Induction of GLUT-1 protein in adult human skeletal muscle fibers

M. GASTER,1,2 J. FRANCH,1 P. STAEHR,2 H. BECK-NIELSEN,2 T. SMITH,3 AND H. D. SCHRODER1
Departments of 1Pathology, 2Endocrinology, and 3Neurology, Odense University Hospital, DK-5000 Odense; and 4Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, DK-5230 Odense, Denmark

Received 19 April 2000; accepted in final form 8 June 2000

Gaster, M., J. Franch, P. Staehr, H. Beck-Nielsen, T. Smith, and H. D. Schroder. Induction of GLUT-1 protein in adult human skeletal muscle fibers. Am J Physiol Endocrinol Metab 279: E1191–E1195, 2000.—Prompted by our recent observations that GLUT-1 is expressed in fetal muscles, but not in adult muscle fibers, we decided to investigate whether GLUT-1 expression could be reactivated. We studied different stimuli concerning their ability to induce GLUT-1 expression in mature human skeletal muscle fibers. Metabolic stress (obesity, non-insulin-dependent diabetes mellitus), contractile activity (training), and conditions of de- and reinnervation (amyotrophic lateral sclerosis) could not induce GLUT-1 expression in human muscle fibers. However, regenerating muscle fibers in polymyositis expressed GLUT-1. In contrast to GLUT-1, GLUT-4 was expressed in all investigated muscle fibers. Although the significance of GLUT-1 in adult human muscle fibers appears limited, GLUT-1 may be of importance for the glucose supplies in immature and regenerating muscle.

Among the members of the glucose transporter family, a group of transmembrane proteins that mediate facilitated transport of glucose are GLUT-1 and GLUT-4 (5). GLUT-1 has been shown to be expressed in various tissues in humans and rodents, i.e., brain, placenta, kidneys, and fatty tissue. In human skeletal muscle, GLUT-1 is expressed during gestation, but its expression is markedly reduced around birth, and GLUT-1 becomes undetectable within the 1st yr of life (9). In adult human skeletal muscle fibers, only the GLUT-4 isoform is found. However, increased GLUT-1 expression in human skeletal muscles has been discussed in connection with metabolic and pathophysiological stress. Thus Miele et al. (24) described increased basal GLUT-1 content in the plasma membrane of skeletal muscle of non-insulin-dependent diabetes mellitus (NIDDM) patients and nondiabetic obese subjects. Prolonged endurance training in humans has also been found to induce an increment in GLUT-1 protein (28). However, the source of biochemically verified GLUT-1 under these pathophysiological conditions has not been resolved, inasmuch as these studies did not discriminate between muscle-derived GLUT-1 and GLUT-1 from other sources, i.e., erythrocytes or perineurial sheaths. On the basis of our own findings on the expression of GLUT-1 in fetal and adult muscle fibers, we would expect that GLUT-1 could be expressed in adult muscle fibers under myogenic (re)construction.

The present study therefore was initiated to investigate the GLUT-1 pattern in human skeletal muscle fibers under different conditions of metabolic stress (obesity and NIDDM-obesity), hypertrophy (training), de- and reinnervation [amyotrophic lateral sclerosis (ALS)], and damage and regeneration (inflammatory myopathies). Immunocytochemistry was used to bypass problems inherent in techniques using tissue homogenates (9).

METHODS

Study Subjects

Obese and NIDDM patients. None of the diabetic patients were treated with insulin (Table 1). Three patients were treated with diet alone, and five were treated with oral administration of metformin or sulfonylurea. Medication was withdrawn 4 days before the study. The patients did not exhibit signs of diabetic retinopathy (apart from simplex retinopathy), nephropathy, neuropathy, or macrovascular complications.

