Functional Electrical Stimulation Exercise Increases GLUT-1 and GLUT-4 in Paralyzed Skeletal Muscle

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The study purpose was to determine the effect of functional electrical stimulation (FES)-leg cycle ergometer training (30 minutes on 3 d/wk for 8 weeks) on the GLUT-1 and GLUT-4 content of paralyzed skeletal muscle. Biopsy samples of vastus lateralis muscle were obtained pre- and post-training from five individuals with motor-complete spinal cord injury ([SCI] four men and one woman aged 31 to 50 years, 3 to 25 years postinjury involving C5-T8). Western blot analysis indicated that GLUT-1 increased by 52% and GLUT-4 increased by 72% with training ($P < .05$). This coincided with an increase in the muscle oxidative capacity as indicated by a 56% increase in citrate synthase (CS) activity ($P < .05$) and an improvement in the insulin sensitivity index as determined from oral glucose tolerance tests ($P < .05$). It is concluded that FES endurance training is effective to increase glucose transporter protein levels in paralyzed skeletal muscle of individuals with SCI.

SKELETAL MUSCLE is the primary site of glucose disposal in the body and is therefore an important factor in the regulation of blood glucose levels.[1] Glucose transport from the blood across the muscle cell membrane is the rate-limiting step in glucose utilization and is dependent on facilitated diffusion involving glucose transporter proteins.[1] Two glucose transporter isoforms are present in skeletal muscle. GLUT-4 is involved in insulin- and exercise-stimulated glucose uptake,[2-4] and GLUT-1 is involved in glucose uptake in the basal, non–insulin-stimulated state.[1]

Endurance exercise training in humans has been shown to increase GLUT-1[5] and GLUT-4[5-7] in skeletal muscle. GLUT-1[8,9] and GLUT-4[8-11] levels in the skeletal muscle of animals have also been shown to increase in response to chronic electrical stimulation. The purpose of the present study was to determine whether GLUT-1 and GLUT-4 increase in response to functional electrical stimulation (FES) endurance exercise training in upper–motor neuron paralyzed skeletal muscle in individuals with spinal cord injury (SCI).

Chronic electrical stimulation or FES exercise training of paralyzed human muscle results in an increase in muscle oxidative capacity, as indicated by an increase in oxidative enzymes.[12-14] Studies of humans[5] and animals[15] have indicated that changes in glucose transport protein levels precede changes in oxidative enzymes. Over the long-term, metabolic pathways involved in glucose transport and oxidation appear to be regulated in parallel, as indicated by significant correlations between GLUT-4 and oxidative enzymes in both humans[5,6,16] and animals.[10,17,18] Since electrical stimulation is effective for increasing muscle oxidative capacity,[12-14] we hypothesized that 8 weeks of FES exercise training of paralyzed muscle would be a sufficient stimulus for an increase in glucose transporters in conjunction with an increase in muscle oxidative capacity.

SUBJECTS AND METHODS

Subjects

Five individuals (four males and one female aged 31 to 50 years) with motor-complete SCI (3 to 25 years postinjury involving levels C5-T8) volunteered for the study. Informed consent was obtained from each subject, and the project received the approval of the University of Alberta Ethics Committee for Research on Human Subjects and the Glenrose Rehabilitation Hospital Ethics Review Committee. All subjects underwent a medical examination by a physician prior to participation in the study. The exclusion criteria excluded subjects with pacemakers, uncontrolled arrhythmia, angina, congestive heart failure, current deep venous thrombosis or pulmonary emboli, severe autonomic dysreflexia response to electrical stimulation, less than 90° flexion range of motion of the hips and knees, flaccid paralysis nonresponsive to FES, or severe lower-extremity spasticity and those who were currently participating in regular exercise.

