Skeletal muscle signaling associated with impaired glucose tolerance in spinal cord-injured men and the effects of contractile activity

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Yarar-Fisher C, Bickel CS, Windham ST, McLain AB, Bamman MM. Skeletal muscle signaling associated with impaired glucose tolerance in spinal cord-injured men and the effects of contractile activity. J Appl Physiol 115: 756–764, 2013. First published June 13, 2013; doi:10.1152/japplphysiol.00122.2013.—The mechanisms underlying poor glucose tolerance in persons with spinal cord injury (SCI), along with its improvement after several weeks of neuromuscular electrical stimulation-induced resistance exercise (NMES-RE) training, remain unclear, but presumably involve the affected skeletal musculature. We, therefore, investigated skeletal muscle signaling pathways associated with glucose transporter 4 (GLUT-4) translocation at rest and shortly after a single bout of NMES-RE in SCI (n = 12) vs. able-bodied (AB, n = 12) men. Subjects completed an oral glucose tolerance test during visit 1 and ∼90 NMES-RE isometric contractions of the quadriceps during visit 2. Muscle biopsies were collected before, and 10 and 60 min after, NMES-RE. We assessed transcript levels of GLUT-4 by quantitative PCR and protein levels of GLUT-4 and phosphorylated- and total AMP-activated protein kinase (AMPK)-α, CaMKII, Akt, and AS160 by immunoblotting. Impaired glucose tolerance in SCI was confirmed by higher (P < 0.05) plasma glucose concentrations than AB at all time points after glucose ingestion, despite equivalent insulin responses to the glucose load. GLUT-4 protein content was lower (P < 0.05) in SCI vs. AB at baseline. Main group effects revealed higher phosphorylation in SCI of AMPK-α, CaMKII, and Akt (P < 0.05), and Akt phosphorylation increased robustly (P < 0.05) following NMES-RE in SCI only. In SCI, low skeletal muscle GLUT-4 protein concentration may, in part, explain poor glucose tolerance, whereas heightened phosphorylation of relevant signaling proteins (AMPK-α, CaMKII) suggests a compensatory effort. Finally, it is encouraging to find (based on Akt) that SCI muscle remains both sensitive and responsive to mechanical loading (NMES-RE) even ∼22 yr after injury.

spinal cord injury; skeletal muscle; glucose uptake signaling; neuromuscular electrical stimulation; resistance exercise

WHILE TODAY INDIVIDUALS WITH spinal cord injury (SCI) tend to live much longer than in past decades, the protracted life expectancy is associated with increased risk of cardiometabolic disease. With longstanding loss of mobility and extreme skeletal muscle atrophy (19, 42), glucose intolerance and peripheral insulin resistance develop, and these metabolic abnormalities are thought to contribute to the increased cardiovascular disease risk in individuals with SCI (7). Cardiometabolic disorders typically manifest in the 5th or 6th decade in the general population, while the average age of SCI patients at time of injury is 41 yr (2). Therefore, individuals with SCI, while relatively young, are at high risk for developing insulin resistance and type II diabetes soon after the onset of injury (5).

Skeletal muscle contractile activity has been shown to improve glucose homeostasis via increased skeletal muscle glucose uptake in both healthy (16, 32) and insulin-resistant able-bodied (AB) individuals (28, 52). The enhanced muscle glucose uptake is mainly due to the translocation of more glucose transporter (GLUT)-4, from cytosolic vesicles to the cell surface in response to contractions. This increase in muscle glucose uptake does not require protein synthesis (23). Contraction-induced translocation of GLUT-4 is thought to be regulated, at least in part, by activation of AMP-activated protein kinase (AMPK) (45, 48) and Ca2+/calmodulin-dependent protein kinase (CaMK)-mediated signaling pathways (40, 51). In addition, AS160 (Akt substrate of 160 kDa, Rab GTPase-activating protein) has been shown to mediate both insulin and contraction-stimulated GLUT-4 translocation in skeletal muscle (12).

