Fiber Type–Specific Expression of GLUT4 in Human Skeletal Muscle

Influence of Exercise Training

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The fiber type–specific expression of skeletal muscle GLUT4 and the effect of 2 weeks of low-intensity training were investigated in 8 young untrained male subjects. Single muscle fibers were dissected from a vastus lateralis biopsy sample. Based on myosin heavy chain (MHC) expression, fibers were pooled into 3 groups (MHC I, MHC IIA, and MHC IIX), and the GLUT4 content of 15–40 pooled fibers was determined using SDS-PAGE and immunological detection. The GLUT4 content in pooled muscle fibers expressing MHC I was ~20% higher ($P < 0.05$) than that in muscle fibers expressing MHC IIA or MHC IIX. No difference in GLUT4 could be detected between fibers expressing MHC IIA or MHC IIX. Two weeks of exercise training increased ($P < 0.05$) the peak power output of the knee extensors by 13%, the maximal activities of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase by 21 and 18%, respectively, and the GLUT4 protein content by 26% in a muscle homogenate. Furthermore, a 23% increase ($P < 0.05$) in GLUT4 was seen in fibers expressing the MHC I isoform after exercise training for 2 weeks. No change was seen in fibers expressing MHC IIA or MHC IIX. In conclusion, our data directly demonstrate that GLUT4 is expressed in a fiber type–specific manner in human skeletal muscle, although fiber type differences are relatively small. In addition, low-intensity exercise training recruiting primarily fibers expressing MHC I increased GLUT4 content in these fibers but not in fibers expressing MHC IIA or MHC IIX, indicating that GLUT4 protein content is related more to activity level of the fiber than to its fiber type, which is defined by expression of contractile protein. *Diabetes* 49:1092–1095, 2000

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CS, citrate synthase; ECL, enhanced chemiluminescence; HAD, 3-hydroxyacyl-CoA dehydrogenase; MHC, myosin heavy chain.

In both rats and humans, glucose transport has been shown to be the rate-limiting step in muscle glucose uptake under most conditions (1,2). Glucose transport in mammalian skeletal muscle is almost exclusively mediated by the insulin- and contraction-regulatable glucose transporter GLUT4 (3). In both rodents and humans, different muscles have been shown to exhibit large differences in their GLUT4 content (4–9), and in rats, this variation is often associated with differences in insulin-stimulated glucose uptake (4–6,8). Because different muscles are composed of a mixture of several different muscle fiber types in both rats and humans (10), it is possible that a relationship exists between fiber type composition and GLUT4 content. Such a relationship has been proposed in human muscle based on indirect evidence (11,12), but in reality, it is unknown at present if the GLUT4 content differs between fiber types in human skeletal muscle.

It is also possible that the differences in GLUT4 content and insulin-stimulated glucose uptake are more related to activity level. Changes in the skeletal muscle activity level have been shown to be important regulators of the GLUT4 content in rats (13–16). In humans, athletes have more GLUT4 than untrained age-matched control subjects (11,17,18), and in both normal healthy control subjects and individuals with decreased insulin-stimulated glucose uptake, physical exercise training has consistently been shown to increase GLUT4 content (19–22). Furthermore, a decrease in activity level will decrease GLUT4 content (23,24). Finally, changes in physical activity and GLUT4 content have been shown to be associated with changes in insulin-stimulated glucose uptake (22,24).

To study the association between muscle fiber composition and GLUT4, we investigated the amount of GLUT4 in pools of different fiber types in human skeletal muscle. Furthermore, we studied the effect of 2 weeks of low-intensity training on the amount of GLUT4 in the different fiber types.

RESEARCH DESIGN AND METHODS

Subjects. Eight healthy men gave their informed consent to participate in the study, which was approved by the Municipal Ethics Committee for Copenhagen and Frederiksberg (KF 01-047/98). Age, weight, height, and BMI were 24 ± 1 years (mean ± SE), 92.0 ± 6.0 kg, 186 ± 2 cm, and 26.5 ± 1.5 kg/m^2, respectively. None of the subjects had a family history of diabetes or other endocrine disorders, and none were taking medication. All of the subjects used their bicycles for local transportation but did not participate in sports on a regular basis. The subjects' maximal pulmonary oxygen consumption determined during an incremental bicycle ergometer test was 44.8 ± 2.2 ml · kg^{-1} · min^{-1}.

