Long YC, Kostovski E, Boon H, Hjeltnes N, Krook A, Widegren U. Differential expression of metabolic genes essential for glucose and lipid metabolism in skeletal muscle from spinal cord injured subjects. J Appl Physiol 110: 1204–1210, 2011. First published March 10, 2011; doi:10.1152/japplphysiol.00686.2010.—Skeletal muscle plays an important role in the regulation of energy homeostasis; therefore, the ability of skeletal muscle to adapt and alter metabolic gene expression in response to changes in physiological demands is critical for energy balance. Individuals with cervical spinal cord lesions are characterized by tetraplegia, impaired thermoregulation, and altered skeletal muscle morphology. We characterized skeletal muscle metabolic gene expression patterns, as well as protein content, in these individuals to assess the impact of spinal cord injury on critical determinants of skeletal muscle metabolism. Our results demonstrate that mRNA levels and protein expression of skeletal muscle genes essential for glucose storage are reduced, whereas expression of glycolytic genes is reciprocally increased in individuals with spinal cord injury. Furthermore, expression of genes essential for lipid oxidation is coordinately reduced in spinal cord injured subjects, consistent with a marked reduction of mitochondrial proteins. Thus spinal cord injury resulted in a profound and tightly coordinated change in skeletal muscle metabolic gene expression program that is associated with the aberrant metabolic features of the tissue.}

SKELETAL MUSCLE ACCOUNTS FOR a substantial proportion of lean body mass and energy consumption. Given its high energy expenditure, skeletal muscle plays a pivotal role in the regulation of thermogenesis and energy homeostasis. The capacity of skeletal muscle tissue to support energy homeostasis is attributed to its ability to alter metabolic fuel selection in response to changes in the supply of fuel substrates and/or physiological conditions (7, 13).

Glucose and fatty acids (FAs) are the major fuel substrates of skeletal muscle, and the utilization is tightly coordinated to enhance efficient substrate metabolism. Healthy skeletal muscle adapts to fed and fasting conditions by shifting reliance from glucose to lipid utilization, which has been referred to as metabolic flexibility (13, 27). Thus, during fed conditions when there is an increase in plasma glucose, insulin stimulates skeletal muscle glucose uptake and oxidation, as well as storage of glucose as glycogen. During fasting, when there is an increase in plasma FAs, skeletal muscle exhibits a greater reliance on lipid fuel substrate (5), which supports a plasma glucose-sparing effect. Although skeletal muscle fuel substrate selection is controlled by covalent and allosteric effects (24), as well as hormonal regulation and substrate availability (13), transition of fed to fasting conditions is also accompanied by coordinated changes in gene expression programming in skeletal muscle tissue. We hypothesized that skeletal muscle mRNA and protein expression of key genes regulating these processes may be altered in the context of spinal cord injury, which may impair the ability of skeletal muscle to adapt to fuel supply and demand via alteration in substrate (glucose and lipid) utilization and storage.

Changes in skeletal muscle gene expression programming are also observed in response to increased contractile activity or exercise. In endurance-trained individuals, there is an increase in the contribution of FA to total energy expenditure compared with untrained subjects, which is thought to slow down the depletion of intramuscular glycogen stores, resulting in a delayed onset of exhaustion (21). Such a greater reliance on FA oxidation is associated with a consistent increase in the expression of lipid metabolic gene expression in response to exercise training.

Individuals with complete cervical spinal cord lesions are characterized by tetraplegia, impaired thermoregulation, decreased glucose homeostasis, and altered muscle morphology including severe muscle wasting (2, 3). Loss of muscle mass below the level of the lesion is a relatively rapid event following spinal cord injury (15). Muscle atrophy in subjects with spinal cord injury is also accompanied by reduced energy expenditure compared with able-bodied subjects. Indeed, energy expenditure is reduced in spinal cord injured individuals even after adjusting for fat-free mass, fat mass, and age (36), indicating a fundamental shift in energy balance. The impaired thermoregulation is associated with reduced skeletal muscle uptake of nutrients and decreased whole body specific heat production (2). In subjects with longstanding tetraplegia, the biochemical properties of the skeletal muscle tissue might be altered due to prolonged inactivity. Indeed, immobilization alone has been demonstrated to result in a rapid reduction in myofiber size and vascularization (26). In this study, we hypothesized that the alterations in gene expression are tightly coordinated and that such changes in gene expression programs parallel the aberrant metabolic characteristics of subjects with tetraplegia. Our results provide evidence that skeletal muscle genes involved in glucose storage are reduced, whereas glucose and lipid metabolic gene are reciprocally altered, in spinal cord injured subjects compared with able-bodied individuals.

