Eccentric contractions decrease glucose transporter transcription rate, mRNA, and protein in skeletal muscle

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Kristiansen, Soren, Jared Jones, Aase Handberg, G. Lynis Dohm, and Erik A. Richter. Eccentric contractions decrease glucose transporter transcription rate, mRNA, and protein in skeletal muscle. Am. J. Physiol. 272 (Cell Physiol. 41): C1734–C1738, 1997.—We have recently shown that eccentric contractions (ECs; forced lengthening of active muscle) elicit a delayed decrease in glucose transporter (GLUT-4) protein content in rat skeletal muscle and a decrease in subsequent contraction-stimulated glucose transport. Here, we investigate whether this decrease in total GLUT-4 protein after prior ECs is due to changes in GLUT-4 gene transcription rate and GLUT-4 mRNA level. Furthermore, the effect of prior ECs on sarcolemmal GLUT-4 protein content in plasma membrane (PM) vesicles isolated from contraction-stimulated muscle was determined. Rat gastrocnemius muscle was electrically stimulated for ECs, and the contralateral muscle served as unstimulated control (UC). Two days later, the total GLUT-4 protein content was decreased by 50% (P < 0.05) and 32% (P < 0.05) in the white and red gastrocnemius muscle, respectively. Furthermore, the GLUT-4 mRNA concentration was decreased by 41% (P < 0.05) in both the white and red gastrocnemius muscle. Moreover, the GLUT-4 transcription rate, determined by nuclear run on analysis, was decreased by 75% (P < 0.05) in mixed EC gastrocnemius muscle compared with UC muscle. PM vesicles were isolated from EC and UC muscle after 15 min of isometric contractions. The PM GLUT-4 protein content was reduced by 51% (P < 0.05) in EC muscle compared with UC muscle. In conclusion, 2 days after ECs, the GLUT-4 transcription rate, GLUT-4 mRNA, and GLUT-4 protein content were decreased in rat skeletal muscle. Moreover, the PM GLUT-4 protein content in contraction-stimulated muscle was decreased. We suggest that eccentric muscle contractions decrease muscle GLUT-4 transcription rate, resulting in a lower GLUT-4 protein content, which in turn decreases the number of GLUT-4 transporters translocated to the sarcolemma, ultimately leading to decreased contraction-induced muscle glucose transport.

Endurance training has demonstrated an increased muscle GLUT-4 protein content (8, 12, 19, 22–24) and insulin-induced muscle glucose transport (19, 22, 24), whereas detraining decreases muscle GLUT-4 protein (28) and insulin-induced glucose uptake (28). Thus changing the level of activity causes changes in GLUT-4 protein expression in skeletal muscle. Another way of changing GLUT-4 protein expression in muscle is by eccentric contractions (ECs). We have previously observed that the muscle GLUT-4 protein content is decreased in rat (3, 14) and human (2) muscle 2 days after ECs. In particular, the GLUT-4 protein content was lower 1 and 2 days after ECs, but not immediately or 4 days after ECs (3). The reduction in rat skeletal muscle GLUT-4 protein content was accompanied by a smaller contraction-induced increase in glucose transport (14). With the use of the hyperinsulinemic euglycemic clamp technique, the maximal insulin-stimulated glucose uptake in quadriceps muscle was also found to be reduced 2 days after EC (1), because of a possible effect of a transient reduction in the total GLUT-4 protein content (2). The molecular mechanism behind the decrease in GLUT-4 protein content in muscle after eccentric exercise is not known. The purpose of this study was to determine the effect of 2 days of prior ECs on GLUT-4 transcription rate, GLUT-4 mRNA level, total GLUT-4 protein content, and sarcolemmal GLUT-4 content.