ALS patients. ALS patients consisted of four women and four men, 49–75 yr of age. The clinical diagnosis of ALS required the presence of upper and lower motor neuron signs and evidence of progression. Patients underwent laboratory testing to exclude other causes of neuropathies, i.e., peripheral neuropathies or monoclonal gammopathies. The clinical diagnosis was supported by the finding of widespread lower
motor neuron degeneration on electrodiagnostic testing using Lambert’s criteria (22): normal sensory potentials, 2) normal motor conduction studies allowing for low compound muscle action potential amplitudes with mild prolongations of distal latencies and mild reduction of motor conduction velocities, and 3) needle electromyography showing spontaneous activity in the form of fibrillation potentials or positive sharp waves, fasciculation potentials, and signs of chronic partial denervation in at least three limbs or two limbs and the tongue. All muscle biopsies demonstrated neurogenic changes seen as fiber type grouping and group atrophy.

**Inflammatory myopathies.** Three female patients, 43, 52, and 63 yr of age, and three male patients, 32, 60, and 71 yr of age, were included. All were clinically characterized by proximal, painless weakness. Creatine kinase levels at the time of biopsy were 28, 254, 260, 1,029, 1,453, and 2,013 U/l (reference 150 U/l). All biopsies showed lymphocytic infiltrate, inclusion of muscle fibers by CD8-positive cytotoxic T lymphocytes (2).

The protocol was approved by the local ethical committees of Funen and Vejle Counties, and informed consent was received from all adult subjects before participation.

**Muscle Biopsy**

Muscle biopsies were obtained in the morning according to the method of Bergström (6). Muscle tissue was mounted in Tissue Tek OCT compound (Sakura, Torrance, CA) and frozen.

**Immunocytochemical Techniques**

GLUT-1 in sections of human muscle was visualized by the labeled streptavidin-biotin (LSAB) or the enhanced tyramide signal amplification (TSA) technique.

**LSAB.** Polyclonal anti-GLUT-4 (AB1346, Chemicon, Temecula, CA) was used as primary antibody. Immunolabeling of antigens was performed using the horseradish peroxidase (HRP)-LSAB technique (27). Briefly, unfixed muscle sections were washed in Tris-HCl-buffered saline (TBS), pH 7.4, and pretreated with 2% BSA in TBS. After 30 min of incubation with the primary antibody AB1346 (1:1,600) at room temperature, the sections were incubated with the biotinylated secondary antibody goat anti-rabbit Ig (E0432, Dako) diluted 1:300. Then the sections were incubated for 30 min with HRP-conjugated streptavidin (P0397, Dako) diluted 1:300. After a 5-min reaction with biotinylated tyramide (NEN Life Science), a secondary 30-min incubation with HRP-conjugated streptavidin (Dako P0397) was done. Subsequently, HRP activity was developed for 20 min using 3-amino-9-ethylcarbazol as chromogen. Immunostaining was followed by brief nuclear counterstaining in Mayers hematoxylin. Finally, coverslips were mounted with AquaTex. Thorough rinsing between incubation steps was done in TBS with 0.05% Tween 20 (Sigma Chemical). Antibodies and HRP-streptavidin were diluted in 0.5% blocking reagent (NEN Life Science) in TBS.

**Staining controls.** In control stainings the primary antibodies were omitted or replaced by primary mouse IgG isotype antibodies (Coulter, Miami, FL).

**TSA.** Primary antibodies were rabbit polyclonal anti-GLUT-1 antibody AB1341 (Chemicon) and mouse monoclonal anti-GLUT-1 antibody F18. (The monoclonal antibody F18 is a G protein-purified antibody raised against GLUT-1, purified from human erythrocyte membrane (29).) GLUT-1 immunoreactivity was detected by a modification of Adams’s TSA technique (1). TSA-Indirect (NEN Life Science, Boston, MA) in combination with LSAB was used as the detection system. Briefly, 5-µm cryosections of skeletal muscle biopsies were fixed in 4% buffered formaldehyde for 5 min. After rinse in tap water, blocking of endogenous biotin was achieved using the Dako Kit X0590 (Dako, Glostrup, Denmark). Before incubation with primary antibody, slides were incubated with 0.5% blocking reagent (NEN Life Science) in TBS for 25 min. Incubation with GLUT-1 antibodies was done overnight at 4°C. Anti-GLUT-1 AB1341 and anti-GLUT-1 F18 were used in a 1:10,000 dilution. Primary antibodies were followed by 30-min incubations with biotinylated secondary antibodies. Biotinylated goat anti-rabbit Ig (Dako E0432) and biotinylated goat anti-mouse Ig (Dako E0433) were diluted 1:200. After endogenous peroxidase activity was blocked for 10 min with ChemMate peroxidase blocking solution (Dako S2023), the sections were incubated for 30 min with HRP-conjugated streptavidin (Dako P0397) diluted 1:300. After a 5-min reaction with biotinylated tyramide (NEN Life Science), a secondary 30-min incubation with HRP-conjugated streptavidin (Dako P0397) was done. Subsequently, HRP activity was developed for 20 min using 3-amino-9-ethylcarbazol as chromogen. Immunostaining was followed by brief nuclear counterstaining in Mayers hematoxylin. Finally, coverslips were mounted with AquaTex. Thorough rinsing between incubation steps was done in TBS with 0.05% Tween 20 (Sigma Chemical). Antibodies and HRP-streptavidin were diluted in 0.5% blocking reagent (NEN Life Science) in TBS.