MATERIALS AND METHODS

Subjects. In this study, 9 men with complete chronic (longstanding injury) lesion of the cervical spinal cord (age: 35 ± 2 yr, height: 1.85 ± 0.01 m, weight: 73.4 ± 3.8 kg), and 10 sedentary, not regular physically active, able-bodied male controls (age: 39 ± 2 yr, height:
1.83 ± 0.03 m, weight: 75.1 ± 2.6 kg) participated. A clinical examination of the spinal cord injured individuals, as previously described (20), was undertaken. They were treated against muscle spasm with baclofen (20–25 mg × 2–4), and one subject received additional treatment with diazepam (5 mg × 2). Condom drainage, for bladder emptying, was used in all spinal cord injured subjects. Time since injury ranged from 6 to 23 yr. The study was conducted according to the principles expressed in the Declaration of Helsinki. The procedures were explained, and informed consent was obtained from each subject. The Regional Committee for Medical Ethics at Helseregion East, Norway, and the Regional Ethical Committee at Karolinska Institutet approved the study protocol. None of the study participants were tobacco users or were taking any other medications as described above. Subjects were instructed to avoid any kind of strenuous exercise 48 h before the experimental day.

Muscle biopsy procedure. Muscle biopsies were obtained in the morning after an overnight fast. Muscle biopsy specimens were taken, as described previously (3, 20), while the subjects were under local anesthesia, from the vastus lateralis portion of the quadriceps femoris muscle and frozen immediately in liquid nitrogen.

RNA isolation and cDNA synthesis. Skeletal muscle was homogenized in Trizol reagent (Sigma, St. Louis, MO) by the use of a mechanical homogenizer Polytron (Kinematica). RNA was purified according to recommendations of the manufacturer. Purified RNA was subjected to DNase treatment by means of a DNA-free kit (Ambion, Huntingdon, Cambridgeshire, UK) according to the manufacturer’s protocol. DNase-treated RNA was used for cDNA synthesis by SuperScript first strand synthesis system (Invitrogen, Carlsbad, CA) with oligo (dT) primers. A reaction without reverse transcriptase was included for each sample as a reverse transcriptase-minus control. RNA was subjected to DNase treatment by means of a DNA-free kit (Ambion, Huntingdon, Cambridgeshire, UK) according to the manufacturer’s protocol. DNase-treated RNA was used for cDNA synthesis by SuperScript first strand synthesis system (Invitrogen, Carlsbad, CA) with oligo (dT) primers. A reaction without reverse transcriptase was included for each sample as a reverse transcriptase-minus control.

Quantitative real-time PCR. The quantity of cDNA for each gene of interest was measured using real-time PCR with the ABI PRISM 7000 sequence detector system and fluorescence-based SYBR-green technology (Applied Biosystems, Warrington, UK). PCR was performed in a final volume of 25 μl, consisting of diluted cDNA sample, 1 × SYBR-green PCR Master Mix (Applied Biosystems), primers optimized for each target gene, and nuclease-free water. Primers were designed using Primer Express computer software (Applied Biosystems), based on mRNA sequences obtained from Genbank with the following accession numbers: atrogin-1 (AY059629), cathepsin L1 (CtsL; NM_145918), carnitine palmitoyltransferase 2 (Cpt2; NM_000998), enolase 3 (Eno; NM_053013), glucose transporter 4 (Glut4;Slc24; NM_001042), hexokinase 2 (Hk2; NM_000189), and pyruvate kinase (Pk; NM_118247). Primer sequences for other genes of interest, including carnitine palmitoyltransferase 1 (Cpt1), fatty acid binding protein (Fabp), forkhead box O1 (Foxo1), glycosynen synthase (Gs), hydroxacyl-CoA dehydrogenase (Had), ketoglutarate dehydrogenase (Kgdh), lactate dehydrogenase A (Ldh), and lipoprotein lipase (Lpl), were obtained from published reports (38, 39). All samples were tested in duplicate, and the relative quantities of different mRNA transcripts were calculated after normalization of the data against β-actin, an endogenous control that displayed unaltered expression, using the standard curve method. mRNA expression of Gapdh was also tested and showed similar results as β-actin (Hjeltines N, Krook A, Widegren U, unpublished data). The specificity of the primers was verified by checking the size of the amplicon expected in the real-time PCR reaction.