Given that individuals with SCI are not able to voluntarily activate skeletal muscle, neuromuscular electrical stimulation (NMES) has been developed to allow SCI individuals to exercise their paralyzed limbs and participate in aerobic activities. However, the affected skeletal muscles become highly fatigueable early after SCI (14) and, therefore, may not be suitable for performing aerobic exercise (10). Resistance exercise has been recommended as an alternative approach to aerobic exercise-based recommendations due to its ability to improve metabolic profiles in healthy (30) and insulin-resistant AB individuals (44), as well as in individuals with SCI (35). Resistance exercise is thought to improve the metabolic profile by increasing the amount of skeletal muscle mass, increasing expression of key metabolic genes, and improving skeletal muscle’s capacity to take up and utilize glucose by activating AMPK and CaMK signaling pathways (13, 18). On the other hand, whether severely deconditioned SCI muscle can activate these pathways in response to NMES-induced contractile activity is not known. The purpose of this research was thus to investigate the effects of NMES-induced resistance exercise on signaling processes that promote muscle glucose uptake in SCI vs. AB individuals. We tested the following hypotheses: 1) a single bout of NMES-induced resistance exercise will effectively activate signaling pathways that promote GLUT-4 translocation; and 2) signaling associated with GLUT-4 transloca-
tion will be more responsive to NMES-induced resistance exercise in SCI muscle than in AB muscle because of the greater relative stimulus (since AB muscles undergo regular contractions of varying intensities on a daily basis).

**MATERIALS AND METHODS**

**Subjects**

Twelve men with SCI (injury levels: C5- Ts; American Spinal Injury Association A and B; 22.4 ± 10 yr postinjury; 49.6 ± 10.0 yr of age) and 12 AB men (42.1 ± 11.3 yr of age) completed the study (subject characteristics are included in Table 1, no differences were found in subject characteristics among groups). One SCI individual was excluded from data analysis due to problems with muscle biopsy specimens; therefore, 11 SCI subjects were included for the final analysis. To negate effects of exercise training, AB and SCI subjects had no recent history of leg resistance training. Subjects were excluded for any medical or other health conditions that would be expected to affect testing (e.g., frank diabetes) or for which testing might be contraindicated (e.g., dystrophinopathy). The study was approved by the University of Alabama at Birmingham (UAB) Institutional Review Board. All participants gave written, informed consent after hearing a thorough explanation of study procedures and risks, and after having an opportunity to ask questions. Medications were recorded, and the only medication that was used in either group was anti-spasticity medication (Baclofen) in two of the SCI individuals. The study consisted of a total of two visits to the UAB Center for Clinical and Translational Science (CCTS) Clinical Research Unit. All investigations were performed before noon following an overnight fast. During the first visit, an oral glucose tolerance test (OGTT) was conducted to evaluate subjects’ ability to handle a 75-g oral glucose load. During the second visit, subjects performed NMES-induced resistance exercise, and muscle biopsies were collected pre-, 10 min post-, and 60 min post-NMES-resistance exercise.

** OGTT**

Each subject consumed a 75-g oral glucose load within 5 min. Blood samples were collected immediately before and 60, 90, and 120 min after glucose ingestion for measurement of plasma glucose and plasma insulin. Blood for plasma glucose determination was collected with sodium fluoride, and blood for plasma insulin was collected with heparin. Blood was immediately centrifuged, separated for plasma, and frozen at −80°C until analysis. Assays were performed in the CCTS Metabolism Core. Plasma glucose assays were performed on an automated glucose analyzer (Sirrus analyzer; Stanbio Laboratory, Boerne, TX), and plasma insulin was measured using an immunofluorescent method with an AIA-600 II analyzer (TOSOH Bioscience, South San Francisco, CA) per manufacturers’ instructions. Whole body insulin sensitivity (WBIS) was calculated from OGTT data using Matsuda Index. This index correlates strongly (r = 0.73, P = 0.0001) with the direct measure of insulin sensitivity derived from the euglycemic insulin clamp (38).

