Eur J Appl Physiol (2010) 109:709–720
DOI 10.1007/s00421-010-1392-z

ORIGINAL ARTICLE

Impact of treadmill locomotor training on skeletal muscle IGF1 and myogenic regulatory factors in spinal cord injured rats

Min Liu · Jennifer E. Stevens-Lapsley · Arun Jayaraman · Fan Ye · Christine Conover · Glenn A. Walter · Prodip Bose · Floyd J. Thompson · Stephen E. Borst · Krista Vandenborne

Accepted: 3 February 2010 / Published online: 7 March 2010

Abstract The objective of this study was to determine the impact of treadmill locomotor training on the expression of insulin-like growth factor I (IGF1) and changes in myogenic regulatory factors (MRFs) in rat soleus muscle following spinal cord injury (SCI). Moderate, midthoracic (T_8) contusion SCIs were produced using a NYU (New York University) impactor. Animals were randomly assigned to treadmill training or untrained groups. Rats in the training group were trained starting at 1 week after SCI, for either 3 bouts of 20 min over 1.5 days or 10 bouts over 5 days. Five days of treadmill training completely prevented the decrease in soleus fiber size resulting from SCI. In addition, treadmill training triggered increases in IGF1, MGF and IGFBP4 mRNA expression, and a concurrent reduction of IGFBP5 mRNA in skeletal muscle. Locomotor training also caused an increase in markers of muscle regeneration, including small muscle fibers expressing embryonic myosin and Pax7 positive nuclei and increased expression of the MRFs, myogenin and MyoD. We concluded that treadmill locomotor training ameliorated muscle atrophy in moderate contusion SCI rats. Training-induced muscle regeneration and fiber hypertrophy following SCI was associated with an increase in IGF1, an increase in Pax7 positive nuclei, and upregulation of MRFs.

Keywords Spinal cord injury · Locomotor training · Skeletal muscle · IGF1 · Muscle regeneration

Communicated by Susan Ward.

M. Liu · J. E. Stevens-Lapsley · A. Jayaraman · F. Ye · K. Vandenborne (✉)
Department of Physical Therapy, University of Florida, P.O. Box 100154, Rm 1142 PHHP Bldg, Gainesville, FL 32610, USA
e-mail: kvandenb@phhp.ufl.edu

P. Bose · F. J. Thompson
Department of Neuroscience, University of Florida, Gainesville, FL, USA

G. A. Walter
Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA

J. E. Stevens-Lapsley · P. Bose
North Florida/South Georgia Veterans Health System, Gainesville, FL, USA

C. Conover · S. E. Borst
Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL, USA

C. Conover · S. E. Borst
Geriatric Research, Education and Clinical Center, North Florida/South Georgia Veterans Health System, Gainesville, FL, USA

J. E. Stevens-Lapsley
Physical Therapy Program, Department of Physical Medicine and Rehabilitation, University of Colorado, Aurora, CO, USA

Introduction

Skeletal muscle is a highly dynamic tissue and has an intrinsic ability to adapt to external stimuli such as limb loading and unloading, neural activity, mechanical stimulation, substrate supply or changes in hormonal levels. Following spinal cord injury (SCI), skeletal muscles below the level of injury show a significant reduction in size, loss of strength and a fiber type shift from slow to fast due to the disruption of neural input as well as decreased loading (Hutchinson et al. 2001; Liu et al. 2008; Stevens et al. 2006). However, these changes can be reduced by therapeutic interventions involving exercise and loading

(Dupont-Versteegden et al. 1998; Reese et al. 1994; Roy et al. 1998; Stevens et al. 2006).

Locomotor training is an experimental gait retraining program that incorporates stepping on a treadmill with body weight support and manual assistance, and has emerged as a promising rehabilitation intervention to improve motor recovery after incomplete SCI. The underlying premise of locomotor training relates to the hypothesis that rhythmic loading of the limbs and force feedback from the lower extremity muscles induces task appropriate activity-dependent plasticity. The improved locomotion is also associated with reduced inhibitory spinal neurotransmission (Edgerton et al. 2001) and increased expression of trophic factors in the spinal cord as well as skeletal muscle (Gomez-Pinilla et al. 2001). A number of studies in both animals and individuals with SCI suggest that treadmill locomotor training provides a sufficient training stimulus to improve muscle mass and function (Dupont-Versteegden et al. 1998; Gregory et al. 2007; Reese et al. 1994; Roy et al. 1998; Stevens et al. 2006). In particular extensor muscles, such as the soleus, which are highly recruited during treadmill training have shown muscle growth during treadmill exercise (Dupont-Versteegden et al. 1998; Reese et al. 1994; Roy et al. 1998; Stevens et al. 2006).

Muscle growth and regeneration induced by exercise training occurs as a result of a net protein gain, and involves an increased activation of satellite cells. Myofibers undergoing hypertrophy appear to require new sources of nuclei to maintain a constant myonuclear domain (Chambers and McDermott 1996). As such, the number of nuclei has been suggested to increase to maintain the ratio of DNA to protein (Kadi et al. 1999). Satellite cells are the myogenic progenitor cells and the probable source of new myonuclei in skeletal muscle (see review, Zammit 2008). Increased loading activates satellite cells, which differentiate into myoblasts (Adams et al. 1999; Allen et al. 1995; Bickel et al. 2005) and ultimately fuse with each other to form new myofibers or with existing myofibers to induce muscle hypertrophy. This process is characterized by the expression of a series of myogenic regulatory factors (MRFs) and growth factors. Insulin-like growth factor I (IGF1) plays a primary role in regulating muscle mass and repair (Adams 1998; Zdanowicz et al. 1995). Overexpression of IGF1 in transgenic mice results in marked myofiber hypertrophy (Coleman et al. 1995), while mouse lines lacking IGF1 (and/or its receptor) demonstrate abnormal muscle development (Liu et al. 1993). Similarly, local infusion or overexpression of IGF1 using viral delivery causes significant muscle hypertrophy (Adams 1998), and reverses age-related loss of muscle mass in mice (Barton-Davis et al. 1998). Moreover, IGF1 levels have also been shown to increase in regenerating muscles and muscles undergoing stretch-induced or overloading hypertrophy (Adams and Haddad 1996; Goldspink et al. 1995; Levinovitz et al. 1992). Several studies suggest that IGF1 promotes both proliferation and differentiation of satellite cells and increases protein synthesis (Coleman et al. 1995; Engert et al. 1996; Florini et al. 1996). The actions of IGF1 are regulated and modified by a family of binding proteins (IGFBPs). Although seven different IGFBPs have been discovered, IGFBP4 and IGFBP5 are suggested to be muscle specific (Awede et al. 1999; Florini et al. 1996). It is generally known that these binding proteins can modulate interaction with the IGF1 receptor and act as a localized storage depot for IGFs (Florini et al. 1996).