Western blot analysis. Portions of skeletal muscle biopsies (∼25 mg) were homogenized in 0.5 ml ice-cold lysis buffer containing 20 mM Tris (pH 8.0), 137 mM NaCl, 2.7 mM KCl, 10 mM NaF, 1 mM MgCl2, 1 mM Na3VO4, 0.2 mM PMSF, 10% glycerol, 1% Triton X 100, 1 μg/ml aprotinin, and 1 μg/ml leupeptin by dounce homogenizer. Homogenates were solubilized by end-over-end mixing at 4°C for 60 min and subjected to centrifugation at 12,000 g at 4°C for 10 min. Total protein was determined using a commercially available kit (Pierce), and proteins (50 μg) solubilized in Laemmli sample buffer were separated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Conventional Western blot analysis was performed using the following antibodies: HK2 (102 kDa) and GS (81 kDa), kind gifts from O. Pedersen (Steno Memorial Hospital, Gentofte, Denmark); pyruvate dehydrogenase kinase 4 (PDK4; 46 kDa; Abgent, San Diego, CA); GAPDH (40 kDa; Santa Cruz Bio-technology, Santa Cruz, CA); and ENO1 (47 kDa) and FOXO1 (70 kDa; Cell Signaling Technology, Danvers, MA). Antibodies against NADH-ubiquinol oxidoreductase, succinate-ubiquinol oxidoreductase, ubiquinol-cytochrome c oxidoreductase subunit II, cytochrome c oxidase subunit I, and ATP synthase subunit (42, 73, 48, 57, and 60 kDa, respectively) were purchased from Molecular Probes (Eugene, OR). Proteins were visualized by chemiluminescence and quantified by densitometry. GAPDH protein was used as a loading control in Western blots instead of β-actin due to more consistent levels of GAPDH than β-actin in the protein extract of control and spinal cord injured subjects.

Statistical analysis. Data are reported as means ± SE. Differences between the two groups were determined by unpaired Student’s t-test. Significance was accepted at P < 0.05.

RESULTS

Spinal cord injury is associated with changes in skeletal muscle mRNA expression of genes regulating glucose metabolism. Transport of glucose into the cytoplasm and further storage involves facilitative transport by the insulin-sensitive GLUT4 and subsequent phosphorylation by HK2. A tendency of Glut4 mRNA levels (27% reduction; P = 0.07) and significant reduction of Hk2 mRNA levels (68%; P < 0.05) was noted in skeletal muscle from spinal cord injured subjects compared with able-bodied control subjects (Fig. 1A). Furthermore, mRNA expression of Gs was reduced by 56% (P < 0.05) in spinal cord injured subjects compared with able-bodied individuals. Additionally, other genes that are critical for glucose utilization were also reduced in a coordi-
nated fashion in the spinal cord injured subjects. In skeletal muscle from spinal cord injured individuals, the mRNA expression of skeletal muscle Eno3 was increased by 69% ($P < 0.001$), and Pk and Ldha mRNA level were elevated by 42% ($P < 0.05$) and 51% ($P < 0.05$), respectively (Fig. 1B).

**Altered protein content of glucose metabolic enzymes in spinal cord injured subjects.** Spinal cord injury resulted in a profound reduction in protein content of skeletal muscle HK2 (89%; $P < 0.005$), while protein content of GS was less, but significantly, reduced by 21% ($P < 0.05$). Furthermore, a striking reduction of 83% ($P < 0.005$) in PDK4 protein content was observed in skeletal muscle from subjects with spinal cord injury compared with able-bodied controls. In accordance with Eno3 mRNA expression, there was a trend ($P = 0.1$) for increased enolase protein expression in the spinal cord injured individuals (Fig. 2).