Before the start of the 2-week training program (see training protocol below), the subjects were allowed to become accustomed to using the one-leg dynamic knee-extensor apparatus (25). Half of the subjects exercised with the dominant leg, and the other half of the subjects exercised with the nondominant leg. Thigh mass was estimated from the thigh volume, as described by Bangsbo et al. (26). An incremental knee extensor test was performed to determine the peak work capacity of the knee extensor. Pulmonary oxygen uptake was measured, and peak workload was defined as the workload when the initial linear relationship between workload and pulmonary oxygen uptake changed to an exponential relationship, indicating the recruitment of accessory muscles to stabilize the body at high workloads (25). The peak workload was also determined after completion of the 2-week training program.

Before and 38–48 h after the last training session, needle biopsies were obtained under local anesthesia from the vastus lateralis using a Bergström needle (27). One incision was made in the thigh, and 2 biopsies were obtained through the incision, with the needle pointed distally during the first biopsy and proximally during the second biopsy. The majority of the obtained skeletal muscle tissue was immediately frozen in liquid nitrogen, and a small part of the tissue was embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, the Netherlands) and frozen in isopentane cooled in liquid nitrogen. Muscle samples were stored at –80°C until analyzed.

Training protocol. Training was performed on the one-leg dynamic knee-extensor apparatus. Subjects exercised every day but 1 day for 2 weeks at 65% of peak workload. The first 3 sessions lasted for 1 h, the next 4 sessions 1.5 h, and the last 6 sessions 2 h.

Analytical procedures. Before analysis, the frozen biopsies were freeze-dried and subsequently dissected free of visual blood and connective tissue. Single fibers were obtained and cut into 2 pieces. Each piece was numbered, and 1 was used for determination of myosin heavy chain (MHC) composition; the other was used for determination of GLUT4 content in pools of the same type of single fibers.

Single-fiber MHC analysis was performed on the muscle samples using SDS-PAGE. The protocol for analyzing the samples was based on the procedure described by Betto et al. (28). Briefly, each fiber was placed in 20–30 µl lysing buffer consisting of 10% glycerol (wt/vol), 5% 2-mercaptoethanol (wt/vol), and 2.3% SDS (wt/vol) in 62.5 mmol/l Tris buffer (pH 6.8). Then, 5–10 µl was loaded on SDS-PAGE gels containing 6% polyacrylamide and 37.5% glycerol. Running conditions were 20 h at 70 V followed by 4 h at 200 V. Proteins were silver-stained using a kit from Amersham Pharmacia Biotech AB (Uppsala, Sweden), and the MHC isoform was determined using a standard known to contain all 3 human MHC isoforms. Altogether the MHC isoforms of 2,292 fibers were determined. Of these fibers, 48.3 ± 1.8% were MHC I fibers, 3.1 ± 0.6% were fibers coexpressing MHC I and MHC IIA, 32.3 ± 1.6% were MHC IIA fibers, 7.7 ± 1.4% were fibers coexpressing MHC IIA and MHC IIX, and finally, 8.7 ± 2.3% were MHC IIX fibers. Studies have shown that human IIB fibers contain MHC transcripts homologous to rat MHC IIX (29). Thus, human IIB fibers may correspond to IIX fibers; therefore, we have chosen MHC IIX to designate the fastest human isoform in the present article. MHC composition was also determined in muscle in homogenates by gel electrophoresis, as already described above.