Exercise Training

Subjects trained using a computer-controlled FES-leg cycle ergometer (ERGYS II; Therapeutic Alliance, Fairborn, OH) for a cumulated duration of 30 minutes of cycling 3 days per week for 8 weeks. With the ERGYS II system, electrical stimulation was applied through surface electrodes to the gluteal, hamstring, and quadriceps muscle groups in a computer-controlled sequence to allow pedaling of the cycle ergometer. In preparation for the exercise sessions, carbon-filled silastic electrodes were placed over motor points of the quadriceps, hamstring, and gluteal muscle groups. One active electrode and one reference electrode was placed on each muscle group. Computer-adjusted electrical stimulation (30 Hz, 10 to 140 mA) was applied to the muscles so that the subjects were able to pedal at 50 rpm. Each training session began with a warm-up of 1 minute of technician-assisted pedaling. The intensity was initially set at the inherent resistance in the ergometer alone (~6 W), and an attempt was made to progressively increase the resistance over the 8-week period. Following the warm-up, electrical stimulation intensity was progressively increased until the subjects were pedaling unassisted at 50 rpm. If the subject could not maintain a pedal cadence of 45 rpm with maximal stimulation, the resistance was decreased for the subject to pedal at 50 rpm. If the pedaling rate decreased to less than 40 rpm, assistance was provided to finish the next time interval divisible by 10 minutes. For example, if the subject was unable to maintain a cadence of 40 rpm after 7 minutes of exercise, assistance was provided until 10 minutes. After each exercise interval, a 2-minute cool-down (technician-assisted pedaling at 45 rpm) and 5-minute monitored rest

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period was provided. The training sessions had a maximum of three exercise intervals to complete 30 minutes of exercise.

Muscle Biopsies

Skeletal muscle biopsies, adapted for suction, were taken from the vastus lateralis muscle at rest before and after the 8-week training program. Post-training biopsies were obtained within 48 hours of the last FES training session. The tissue was frozen in liquid nitrogen and stored at −80°C for later analyses.

GLUT-4 and GLUT-1 Assays

For measurement of GLUT-4 and GLUT-1 protein levels, muscle samples were transferred to microcentrifuge tubes and homogenized using a Teflon pestle in a 1:10 (wt/vol) buffer (pH 7.4) containing 10 mmol/L Tris hydrochloride, 1 mmol/L EGTA, 250 mmol/L sucrose, 1 mmol/L phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, and 1% (vol/vol) Tergitol (Nonidet P-40).

GLUT-4 and GLUT-1 were determined by Western blot analysis. Protein (75 μg) was mixed with Laemmli sample buffer to a total volume of 20 μL. If necessary, samples were diluted with 1% sodium dodecyl sulfate (SDS). Samples were separated by SDS–polyacrylamide electrophoresis on a 10% separating gel. Pre- and post-training muscle samples from each subject were placed on the same gel. Kaleidoscope molecular weight markers (Bio-Rad, Mississauga, Ontario, Canada), mixed with an equal volume of sample buffer (20 μL total) were applied to each gel to estimate the apparent molecular weight of GLUT-4 and GLUT-1. Proteins were transferred to two supported nitrocellulose membranes by wet electromembrane transfer at 20 mA overnight, and then blocked in 6% bovine serum albumin (BSA)/Tween-Tris–buffered saline (TTBS) for 1 hour. Membranes were incubated separately with either 2 μL polyclonal rabbit GLUT-4 antibody, specific for the carboxy-terminal region of GLUT-4 (Cedar Lane Laboratories, Hornby, Ontario, Canada), in 1.5 mL 3% BSA/TTBS or 10 μL polyclonal rabbit GLUT-1 antibody, specific for the carboxy-terminal region of GLUT-1 (Cedar Lane Laboratories), in 2 mL 3% BSA/TTBS at 4°C overnight. The membranes were washed separately (three times for 20 minutes) in 6% BSA/TTBS and incubated with 2 μL donkey anti–rabbit immunoglobulin G horseradish peroxidase–linked secondary antibody (Amersham, Oakville, Ontario, Canada) in 4 mL 3% BSA/TTBS at 4°C for 1 hour. The membranes were washed (four times for 45 minutes) in 6% BSA/TTBS. GLUT-4 and GLUT-1 proteins were visualized using an enhanced chemiluminescence detection system (Amersham) according to the manufacturer's instructions. Western blots were quantified by scanning with a Bio-Rad 6S670 densitometer. Optical densities of the Western blots were measured using image-analysis software (Molecular Analyst; Bio-Rad). GLUT-4 and GLUT-1 protein levels were expressed in relative optical density units. Blank and preabsorption controls were used to verify the specificity of band detection (results not shown).