The purpose of this study was twofold. The first objective of this study was to determine the impact of treadmill locomotor training on the expression of IGF1 and its associated receptor and binding proteins in the rat soleus muscle following moderate midthoracic (T_8) spinal cord contusion injury. A second objective was to monitor muscle regeneration and changes in MRFs in rats following SCI with and without treadmill locomotor training.

Materials and methods

Animals

Thirty adult Sprague-Dawley rats (female, 16–20 weeks, weighing 260–300 g at the start of this study; Charles Rivers Laboratory) were studied. All procedures were performed in accordance with the US Government Principle for the Utilization and Care of Vertebrate Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida. Rats were randomly assigned to one of the five groups.

- Control
- SCI-8D (sacrificed 8 days after SCI)
- SCI-8D-TM (sacrificed 8 days after SCI and performing three 20 min bouts of treadmill training over the previous 1.5 days)
- SCI-14D (sacrificed 14 days after SCI)
- SCI-14D-TM (sacrificed 14 days after SCI and performing ten 20-min bouts of treadmill exercise over the previous 5 days)

Spinal cord injury

Spinal cord contusion injuries were produced using an NYU (New York University) impactor as described previously (Stevens et al. 2006). Briefly, a 10 g weight was dropped from a 2.5-cm height onto the T_8 segment of the spinal cord which was exposed by laminectomy. The entire procedure was carried out under sterile conditions. All

injuries were performed under ketamine (100 mg/kg)–xylazine (6.7 mg/kg) anesthesia. Animals received two doses of Ampicillin (100 mg/kg) per day for 5 days starting on the day of surgery. To prevent dehydration, subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given buprenorphine (0.05 mg/kg) and ketoprofen (5.0 mg/kg subcutaneously) for pain and inflammation over the first 36 h after SCI. The animals were kept under vigilant postoperative care, including daily examination for signs of distress, weight loss, dehydration, and bladder dysfunction. Manual expression of bladders was performed 2–3 times daily until spontaneous voiding returned, and animals were monitored for the possibility of urinary tract infection. Animals were housed in pairs with the exception of the first few hours following surgery.

At post-operative day 7, open field locomotion was assessed using the Basso–Beattie–Bresnahan (BBB) locomotor scale (Basso et al. 1995) to determine if the animals met inclusion criteria for this study. This was done to ensure a relative homogenous animal study group (i.e. animals that did not fall within a preset range (scores 3–7) were regarded as too mildly or severely injured and were excluded from the study).

The BBB scale is an operationally defined ordinal scale that measures locomotor behavior. Each of the components of the 21-point scale is based on specific features of locomotor recovery after spinal cord contusion including joint movement, trunk posture, stepping and weight support ability, paw position, and tail position. The score order from 0 to 21 is based on the observation that after spinal cord contusion injury, rats progress through three general phases of recovery. The early phase is characterized by little or no hindlimb joint movement (scores 0–7). The intermediate phase includes bouts of uncoordinated stepping (scores 8–13). The late phase involves fine details of locomotion such as dragging of the toes and tail, trunk instability and rotation of the paws (scores 14–21). To perform the test, the animal was placed in a test apparatus, observed for 4 min, and scored in real time by two-blinded observers.

Treadmill locomotor training

Treadmill locomotor training began on post-operative day 7 (Kunkel-Bagden et al. 1993). There were two reasons for this. First, on day 7 the surgical staples were removed and soft tissue had healed sufficiently so that trauma was avoided at the incision site. Second, occasional appearance of red porphyrin expression around the eyes, a symptom associated with stress, disappeared within a week post-SCI. Therefore, animals could be trained without apparent discomfort and stress at this time.

Training consisted of assisted quadripedal treadmill stepping. Animals were trained twice a day for 20 min (morning and afternoon) with a minimum interval of 2 h between training sessions. Animals were given 5 min to explore the treadmill on the first training day and then encouraged to walk on the moving treadmill (11 m/min) (Kunkel-Bagden et al. 1993). Two trainers performed the animal treadmill training for the entire study. Every effort was made to standardize the training technique prior to implementation of this intervention. Body weight support was provided manually (base of tail) and consistently targeted to be the least possible amount needed for ambulation. The support was specifically adjusted to insure that the animals' hindlimbs did not collapse during locomotion. Typically, when rats had profound paraplegia, gait assistance was provided to place the rat hind paws in plantar stepping position during training.

Tissue harvest

At the time points indicated previously, the soleus muscles of both legs were dissected, carefully rinsed in cold PBS to remove excess blood, and snap-frozen at resting length in melting isopentane, pre-cooled in liquid nitrogen and stored at −80°C.