After MHC determination, single skeletal muscle fibers were pooled in 3 groups—MHC I, MHC IIA, and MHC IIX—for determination of GLUT4 content. GLUT4 content was not determined in fibers expressing more than 1 MHC isoform. After 15–40 fibers were dissolved in 50 µl 2% SDS in 10 mmol/l Tris-HCl and 0.1 mmol/l EDTA, protein concentrations were determined in triplicate using bicinchonic acid reagent (Pierce, Rockford, IL) and bovine serum albumin as standard. Samples were diluted to the same protein concentration (0.25 µg/µl) with Laemmli sample buffer and then subjected to SDS-PAGE and immunoblotting as previously described (30). The primary antibody was a goat polyclonal antibody produced against a synthetic peptide corresponding to the amino acids 490–509 in the COOH-terminal end of GLUT4 (Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibody was a horseradish peroxidase–labeled rabbit anti-goat antibody (Dako A/S, Glostrup, Denmark). Antibody-antigen complexes were visualized by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Uppsala, Sweden). Exposures were obtained on Hyperfilm-ECL (Amersham Pharmacia Biotech) films and transferred to a computer using a video camera. A representative blot is shown in Fig. 1. Densitometric scanning was then performed using CREAM (Kem-En-Tec Software Systems, Copenhagen, Denmark).

Total crude membrane GLUT4 in muscle was determined as previously described (30). Briefly, 20–30 mg vastus lateralis muscle was homogenized, and total crude membranes were recovered by ultra centrifugation. The pellet was solubilized and stored in aliquots at –80°C for subsequent determination of protein concentration and Western blotting. Protein concentrations were determined, and electrophoresis and immunoblots were performed as already described. The maximal activities of citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) were determined using NAD-NADH enzymatic fluorometric assays according to Lowry and Passonneau (31).

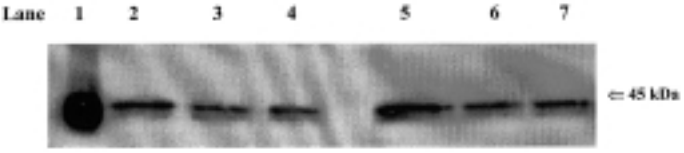

FIG. 1. Representative Western blot of GLUT4 protein in pooled single fibers in vastus lateralis muscle of 1 subject before and after one-legged exercise training for 2 weeks. Lane 1 shows GLUT4 content in a rat heart standard. Lanes 2, 3, and 4 show GLUT4 content before training in pools of MHC I, MHC IIA, and MHC IIX fibers, respectively. Lanes 5, 6, and 7 show GLUT4 content after 2 weeks of training in pools of MHC I, MHC IIA, and MHC IIX fibers, respectively. Note: An equal amount (micrograms protein) of pooled single fibers was loaded on the gel, but twice as much of the rat heart standard was loaded.

To determine pulmonary oxygen uptake, expired air was collected in locally constructed Douglas bags, and volume was measured in a Tissot type spirometer (Collins, Braintree, MA). Oxygen and carbon dioxide contents were determined with a Servomex paramagnetic oxygen analyzer and a Beckman infrared carbon dioxide analyzer (Beckman Instruments, Fullerton, CA), respectively.

Statistical analysis. Sigma Stat version 2.01 (Jandel Corporation, San Rafael, CA) was used for statistical analysis. Data in the text, tables, and figures are given as means ± SE. Nonparametric tests, Wilcoxon's signed-rank test (paired data), and Mann-Whitney rank-sum test (unpaired data) were used where appropriate. P values <0.05 were considered statistically significant.

RESULTS

Both before and after training, the GLUT4 content in muscle fibers expressing the MHC I isoform was slightly higher ($P < 0.05$) than in muscle fibers expressing the MHC IIA isoform (Fig. 2). No difference could be detected in the amount of GLUT4 when pooled MHC IIA fibers were compared with MHC IIX fibers either before or after training (Fig. 2).

Two weeks of exercise training significantly ($P < 0.05$) increased total GLUT4 protein in a muscle homogenate from 81.4 ± 8.1% of rat heart standard before training to 102.2 ± 7.3% of rat heart standard after training ($n = 8$). Furthermore, an