**NMES-induced Resistance Exercise**

Bipolar 8 × 10 self-adhesive NMES electrodes were placed over the distal-lateral and proximal-lateral portion of the quadriceps femoris muscle group, as previously described (8, 9). Subjects were seated in a custom-built chair with the hip and knee secured at ~70° of flexion. The leg was firmly secured to a rigid lever arm with an inelastic strap to ensure that the knee extendors could only perform isometric contractions. The moment arm was established by placing a load cell (model 2000A, Rice Lake Weighing Systems, Rice Lake, WI) parallel to the line of pull and perpendicular to the lever arm. Torque was recorded from the load cell via a PowerLab analog-to-digital converter (model ML870, ADInstruments, Milford, MA), sampling at 1,000 Hz, and interfaced with a portable computer. A commercial stimulator (TheraTouch model 4.7, Rich-Mar, Inola, OK) was utilized for NMES. The AB individuals performed a maximum voluntary contraction (MVC) for isometric knee extension before NMES. After that, the electrical current sufficient to elicit 30% of the observed MVC was determined and used for the subsequent NMES protocol. It has been previously reported that, when applied over the motor points of the vastus lateralis (VL; the biopsied muscle), a contraction that elicits 30% of maximum knee extension force is sufficient to induce 100% activation of the VL (1). For SCI subjects, the torque was determined by increasing current incrementally until torque no longer increased (i.e., electrically evoked maximum). To approximate the same relative intensity with AB subjects, in SCI the electrical current sufficient to elicit 30% of the electrically evoked maximum was determined and used for the subsequent NMES protocol, as our laboratory has done previously (9). The NMES protocol consisted of 5-s contractions separated by 15 s for ~30 min at previously determined current levels. For both groups, contractions were evoked with 50-Hz trains of 450-μs biphasic pulses.

**Muscle Biopsy**

Muscle samples were collected from the VL before and 10 and 60 min after NMES-induced resistance exercise via our laboratory’s established percutaneous needle biopsy procedure (3). Briefly, biopsies were performed under local anesthetic (1% lidocaine) using a

<table>
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<th>Subject No.</th>
<th>Sex</th>
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<th>No. Years Postinjury</th>
<th>Weight, kg</th>
<th>Height, m</th>
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SCI, spinal cord injury; AB, able-bodied; M, male; F, female; BMI, body mass index; ASIA, American Spinal Injury Association Impairment Scale.

Table 1. Characteristics of the study participants

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5-mm Bergstrom type biopsy needle under suction, as previously described. Approximately 50–70 mg of muscle for immunohistochemistry were mounted cross-sectionally and frozen in liquid nitrogen-cooled isopentane. Remaining tissue was snap frozen in ~30-mg portions for biochemical assays.

**Determination of Myofiber-type Distribution**

The relative distributions of myofiber types I, IIa, and IIax/IIX were determined immunohistochemically using our laboratory’s well-established protocol (29, 31). An exception in the present analysis was the pooling of hybrid IIax fibers with type IIx fibers. This was deemed necessary because of the remarkably high percentage of IIax myofibers noted in the SCI muscle samples.

**Immunoblotting**

Mixed muscle protein lysate was prepared utilizing established methods in our laboratory (39). Briefly, muscle samples (~30 mg) were homogenized after a 15-min preincubation in 6 μL/mg muscle of ice-cold lysis buffer with protease and phosphatase inhibitors and then centrifuged at 15,000 g for 40 min at 4°C. Supernatant was stored at ~80°C until assayed for protein content using the bicinchoninic acid technique with BSA as a standard. The content and phosphorylation of proteins associated with skeletal muscle GLUT-4 translocation were assessed. Additionally, the phosphorylation of p44–42 extracellular signal-regulated kinase (ERK)-1/2 was assessed for gaining an insight into the sensitivity of SCI muscle in response to mechanical stimulus. Twenty-five micrograms of skeletal muscle mixed protein lysate were resolved on 4–12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes as our laboratory has described previously (39, 46). Equal loading was verified on Ponceau S stained membranes before any antibody (Ab) treatments. All gels contained samples from both AB and SCI subjects loaded in series. Primary Abs were purchased from Cell Signaling Technologies (Danvers, MA) and used at 1:1,000 dilution in 5% goat serum (monoclonal Abs) or 2% milk + 2% BSA (polyclonal Abs) against the following: GLUT-4 transporter, total AMPK-α, phospho (Thr172)-AMPK-α, total CaMKII, phospho (Thr286)-CaMKII, total Akt, phospho (Ser473)-Akt, total AS160, phospho (Thr642)-AS160, and phospho-p44/42 MAPK (Erk-1/2) (Th202/Tyr204). Horseradish peroxidase-conjugated secondary Abs were used at 1:50,000 followed by chemiluminescent detection (SuperSignal West Dura Chemiluminescent Substrate, Thermo Scientific, Rockford, IL) in a BioRad ChemiDoc imaging system with band densitometry performed using BioRad Quantity One software (version 4.5.1). Parameters for image development in the ChemiDoc were consistent across all membranes using predefined saturation criteria for the charge-coupled device camera, as previously described (3).