Immunohistochemistry and fiber size measurements

Cryostat sections (10 μm) in a transverse plane were prepared from the central portion of each muscle and mounted serially on gelatin-coated glass slides. Immunocytochemical reactions were performed on serial cryostat sections with anti-laminin and anti-MHC antibody at various dilutions. Rabbit anti-laminin (Neomarker, Labvision, Fremont, CA) was used to outline the muscle fibers for cross-sectional area (CSA) quantification. Four anti-MHC monoclonal antibody mAbs (BA-D5, SC-71, BF-F3, and BF-35) were selected on the basis of their reactivity toward adult MHC. Sections were incubated with rabbit anti-laminin and one of the anti-MHC antibodies (4°C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and FITC-conjugated anti-mouse IgG (Nordic Immunological Laboratories, Westbury, NY). Stained sections were mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4°C to diminish fading. Stained cross sections were photographed (10× magnification) by using a Leica DM LB fluorescence microscope with a digital camera (Leica Microsystems, Solms, Germany). The soleus fiber CSAs were analyzed using The NIH image J program (version 1.62; http://rsb-web.nih.gov/ij/) using immunocytochemical reactions with anti-laminin and rhodamine-conjugated anti-rabbit IgG. The pixels setting used for conversion of pixels to micrometers was 1.50 pixels–1 μm^2 for a 10× objective. The average fiber CSA of fast and slow fibers studied was determined.

To study soleus muscle regeneration, cryostat sections were incubated with rabbit anti-laminin and mouse anti-Embryonic myosin antibodies (DSHB, Iowa City, IA, 1:20) or mouse anti-Pax7 antibody (R&D System, Minneapolis, MN, 1:50) (4°C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and FITC-conjugated anti-mouse IgG. The average positive number per 100 muscle fibers was determined from a sample of 150–250 fibers selected across one muscle slice, acquired from the belly of each muscle.

RNA extraction, reverse transcription and TaqMan quantitative PCR analysis

RNA extraction

Total RNA was extracted from frozen soleus muscle samples using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the company's protocol. Briefly, approximately 30 mg of muscle was disrupted in 1.0 ml of TRIzol Reagent using a blade homogenizer. RNA was precipitated with isopropanol, washed with 75% ethanol and, after drying, suspended in DEPC-treated water and stored at −80°C. RNA was quantified by optical density at 260 nm and expressed as total RNA/tissue.

Reverse transcription

One microgram of total RNA was reverse transcribed using the SuperScript III First-Strand Synthesis for RT-PCR (Invitrogen Life Technologies, Carlsbad, CA). Since 18S served as internal standard, a mix of random primers (200 ng/reaction) and Oligo(dt) (100 ng/reaction) was used in the RT reaction in a 20 μl total reaction volume at 500°C for 50 min according to the company's protocol. At the end of the RT reaction, the tubes were heated at 85°C to terminate the reaction and then stored at −80°C for PCR reactions.

TaqMan quantitative PCR

cDNA was amplified in duplicate in SYBR Green PCR Master Mix (SuperArray Bioscience). The thermal cycling conditions included 94°C for 5 min, followed by 40 cycles of amplification at 94°C for 30 s and 60°C for 1 min. Primer sequences are provided in Table 1. Estimation of amplified gene products were normalized to 18S (housekeeping gene), compensating for variations in quantity as well as for differences in RT efficiency. Primers were tested for nonspecific amplicons and primer dimers by visualizing PCR products on 2% agarose gels before performing PCR with the same thermal cycler used for quantitative PCR. Only primers that did not generate nonspecific products and primer dimers were used for the qRT-PCR assay. The final primer concentration used in the amplification reaction was 500 nM.

Table 1 Primer sequences used for quantitative RT-PCR

Primer	Sequence	Genebank number
IGF1 (total)		
Forward	GGGCATTGTGGATGAGTGTTGCTT	NM_178866
Reverse	TGGAACGAGCTGACTTTGTAGGCT	
MGF		
Forward	GGAGGCTGGAGATGTACTGTGCT	X06108
Reverse	TCCTTTGCAGCTTCCTTTTCTTG	
IGF1R		
Forward	AGAGCGAGCTTCCTGTGAAAGTGA	NM_052807
Reverse	GTGCCACGTTATGATGATGCGGTT	
IGFBP4		
Forward	TCGGAAATCGAAGCCATCCAGGAA	NM_010517
Reverse	GGGTTGAAGCTGTTGTTGGGATGT	
IGFBP5		
Forward	ACGGCGAGCAAACCAAGATAGAGA	NM_012817
Reverse	TTCTGCGATCCTTCTTCACAGCCT	
MyoD		
Forward	CTACAGCGGCGACTCAGACG	M84176
Reverse	TTGGGGCCGGATGTAGGA	
Myogenin		
Forward	TGGTCCCAACCCAGGAGATCATTT	M24393
Reverse	ACATATCCTCCACCGTGATGCTGT	
Myf5		
Forward	CCCGAAAGAACAGCAGCTTTGACA	NM_008656
Reverse	AGACGTGATCCGATCCACAATGCT	

Detection of IGF1 protein concentration

Frozen soleus muscle tissue (15–20 mg) was very briefly rinsed with cold PBS to remove excess blood, homogenized in 0.5 mL of 1× PBS using FastPrep Homogenizer and Isolation System (Thermo Fisher Scientific, Franklin, MA) and stored overnight at −20°C. The homogenates were then centrifuged for 5 min at 5,000×*g*. IGF1 protein levels were measured in the supernatants using a commercially available ELISA kit specific for rodent IGF1 protein (R&D Systems, Minneapolis, MN). The IGF1 concentration was calculated based on a standard curve generated from recombinant mouse IGF1. This kit has been validated for the determination of rat IGF1 at 30–3,000 pg/ml with an intra-assay precision of ~4.3% and an inter-assay precision of ~6.0% (manufacturer's manual). All samples were measured on a micro-plate reader (Bio-Rad Laboratories, CA) at 450 nm. All samples were measured in duplicate.

