Altered content of AMP-activated protein kinase isoforms in skeletal muscle from spinal cord injured subjects

Emil Kostovski,1,2,3 Hanneke Boon,4 Nils Hjeltens,1 Leonidas S. Lundell,5 Maria Ahlsén,5 Alexander V. Chibalin,4 Anna Krook,4,5 Per Ole Iversen,2,6 and Ulrika Widegren4

1Section for Spinal Cord Injury, Sunnaas Rehabilitation Hospital, Nesoddtangen, Norway; 2Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; 3Institute of Clinical Medicine, University of Oslo, Oslo, Norway; 4Departments of 4Medical Medicine and Surgery, and 5Physiology and Pharmacology, Section for Integrative Physiology, Karolinska Institutet, Stockholm, Sweden; and 6Department of Hematology, Oslo University Hospital, Oslo, Norway

Submitted 11 March 2013; accepted in final form 4 September 2013

AMP-activated protein kinase (AMPK) is a key regulator of cellular energy homeostasis, coordinating metabolic pathways that balance the supply of nutrients with energy demand (30). Activation of AMPK protects skeletal muscle against an energy deficiency by promoting glucose transport and fatty acid oxidation and by inhibiting acetyl-CoA carboxylase (ACC) (2, 6, 10, 27, 44). In addition to these metabolic actions, AMPK has been implicated in the control of gene transcription and subsequent mRNA expression (28, 48–50). AMPK is a trimERIC protein kinase consisting of a catalytic (α) subunit and two regulatory subunits (β and γ), all of which are necessary for full kinase activity. There are two isoforms of the α- (α1 and α2) and the β- (β1 and β2) subunits, and three isoforms of the γ-subunit (γ1, γ2, and γ3) (18, 24). Expression of different AMPK isoforms is dependent on the metabolic characteristics of skeletal muscle and consequently, most likely also influenced by the level of physical activity (46). Thus, changes in relative expression of different AMPK isoforms may partially regulate changes in metabolic characteristics of a decentralized skeletal muscle and may explain the increased risk for metabolic disturbances after spinal cord injury.

The physiological factors that regulate the relative expression of AMPK isoforms in human skeletal muscle has not yet been fully identified. Regular physical activity in the form of endurance training has been associated with reduced expression of the γ3 isoform and increased expression of the α2, β2, and γ1 isoforms (13). After prolonged inactivity, skeletal muscle fiber type composition shifts so that type IIb/x fibers predominate (5, 7, 31). In accordance, expression of the

mecles typically contain relatively large amounts of small type IIx fibers, some type IIa fibers, and virtually no type I fibers, as determined by mATPase staining (17). Furthermore, increased expression of myosin heavy chain (MHC) II is also observed (41). This is in accord with rodent studies, although fiber type composition and rate of transformation differ between species (8, 14, 16, 22, 33, 40).

In recent years, incomplete tetraplegia has become the most frequent neurological deficit following traumatic spinal cord injury, which has led to an increased focus on the clinical rehabilitation of this patient group (37). Depending on the degree of injury, some people with incomplete injury, who are initially almost totally paralyzed, are capable of standing and/or walking one year postinjury. Whether this limited range of movement is sufficient to maintain the molecular integrity of skeletal muscle and prevent the dramatic changes in cellular energy homeostasis that occur with complete spinal cord injury is unknown.

AMP-activated protein kinase (AMPK) is a key regulator of cellular energy homeostasis, coordinating metabolic pathways that balance the supply of nutrients with energy demand (30). Activation of AMPK protects skeletal muscle against an energy deficiency by promoting glucose transport and fatty acid oxidation and by inhibiting acetyl-CoA carboxylase (ACC) (2, 6, 10, 27, 44). In addition to these metabolic actions, AMPK has been implicated in the control of gene transcription and subsequent mRNA expression (28, 48–50). AMPK is a trimERIC protein kinase consisting of a catalytic (α) subunit and two regulatory subunits (β and γ), all of which are necessary for full kinase activity. There are two isoforms of the α- (α1 and α2) and the β- (β1 and β2) subunits, and three isoforms of the γ-subunit (γ1, γ2, and γ3) (18, 24). Expression of different AMPK isoforms is dependent on the metabolic characteristics of skeletal muscle and consequently, most likely also influenced by the level of physical activity (46). Thus, changes in relative expression of different AMPK isoforms may partially regulate changes in metabolic characteristics of a decentralized skeletal muscle and may explain the increased risk for metabolic disturbances after spinal cord injury.