**Real-time Quantitative Polymerase Chain Reaction**

RNA isolation. RNA was isolated and purified from the frozen muscle samples using Tri-Reagent (Molecular Research Center, Cincinnati, OH) and RNeasy Mini Kits (QIAGEN, Valencia, CA), following the manufacturer’s instructions. RNA quantity and quality were determined using a spectrophotometer to measure absorbance at 260 nm and the 260:280 ratio, respectively (Nanodrop ND-1000, ThermoScientific, Rockford, IL).

Real-time quantitative polymerase chain reaction. Muscle mRNA expression of GLUT-4 was measured in the SCI and AB tissue samples using real-time quantitative polymerase chain reaction (StepOne System, Applied Biosystems, Foster City, CA). cDNA was synthesized using the SuperScript VILO cDNA Synthesis kit (Invitrogen, Carlsbad, CA). SLC2A4 (GLUT-4, Hs00168966_m1) TaqMan Gene Expression Assay (Applied Biosystems) was used for analysis of mRNA expression. GAPDH (Hs00275891_g1) was used as an endogenous control. All amplifications were run in 10 μl of total volume using TaqMan Fast Advanced Master Mix (Applied Biosystems), following the manufacturer’s instructions using the StepOne Real-Time PCR System (Applied Biosystems). All samples were run in triplicate. Relative amounts of target mRNA were determined using the comparative threshold cycle method. Expression of GLUT-4 was normalized to the corresponding expression level of GAPDH, which did not differ between groups. The data were analyzed using StepOne software version 2.2.2 (Applied Biosystems), and the results are expressed relative to the AB group at baseline.

**Statistics**

For dependent variables with repeated measures, group × time repeated-measures ANOVA was used to test group × time interactions, and main effects of time and group. Least significant difference post hoc tests were performed as appropriate. An independent-samples t-test was conducted to compare baseline GLUT-4 mRNA between SCI and AB groups. Data are presented as means ± SE. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Torque Measurements**

Analysis of the torque output indicated that the AB participants were stimulated to produce 32 ± 10% of their previously measured MVC (206 ± 71 Nm). Over the course of the NMES-induced exercise, the torque of the AB participants decreased by 57 ± 24%, whereas the individuals with SCI demonstrated a 76 ± 15% decline.

**Plasma Glucose and Insulin Responses to OGTT**

Blood glucose and insulin levels during the OGTT are shown in Fig. 1. In the fasted state, all subjects were euglycemic and had insulin levels in the normal range. In response to the glucose challenge, however, significant group × time interaction (P < 0.05) and main effects of time (P < 0.0001) and group (P < 0.05) were found for plasma glucose levels. SCI had elevated plasma glucose concentrations at 60 (P < 0.0001), 90 (P < 0.05), and 120 min (P < 0.05), and, at each postingestion time point, SCI glucose levels were higher than AB. A main effect of time (P < 0.0001) was found for insulin concentrations, with the expected increases at all time points following glucose ingestion. Within groups, plasma insulin levels were elevated (P < 0.05) at all three time points after glucose ingestion in SCI, while a statistically significant elevation in AB was noted only at 60 min postingestion. No significant differences were found in WBIS levels between groups (Fig 2).

