicate that the 40 concentric tetanic contractions are insufficient to increase GLUT-4 protein content or that the effect is transient and lasts <48 h. Alternatively, the increase in GLUT-4 requires whole body exercise with its changes in humoral milieu. Arguing against this latter possibility are studies in which chronic electrical stimulation of a small muscle group increases GLUT-4 protein within 1 day (18).

Both types of stimulation increased the water content to the same extent in all fiber types immediately after the bout. The content remained elevated in the eccentric damaged muscles for >4 days, whereas it had returned to control values in <1 day after the bout in the eccentric soleus and in all the concentric muscles. In the eccentric soleus, there was a slight increase on day 4, and this in addition to the delayed decrease in glycogen concentration could indicate that the effects are delayed in this fiber type. Acute accumulation of water in intensely exercised muscles has been described previously (29) and is probably due to changes in osmotic forces, hydrostatic pressure, and in the capillary surface area. Unpublished data from our laboratory have shown that the extracellular (mannitol) space is increased in the eccentric damaged muscles 2 days after the bout, indicating that part of the water excess is located as extracellular edema. The reasons for this sustained elevated water content in the eccentric dam-

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<th>Concentric</th>
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<td></td>
<td>Post</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>WG-C</td>
<td>76.92±0.37</td>
<td>76.08±0.10</td>
<td>76.23±0.22</td>
</tr>
<tr>
<td>WG-E</td>
<td>80.47±0.36*</td>
<td>76.33±0.18</td>
<td>76.75±0.21</td>
</tr>
<tr>
<td>RG-C</td>
<td>77.07±0.21</td>
<td>75.46±0.22</td>
<td>76.00±0.15</td>
</tr>
<tr>
<td>RG-E</td>
<td>78.52±0.54*</td>
<td>74.83±0.47</td>
<td>76.22±0.31</td>
</tr>
<tr>
<td>S-C</td>
<td>77.96±0.97</td>
<td>76.50±0.15</td>
<td>76.63±0.19</td>
</tr>
<tr>
<td>S-E</td>
<td>77.97±0.29*</td>
<td>76.81±0.20</td>
<td>76.72±0.30</td>
</tr>
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</table>

Values are means ± SE of 6–8 observations in each group. Water content measured in %.

Table 4. Total water content in the different muscle fiber types after one logged concentric or eccentric contractions

<table>
<thead>
<tr>
<th></th>
<th>Eccentric</th>
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<tbody>
<tr>
<td></td>
<td>Post</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>WG-C</td>
<td>77.68±0.35</td>
<td>77.25±0.60</td>
<td>76.82±0.26</td>
</tr>
<tr>
<td>WG-E</td>
<td>81.97±0.31*</td>
<td>82.08±0.76*</td>
<td>79.55±0.62*</td>
</tr>
<tr>
<td>RG-C</td>
<td>77.32±0.49</td>
<td>76.52±0.05</td>
<td>76.72±0.42</td>
</tr>
<tr>
<td>RG-E</td>
<td>79.72±0.85*</td>
<td>78.95±0.97*</td>
<td>78.57±0.82*</td>
</tr>
<tr>
<td>S-C</td>
<td>78.12±0.17</td>
<td>78.02±0.14</td>
<td>77.86±0.17</td>
</tr>
<tr>
<td>S-E</td>
<td>78.97±0.23*</td>
<td>78.72±0.27</td>
<td>77.84±0.23</td>
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</table>
-aged muscles are obscure but might be caused by the loss of skeletal muscle membrane integrity and leakage of intracellular proteins, followed by a higher intratiss
tial osmotic pressure. Interestingly, increased muscle water content and accumulation of inflammatory cells persisted on day 4, whereas GLUT-4 content was re-
stored. This shows that the time course of the different processes set off by eccentric contractions is quite vari-
able.

The principal findings in this study are that eccentric muscle damage causes a transient decrease in muscle GLUT-4 protein content. This change did not simply reflect unspecific muscle damage, since the maximal enzyme activity of another of the insulin-regulatable proteins, namely glycogen synthase, was unchanged by eccentric muscle activity. Although the electrical stimulation in the present study obviously is different from voluntary motor unit recruitment, the results are applicable to eccentric exercise in vivo, since eccentric exercise also in humans transiently decreases muscle GLUT-4 protein content (4).

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