The physiological factors that regulate the relative expression of AMPK isoforms in human skeletal muscle has not yet been fully identified. Regular physical activity in the form of endurance training has been associated with reduced expression of the γ3 isoform and increased expression of the α2, β2, and γ1 isoforms (13). After prolonged inactivity, skeletal muscle fiber type composition shifts so that type IIb/x fibers predominate (5, 7, 31). In accordance, expression of the...
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>BMI, kg/m²</th>
<th>ASIA Motor score</th>
<th>Injury level</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB SCI</td>
<td>33 (2)</td>
<td>25 (1)</td>
<td>NA</td>
<td>C7</td>
</tr>
<tr>
<td>SCU COMPLETE</td>
<td>33 (4)</td>
<td>24 (1)</td>
<td>26 (4)</td>
<td>1</td>
</tr>
<tr>
<td>SCI INCOMPLETE</td>
<td>33 (4)</td>
<td>24 (0.4)</td>
<td>19 (4)*</td>
<td>0</td>
</tr>
<tr>
<td>SCI COMPLETE</td>
<td>49 (5)*</td>
<td>24 (0.8)</td>
<td>21 (5)*</td>
<td>6</td>
</tr>
<tr>
<td>SCI INCOMPLETE</td>
<td>33 (3)</td>
<td>24 (0.4)</td>
<td>24 (5)*</td>
<td>4</td>
</tr>
<tr>
<td>SCI COMPLETE</td>
<td>49 (12)</td>
<td>72 (6)</td>
<td>40 (12)</td>
<td>1</td>
</tr>
<tr>
<td>SCI INCOMPLETE</td>
<td>33 (3)</td>
<td>25 (0.4)</td>
<td>25 (0.4)</td>
<td>2</td>
</tr>
<tr>
<td>SCI COMPLETE</td>
<td>49 (12)</td>
<td>81 (2)</td>
<td>72 (6)</td>
<td>1</td>
</tr>
</tbody>
</table>

Subject characteristics of able-bodied (AB), recent longstanding complete spinal cord injured (SCI), and recent complete and incomplete SCI subjects. In the recently injured individuals, each time point (1, 3, and ≥12 mo) were compared between individuals with complete and incomplete spinal cord injured individuals. BMI, body mass index. NA, not assessed. The motor scores were evaluated according to international standards for classification of spinal cord injury; ASIA (28). Values are means (SE). *P < 0.05.

AMPK<sub>y3</sub> isoform has a high degree of specificity for glycolytic fast-twitch skeletal muscle (32). Given that nerve activity regulates many metabolic and morphological properties of skeletal muscle, we tested the hypothesis that spinal cord injury is accompanied by alterations in the relative expression of skeletal muscle AMPK isoforms. Thus, the aim of this study was to determine AMPK isoform protein abundance in human skeletal muscle after spinal cord injury. Specifically, we compared individuals with extreme muscle disuse to characterize the influence of long-standing and recent spinal cord injury on AMPK isoform abundance. Furthermore, the effect of electrically stimulated leg cycling (ESLC) on mRNA expression of AMPK isoforms was assessed in a subgroup of individuals with long-standing spinal cord injury.