**RESULTS**

**GLUT-1 Immunoreactivity in Normal Adult Muscle**

Applying the TSA technique on cryosections of adult human skeletal muscles, we found GLUT-1 immunoreactivity in the perineurial sheaths, capillaries, and erythrocytes (Fig. 1a).

Table 2. Clinical data for sedentary control subjects and trained athletes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25.1 ± 1.3</td>
<td>28.3 ± 1.8</td>
</tr>
<tr>
<td>Endurance training, yr</td>
<td>6.5 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.7 ± 3.6</td>
<td>73.4 ± 1.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5 ± 1.0</td>
<td>22.7 ± 0.6</td>
</tr>
<tr>
<td>V\text{O}_\text{max}, l/min</td>
<td>3.62 ± 0.14</td>
<td>4.63 ± 0.13</td>
</tr>
<tr>
<td>V\text{O}_\text{max}, ml·min^{-1}·kg^{-1}</td>
<td>49.9 ± 1.5</td>
<td>64.3 ± 1.2</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no/group. V\text{O}_\text{max}, maximal oxygen uptake. *P < 0.05 vs. control by Mann-Whitney test.
GLUT-1 Immunoreactivity in the Study Group

The effect of physical training was observed as increased muscle fiber diameters. However, the physical stress did not induce GLUT-1 expression in muscle fibers. Muscle fibers from obese patients and patients with NIDDM were GLUT-1 negative (Fig. 1b). All patients with polymyositis had muscle fibers infiltrated with CD8-positive lymphocytes (not shown) and expressed regenerating muscle fibers. In five patients, GLUT-1 immunoreactivity was found in regenerating fibers (Fig. 1c). In the muscle biopsy of the sixth patient, muscle fibers were invaded by lymphocytes, but there were only a few regenerating fibers, none of which expressed GLUT-1. In muscle from patients with ALS, neither fibers with normal dimensions nor atrophic fibers expressed GLUT-1 (Fig. 1d). In all study groups, capillaries, erythrocytes, and perineurial sheaths were GLUT-1 immunoreactive, as shown in controls (Fig. 1a).

Muscle Fibers and GLUT-4

The GLUT-4 immunoreactivity in striated muscle was confined to the muscle fibers. The GLUT-4 immunoreactivity was similar in fibers obtained from controls and the study groups. A scattered, distinct granular reaction was seen in association with the cell surface of all muscle fibers, and fewer grains were found in deeper parts of the sarcoplasm (Fig. 1e).

DISCUSSION

GLUT-1 expression in adult human skeletal muscles has been discussed in connection with various pathophysiological conditions. In the present study we show that GLUT-1 expression can be induced in adult muscle fibers regenerating after damage. Other stress conditions did not result in expression of GLUT-1. In accordance with our previous study on healthy human muscles (9), we bypass the inherited problems by using tissue homogenates. The use of
immunohistochemistry allows us to discriminate between muscle fiber-derived GLUT-1 and GLUT-1 from other sources, i.e., erythrocytes and perineurial sheaths (9, 12, 21, 23).