Citrate Synthase Assay

The maximal activity of citrate synthase (CS) was determined by the method of Srere.[19] All enzyme assays were performed at the same time to minimize interassay variability.

Oral Glucose Tolerance Test

Plasma glucose and insulin responses to an oral glucose load were used as an index of relative insulin sensitivity. Subjects consumed at least 200 g carbohydrate daily for 3 days prior to the test. All testing occurred at 8 AM after a 12-hour fast. Venous blood samples were obtained via an indwelling catheter at rest and at 30, 60, 90, and 120 minutes after consumption of a 75-g glucose load. The samples were anticoagulated with EDTA. Plasma was separated by centrifugation at 0°C. Plasma glucose was determined with a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA), and plasma insulin was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Tests were performed before and after the training program, with post-training tests performed 48 hours after the final training session. An insulin sensitivity index was calculated from the incremental areas under the curves above fasting glucose and fasting insulin levels according to the procedures of Cederholm and Wibell.[20] This index is an indication of insulin release and relative peripheral sensitivity, with higher values indicating enhanced peripheral insulin action.[20-22]

Statistics

All results are expressed as the mean ± SEM. Changes in the work rate and duration during cycle ergometer exercise and changes in GLUT-1, GLUT-4, and CS levels and in the insulin sensitivity index were analyzed by within-subject t tests. Significance was preset at an α level of .05.

RESULTS

Continuous Exercise

Training resulted in an increase in the work rate during continuous exercise in three of five subjects. The mean work rate for all subjects was 6 W at the beginning of the program and 8.5 ± 1.5 W ($P > .05$) following 8 weeks of training. Prior to training, the duration for which subjects were able to continuously pedal the ergometer without assistance was 4.4 ± 0.9 minutes. Following 8 weeks of training, the duration of exercise increased to 24.6 ± 5.4 minutes ($P < .05$). The total work output during the exercise sessions increased in all five subjects from 1.6 ± 3.1 kJ to 13.4 ± 4.1 kJ ($P < .05$; kJ = watts × time(s)/1,000).

GLUT-4 and GLUT-1 Content

Western blots for GLUT-4 and GLUT-1 pre- and post-training are shown in Figs 1A and 2A, respectively. GLUT-4 content increased by 72% ($P < .01$; Fig 1B) and GLUT-1 content increased by 52% ($P < .01$; Fig 2B) following 8 weeks of training.

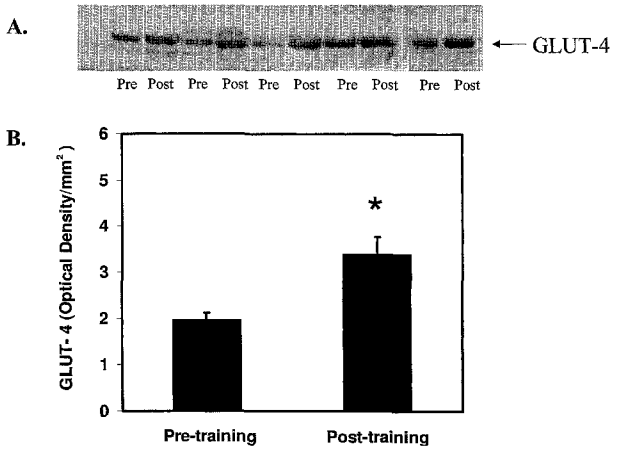

Fig 1. (A) Western blots of GLUT-4 pre- and post-training. (B) GLUT-4 levels in paralyzed skeletal muscle pre- and post-training with FES. *Significantly different post- v pre-training ($P < .01$).