MATERIALS AND METHODS

Subjects. Nineteen individuals with spinal cord injury, who were otherwise healthy, were included in this study. The subject characteristics are presented in Tables 1 and 2. The participants were divided into different groups: six males with a longstanding complete injury of the cervical spinal cord (time since injury 17–31 yr); seven subjects (one woman and six men) with a recent (<2 mo at the time of inclusion) complete injury of the cervical spinal cord; six males with a recent incomplete injury, (Table 1), and a subgroup of long-standing spinal cord injured subjects (n = 4) that participated in exercise training, ESLC, for 8 wk (Table 2). The training period consisted of seven exercise sessions per week; one session per day for 3 days and two sessions per day for 2 days. A detailed description of the training protocol has been previously reported (21). The training bouts were carried out on a computer-controlled electrical stimulation exercise ergometer (ERGYS-I-Clinical Rehabilitation System; Therapeutic Alliance, Fairborn, OH). All ESLC sessions were supervised by a physician and a physiotherapist. No ESLC bouts were performed during the 48 h before muscle biopsies were obtained.

The diagnoses of complete and incomplete tetraplegia were based on internationally accepted criteria (11). The subjects with an incomplete cervical lesion scored 4 or better according to the American Spinal Cord Injury Association (ASIA) classification (34) when tested for muscular force of the quadriceps femoris muscle 12 mo or more postinjury. Seven healthy, able-bodied males matched for body mass index (BMI) and age were included as controls.

On the experimental day, the spinal cord injured subjects underwent a thorough clinical examination, a muscle biopsy was obtained, and routine blood and urine chemistry analyses were performed. The procedures were explained, and informed consent was obtained from each study participant. The Regional Committee for Medical Ethics at Helse Sør-Ost, Norway, and the Regional Ethics Committee at Karolinska Institutet approved the study protocol. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Muscule biopsy procedure. All muscle biopsies were obtained in the postprandial state. Muscle biopsies from the recent spinal cord injured individuals were obtained approximately at month 1 (31 ± 3 days); month 3 (110 ± 22 days); and ≥12 mo postinjury (461 ± 155 days). Muscle biopsy specimens were obtained from the vastus lateralis portion of the quadriceps femoris muscle under local anesthesia (Lidocaine 5 mg/ml) and immediately frozen in liquid nitrogen.

Muscle preparation and Western blot. Portions (30–60 mg) of muscle biopsies were freeze-dried for 24 h. Samples were transferred to room temperature and dissected by the use of a microscope and fine jeweler’s forceps to remove fat, blood, and connective tissue. Muscles were then homogenized in 0.6 ml of ice-cold buffer containing 137 mM NaCl, 1 mM MgCl<sub>2</sub>, 2.7 mM KCl, 1 mM EDTA, 20 mM Tris, pH 7.8, 5 mM Na pyrophosphate, 10 mM NaF, 1% Triton X-100, 10% (vol/vol) glycerol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 0.5 mM Na<sub>3</sub>VO<sub>4</sub> and 1× protease inhibitor cocktail Set 1 (Calbiochem, EMD Biosciences, San Diego, CA). Insoluble material was removed by centrifugation at 12,000 g for 10 min at 4°C. Protein was determined using a commercially available assay (Pierce BCA protein assay kit; Thermo Scientific, Rockford, IL). Equal amounts of protein were suspended in Laemmli sample buffer. Samples were separated in parallel by SDS-PAGE (Criterion XT Precast gel; Bio-Rad, Hercules, CA), and proteins were transferred to PVDF membranes. Equal loading was confirmed by Ponceau staining. The membranes were blocked with 7.5% nonfat dry milk for 1 h at room temperature and incubated overnight at 4°C with appropriate primary antibodies against AMPK<sub>a1</sub> (62 kDa, #07–350; Millipore, Billerica, MA), AMPK<sub>a2</sub> (62 kDa, #07–363; Millipore), AMPK<sub>b1</sub> (38 kDa, #1604–1; Epitomics Burlingame, CA), AMPK<sub>b2</sub> H-75 (34 kDa, #sc20146; Santa Cruz Biotechnology, Santa Cruz, CA), AMPK<sub>y1</sub> (37

Table 2. Characteristics of a subgroup of 4 spinal cord subjects participating in an 8-wk training program