EXPERIMENTAL PROCEDURES

Materials. Hypnorm was from Janssen Pharmaceutical (Oxford, UK). Dormicum was from Roche (Basel, Switzerland). All radiolabeled compounds were purchased from Du Pont-New England Nuclear (Boston, MA). Nonradiolabeled nucleotides ATP, CTP, and GTP were obtained from Pharmacia (Piscataway, NJ). All restriction enzymes, RQI deoxyribonuclease, and RNasin were purchased from Promega (Madison, WI). The enhanced chemiluminescence (ECL) kit, Hyperfilm, and Hybond-N membrane were from Amersham. Percoll and sodium dodecyl sulfate (SDS) ExcelGel were from Pharmacia (Uppsala, Sweden). Phenol and nycodenz were from Gibco Life Technologies (Gaithersburg, MD). Immobilon membranes were from Millipore (Bedford, MA). Horseradish peroxidase-labeled goat anti-mouse were from Dakopatt (Glostrup, Denmark). All other chemicals, unless otherwise specified, were of molecular biology grade and purchased from Sigma.

Stimulation protocol. The ECs were induced by electrical stimulation of one lower leg, as previously described in Refs. 3 and 14. The contralateral calf muscle served as an unstimulated control (UC). After the stimulation protocol, the rats...
were rested for 2 days and fed rat chow ad libitum. After the second day of rest, the EC and UC muscles were electrically stimulated to isometric muscle contractions (11), and the EC and UC muscles were subsequently used for production of plasma membrane vesicles, as previously described (20). Furthermore, resting EC and UC muscles were also excised and used for isolation of nuclei (see Isolation of nuclei and run-on transcription analysis) or the muscles wereclamped frozen and stored at -80°C until they were used for total crude membrane (TCM) preparation (20) or RNA isolation (see RNA isolation and GLUT-4 mRNA detection).

**SDS-polyacrylamide gel electrophoresis and Western blot.** The frozen plasma membrane vesicles and TCM samples were thawed for subsequent determination of protein concentration (25) and Western blotting (20). In brief, samples intended for comparison were loaded with equal amounts of protein in each lane and run together on the same gel. The GLUT-4 antibody was a mouse monoclonal antibody produced against the COOH-terminal amino acids of GLUT-4 (20). The Ca2+-Mg2+-ATPase antibody was a mouse monoclonal antibody produced against the skeletal and cardiac Ca2+-Mg2+-ATPase. The antibody against the dihydropyridine receptor was a mouse monoclonal immunoglobulin G antibody (Upstate Biotechnology, Lake Placid, NY). Antibody-antigen complexes were visualized within the linear response by the ECL detection kit (Amersham, Buckinghamshire, UK). The antibody signal was expressed in arbitrary units per micrograms protein relative to a rat heart TCM standard run on all gels.

**RNA isolation and GLUT-4 mRNA detection.** Total RNA was extracted from 50–100 mg of muscle with a mutant solution of phenol and guanidine isothiocyanate (5, 9). RNA samples were separated on a 1% agarose gel, ethidium bromide stained, and monitored under ultraviolet light to verify RNA integrity and absence of RNA degradation. The remaining RNA samples were stored at -80°C overnight. The GLUT-4 mRNA concentration was detected, as previously described by Dela et al. (9). In brief, 15 μg of RNA were microfiltered (Bio-Dot, Bio-Rad) to a Hybond-N+ membrane. A full-length GLUT-4 cDNA fragment was also applied on the membrane as a standard (13). This dot-blot technique permitted the detection of as many as 96 RNA samples simultaneously. Signals were visualized within the linear range by autoradiography, quantitated by densitometric scanning (Kem-En-Tec Software Systems, Copenhagen, Denmark), and hybridization was done for 3 days at 47°C after which the filters were washed and dried, and the membranes were placed on a PhosphorImager screen for 3 days. All bands were visualized and quantitated with PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA).