**Spinal cord injury suppressed mRNA expression of genes critical for lipid metabolism.** The transport of free FA into the cytoplasm involves LPL, which liberates FA from lipoprotein in the vascular space, for further transport across the plasma membrane into the cytoplasm where FA binds to FABP. In skeletal muscle from spinal cord injured subjects, mRNA abundance of Lpl and Fabp was reduced by 42% ($P < 0.05$) and 34% ($P < 0.05$), respectively (Fig. 3A). Distal steps of lipid oxidation include transfer of fatty acyl-CoA into the mitochondria, by CPTI and CPTII, and β-oxidation, which involves 3-HAD. Spinal cord injury caused a consistent down-regulation in the mRNA abundance of Cpt1 (32%; $P < 0.05$; Fig. 3A), Cpt2 (27%; $P < 0.05$), and Had (46%; $P < 0.005$). Consistent with the decreased expression of lipid-oxidative genes, the mRNA level of Kgdh was reduced 48% ($P < 0.05$) in spinal cord injured subjects relative to the able-bodied controls (Fig. 3B).

**Reduction in protein content of oxidative phosphorylation enzymes in spinal cord injured subjects.** Spinal cord injury led to a marked reduction in the protein content of enzymes critical for oxidative phosphorylation. There was a coordinated striking reduction in mitochondrial proteins for oxidative phosphorylation in spinal cord injured individuals relative to control (Fig. 4), including NADH-ubiquinol oxidoreductase (90%; $P < 0.001$), succinate-ubiquinol oxidoreductase (66%; $P < 0.001$), ubiquinol-cytochrome c oxidoreductase subunit II (91%; $P < 0.001$), cytochrome c oxidase subunit I (70%; $P < 0.0001$), and ATP synthase subunit (65%; $P < 0.0001$).

**Spinal cord injury associates with an enhanced FOXO1 mRNA and protein expression.** FOXO transcription factors have been implicated in the development of skeletal muscle atrophy. Consistent with this notion, there was a marked elevation in Fxo1 mRNA (223%; $P < 0.005$; Fig. 5A) and FOXO1 protein expression (326%; $P < 0.005$; Fig. 5B) in skeletal muscle from spinal cord injured individuals. However, the mRNA abundance of atrogin-1 (also known as muscle

---

Fig. 2. Altered protein content of enzymes involved in glucose utilization. Protein content of HK2, GS, pyruvate dehydrogenase kinase 4 (PDK4), and ENO in skeletal muscle from able-bodied control subjects (closed bars) and subjects with spinal cord injury (open bars) was determined by Western blot analysis. Representative Western blots of protein content are presented at top. Equal sample loading was confirmed by blotting for GAPDH. Data are expressed as percentage of protein content in control subjects. Data are means ± SE for $n = 9–10$ muscles. *$P < 0.05$, **$P < 0.005$, compared with control.

Fig. 3. Expression profile of regulatory genes for lipid metabolism. Level of mRNA expression of genes critical for lipid metabolism in skeletal muscle from able-bodied control subjects (closed bars) and subjects with spinal cord injury (open bars) was determined by real time PCR. Data are expressed as percentage of mRNA level of able-bodied control subjects. A: Lpl, lipoprotein lipase; Fabp, fatty acid binding protein; Cpt1, carnitine palmitoyltransferase 1; B: Cpt2, carnitine palmitoyltransferase 2; Had, hydroxyacyl-CoA dehydrogenase; Kgdh, ketoglutarate dehydrogenase. Data are means ± SE for $n = 9–10$ muscles. *$P < 0.05$, **$P < 0.05$, compared with control.