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Time Since Injury (yr)</th>
<th>Injury Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44</td>
<td>1.87</td>
<td>87.5</td>
<td>25.0</td>
<td>23</td>
<td>C7</td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>1.86</td>
<td>69</td>
<td>19.9</td>
<td>11</td>
<td>C6</td>
</tr>
<tr>
<td>C</td>
<td>38</td>
<td>1.85</td>
<td>80</td>
<td>23.4</td>
<td>7</td>
<td>C5</td>
</tr>
<tr>
<td>D</td>
<td>28</td>
<td>1.86</td>
<td>64</td>
<td>18.5</td>
<td>6</td>
<td>C6</td>
</tr>
</tbody>
</table>
kDa, #1592–1; Epitomics), AMPKγ3 (54 kDa; #HPA004909, Sigma-Aldrich, MO), phospho-AMPKα Thr172 (62 kDa, #2531; Cell Signaling Technology), phospho-ACC Ser79 (280 kDa, #3661; Cell Signaling Technology), MitoProfile Total OXPHOS Human WB Antibody Cocktail (Complexes I: 18 kDa, II: 29 kDa, III: 48 kDa, IV: 22 kDa, and V: 55 kDa; #ab110411; Abcam, Cambridge, UK), and myosin heavy chain (MHC) I, IIa, and IIx (kind gifts from Stefano Schiaffino), respectively. Membranes were washed and incubated with the appropriate secondary antibodies as recommended by the supplier (Amersham, Arlington, IL). Proteins were visualized by enhanced chemiluminescence and quantified by densitometry.

RNA extraction. Portions of skeletal muscle biopsies (25–35 mg) were homogenized using a polytron mixer in 1 ml Tri Reagent (Sigma, St. Louis, MO). Extraction of total RNA and cDNA synthesis was carried out as described (25), diluted, and stored in aliquots at −20°C.

Analysis of mRNA expression. Real-time PCR (ABI PRISM A 7000 Sequence Detector; PerkinElmer Applied Biosystems, Foster City, CA) was used for quantification of specific mRNAs. Data were collected and analyzed by ABI Prism 7000 SDS Software v. 1.1. Oligonucleotide primers and TaqMan probes (FAM-MGB) for human AMPK isoforms α1, α2, β1, β2, γ1, γ2, and γ3 were purchased as Assays-on-Demand from Applied Biosystems (Assay IDs: Hs00178893_m1, Hs00178903_m1, Hs00271294_m1, Hs00176952_m1, Hs00211903_m1, Hs00179660_m1). Human β-actin was used as endogenous control to correct for potential variation in cDNA loading and quantity. β-Actin primers and TaqMan probe (VIC-TAMRA) were

![Figure 1](image-url)
purchased from Applied Biosystems (part no. 4310881E). All reactions were performed in 96-well MicroAmp optical plates with a sample volume of 25 μl. Amplification mixes (25 μl) contained the diluted cDNA sample, TaqMan Universal PCR Mastermix, forward and reverse primers, and probe for the target gene, as well as β-actin. Thermal cycling conditions included 2 min at 50°C and 10 min at 95°C before the onset of the PCR cycles, which consisted of 40 cycles at 95°C for 15 s and 65°C for 1 min. Target gene and β-actin were amplified in multiplex reactions in triplicate. All subjects were analyzed simultaneously in one assay run, i.e., one 96-well plate per gene of interest.

The efficiencies of primers and probes for the AMPK isoforms and β-actin were within similar range, allowing for the use of the comparative Ct method, where the relative quantities of different mRNA transcripts were calculated after normalization of the data against β-actin (45). Normalization of AMPK isoform expression to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression did not change the expression pattern.

Statistics. Data are presented as means ± SE. Student’s t-test was used to analyze differences in expression of AMPK and MHC isoforms between able-bodied and longstanding spinal cord injured subjects. Changes of respective AMPK and MHC protein isoforms were evaluated by mixed-models analyses. The statistical analyses were performed with SPSS v. 18.0 (Chicago, IL) and the MedCalc Software (Mariakerke, Belgium). P values of <0.05 were considered to indicate statistical

---

**Fig. 2.** Skeletal muscle protein abundance of AMPK α-, β-, and γ-isforms from individuals with recent complete cervical spinal cord injury at -1 mo (open columns), 3 mo (gray columns), and ≥12 mo (filled columns) after injury. *P <0.05. Representative Western blots are presented above each graph. Values are presented as means ± SE AU; n = 7.