Protein extraction and western analysis

Soleus muscles were homogenized in 10 volumes/muscle wet weight of modified lysis buffer (50 mM Tris–HCl pH 7.4, 1% wt/vol Triton X-100, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM $NaVO_4$, 1 mM NaF, 1 mM EGTA). Homogenates were centrifuged and the total protein was measured in the supernatant. Equal amounts of protein from each muscle lysate were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Immobilon-P, Millipore, Bedford, MA). Membranes were incubated in a blocking buffer [5% nonfat dry milk in Tris-buffered saline (TBS) plus 0.1% Tween 20 (5% milk-TTBS)] and then incubated in primary antibody diluted in 5% milk-TTBS overnight at 4°C. The following antibodies were used: MyoD (Sc-318), myogenin (Sc-576), and Myf5 (Sc-302) (Cell Signaling, Beverly, MA). Membranes were then washed in 5% milk-TTBS and incubated with horseradish peroxidase-conjugated secondary antibody. After a series of washes in 5% milk-TTBS, TTBS, and TBS, protein detection was performed using enhanced chemiluminescence and the Kodak mm4000 detection system. Analysis of the band intensity was performed using Image J.

Data analysis

All statistical analysis was performed using SPSS for Windows (Version 11.1.0). Results were expressed as mean ± standard error of mean (SEM). Research hypotheses were tested at an alpha level of 0.05. One-way analysis of variance was performed to determine differences between groups. Post hoc tests were performed using the Bonferroni–Dunn procedure for multiple pair-wise comparisons.

Results

IGF1 expression following SCI and treadmill locomotor training

We previously monitored the lower extremity muscles of moderate contusion injured SCI animals using MRI and measured significant muscle atrophy in all the lower hindlimb muscles (Liu et al. 2008). Similarly, using histological analysis in the current study, we found that at 2 weeks following moderate T8-contusion SCI, the cross-sectional area of type I and II fibers in the soleus muscle of untrained animals (SCI-14D) were significantly decreased (16.2–20%) compared to controls ($P < 0.05$, Table 2). However, no differences were found between the soleus fiber CSAs in trained SCI animals (SCI-14D-TM) and controls, indicating that treadmill training effectively ameliorated the decrease in fiber size following moderate contusion SCI.

Table 2 Soleus fiber type specific cross-sectional area (μm^2) in control and SCI animals (trained and untrained) at 14 days post-SCI

	CON	SCI-14D	SCI-14D-TM
Type I fibers	2,709 ± 205	2,122 ± 270*	2,795 ± 153
Type II fibers	1,624 ± 52	1,366 ± 81*	1,674 ± 96

* Significantly lower fiber cross-sectional areas were found in SCI-14D animals compared to SCI-14D-TM and control, $P < 0.05$

To further examine the role of IGF1 in regulating muscle mass and muscle regeneration, we measured the expression of IGF1 and its associated receptor and binding proteins after SCI and treadmill training. Total IGF1 and MGF (IGF1b) mRNA levels were unchanged at 8 days post-SCI, but significantly elevated at 2 weeks following SCI alone. At the same time points, mRNA for IGF1R and IGFBP4 mRNA were not significantly altered. In contrast, IGFBP5 mRNA progressively increased after SCI, with 2.5-fold higher values ($P < 0.05$) at 2 weeks post-SCI, compared to controls (Fig. 1).

One week of treadmill training had a significant effect on mRNA for IGF1 (total), MGF, IGF1R as well as the IGF1 binding proteins (Fig. 1) compared with untrained SCI controls. Specifically, quantitative RT-PCR showed a 2–3-fold increase ($P < 0.05$) in mRNA for IGF1 in the SCI-14D-TM animals and a tenfold increase in MGF mRNA in both SCI-8D-TM and SCI-14D-TM animals, compared to non-trained SCI animals. In addition, IGFBP4 and IGF1R mRNA expression were significantly elevated in the SCI locomotor-trained animals, while mRNA for IGF-BP5 was significantly decreased in the SCI-8D-TM and SCI-14D-TM animals. Interestingly, soleus muscle IGF1 protein levels, as measured by ELISA, were increased 2–3-fold in the SCI-8D and SCI-14D animals, and 8–9-fold in the SCI-8D-TM and SCI-14D-TM animals, compared to controls (Fig. 2).

Muscle regeneration and myogenic regulatory factors

To evaluate the impact of treadmill training on muscle regeneration after SCI, we stained the soleus muscles for embryonic myosin and paired box transcription factor seven (Pax7). In addition, we performed RT-PCR and western blotting of several MRFs. Immunolabeling of the soleus muscle revealed a significant increase in the incidence of small (fibers less than 700 μm^2) muscle fibers expressing embryonic myosin in the locomotor-trained SCI rats. As shown in Fig. 3, the proportion of fibers expressing embryonic myosin was 1.6 ± 0.9 and 6.4 ± 2.0% in the SCI-14D