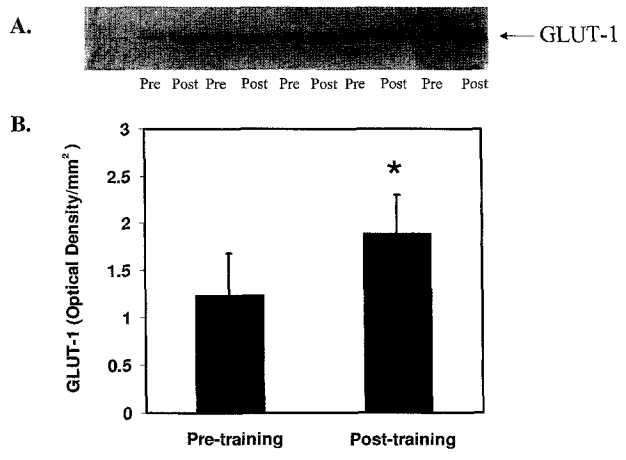

Fig 2. (A) Western blots of GLUT-1 pre- and post-training. (B) GLUT-1 levels in paralyzed skeletal muscle pre- and post-training with FES. *Significantly different post- *v* pre-training ($P < .01$).

CS Activity

CS activity increased by 56% with training (Fig 3). Changes in CS activity were not significantly correlated with changes in GLUT-4 or GLUT-1.

Response to Oral Glucose Tolerance Test

Mean glucose and insulin responses to the oral glucose tolerance test are shown in Fig 4A and B. The insulin sensitivity index was significantly elevated post-training, indicating enhanced peripheral insulin action (Fig 4C). There were no significant correlations between the changes in resting glucose levels and changes in GLUT-1 and between the changes in the insulin sensitivity index and changes in GLUT-4.

DISCUSSION

The main finding of this study is that FES exercise training is effective for increasing glucose transport proteins (GLUT-1 and GLUT-4) in conjunction with muscle oxidative capacity in

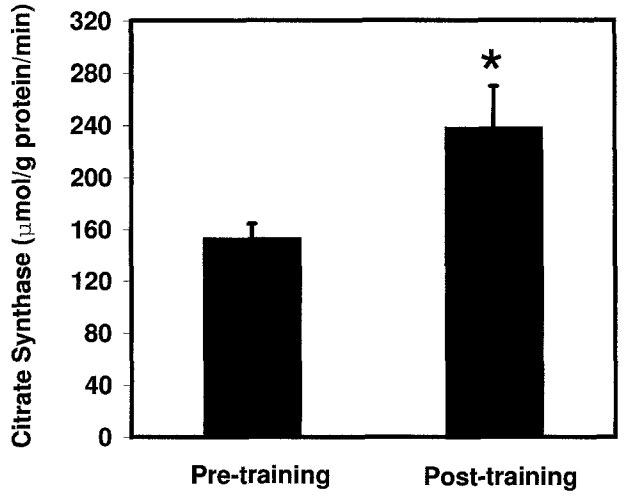

Fig 3. Citrate synthase levels in paralyzed skeletal muscle pre- and post-training with FES. *Significantly different post- *v* pre-training ($P < .05$).