**Skeletal Muscle Intracellular Signaling**

The phosphorylation states of AMPK-α, CaMKII, and Akt are shown in Figs. 3–5. For all three signaling proteins, significant main group effects (P < 0.05) indicated higher states of activation in SCI vs. AB. Within time points, post hoc testing indicated higher AMPK phosphorylation (Thr172) in SCI vs. AB at the 10-min post-NMES time point (Fig. 3), while CaMKII phosphorylation (Thr286) was higher in SCI vs. AB at both time points (P < 0.05) after NMES resistance exercise (Fig. 4). A trend (P = 0.06) toward a higher CaMKII phosphorylation was observed at baseline in SCI vs. AB. A group × time interaction (P < 0.05) and main effects of time (P < 0.0001) and group (P < 0.05) were noted for Akt phosphorylation.
In SCI, Akt phosphorylation was elevated above baseline 60 min after NMES resistance exercise. In addition, Akt phosphorylation was higher in SCI vs. AB at both time points after NMES. Similar to CaMKII phosphorylation, a strong trend ($P = 0.052$) toward higher Akt phosphorylation was observed at baseline in the SCI vs. AB. Total protein levels for AMPK, CaMKII, and Akt were not different between groups and did not change over time (not shown). No group or time effects were found for phosphorylation or total levels of AS-160 (not shown). In SCI, p44-42 ERK phosphorylation was elevated above baseline at 10 and 60 min after NMES resistance exercise. In addition, p44-42 ERK phosphorylation was higher in SCI vs. AB at 60 min after NMES (see Fig. 8).

**mRNA Expression and Total Protein Content of GLUT-4**

No difference was evident in mRNA expression of GLUT-4 among groups (not shown). However, GLUT-4 total protein content was 25% lower ($P < 0.05$) in the SCI group at baseline compared with AB (Fig. 6).

**Myofiber-type Distribution**

As shown in Fig. 7, we found significant differences in fiber-type distribution among groups. Compared with AB, SCI had far fewer type I fibers ($P < 0.05$) and a predominance of IIa + IIx fibers ($P < 0.05$). In SCI, the number of IIax hybrids was unusually high; thus we counted and combined them with IIx fibers.

**DISCUSSION**

The aim of the present study was to investigate the effects of NMES-induced resistance exercise on signaling processes that are associated with GLUT-4 translocation in the skeletal muscles of SCI vs. AB individuals. The primary and novel finding is that GLUT-4 protein content was lower in resting muscles of SCI, while, on the other hand, the phosphorylation states of two signaling proteins, AMPK-α and CaMKII, that stimulate muscle glucose uptake, along with Akt, which is a central regulator of numerous cellular processes, including glycogen synthesis and protein synthesis, were generally elevated in SCI vs. AB (Figs. 3–5). While we can only speculate as to mechanisms underlying these observations, it is possible that heightened intracellular signaling in SCI muscle is an attempt to...
compensate for low GLUT-4 levels and/or impaired anabolism. We were also encouraged to find that SCI muscle sustains the ability to activate intracellular signaling (e.g., Akt) in response to NMES-induced contractions even ~22 yr after the individual’s injury. Finally, as shown previously (6), we found that glucose tolerance is substantially impaired in long-term SCI victims, which may play a role in secondary health complications (metabolic syndrome) associated with SCI. That SCI muscles were actually more sensitive to NMES contractions than AB muscles (in terms of protein phosphorylation) could reflect differences in the relative stimulus (i.e., unlike SCI, ambulatory muscles of AB contract regularly).