**Functional role of decreased GLUT-4 protein in muscle.** We have previously observed that the contraction-induced increase in glucose transport in perfused rat skeletal muscle was decreased when intermittent isometric contractions were performed 2 days after ECs (14). To study the molecular mechanism behind this decrease in contraction-induced glucose transport, the GLUT-4 protein content was also measured in plasma membrane vesicles isolated from contraction-stimulated UC and EC muscle. As shown in Fig. 2, the GLUT-4 content was significantly lower in plasma membrane vesicles produced from contraction-stimulated UC and EC muscle. The GLUT-4 cDNA was cross-linked to a Hybond-N+ membrane, and hybridization was done for 3 days at 47°C, after which filters were washed and dried, and the membranes were placed on a PhosphorImager screen for 3 days. All hands were visualized and quantitated with PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA). The GLUT-4 cDNA was cross-linked to a Hybond-N+ membrane, and hybridization was done for 3 days at 47°C, after which filters were washed and dried, and the membranes were placed on a PhosphorImager screen for 3 days. All hands were visualized and quantitated with PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA).
The present findings demonstrate that the GLUT-4 protein expression in skeletal muscle is profoundly affected by ECs. First, the total GLUT-4 protein content was decreased in the white gastrocnemius muscle as well as in the red gastrocnemius muscle 2 days after ECs (Fig. 1). The decrease in total GLUT-4 was reflected by a decrease in GLUT-4 in plasma membrane vesicles isolated from contraction-stimulated muscle (Fig. 2). Second, the GLUT-4 mRNA was also decreased in the white and red gastrocnemius muscle (Fig. 3). Finally, prior ECs also induced a decrease in the muscle GLUT-4 gene transcription rate measured by nuclear run-on analysis (Fig. 4).

We have previously observed that muscle GLUT-4 protein content is decreased in white and red gastrocnemius rat muscle (3, 14) and in human mixed muscle (2) 2 days after ECs. The reduction in rat skeletal muscle GLUT-4 protein content on day 2 was accompanied by a lower rate of contraction-induced glucose transport, as measured by the isolated hindlimb perfusion technique.
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Fig. 4. Nuclear run-on analysis and quantification of the relative levels of GLUT-4 transcription from mixed skeletal muscle 2 days after EC (solid bar) compared with contralateral UC (open bar) muscle. Nuclei were isolated from mixed calf muscle and subjected to nuclear run-on analysis as described in EXPERIMENTAL PROCEDURES. A: representative signals detected on autoradiogram showing hybridization of in vitro-transcribed RNA to the indicated cDNA. Bands were quantitated by PhosphorImager analysis and normalized to genomic DNA (G-DNA). B: summary of GLUT-4 transcript densitometric analysis (n = 6; presented as means ± SE). *Significantly (P < 0.05) different from control.

(14). Furthermore, with the use of the hyperinsulinemic euglycemic clamp technique, it was also demonstrated that maximal insulin-stimulated glucose uptake was decreased in human quadriceps muscle 2 days after ECs (1). Such decreases in glucose uptake might be explained by the reduction in total GLUT-4 protein content (2). Thus, when the present data and data from the previous studies with rat (3, 14) and human skeletal muscle (1, 2) are synthesized, a scenario emerges: independent of the eccentric stimulation technique (electrically induced ECs of rat muscle or voluntary eccentric exercise in humans), unaccustomed eccentric exercise apparently induces a decrease in GLUT-4 transcription in human muscle (2). Thus, the observations (6, 26, 27) that cytokines may induce a decrease in GLUT-4 transcription in 3T3-L1 adipocytes and L6 myotubes may also provide a rationale for the present findings.

ECs are known to cause muscle damage (15). It could therefore be speculated that the decrease in muscle GLUT-4 transcription rate was a result of unspecific muscle damage. However, it was found that the total RNA yield per milligram EC muscle was not different from UC muscle (see RESULTS). Furthermore, it was found that there was a positive correlation between the added 32P activity and genomic signal (r = 0.657, P < 0.05) in the nuclear run-on assay. Thus the total DNA transcription rate and RNA concentration appeared unaffected in EC muscle. Second, the protein recovery in the EC TCM preparation was not different from UC preparations (data not shown). These TCM samples were also immunoblotted against the α1-subunit of the dihydropyridine receptor (T tubule marker) and the Ca2+-Mg2+-ATPase (marker for the sarcoplasmic reticulum). The concentrations of these membrane markers were not affected by prior ECs (P > 0.05, n = 3). The K+−activated p-phenylphosphatase (sarcolemmal marker) and succinate dehydrogenase (marker for mitochondrial membrane) activities were assayed in TCM samples made from white gastrocnemius muscle. The activities of these two membrane markers did not reveal any significant change in activity in response to prior EC contractions (see Table 1; P > 0.05, n = 3).