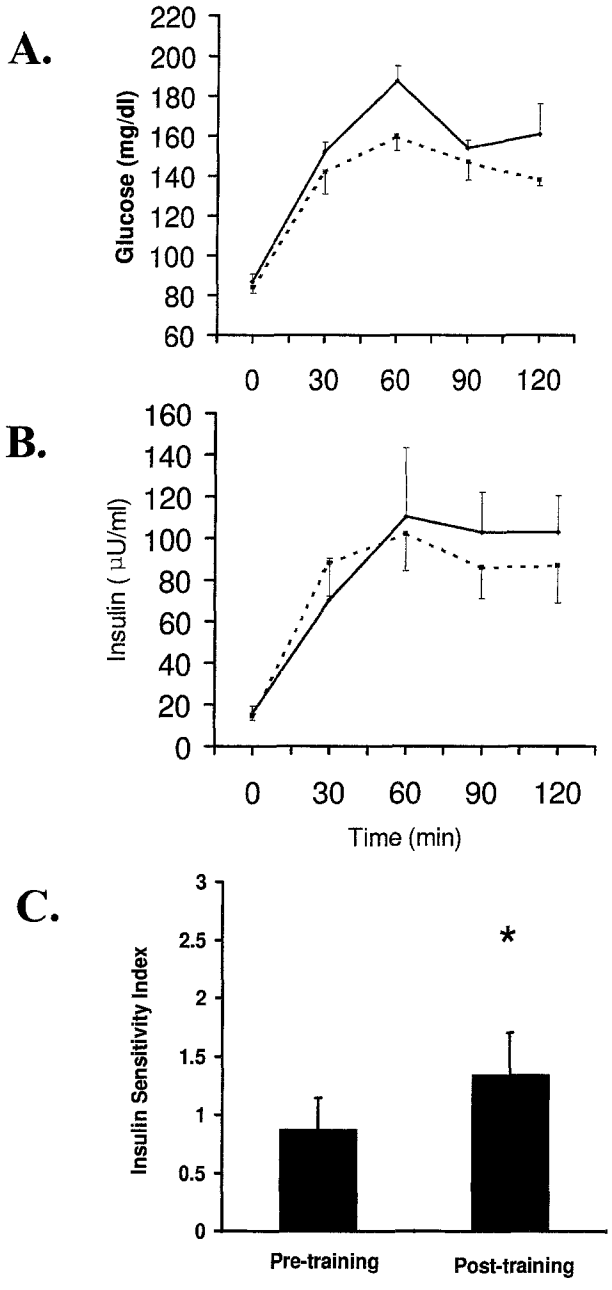

Fig 4. (A) Glucose and (B) insulin responses to oral glucose tolerance tests. (— —) Response pre-training; (---) response post-training. (C) Insulin sensitivity index pre- and post-training. *Significantly different post- *v* pre-training ($P < .05$).

upper–motor neuron paralyzed human skeletal muscle. This confirms our hypothesis which is based on the findings that FES training and other electrical stimulation protocols increase muscle oxidative capacity[12-14] and that in normal healthy skeletal muscle the glucose transport protein concentration is related to muscle oxidative capacity.[4-6,10,16-18,23] Similar to our results, Hjeltnes et al[24] recently found that GLUT-4 is increased with FES training of paralyzed human skeletal muscle. In contrast to our results, they did not find an increase in muscle oxidative capacity (as measured by CS activity). The subjects in

their study had a reduced training volume in the last 2 weeks of training (in an 8-week program) due to various complications; this may have attenuated any gain in CS activity during their program. The fact that GLUT-4 remained elevated in their subjects indicates that perhaps changes in glucose transport proteins do not always occur with changes in muscle oxidative capacity.[5,15] This could explain the lack of correlation between the changes in these variables in the present study.