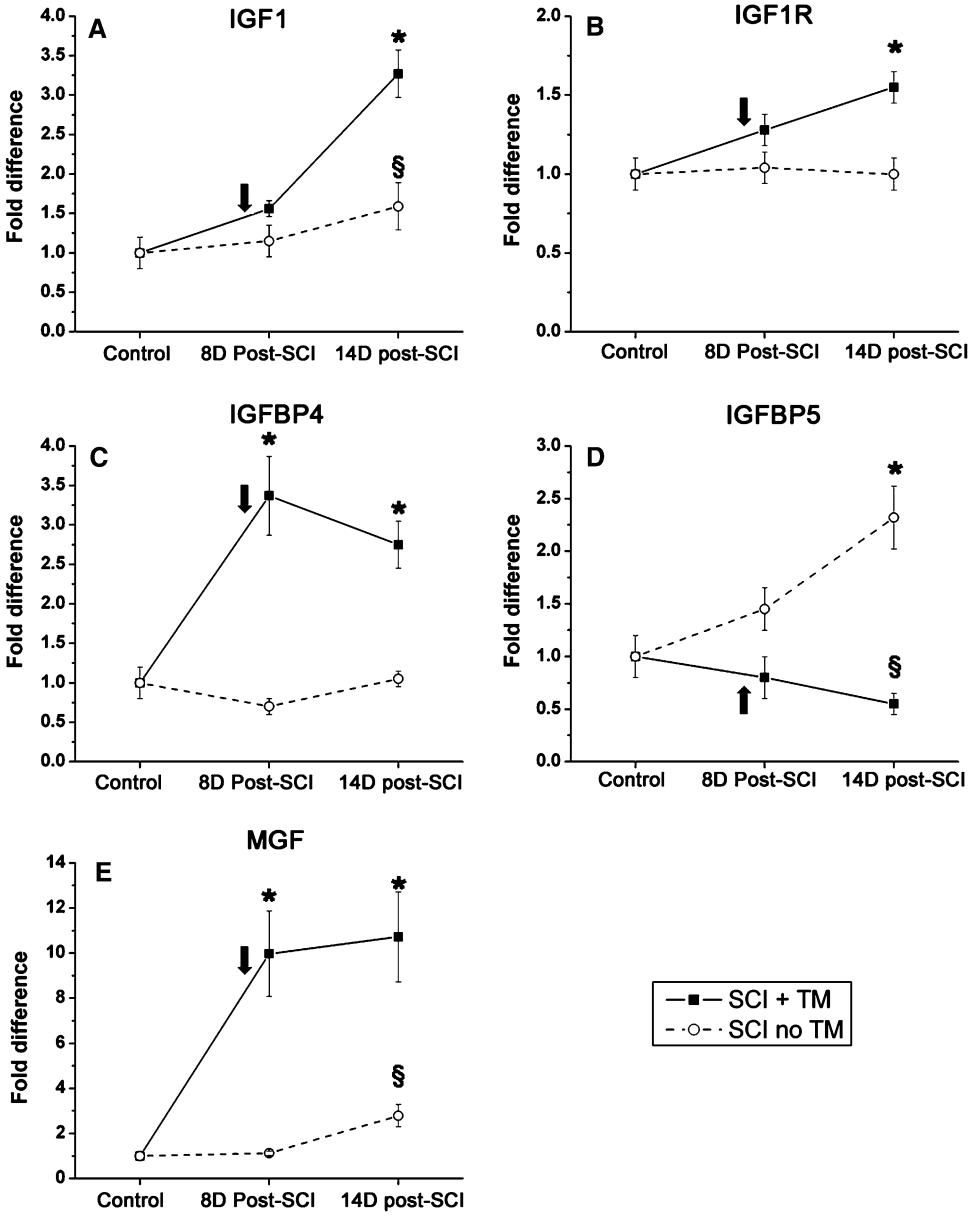

Fig. 1 IGF1 (total) (**a**), IGF1R (**b**), IGFBP4 (**c**), IGFBP5 (**d**) and MGF (**e**) mRNA expression in the soleus muscle of controls and SCI animals (trained = TM and untrained = no TM) at 8 days and 14 days post-SCI. *Significantly different compared to both control and untrained injured animals. §Significantly different compared to control animals. *Arrows* indicate when the training was started

and SCI-14D-TM animals, respectively. In contrast, we did not find any embryonic myosin positive fibers in the control soleus muscle fibers.

In muscle, Pax7 is specific for satellite cells (Allouh et al. 2008; Day et al. 2007). Pax7 is expressed by quiescent, active, and proliferating satellite cells (Relaix et al. 2005; Shefer et al. 2006). As shown in Fig. 4, the mean frequency of Pax7 positive myonuclei was ~5 per 100 fibers in normal control soleus muscles. The number of Pax7 positive myonuclei was increased in both the SCI-14D and SCI-14D-TM animals. However, greatest number of nuclei staining positive for Pax7 was noted after locomotor training with the number of myonuclei expressing Pax7 elevated fourfold in the SCI-14D-TM animals compared to controls.

To investigate the effect of SCI and treadmill training on MRFs in skeletal muscle, we first examined the expression of MyoD, myogenin and myf5 in the soleus muscle. As shown in Table 3, mRNA expression of MRFs in the soleus were not significantly altered following SCI alone, except for MyoD which showed an approximate twofold increase at 2 weeks post-SCI. However, treadmill training had a significant impact on both MyoD and myogenin gene expression in the spinal cord injured rats. The most prominent effect, a sixfold increase in MyoD mRNA, was noted at 8 days post-SCI after three bouts of locomotor training (SCI-8D-TM). In contrast, Myf5 expression did not show significant changes following treadmill training in SCI animals.

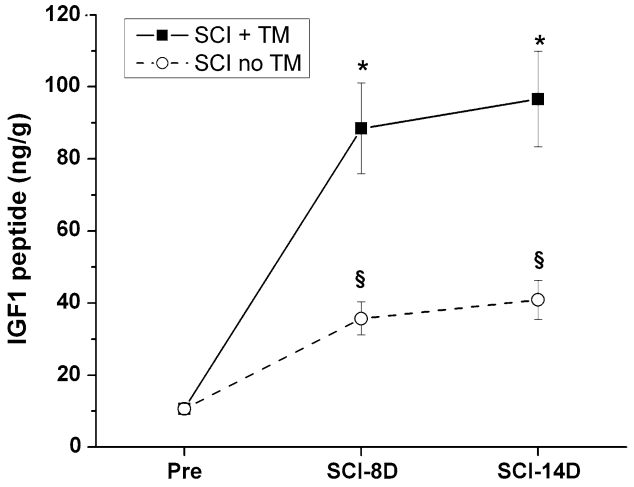

Fig. 2 IGF1 peptide concentration in soleus muscle for control and SCI animals at 8 days and 2 weeks post-SCI. *Significantly different compared to both control and untrained injured animals. §Significantly different compared to control animals

Protein levels of MyoD, myogenin and Myf5 were measured by immunoblotting of soleus muscle homogenates at 2 weeks post-SCI. As shown in Table 4, protein levels of MyoD and Myf5 were not altered following SCI without training, while myogenin levels were elevated ($P < 0.05$). Interestingly, myogenin levels were elevated ($P < 0.05$) in both the untrained and trained SCI animals. SCI-14D-TM animals also showed a significant increase in Myf5 levels, but not MyoD, as compared to control animals.