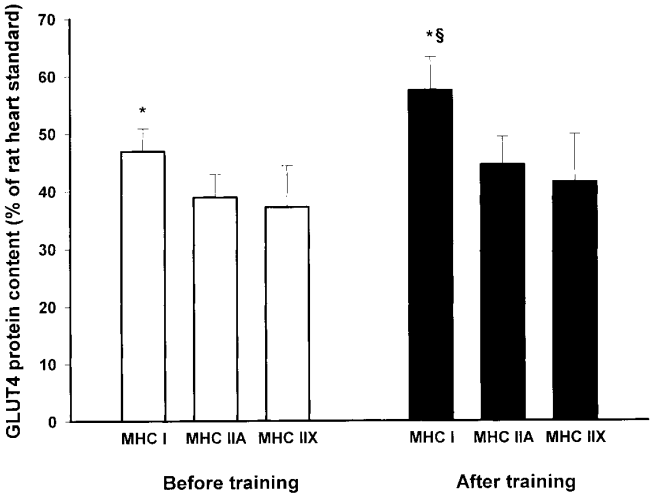

FIG. 2. GLUT4 protein content in individual fiber types before and after exercise training for 2 weeks. Values are means ± SE for 8 subjects, except for GLUT4 in MHC IIX fibers, which are values for 4 subjects. *$P < 0.05$, significantly different from GLUT4 in MHC IIA fibers; §$P < 0.05$, significantly different from before training.

TABLE 1
Effects of 2 weeks of training on thigh muscle volume, peak power output, and maximal enzymatic activity of CS and HAD

	Before training	After training
Volume of vastus lateralis (l)	3.6 ± 0.2	3.4 ± 0.2
Peak power output (W)	56.9 ± 3.4	64.4 ± 5.1*
CS (μmol · g^{-1} · min^{-1})	32.0 ± 2.0	38.6 ± 3.5*
HAD (μmol · g^{-1} · min^{-1})	30.0 ± 1.5	35.3 ± 2.4*

Data are means ± SE for 7 subjects, except for thigh muscle volume, which are values for 6 subjects, and peak power output, which are values for 8 subjects. *$P < 0.05$, significantly different from before training.

TABLE 2
Effects of 2 weeks of training on MHC composition in a muscle homogenate

	Before training	After training
MHC I (%)	52.0 ± 2.4	53.4 ± 3.5
MHC IIA (%)	40.0 ± 2.2	36.1 ± 2.0
MHC IIX (%)	8.0 ± 2.0	11.1 ± 1.7

Data are means ± SE for 8 subjects.

increased ($P < 0.05$) amount of GLUT4 was seen in the pooled fibers expressing the MHC I isoform after exercise training for 2 weeks (Fig. 2). Interestingly, in the pooled fibers expressing the MHC IIA isoform and in the pooled fibers expressing MHC IIX, no significant increase in the GLUT4 content was seen after the training (Fig. 2).

Two weeks of exercise training increased ($P < 0.05$) the maximal activities of CS and HAD by 21 and 18%, respectively (Table 1), and the peak power output of the knee extensors was increased to 13% ($P < 0.05$) (Table 1). Exercise training for 2 weeks had no effect on either the thigh volume (Table 1) or the amount of MHC I, MHC IIA, and MHC IIX in a muscle homogenate (Table 2).

DISCUSSION

We studied the fiber type–specific expression of GLUT4 in human skeletal muscle and found that the amount of GLUT4 in fibers expressing MHC I is only slightly higher than that in fibers expressing MHC IIA and MHC IIX (Fig. 2). Furthermore, there was no difference in the amount of GLUT4 in fibers expressing MHC IIA compared with fibers expressing MHC IIX (Fig. 2).

In rat skeletal muscle, the amount of GLUT4 is related to the muscle fiber composition, with more GLUT4 in muscles composed primarily of oxidative fibers compared with muscles composed primarily of glycolytic fibers (4–6,8). Because large proportions of the skeletal muscles in the rat have a relatively homogenous fiber type composition and because analysis can be done on the entire muscle, this approach is probably valid in this animal. However, great caution should be taken in interpretation because fibers of one type in one muscle can be very different from fibers of the same type in another muscle (32–34). This is well illustrated in the study by Chi et al. (32), who found 2–3 times higher activity of both glycolytic and oxidative enzymes in type I fibers from rabbit tibialis-anterior muscle compared with type I fibers from the soleus muscle.