**Fig. 3.** Skeletal muscle protein phosphorylation of AMPK Thr172 and acetyl-CoA carboxylase (ACC) Ser79 obtained from individuals at -1 mo (open columns), 3 mo (gray columns), and ≥12 mo (filled columns) after a complete cervical spinal cord injury. *P < 0.05. Representative Western blots are presented above each graph. Values are presented as means ± SE AU; n = 6–7.
significance. Individual values \((n = 4)\) of AMPK isoform expression before and after ESLC training are illustrated in scatter diagrams.

**RESULTS**

**Skeletal muscle protein abundance of AMPK \(\alpha\)-subunits after spinal cord injury.** Skeletal muscle AMPK\(\alpha\)1 and -\(\alpha\)2 protein abundance did not differ between able-bodied and longstanding complete cervical spinal cord injured individuals (Fig. 1). However, in individuals with recent complete cervical spinal cord injury, AMPK\(\alpha\)2 protein abundance decreased by 25% during the first year after injury \((P < 0.05)\), whereas no significant change in AMPK\(\alpha\)1 content was detected (Fig. 2).

**Skeletal muscle protein abundance of AMPK \(\beta\)-subunits after spinal cord injury.** Skeletal muscle AMPK\(\beta\)1 and -\(\beta\)2 protein abundance was unaltered between able-bodied and longstanding complete cervical spinal cord injured individuals (Fig. 1). However, in individuals with recent complete spinal cord injury, AMPK\(\beta\)1 content declined by 45% during the first year after injury \((P < 0.05)\), whereas no change in AMPK\(\beta\)2 protein abundance was detected (Fig. 2).

**Altered skeletal muscle AMPK\(\gamma\)1 and -\(\gamma\)3 protein abundance after spinal cord injury.** In individuals with longstanding complete cervical spinal cord injury, skeletal muscle AMPK\(\gamma\)1 protein abundance was 80% \((P = 0.02)\) lower compared with able-bodied individuals (Fig. 1). Conversely, AMPK\(\gamma\)3 protein...
abundance was nearly threefold higher (Fig. 1; *P < 0.05). Accordingly, in individuals with recent complete spinal cord injury, protein abundance of AMPKγ1 declined 40% (Fig. 2; *P < 0.02), and AMPKγ3 was augmented 45% (Fig. 2; *P < 0.02) during the first year after injury.

**Phosphorylation of AMPK Thr172 and ACC Ser79 is altered after spinal cord injury.** AMPK phosphorylation on Thr172 was significantly decreased during the first year post-spinal cord injury (Fig. 3). In accordance, Ser79 phosphorylation of ACC was also decreased (Fig. 3; *P < 0.05 vs. month 1).

**Complexes I–V.** In recent spinal cord injured individuals, mitochondrial protein abundance of complexes I and III were significantly decreased during the first year (Fig. 4; *P < 0.05). This was also noticed for complex II although not significant (Fig. 4; *P = 0.05). In contrast, complexes IV and V remained unchanged.

**Altered MHC isoform protein abundance in complete spinal cord injured subjects.** Skeletal muscle MHC I protein abundance was lower in longstanding complete cervical spinal cord injured (P < 0.001) compared with able-bodied individuals (Fig. 5). Conversely, protein abundance of MHC Ila and MHC IIX was markedly elevated (P < 0.001) after longstanding spinal cord injury (Fig. 5). These changes were apparent in the early phase following complete spinal cord injury, since protein abundance of MHC I declined by 60% (P < 0.001), whereas MHC Ila was augmented by 50% (P < 0.001), and MHC IIX was unchanged (Fig. 6).