Discussion

Treadmill locomotor training significantly attenuates soleus muscle atrophy after SCI in rats. As expected, the soleus fiber CSA was significantly reduced 2 weeks following a moderate mid-thoracic contusion SCI. Five days of treadmill training completely prevented the reduction in fiber CSA, such that muscle fiber sizes were comparable between trained SCI and control animals. In addition, treadmill locomotor training triggered increases in IGF1, MGF, IGF1R and IGFBP4 mRNA expression, and decreased IGFBP5 expression in soleus muscle following SCI. Locomotor training also caused an increase in the number of small fibers expressing embryonic myosin, number of nuclei expressing Pax7, and increased mRNA expression of the MRFs myogenin and MyoD.

Skeletal muscle undergoes rapid atrophy following SCI. Using a rat spinal cord contusion injury model, we have previously found significant muscle atrophy in all rat hindlimb muscles (Stevens et al. 2006). In addition, muscle atrophy is more pronounced in paralyzed muscles that contain a large proportion of slow fatigue-resistant muscle fibers and are largely responsible for maintaining posture and bearing weight, such as the soleus muscle. However, muscle atrophy and dysfunction can be effectively reduced or reversed by inducing weight-bearing in spinal cord injured animals while they are stepping on a treadmill. A number of studies suggest that exercise (such as,

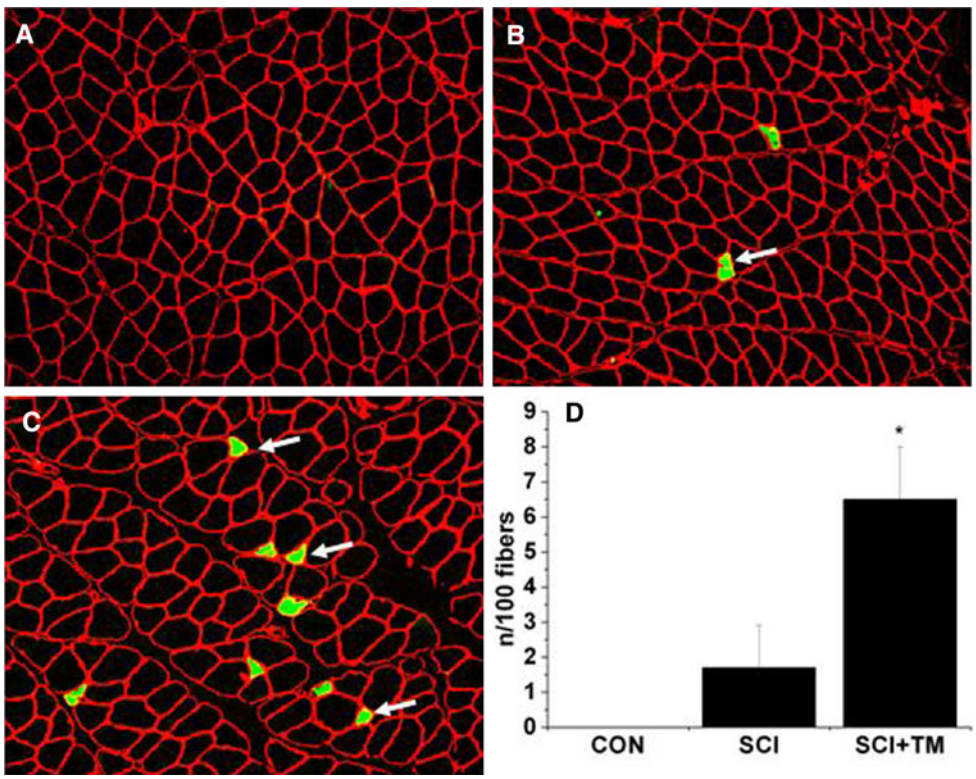

Fig. 3 Cross-section of soleus muscle stained with monoclonal antibody against embryonic myosin isoform. **a** Control soleus, **b** SCI-14D soleus, **c** SCI-14D-TM soleus, **d** Relative number of muscle fibers expressing embryonic myosin isoform. *Significantly different compared to untrained SCI animals. *Arrows* indicate positive fibers stained with monoclonal antibody against embryonic myosin isoform

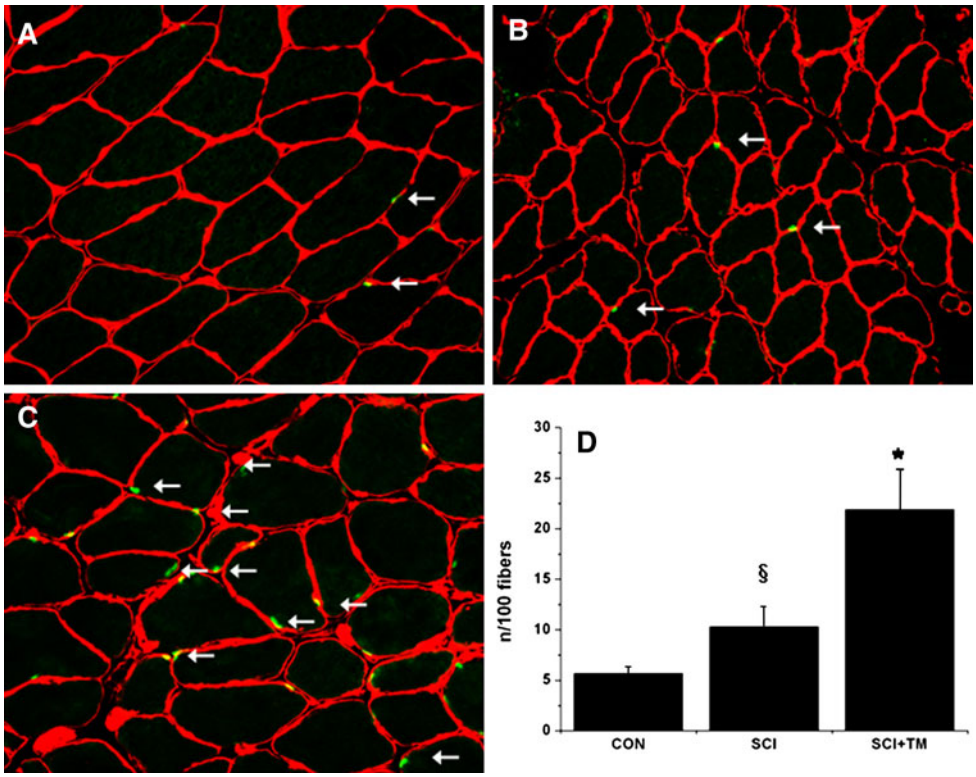

Fig. 4 Cross-section of soleus muscle stained with monoclonal antibody against Pax7. **a** Control soleus, **b** SCI-14D soleus, **c** SCI-14D-TM soleus, **d** Relative number of muscle fibers expressing Pax7. *Significantly different compared to both control and untrained injured animals. §Significantly different compared to control animals. *Arrows* indicate positive nuclei stained with monoclonal antibody against Pax7