Also in rabbit skeletal muscle, it has been possible to study the fiber type–specific expression of GLUT4 in single fibers. The results reported by Kong et al. (9) are in accordance with our results in human subjects, since they observed a higher amount of GLUT4 in fibers classified as slow oxidative fibers compared with fibers classified as fast oxidative glycolytic fibers. However, in the same study, it is reported that fibers classified as fast oxidative glycolytic fibers have a higher amount of GLUT4 than fibers classified as fast glycolytic fibers (9). The discrepancy could relate to the fact that compared with the human, one additional myosin isoform is present in rabbit (and rat) skeletal muscle. This isoform, designated type IIB, is characterized as being the fastest and most glycolytic fiber known, and this fiber type dominates in fast glycolytic rabbit and rat muscle (10).

Several studies have investigated the possible relationship between muscle fiber type composition and GLUT4 in human skeletal muscle using an indirect approach, whereas histochemically determined fiber type compositions have been related to GLUT4 contents in muscle homogenates (7,11, 12,17,22). We used a more direct approach, and because the GLUT4 content at present cannot be determined in a single human skeletal muscle fiber because of very low amounts of GLUT4, we studied pooled single muscle fibers. It cannot readily be determined if the increased amount of GLUT4 in MHC I fibers compared with MHC II fibers reflects a true fiber type–specific difference or is related to the recruitment pattern of muscle fibers in everyday life because recruitment pattern is related to both MHC composition and GLUT4 (see below). In any case, the difference in GLUT4 among human fiber types seems to be minute, and it is therefore impressive that a relationship between muscle fiber composition and GLUT4 actually has been demonstrated (11,12). However, most studies do not find a relationship between muscle fiber type composition and GLUT4 in human skeletal muscle (7,17,22).

After 2 weeks of one-legged low-intensity training, we observed a 26% increase in total skeletal muscle crude membrane GLUT4 content. Previous studies using untrained young subjects have reported anywhere from a 26% (19) to a 166% increase (20) in GLUT4 content after one-legged training for 70 days and endurance exercise training for 7 days, respectively. Thus, compared with most other longitudinal studies, our 26% increase is low. This can probably be explained by the relatively low intensity of the exercise training (65% of one-legged peak power output) and by the relatively short duration of the training period (2 weeks). The increase in GLUT4 content is accompanied by similar increases in the maximal activities of CS and HAD (Table 1). Our 20% increase in CS corresponds well with the 16 and 20% increase in CS observed after one-legged exercise training for 3 weeks (35,36). To our knowledge, the effect of one-legged exercise training for 2 weeks on maximal activity in HAD has never previously been reported, but Kiens et al. (37) have showed that one-legged training for 8 weeks increases maximal HAD activity by 38%. Because CS is a mitochondrial marker and HAD is a marker of fat oxidative pathways, our data reflect the training effect that was put primarily on the oxidative energy delivering system.

In this article, we show that 2 weeks of low-intensity exercise primarily increases the amount of GLUT4 in fibers expressing MHC I (Fig. 2). It has been shown previously that

in low-intensity knee extensor exercise for up to 2 h, the so-called slow-twitch fibers (MHC I) are primarily recruited and the fast-twitch fibers (MHC II) are recruited much less (37). Interestingly, this recruitment pattern seems to be reflected in our GLUT4 data (Fig. 2). This result could mean that increasing the training period would not lead to increased amounts of GLUT4 in MHC II fibers. In contrast, increasing the exercise intensity, which would recruit MHC II fibers, could possibly lead to marked increases in the expression of GLUT4 in the MHC II fibers. Taking this hypothesis further, it would mean that to achieve the maximal increase in muscle GLUT4, and thereby insulin sensitivity (22,24), exercise training should involve recruitment of all muscle fiber types. Future studies will reveal if this hypothesis is true.

In the present study, we have shown for the first time in human skeletal muscle that the amount of GLUT4 is higher in muscle fibers expressing MHC I than in those expressing MHC IIA and MHC IIX but that the magnitude of the difference is surprisingly small. We have also shown that 2 weeks of low-intensity exercise, recruiting primarily MHC I fibers, increases the amount of GLUT4 in these fibers but not in fibers expressing either MHC IIA or MHC IIX, which indicates that those muscle fibers recruited during the training session have an increase in GLUT4. Taken together, the data imply that the GLUT4 protein content of an individual muscle fiber is related more to the activity level of the fiber than to its actual type, as defined by its expression of contractile protein.

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