The relative changes in glucose transporters with the training protocol of the present study are similar to changes shown with training of nonparalyzed human or animal muscle. A number of studies in humans have shown that endurance training of short (5 days) to moderate (~14 weeks) duration increases GLUT-4 levels up to twofold.[5-7] Similarly, endurance training of animals of short (1 day) to moderate (~5 weeks) duration increases GLUT-4 by approximately the same relative amount.[15,18,25] Only one study involving humans has measured GLUT-1 changes with training. Phillips et al[5] found that GLUT-1 levels increased 131% after 31 days of 2-h/d training, a much larger increase than the 52% found with 8 weeks of training using the protocol of the present study. In contrast, the results of animal studies have been mixed with respect to the effectiveness of training on GLUT-1 levels. One study showed a proportional increase in GLUT-1 and GLUT-4,[26] whereas two others have shown that increases in GLUT-4 are unaccompanied by increases in GLUT-1.[27,28] The differences between studies may be related to different animal training protocols; the former used swimming and the latter two used voluntary wheel-running.

The present study is the first to demonstrate increases in GLUT-1 with electrical stimulation of paralyzed human muscle and corroborates the recent study by Hjeltnes et al,[24] who found increases in GLUT-4 with FES training of tetraplegics. The relative increase of GLUT-4 in their study was greater than that in the present study (378% *v* 72%). The training duration of both studies was similar (8 weeks), but the frequency of training in the study by Hjeltnes et al was greater than ours (7 d/wk *v* 3 d/wk). This greater training frequency may account for their greater increase in GLUT-4. Several others have demonstrated a rapid increase in glucose transporters with chronic low-frequency electrical stimulation of animal muscle. GLUT-4 levels increase up to twofold following 1 or more days of chronic electrical stimulation.[9-11] Also, GLUT-1 mRNA and protein levels increase 24 hours after stimulation of animal muscle.[8,9] Our finding that the glucose transporter level in paralyzed muscle increases with electrical stimulation is similar to the finding of Castello et al,[8] who demonstrated that direct electrical stimulation of rabbit tibialis anterior muscle prevents a decrease in GLUT-4 mRNA levels following paralysis induced by peripheral denervation. It therefore appears that the expression of muscle glucose transporters is very sensitive to the electrical and/or contractile activity experienced by skeletal muscle.[4] Studies using electrical stimulation of animal muscle involve neural stimulation, whereas the current study used direct stimulation of muscle. This suggests that contraction per se stimulates GLUT-4 and GLUT-1 regulation rather than regulation via neural activity.

There are several possible benefits of a higher number of glucose transporter proteins in skeletal muscle. The most obvious benefit of increased GLUT-4 levels would be an enhanced rate of glucose clearance in response to insulin or exercise.[27,28] A benefit of increased GLUT-1 levels would be an enhanced rate of glucose clearance in the basal, non–insulin-stimulated state.[1] This may be of importance for individuals with SCI, because they generally have an increased incidence of glucose intolerance as compared with the general population.[29-31] Initial glucose transporter numbers are not lower in individuals with SCI compared with healthy controls; therefore, this does not seem to be a contributing factor for their glucose intolerance.[29] However, an increase in glucose transporter levels with training may contribute to an improvement in insulin action in individuals who are initially insulin-resistant.[7] The subjects of the present study had an increase in the insulin sensitivity index as calculated from the oral glucose tolerance test (Fig 4), indicating an enhanced peripheral insulin action. The findings that the changes in GLUT-4 did not correlate with changes in the insulin sensitivity index and that the changes in GLUT-1 did not correlate with changes in resting glucose levels indicate that perhaps multiple factors in addition to glucose transporter proteins contribute to improvements in insulin sensitivity and resting glucose levels. These may include enhancements in muscle mass, muscle capillary formation, and enzymes (ie, hexokinase) involved in glucose translocation, all of which are improved with FES exercise training.[13,24,32]

A second possible benefit of higher glucose transporter levels is an enhanced ability to store glycogen in skeletal muscle.[33,34] Higher glycogen levels increase the endurance exercise time before exhaustion.[35] This may account for the nearly sixfold improvement in the exercise endurance time of subjects in the present study.

In summary, FES endurance exercise training results in an increase in GLUT-1 and GLUT-4 levels and in the oxidative capacity of paralyzed skeletal muscle in individuals with SCI.

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