Table 3 mRNA expression of MyoD, myogenin and Myf5 in the soleus muscle at 8 days and 2 weeks following SCI

	Control	No training		Treadmill training	
		SCI-8D	SCI-14D	SCI-8D	SCI-14D-TM
MyoD	1	−1.28	1.93*	6.42*	1.51*
Myogenin	1	−1.02	1.38	1.65*	1.97*
Myf5	1	−1.27	−1.19	1.37	1.22

Expressed as fold difference compared to the control muscle

"−" down-regulation

* Significantly different compared to control animals

Table 4 Protein levels of MyoD, myogenin and Myf5 in soleus muscle in control animals and at 2 weeks following SCI with or without training (n = 6/group)

	Control	SCI-14D	SCI-14D-TM
MyoD	0.63 ± 0.19	0.36 ± 0.12	0.60 ± 0.10
Myogenin	0.41 ± 0.11	1.00 ± 0.23*	0.99 ± 0.15*
Myf5	0.64 ± 0.12	0.85 ± 0.10	1.00 ± 0.13*

Expressed as ratio over GAPDH (internal standard)

* Significantly different compared to control animals

pedaling, stepping, and resistance training) can dramatically improve muscle size and function in spinal cord injured animals (Dupont-Versteegden et al. 1998; Reese et al. 1994; Roy et al. 1998; Stevens et al. 2006).

IGF1 has been shown to stimulate anabolic and myogenic processes and to exert a primary role in modulating muscle size during muscle growth. IGF1 stimulates protein metabolism in existing myofibers and increases the myonuclear number via the proliferation, differentiation and fusion of satellite cells (Glass 2005). However, the role of IGF1 in modulating muscle size during disuse or SCI has been less clear. To better understand the role of IGF1 in mediating muscle plasticity in paretic muscles following SCI and locomotor training, we measured the expression of IGF1 mRNA levels, as well as its associated receptor and binding proteins. We observed increases in both IGF1 mRNA and mRNA for MGF, the loading-sensitive isoform of IGF1 (Goldspink 1999) at 14 days post-SCI. In the meanwhile, we found around twofold increase in IGF1 peptide levels. Our findings are similar to that of Reardon et al. (2001) who reported that IGF1 mRNA is upregulated in human muscle during chronic disuse, and Kim et al. (2008) who found higher IGF1 and MGF mRNA levels in the soleus muscle at 5 days after spinal cord isolation. These results are somewhat different than those of Awede et al. (1999), who reported a transient decrease (30%) in soleus muscle IGF1 mRNA following 2 days hindlimb suspension in mice. Additionally, Zeman et al. (2009) recently found that IGF1 mRNA expression was unchanged in both EDL and soleus muscle after spinal cord transaction. Therefore, our findings further support the concept that muscle atrophy pathways do not necessarily mirror pathways involved in

muscle hypertrophy. This does, however, not imply that upregulating IGF1 in paretic muscles does not have therapeutic value for the preservation or restoration of muscle function.

In the present study, we determined that treadmill locomotor training significantly increased IGF1 and MGF expression in the soleus muscle after SCI. We found 2–3-fold higher levels of IGF1 mRNA and a 5–10-fold increase in mRNA for MGF in trained SCI animals compared to non-trained animals. In addition, treadmill-trained SCI animals showed approximately ninefold increases in IGF1 protein levels after three bouts and 5 days of training. The increase in MGF mRNA in the treadmill-trained animals was already prevalent after only three bouts of training (SCI-8D), while total IGF1 mRNA levels failed to show a significant change at this time point. MGF is known to be expressed locally in skeletal muscle through alternative splicing of the IGF1 gene, which occurs in up to 10% of the IGF-I transcripts (see review, Barton 2006). MGF plays a very important role in many biological aspects of muscle, including hypertrophy and muscle repair. Recent evidence suggests that MGF may act through a novel receptor, and its expression following exercise may be carried out in a growth hormone independent manner (Goldspink 2005). Yang et al. found that transfection of MGF into C2C12 cells increased cell proliferation, but it completely inhibited myotube formation (Yang and Goldspink 2002). Interestingly, MGF still increased myoblast proliferation after selective blocking of the IGF-I receptor, suggesting that MGF does not share the same receptor or regulatory pathway with IGF-IEa (Yang and Goldspink 2002). Other studies provide evidence that alterations in MGF expression in response to loading may occur earlier than changes in the IGF-1Ea isoform (Li et al. 2009; Yang et al. 1996).