Endurance training has been described to increase GLUT-1 expression in human muscle (28). We investigated skeletal muscle fibers from trained subjects and found no induction of GLUT-1 in the muscle fibers. Consistent with this finding, exercise has failed to induce changes in GLUT-1 content in rodents (31). Furthermore, there is convincing evidence that GLUT-4 expression is increased by training (18–20, 28) and that a reciprocal regulation of GLUT-1 and GLUT-4 exists in satellite cell cultures (25, 34) and in developing rat muscles (30, 33). On the basis of this inverse relationship, an exercise-induced increased GLUT-4 expression should be paralleled by a diminished GLUT-1 expression.

We studied GLUT-1 expression in muscle fibers from obese subjects and obese NIDDM patients. We did not find GLUT-1 immunoreactivity localized to the muscle fiber, but to capillaries and perineurial sheaths. The increased GLUT-1 expression in obesity and NIDDM described by Miele et al. (24) seems, on the basis of our findings, to originate from capillaries and perineurial sheaths. Vogt et al. (37) and Pedersen et al. (26), studying human muscle, found no differences in GLUT-1 expression between NIDDM patients and control subjects. Furthermore, the GLUT-1 gene locus shows no linkage with NIDDM (4).

The expression pattern of GLUT-1 in denervated human muscle is unknown. We did not find GLUT-1 expression in denervated muscle fibers from ALS patients. However, increased GLUT-1 expression is seen in denervated rat muscles (13).

The processes of myogenesis and regeneration and satellite cells in culture share many similarities. In all three conditions, mitotically competent myoblast/satellite cells proliferate, fuse, and differentiate into muscle fibers, replacing or building up muscle fibers (3, 7, 15). GLUT-1 protein expression has been shown in fetal muscle (9) and human satellite cell cultures (8, 34). We expected GLUT-1 expression in adult muscle fibers during part of the myogenic reconstruction. In this report we show that regenerating muscle fibers in patients with polymyositis express GLUT-1 immunoreactivity.

The mechanisms responsible for GLUT-1 expression in particular regenerating muscle fibers are unclear. Damaged muscle fibers are restored by satellite cells, which in cultures are GLUT-1 immunoreactive (10, 16, 34). Regeneration of damaged tissue is followed by an inflammatory response, which includes release of various factors, i.e., growth factors. GLUT-1 expression in cell cultures can be increased by serum (17), hormones (35, 36), and growth factors (17, 32) and possibly by other yet unknown factors accompanying the inflammatory process. It could be speculated that satellite cell proliferation and fusion in combination with local external factors are necessary, inasmuch as muscle hypertrophy is not followed by GLUT-1 expression.

The basal glucose transport is increased in transgene mice overexpressing GLUT-1 (11, 14) compared with their nontransgene littermates. Increased GLUT-1 expression under conditions of growth, proliferation, and regeneration seems to ensure an insulin-independent glucose uptake that can be utilized as energy fuel or as biosynthetic precursors needed under proliferation and regeneration.

GLUT-4 is the only immunocytochemically detectable glucose transporter isoform in adult skeletal muscle fibers (9). We found GLUT-4 expression in all investigated muscle fibers. This indicates that GLUT-4 is the main glucose transporter in muscle fibers from obese patients, NIDDM patients, controls, and trained subjects. These findings also support our hypothesis that GLUT-4 also mediates the basal glucose transport in muscle fibers (9).

In summary, we investigated different conditions for their ability to induce GLUT-1 expression in human skeletal muscle fibers. Only regenerating muscle fibers express GLUT-1. The stimuli responsible for GLUT-1 expression in myofibers in this particular situation are unclear. In contrast, GLUT-4 was expressed in all investigated muscle fibers. The physiological significance of GLUT-1 in adult human muscle fibers seems limited, but it may be of importance in immature and regenerating skeletal muscle.

Irene Lynfort, Kirsten Dahl, and Ole Nielsen provided excellent technical assistance.

The Clinical Research Institute, Odense University Hospital, and the County of Funen provided financial support.

REFERENCES