AMPK is a serine/threonine protein kinase that functions as a fuel-sensing enzyme activated by depletion of high-energy molecules (i.e., ATP/GTP) and the accumulation of AMP in the mammalian cell (26). Activation of AMPK initiates a series of signaling events that trigger increases in the uptake and oxidation of substrates important for ATP synthesis. For example, AMPK promotes GLUT-4-dependent glucose transport in skeletal muscle in response to contractile activity (4). In the present study, higher AMPK phosphorylation was evident in the SCI group at 10 min post-NMES resistance exercise compared with the AB group (Fig. 3). This apparent difference in AMPK phosphorylation between groups may reflect a higher relative exercise intensity for the SCI muscle, since the VL in the SCI group had been relatively quiescent for, on average, more than 2 decades. The magnitude of AMPK activation may also have been influenced by muscle fiber-type distribution. Fiber-type-specific differences in AMPK activation in human skeletal muscle have been shown at rest and after an acute bout of exercise (33). Resting AMPK phosphorylation appears to be greatest in IIa fibers, while an acute bout of exercise increased AMPK phosphorylation in all fibers, with the greatest increase taking place in IIX fibers. The VL contains a mixture of type I, IIa, and IIX fibers in untrained AB individuals, whereas SCI muscles display a fiber-type shift toward a predominance of type II fibers, particularly type IIX fibers (15, 36), as early as 6 mo after the injury. Our myofiber type distribution findings are consistent with the previous work (14). Compared with AB, SCI had a predominance of IIX fibers (Fig. 7); therefore, it is certainly possible that higher AMPK phosphorylation in the SCI muscle may have been due to the remarkable group differences in fiber-type distribution.
Although it has been well established that activation of AMPK is involved in mediating the stimulation of glucose uptake by contractile activity, it does not appear to account for all of the increase in glucose uptake in skeletal muscle. Findings from previous work suggest that the increase in cytosolic Ca\(^{2+}\) during contractile activity also contributes to pathways involved in increased muscle glucose uptake (40, 41). The stimulation of glucose transport by Ca\(^{2+}\) involved in increased muscle glucose uptake (40, 41). The Ca\(^{2+}\) sient in skeletal muscle. When stimulated at physiological stimulation of glucose transport by Ca\(^{2+}\) has been shown to be mediated by activation of CaMK, specifically CaMKII, which appears to be the major CaMK isoform expressed in skeletal muscle (43). While previous work has shown that CaMKII regulates glucose uptake via an AMPK-dependent way (25), a growing body of evidence suggests that CaMKII-dependent signaling regulates skeletal muscle glucose uptake independent of AMPK, Akt, and AS160 phosphorylation (49, 50); which is consistent with the hypothesis that contraction-mediated glucose uptake is mediated via multiple signaling pathways. We found that CaMKII phosphorylation was significantly higher in the SCI group vs. AB, and particularly after NMES resistance exercise. To our knowledge, the fiber-type-specific response of CaMKII has not been investigated; however, motor unit firing frequency has been indirectly linked to modulation of CaMKII phosphorylation. For example, motor unit firing frequency determines both the amplitude and duration of the Ca\(^{2+}\) transient in skeletal muscle. When stimulated at physiological frequencies, intracellular Ca\(^{2+}\) in isolated skeletal muscle is ~10 times higher in type II myofibers (1–2 \(\mu\)M) compared with type I fibers (100–300 nM) (22). These marked changes in intracellular Ca\(^{2+}\) concentration are thought to activate CaMK-dependent pathways (20). Therefore, it is possible that the higher activation of CaMKII in the SCI group was due to a much higher proportion of type II myofibers.

Akt phosphorylation (Ser473) significantly increased by 60 min post-NMES-induced resistance exercise in the SCI group, yet no significant change was observed in the AB group. Our results in the SCI group are consistent with previous studies in AB individuals (11, 17), showing elevated Akt phosphorylation during 60-min recovery after an acute bout of resistance exercise in the fasted state. Akt has been implicated as a key signaling protein for activation of protein synthesis and glucose uptake (via GLUT-4 translocation) in skeletal muscle when stimulated by insulin (27). However, our subjects were fasted during data collection; thus insulin is less likely to activate Akt. Previous work has shown that mechanical load via resistance exercise can activate Akt signaling and promoting muscle hypertrophy independent of insulin (21). Therefore, enhanced Akt phosphorylation among SCI is likely attributable to changes in the mechanical environment via NMES-induced contractile activity in the SCI muscle. Surprisingly, we did not find a contraction-induced increase in Akt activation in the AB group. The trend toward higher basal Akt activation in SCI, along with the robust, contraction-mediated induction of Akt signaling after NMES resistance exercise, indicates long-term SCI does not negatively impact the response and may actually lead to a heightened sensitivity to contractions and/or a greater relative stimulus in SCI (as AB muscles are accustomed to daily contractions of varying intensities). Our findings of higher contraction-mediated induction of p44/42 ERK signaling in the SCI group supports (Fig. 8) this hypothesis, as ERK is known to be involved in mechanically induced signaling in skeletal muscle, where ERK activation has been reported to increase in response to contraction and passive stretch (37). On the other hand, it is possible that this heightened Akt activation in SCI muscle may be a failing attempt at compensation, since we do not know whether it leads to biologically meaningful events that improve homeostasis.