**Recent incomplete spinal cord injury.** To further investigate the role of physical/neuromuscular activity on AMPK protein abundance, a subgroup of individuals with recent incomplete spinal cord injury was included. In these individuals, skeletal muscle AMPKγ1 protein content was reduced by 10% 1 yr postinjury (Fig. 7; *P < 0.05); protein abundances of AMPKβ1 and γ3 were unaltered (Fig. 7).

To determine the effect of spinal cord injury on muscle fiber type, we also assessed protein abundance of MHC isofoms in the subgroup of incomplete spinal cord injured individuals. In contrast to a recent complete spinal cord injury, MHC I was unchanged and MHC Ila protein abundance was augmented by 30% (Fig. 8; *P < 0.05). The MHC IIX protein content, though, was unchanged, similar to the early phase following complete spinal cord injured subgroup (Fig. 6).

**Effects of exercise training on mRNA expression of AMPK isofoms.** A subgroup (n = 4) of spinal cord injured individuals participated in an 8-wk exercise training program (ESLC). AMPKγ1 mRNA expression was decreased after ESLC training in two subjects and increased in the remaining subjects (Fig. 9). Exercise increased AMPKγ2 mRNA expression in all subjects. AMPKγ3 expression was slightly reduced in two subjects and increased in the remaining two. Separate symbols and connecting lines are representing each individual.

**Expression patterns of mRNA isofoms in human skeletal muscle.** In skeletal muscle from able-bodied control subjects, AMPKα2 was the predominant α-subunit, with an average relative expression of 14-fold at the mRNA level compared with AMPKα1 (Fig. 10, left). AMPKβ2 was the predominant β-subunit, with an average of 14-fold higher mRNA expression compared with AMPKβ1. The γ-subunit mRNA was
expressed at a ratio of 1:0.6:3.3 for γ1:γ2:γ3, respectively, in able-bodied subjects.

The relative expression of AMPK subunits in spinal cord injured subjects was 1:6 for α1:α2, 1:17 for β1:β2, and 1:0.3:3.6 for γ1:γ2:γ3 (Fig. 10, middle). In able-bodied subjects, the relative expression of α2, β2, and γ3 was 1:1.3:0.3. In skeletal muscle from spinal cord injured subjects, this relationship was altered due to the reduction in α2 and β2 mRNA expression, and the increase in γ3 mRNA expression, (1:2.3:3.4), which was partially normalized following exercise (1:3.7:2.1; Fig. 10, right).

DISCUSSION

In this study, we determined the effects of longstanding and recent cervical spinal cord injury on AMPK isoform protein abundance in skeletal muscle. Although downstream targets of AMPK are widely characterized, the physiological factors that govern AMPK protein kinase abundance in human skeletal muscle are only partly known. Nerve activity and contraction have a major impact on skeletal muscle metabolic and morphological properties and thus are likely to influence the expression of AMPK.

AMPK has profound effects on skeletal muscle oxidative capacity and mitochondrial biogenesis. We have recently reported that spinal cord injury is associated with a marked downregulation of genes that are critical for lipid metabolism and glycogen storage in skeletal muscle (29, 30). In this study, we also provide evidence for an altered content of mitochondrial proteins (15), complexes I-III, due to spinal cord injury. Furthermore, phosphorylation of AMPK Thr172 and its downstream targets, ACC Ser79, was decreased during the first year of a spinal cord injury, indicating a disregulated fatty acid oxidation (2, 6, 10, 26, 43).