Fig. 4. Protein content of enzymes that are critical for mitochondrial oxidative metabolism in skeletal muscle from able-bodied control subjects (closed bars) and subjects with spinal cord injury (open bars) was determined by Western blot analysis. Data are expressed as percentage of protein content in able-bodied control subjects. Data are means ± SE for $n = 9–10$ muscles. Spinal cord injury resulted in reduction of mitochondrial protein. Representative Western blots of protein content for NADH-ubiquinol oxidoreductase (NUO), succinate-ubiquinol oxidoreductase (SUO), ubiquinol-cytochrome c oxidoreductase subunit II (COREII), cytochrome c oxidase subunit I (COX1), and ATP synthase α-subunit in skeletal muscle from able-bodied control subjects and subjects with spinal cord injury are presented at top. Equal sample loading was confirmed by blotting for GAPDH.
in spinal muscle from able-bodied control subjects (open bars) was determined by real-time PCR. In Fig. 5A, we report that spinal cord injury is associated with marked reduction in mRNA and protein content of enolase 2 (ENO2) and cathepsin L1 (Cts), target genes of FOXO1. Expression of the atrophy-related FOXO1 (Foxo1), and cathepsin L1 (Cts), target genes of FOXO1, was decreased in skeletal muscle from subjects with spinal cord injury (open bars) compared with able-bodied control subjects. Thus the coordinated reduction in mRNA and protein content of enzymes critical for lipid-oxidative metabolism in spinal cord injured subjects parallels the aberrant body composition, which together with increased expression of uncoupling proteins, may explain the aberrant thermoregulation observed in these subjects.

Fig. 5. Expression of genes involved in skeletal muscle atrophy. A: level of mRNA expression of forkhead box O1 (Foxo1), atrogin-1, and cathepsin L1 (Cts) in skeletal muscle from able-bodied control subjects (closed bars) and subjects with spinal cord injury (open bars) was determined by real-time PCR. B: protein content of FOXO1. Data are expressed as percentage of mRNA or protein levels of able-bodied control subjects. Data are means ± SE for n = 9–10 muscles. **P < 0.005, compared with control.

Atrophy F-box; MAFbx) and Cts, target genes of FOXO1, was unaltered in the spinal cord injured subjects compared with able-bodied individuals (Fig. 5A).

DISCUSSION

In this study, we report that spinal cord injury is associated with a marked downregulation of the expression of genes that are critical for lipid oxidation and glycogen storage in skeletal muscle. Furthermore, spinal cord injury also leads to a profound reduction in mitochondrial proteins that are essential for oxidative phosphorylation. The coordinated downregulation of gene expression was accompanied by consistent upregulation of glycolytic genes and marked increase in the gene and protein expression of the atrophy-related FOXO1 (Foxo1).

Metabolic gene programming in skeletal muscle is highly flexible, and the expression profile changes in response to physiological and pathological conditions (7). During fasting conditions when FA oxidation is increased, mRNA expression of Lpl (10, 17, 33, 38) and Cpt1 (17, 33, 38), genes that are essential for FA metabolism, increases in skeletal muscle. Augmented mRNA expression and protein content of Lpl (28, 42) and Cpt1 (44) have also been observed following exercise, consistent with a greater reliance of skeletal muscle on FA metabolism. In contrast, data presented in this study demonstrate that genes involved in FA metabolism were coordinately downregulated in subjects with chronic spinal cord injury, characterized by physical inactivity. The genes investigated are involved in multiple steps of skeletal muscle FA metabolism, including transport of FA into the myofibers (LPL and FABP), transport of FA into the mitochondria (CPT1 and CPT2), as well as oxidation of FA (HAD and KGDH). Therefore, expression of genes along the FA metabolic pathway is remarkably adaptive to nutrients and functional cues, and skeletal muscle disuse and inactivity in subjects with spinal cord injury are associated with marked reduction in the expression of lipid metabolic genes.

The capacity of FA to serve as energy substrate relies on mitochondrial oxidative phosphorylation to generate ATP from NADH and flavin adenine dinucleotide 2 derived from FA oxidation. In parallel with the coordinated downregulation of lipid metabolic genes, mitochondrial proteins of oxidative phosphorylation are consistently and markedly reduced for complex I through V. Given the central role of mitochondria in lipid metabolism and energy production, the current results of a consistent reduction in lipid metabolic genes and mitochondrial protein content coincide with the findings that individuals with chronic tetraplegia are characterized by increased body fat (3, 18) and reduced whole body specific heat production (2). Interestingly, we (19) have previously shown that mRNA expression of two other mitochondrial proteins, uncoupling protein 2 and 3, was increased in skeletal muscle from tetraplegic individuals compared with able-bodied subjects. Thus the coordinated reduction in mRNA and protein content of enzymes critical for lipid-oxidative metabolism in spinal cord injured subjects parallels the aberrant body composition, which together with increased expression of uncoupling proteins, may explain the aberrant thermoregulation observed in these subjects.