Finally, we have previously observed that the glycogen synthase activity was not decreased 2 days after eccentric muscle contraction (3). Interestingly, we have also observed that the 40-kDa plasma membrane fatty acid binding protein was increased in the white gastrocnemius muscle (where the GLUT-4 protein was de-

60% suppression of the GLUT-4 mRNA cell content, whereas the GLUT-1 mRNA content increased markedly above basal (6). In accordance with this reciprocal regulation of the two major glucose transporter proteins, we show, in the present study, a decrease in muscle GLUT-4 gene transcription and have previously shown an increase in GLUT-1 protein content after ECs (14). Thus the observations (6, 26, 27) that cytokines may induce a decrease in GLUT-4 transcription in 3T3-L1 adipocytes and L6 myotubes may also provide a rationale for the present findings.

Table 1. Membrane marker protein analysis

<table>
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<tr>
<th>Protein</th>
<th>Control Muscle</th>
<th>Eccentric Muscle</th>
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<tbody>
<tr>
<td>p-Phenyl phosphatase, nmol·mg protein−1·h−1</td>
<td>52.9 ± 7.0</td>
<td>56.7 ± 12.4</td>
</tr>
<tr>
<td>Succinate dehydrogenase, nmol·mg protein−1·h−1</td>
<td>13.1 ± 5.6</td>
<td>11.0 ± 5.8</td>
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<tr>
<td>Dihydropyridine receptor, arbitrary units</td>
<td>0.55 ± 0.19</td>
<td>0.55 ± 0.14</td>
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<tr>
<td>Ca2+−Mg2+−ATPase, arbitrary units</td>
<td>1.07 ± 0.17</td>
<td>1.10 ± 0.31</td>
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Values are means ± SE, n = 3. Total crude membrane was isolated from white gastrocnemius muscle subjected to eccentric contractions and unstimulated control white gastrocnemius muscle and assayed for K+−activated p-phenyl phosphatase (plasma membrane marker) and succinate dehydrogenase (mitochondrial membrane marker) activity, or the concentrations of the α-subunit of the dihydropyridine receptor (marker for the T tubules) and Ca2+−Mg2+−ATPase (marker for the sarcoplasmic reticulum) were determined by Western blot analysis.
increased the most) 2 days after ECs (S. Kristiansen, L. P. Turcotte and E. A. Richter, unpublished observations). Together, these independent observations suggest that total DNA, RNA, and marker protein concentrations and/or activities are not affected by prior ECs, suggesting that the decrease in GLUT-4 transcription rate is not due to unspecific muscle damage.

In conclusion, 2 days after ECs, the muscle GLUT-4 gene transcription rate, the GLUT-4 mRNA, and total GLUT-4 protein content were decreased. Furthermore, the sarcolemmal GLUT-4 protein content was lower in plasma membrane vesicles produced from contraction-stimulated eccentric muscle compared with contraction-stimulated control muscle. We suggest that unaccustomed eccentric muscle contractions lead to a transient decrease in GLUT-4 gene transcription rate, resulting in a transient decrease in muscle GLUT-4 protein content. The functional consequence of this is decreased contraction-induced muscle glucose transport due to decreased sarcolemmal GLUT-4 protein content.

We thank Dr. Per Norup Jørgensen, Nordio-Nordik, Bageværd, Denmark, for the gift of the GLUT-4 antibody. The technical assistance of Karin Clante, Department of Medical Physiology, Panum Institute, Copenhagen, Denmark, is gratefully acknowledged.

The study was supported by the Danish National Research Foundation (Grant 504–14) and the Danish Natural Science Research Council (Grant 11–0088). S. Kristiansen was supported by the Danish Research Council (Grant 9600422).

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Received 22 August 1996; accepted in final form 13 December 1996.

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