Extreme skeletal muscle inactivity due to a complete cervical spinal cord injury is accompanied by elevated AMPKγ3 and reduced AMPKγ1 protein abundance compared with able-bodied controls. These γ-isofrom changes are rapidly established and apparent at 3 and 12 mo after complete spinal cord injury. Conversely, these changes were attenuated after an incomplete cervical injury. Expression of the AMPKγ3 isofrom displays a high degree of specificity for white fast-twitch skeletal muscle (4, 32). Moreover, a possible role of AMPKγ3 in the regulation of skeletal muscle metabolism, specifically in the form of glycogen supercompensation, has been proposed from studies of pigs that carry a natural occurring mutation of γ3 (12, 35). Thus, the AMPKγ3 isoform is likely to play a specific role in mediating metabolic responses in fast glycolytic muscle. Moreover, the expression of the γ3 isoform may be altered by slow to fast twitch reprogramming, which in turn could alter AMPK-mediated metabolic responses.
In skeletal muscle that is inactivated for an extended period of time, the fiber type composition shifts so that the type IIb/x fibers predominate (5, 9, 31). Our findings are consistent with a shift in muscle fiber type in spinal cord injury, since we observed a high proportion of type II fibers expressed after longstanding spinal cord injury. The difference in the MHC protein abundance profiles was apparent for MHC I and IIA isoforms in the individuals with a recent complete spinal cord injury, but only to a lesser extent for the MHC IIA isoform in individuals with incomplete injury. Thus, longstanding decentralized skeletal muscle in spinal cord injured (tetraplegic) subjects is characterized by IIb/x fibers (17, 21, 31) compared with a mixed fiber type composition as typically seen in able-bodied subjects (1). This is supported by our finding of a less pronounced change in MHC isoforms in the incomplete spinal cord injured group. Hence, differences in AMPK isoform protein abundance in spinal cord injured vs. able-bodied subjects may reflect the high proportion of type II fibers expressed in these individuals. Furthermore, this is also supported by the finding of a relative increase in skeletal muscle mRNA expression of AMPKγ3 in the subgroup of longstanding spinal cord injured subjects.

There are seven different genes encoding the subunits of the AMPK trimer, making a total of 12 possible AMPK heterotrimer combinations. We and others have reported that in skeletal muscle the predominant AMPK species are α2, β2, and γ3 (4, 32). In this study, we observed that α2, β2, and γ3 mRNA represent the most abundant variant of each isoform in skeletal muscle from able-bodied controls, which is also in agreement with earlier work. More importantly, despite the loss of neuronal input, α2, β2, and γ3 mRNA remained the most abundant variant of each isoform in skeletal muscle of spinal cord injured subjects, suggesting a critical role of these isoforms in skeletal muscle. Changes in relative expression of the α-subunits may confer different sensitivities to exercise-mediated glucose uptake, as the two isoforms have been shown to respond differently to activation with the pharmacological activators of AMPK (23).

The expression of the AMPKγ3 isoform has a high degree of specificity for white fast-twitch (type IIb/x) skeletal muscle. When skeletal muscle is inactivated for an extended period of time, the muscle fiber type composition shifts so that type IIb/x fibers predominate (5, 9, 31). Thus, longstanding decentralized skeletal muscles in spinal cord injured (tetraplegic) subjects are dominated by IIb/x fibers (17, 20, 31): 2 ± 1% type I, 35 ± 7% type IIA, and 64 ± 8% type IIb (20) compared with a mixed fiber type composition in able-bodied subjects (33% type I, 45% type IIA, 32% type IIb) (1).

Our findings provide evidence that skeletal muscle abundance of AMPK γ-isoforms is related to physical activity and/or muscle fiber type, which is further underscored by our finding of more modest changes in AMPK isoform abundance after an incomplete spinal cord injury. Skeletal muscle from untrained subjects have a higher proportion of type IIx fibers compared with endurance-trained subjects, further supporting the relationship between AMPKγ3 expression and metabolic and performance characteristics of skeletal muscle. Accordingly, exercise training status and AMPKγ3 expression is inversely correlated in human skeletal muscle, suggesting that endurance exercise training in able-bodied untrained subjects, also known to change fibers type, reduces AMPKγ3 and increases AMPKγ1 skeletal muscle protein abundance (13). This is further supported by the effects of exercise training on mRNA expression of AMPK isoforms. In spinal cord injured subjects, 8 wk of exercise training results in a fiber type transformation to a more oxidative muscle: 5 ± 2% type I, 55 ± 8% type IIA, and 39 ± 5% type IIb (20) and a reduction in AMPKγ3 mRNA expression. Interestingly, AMPKγ3 protein and mRNA levels are increased in skeletal muscle from untrained compared with trained subjects (38, 47). Taken together with our present results, physical exercise modulates AMPKγ3 expression in skeletal muscle. Studies from genetically modified animals indicate that reduced expression of AMPKγ3 alters the metabolic properties of skeletal muscle (3, 4). Thus changes in AMPKγ3 mRNA expression may mediate training-induced alterations in muscle metabolism.