In skeletal muscle from spinal cord injured subjects, mRNA expression of genes essential for glucose storage (Hk2 and Gs) was consistently downregulated. Conversely, mRNA of genes involved in glucose utilization (Eno, Pk, and Ldh) was increased, which was also supported by a trend for an increased ENO protein expression. The data suggest that at the transcriptional level spinal cord injury alters gene expression patterns favoring reduced glucose storage and an increased glucose utilization. In parallel with the reduced mRNA expression, the protein content of HK2 and GS was reduced in skeletal muscle from spinal cord injured subjects. A marked decrease in the protein content of PDK4, an enzyme that plays a role in the suppression of glucose oxidation through the inhibition of pyruvate dehydrogenase, was also noted. Despite these alterations in key enzymes regulating glucose utilization, skeletal muscle glycogen content was comparable to able-bodied control subjects in a similar cohort of patients with spinal cord injury (3). Our current data and previous findings therefore suggest that transcriptional changes in the glucose metabolic gene program in subjects with spinal cord injury are not sufficient to suppress glycogen storage in skeletal muscle. Furthermore, in vitro glucose transport in vastus lateralis muscle biopsies obtained from subjects with spinal cord injury demonstrates a comparable rate of insulin-stimulated glucose transport compared with able-bodied control subjects (3). Therefore, posttranslational covalent and allosteric regulation of GLUT4, HK2, and GS may be sufficient and/or upregulated in skeletal muscle from individuals with spinal cord injury to maintain normal glycogen storage. Further, HK2 is critical for both glucose utilization and storage, and its reduced gene expression appeared to be inconsistent with increased expres-
sion of glycolytic genes. Nonetheless, mice with 50% reduction of HK (HK+/−) have reduced skeletal muscle glycogen content (14). Despite this, carbohydrate utilization in the HK+/− mice was unaltered under resting condition and even increased during exercise. Thus this mouse model provides evidence that glucose utilization is less sensitive to a reduction in Hk2 gene expression compared with glucose storage.

Our finding of a tight coordination in the expression of glucose and lipid metabolic genes is consistent with the notion that genes along a linear metabolic pathway are coexpressed to favor an efficient control of metabolic flow (23). It has been proposed that such coordination in transcriptional control is achieved by the regulation of gene promoters sharing a common set of transcription effectors (23). Indeed, a wide array of lipid metabolic genes such as Lpl, Cpt1, Cpt2, Had, and Pdk4 are regulated by peroxisome proliferator-activated receptor-α (PPAR) (35). In mouse skeletal muscle, transgenic overexpression of PPARα promotes the expression of Cpt1, Had, and Pdk4 (12). Furthermore, skeletal muscle mitochondrial biogenesis is induced in transgenic mice of either peroxisome-proliferator-activated receptor-γ coactivator 1α (PGC1α; Ref. 32) or activated PPARδ (45). Conversely, genetic ablation of PGC1α impaired expression of genes important for mitochondrial biogenesis (6, 31) and respiration (31). Transgenic or pharmacological activation of PPARδ also induced mitochondrial gene expression in mouse skeletal muscle, including Cpt1 and multiple Cox genes (45). Taken together, these studies in mouse models provided evidence that the expression of skeletal muscle lipid metabolic and mitochondrial genes is regulated by PPARα, PPARδ, and PGC1α. Consistent with our current findings of a striking reduction in lipid metabolic genes and mitochondrial proteins, we have previously reported that the mRNA expression of PPARα, PPARδ, and PGC1α are reduced in skeletal muscle from spinal cord injured subjects (29). Therefore, the alteration in lipid and mitochondrial gene expression is associated with reduced expression of transcriptional effectors that are critical for these genes in spinal cord injured individuals.