AS160 is thought to be a converging point for both insulin-mediated/Akt and contraction-mediated/AMPK signaling pathways on GLUT-4 translocation (12). Under basal states, the Rab GTPase activating protein domain of AS160 stimulates Rab-associated GTP and formation of inactive GDP-bound Rab, which inhibits GLUT-4 translocation. Contraction-induced phosphorylation of AS160 (Thr642), potentially by AMPK, relieves this inhibition by increasing the GTP-bound Rab, which results in

**Fig. 7.** Muscle fiber distribution in individuals with SCI and AB individuals. Values are means ± SE. *P < 0.05, different from AB.
greater GLUT-4 translocation to the plasma membrane (12). Previous studies have shown that muscle AS160 phosphorylation is significantly increased after a bout of moderate-intensity aerobic exercise and remains elevated up to 90 min postexercise (47). In the present study, while AMPK and Akt phosphorylation were higher in the SCI group following exercise, AS160 phosphorylation was unaffected by NMES-induced resistance exercise in either group (not shown). Our results are in agreement with previous studies (11, 24), which have shown phosphorylation of AS160 after prolonged, moderate-intensity cycling exercise, yet not after resistance exercise.

It was surprising to us that the NMES contraction protocol did not alter any measured indexes of intracellular signaling among AB subjects, and only led to a measurable induction of Akt in SCI. The regulation of these signaling processes is, of course, influenced by mode, intensity, and volume of exercise/contraction. Because the NMES protocol elicited maximum activation of the biopsied VL, we are confident that contraction intensity was sufficient. In fact, both groups suffered substantial fatigue, as measured by the differences in torque production between the initial and final contractions; furthermore, during and after the ~90 contractions, several AB subjects reported subjective feelings of significant fatigue and muscle pain/burning. One limiting factor, however, may have been the use of isometric rather than dynamic contractions. When considering the findings in other studies, it is certainly possible that dynamic contractions are a more potent stimulus for these signaling pathways than are isometric contractions. Finally, timing of muscle biopsies is a serious consideration when designing or interpreting acute response studies. We selected 10 and 60 min postexercise because dynamic muscle signaling regulation (phosphorylation) is a common finding within this first hour.

To our knowledge, this is the first study to compare muscle GLUT-4 protein content in SCI vs. AB individuals. While there were no group differences in GLUT-4 mRNA expression, basal GLUT-4 protein content was 25% lower in the SCI group, which may potentially play a role in impaired glucose tolerance and insulin sensitivity associated with SCI. In contrast to our findings, previous work (34) suggests that SCI is associated with a marked downregulation of muscle GLUT-4 mRNA. An important distinction between studies was that only individuals with complete SCIs were recruited for the previous investigation, while we included a mixture of individuals with complete and incomplete injuries. Furthermore, the subject characteristics, such as number of years postinjury, were not clearly identified in the previous investigation; thus it is possible that the differences in subject characteristics, together with differences in completeness of injury, may have contributed to the differences in GLUT-4 mRNA expression among studies.

Lastly, our results support earlier findings (7, 35) showing markedly impaired glucose tolerance in individuals with SCI compared with untrained AB controls, which is likely attributed, at least partially, to the low muscle mass and low protein levels of muscle GLUT-4. We also evaluated WBIS from the data obtained from the OGTT via using Matsuda Index (Fig. 2) (38). The WBIS was ~50% lower in the SCI group vs. AB group. We believe the combination of low levels of muscle GLUT-4 protein and low WBIS potentially play a role in the secondary health complications (metabolic disorders and diabetes) associated with SCI.

In conclusion, the phosphorylation of signaling proteins associated with GLUT-4 translocation (AMPK, CaMKII) are upregulated in SCI muscle even ~22 yr after injury. The enhanced signaling in SCI may be an attempted compensatory response to low-muscle GLUT-4 protein levels. Interestingly, NMES resistance exercise induced Akt phosphorylation in SCI only, suggesting heightened sensitivity to muscle contractions and/or a greater relative stimulus in SCI. It would be of great value in future work to determine whether chronic exposure to NMES resistance exercise training, a putative muscle hypertrophy stimulus, would increase the protein levels of GLUT-4 in concert with muscle mass and correct the metabolic state characterized by low-glucose tolerance and heightened (but perhaps futile) signaling.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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