In response to chronic extreme skeletal muscle inactivity due to a complete cervical spinal cord injury, we noted a nonsignificant trend for elevated AMPKβ1 protein abundance compared with able-bodied controls. However, in recent cervical spinal cord injury individuals, AMPKβ1 protein abundance decreased after 3 and 12 mo postinjury. Skeletal muscle fiber type transformation occurs in stages after spinal cord injury (5). In human single fibers and isolated rat skeletal muscle,
slow-twitch compared with fast-twitch muscle contains more AMPKβ1, which is in accord with our results (36). However, the changes in AMPKβ1 isoform protein abundance in longstanding vs. recent spinal cord injured individuals suggest that recent and longstanding adaptations to immobilization or spinal cord injury have a differential effect on skeletal muscle AMPKβ1. This may also be applicable for the AMPKα2 isoform, as a reduction in AMPKα2 protein content was observed in the recent complete spinal cord injured individuals but not in the longstanding spinal cord injured subjects. To our knowledge, changes in α2 and/or β1 isoforms after exercise or immobilization (13, 38) have not been documented. Our finding that AMPKα1 and β2 isoforms were unaltered are in accord with an earlier study where immobilization-induced muscle inactivity for 2 wk did not alter the protein content of the AMPKα1 or β2 isoforms (11). In contrast, muscle-specific β1/2M-KO mice are exercise intolerant, suggesting that the skeletal muscle AMPK β-isozymes are necessary to meet the increase in metabolic demand with exercise (39). The relative increase in skeletal muscle protein content of AMPKγ3 and the decrease of AMPKβ1 in our present study may therefore reflect a change toward a higher composition of AMPKγ3 and the decrease of AMPKβ1 in our present study may therefore reflect a change toward a higher proportion of type II fibers and a more glycolytic phenotype in the recent spinal cord injured groups (5).

In conclusion, we provide evidence of profound changes in AMPK isoform abundance after longstanding and recent complete spinal cord injury. These changes reflect a transition in skeletal muscle fiber type to a glycolytic fast-twitch phenotype. This may partly mediate some of the metabolic changes observed in spinal cord injured individuals. Furthermore, partial recovery of skeletal muscle function (incomplete cervical lesions) attenuates changes in the AMPK isoform profile, suggesting that physical activity/neuromuscular activity is an important determinant of AMPK isoform profiles. Electrically stimulated exercise training partially normalizes expression of AMPK isoforms. Thus, physical activity affects the isoform composition of AMPK, and this contributes to the metabolic profile of skeletal muscle, which further underscores the need for physical activity as part of the treatment regimen.

Acknowledgments

We thank Dr. Juleen Zierath for helpful discussion and critical reading of the manuscript.

Grants

This study was supported by grants from the Institute International de Recherche de Paralipégie, the Swedish National Center for Research in Sports, the Swedish Medical Association, Magnus Bergvall Foundation, Lars Hierta Foundation, Fredrik and Ingrid Thuring Foundation, Tore Nilson Foundation for Medical Research, Ake Wiberg Foundation, the Swedish Research Council, the Novo Nordisk Foundation, the Strategic Research Program in Diabetes at the Swedish Medical Association, Magnus Bergvall Foundation, Fredrik and Ingrid Thurings Foundation, Tore Nilson Foundation, and the Swedish Research Council.

Disclosures

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

Author Contributions


References


AJP-Endocrinol Metab. doi:10.1152/ajpendo.00132.2013 • www.ajpendo.org