When skeletal muscle is inactivated for an extended period of time, the muscle fiber type composition shifts so that type II fibers predominate (8, 9, 34). Thus longstanding peripheral skeletal muscles in spinal cord injured (tetraplegic) subjects are dominated by IIb fibers (3, 20, 34) [2 ± 1% type I, 35 ± 7% type IIa, and 64 ± 8% type IIb; Ref. 20] or more recently referred to as IIx fibers (4, 11, 43), compared with a mixed fiber type composition in able-bodied subjects (33% type I, 45% type IIa, and 32% type IIb; Ref. 3). The reciprocal increase of glucose metabolic genes and decrease in lipid metabolic genes observed in this study provide evidence for a strong coupling of fiber-type shift and metabolic gene expression program in the spinal cord-injured subjects. This finding is in contrast to most studies involving endurance-trained human subjects that have shown weaker correlation of the two major adaptations of skeletal muscle, whereby increases in metabolic enzyme activities are only coupled with less dramatic changes on the fiber type transformation (37).

Besides a change in the regulation of substrate metabolism, skeletal muscle mass per se has also been implicated as an important factor in the maintenance of glucose homeostasis. A decline in muscle mass decreases substrate storage capacity and decreases energy expenditure, thus potentially worsening an aberrant metabolic phenotype. Thus transcriptional control plays an important role in the regulation of skeletal muscle growth and atrophy (40). The FOXO subfamily has been implicated in control of diverse cell fates, including proliferation, differentiation, and metabolism (1, 16). In many differentiating cells, a transient inhibition of FOXO activity is required to exit from the cell cycle and initiation of terminal differentiation program. In skeletal muscle, FOXO1 plays an important role in regulating skeletal muscle differentiation through the phosphatidylinositol 3-kinase-AKT pathway (22). Mice with muscle-specific expression of the FOXO1 transcription factor display a marked reduction in muscle mass and an elevated expression of the lysosomal proteinase cathepsin L (25). Conversely, inhibition of FOXO transcription factors in myotubes by RNAi or dominant-negative construct rescued the myotubes from glucocorticoid-induced atrophy, as well as suppressed starvation-induced expression of atrogin-1 (41), a ubiquitin ligase involved in proteasomal degradation of protein. Collectively, induction of Foxo transcription factors is associated with skeletal muscle atrophy and the expression of genes such as cathepsin L and atrogin-1 that are involved in lysosomal and proteasomal degradation of skeletal muscle proteins, respectively. In skeletal muscle from subjects with spinal cord injury, there is a marked increase in the FOXO1 protein content, as well as in the mRNA expression of Foxo1, consistent with the phenotype of Foxo1 overexpressing transgenic mice (25). Decreased expression of Foxo1 and atrogin-1 mRNA and protein has been noted in subjects with spinal cord injury (30), and the authors speculate that this may reflect an internal mechanism to prevent further loss of muscle mass. The reason for the different results regarding Foxo1 and atrogin-1 expression compared with those obtained in our current study is not clear. We did not observe the expected increase in the expression of cathepsin L and atrogin-1. Since the subjects studied have a long history of spinal cord injury (6–23 yr), we speculate that cathepsin L and atrogin-1 might be upregulated during the earlier phase of muscle atrophy development, while the transcription regulator Foxo1 remains elevated and sustains the reduced muscle mass.

Our data provide evidence that spinal cord injury is associated with coordinated changes in the expression of genes, including a suppression of genes involved in FA utilization and glucose storage, as well as a reciprocal upregulation of genes involved in glucose utilization. Our results demonstrate that skeletal muscle atrophy caused by spinal cord injury is characterized by an increase in FOXO1 expression. Thus spinal cord injury leads to a profound and tightly coordinated alteration in the metabolic gene expression program in skeletal muscle.

GRANTS

This study was supported by grants from the Swedish National Center for Research in Sports, Swedish Medical Association, Magnus Bergvall Foundation, Lars Hierta Foundation, Fredrik and Ingrid Thuring Foundation, Tore Nilson Foundation for Medical Research, Åke Wiberg Foundation, Swedish Research Council, European Foundation for the Study of Diabetes, European Research Council, Swedish Diabetes Association, Novo Nordisk Research Foundation, and Commission of the European Communities (contract No. LSHM-CT-2004-00272 EXGENESIS and contract No. LSHMCT-2004-512013 EUGENE) supported this research.

Current address for Y. Long: Eli Lilly and Lilly Corporate Center, Indianapolis, IN 46285.