While our study provides further evidence that loading and muscle activity plays an important role in elevating skeletal muscle IGF1 levels, less clear is how the cellular effects of IGF1 may be influenced by its associated receptor and binding proteins. When IGF1 binds to IGF1R, two signaling pathways are activated: the Ras-Raf-MEK-ERK pathway and the PI3K-Akt pathway, both of which are important in regulating muscle mass (Moelling et al. 2002; Murgia et al. 2000). These processes can be potentially mediated by IGF1 binding proteins. Among seven known IGF binding proteins, IGFBP4 and IGFBP5 have been shown to play an important role in skeletal muscle adaptations (Awede et al. 1999; Ewton et al. 1998; Florini et al. 1996). IGFBP4 is suggested to inhibit L6A1 muscle cell proliferation and differentiation induced by IGF1, whereas IGFBP5 exhibits both inhibitory and stimulatory actions (Ewton et al. 1998). Awede et al. (1999) reported that overloading-induced muscle hypertrophy of the soleus muscle in mice was associated with a doubling of IGFBP4 and significant down-regulation (~66%) of IGFBP5 mRNA level. Haddad and Adams (2002) reported that resistance exercise induced an increase of IGFBP4, with no changes in IGFBP5 in the rat medial gastrocnemius muscles. Similarly, Bickel et al. (2005) showed an increase in IGFBP4, but not IGFBP5 after a single bout of resistance training in patients with a SCI. Recently, Kim et al. (2008) found that IGFBP4 mRNA and IGFBP5 mRNA in both medial gastrocnemius and soleus muscles in spinal cord isolated animals were similar to control animals after 5 days of brief bouts stimulation. In the present study, IGFBP4 mRNA was significantly increased after three bouts as well as 5 days of treadmill locomotor training. In contrast, IGFBP5 mRNA was down regulated at both time points during locomotor training, with the largest changes after 5 days of training. While, the roles of the specific IGF1 binding proteins are still controversial, the mirror response in IGFBP5 mRNA observed during SCI-induced muscle atrophy and locomotor training, strongly suggests that IGFBP5 may be inhibitory to IGF1 function.

One interesting finding of this study was that locomotor training caused a significant increase in the number of small muscle fibers expressing embryonic myosin, the latter being a hallmark of muscle regeneration (Glass 2005). The proportion of fibers expressing embryonic myosin was 6.4 ± 2.0 and $1.6 \pm 0.9\%$ in the trained and untrained SCI animals, respectively. The size of fibers expressing embryonic myosin varies from 210 to 700 μm^2 compared to the average size of 2,709 μm^2 in the control muscle. It is generally known that mammalian skeletal muscle has the ability to regenerate completely following bouts of contraction-induced muscle damage (see review, Faulkner et al. 1993). However, we previously did not find any histological evidence of muscle injury in the soleus muscle of moderate contusion SCI animals during treadmill locomotor training (Liu et al. 2006).

To further investigate muscle regeneration following SCI and locomotor training, we studied the expression of several myogenic regulators in soleus muscle. The activation of satellite cells is a key event in skeletal muscle regeneration. During regeneration, satellite cells proliferate, differentiate and join to form new myofibers. Satellite cells also fuse with existing myofibers in response to increased loading, and this process appears essential for compensatory muscle hypertrophy (Adams et al. 1999; Dupont-Versteegden et al. 1998). To study the satellite cell pool in the soleus muscle following locomotor training, we examined the frequency of Pax7 positive nuclei. The Pax7 gene is a member of the paired box containing gene family of transcription factors implicated in development of the skeletal muscle. Mice lacking Pax7 appear normal at birth but show 50% decrease in body weight at 7 days compared to the wild-type mice. Pax7 is expressed in both quiescent and

activated muscle satellite cells (Seale et al. 2000). In the present study, we found that 1 week (5 days) of treadmill training dramatically increased (~fourfold) the number of myonuclei expressing Pax7. Interestingly, the soleus muscle of untrained SCI animals also showed a modest increase in the number of Pax7 positive myonuclei at 14 days after SCI. We speculate that the latter may be due to the self training that occurs in the incomplete moderate contusion injury. Basso et al. (1996) showed that after a moderate thoracic spinal cord contusion injury, animals demonstrated hindlimb paralysis until 7 days post injury, which is followed by a progressive recovery in locomotor function over the next 5 weeks. Using the same model, we also found spontaneous reversal of muscle atrophy (Liu et al. 2008). Therefore, the posterior soleus muscle in moderate contusion SCI animals may experience sufficient loading induced by self training to initiate satellite cell activation.

Muscle regeneration is controlled by the MRFs (such as MyoD, myogenin and MRF5). It has been apparent that MRFs play a role in the regulation of muscle responses to changes in limb loading and/or activity. Recently, studies have shown that MRFs can also be altered in adult skeletal muscle under conditions of both increased and decreased activity and can be used to monitor satellite cell activation in response to muscle damage. MyoD has been suggested to be an important regulator involved in the adaptation of the skeletal muscle to exercise. However, the role of MyoD in the plasticity of skeletal muscle still remains unclear (see review, Legerlotz and Smith 2008). Its expression increases after both exercise (Kosek et al. 2006) and denervation (Hyatt et al. 2003). Upon satellite cell activation, MyoD upregulation appears the earliest within 12 h of activation. In this study, we found MyoD mRNA levels were increased significantly (sixfold) after three bouts of training over the course of 36 h but dropped dramatically (still higher than control) after 1 week of training. Similarly, Psilander et al. (2003) also found a transient increase in MyoD expression in response to resistance training. In contrast to MyoD, myogenin seems essential for muscle differentiation, consistent with their later regulatory functions in processes of muscle fiber-type specialization. We found that 1 week of training significantly elevated myogenin expression, which is similar to the data reported by Carson and Booth (1998) who found that myogenin mRNA expression was increased and remained elevated through 21 days of increased loading in birds. Finally, we found increased protein levels of Myf5 content after 1 week of training but no significant change in mRNA levels. The role of Myf5 in postnatal myogenesis is not clearly defined. Myf5 may play a role in facilitating satellite cells self-renewal (Yoshida et al. 1998). Recently, Gayraud-Morel et al. (2007) found that Myf5 null mutants are characterized by progressive muscle regeneration deficits and suggested that Myf5 is necessary for efficient regenerative myogenesis.

One consideration in interpreting the study findings is our use of a moderate contusion SCI model which represents an incomplete SCI. Rats with moderate contusion SCI still have a small area of spared axons observed at the periphery of the cord, indicating that communication between the supraspinal centers and caudal region of the spinal cord is not totally eliminated. Starting from 1 week after SCI, rats can demonstrate a gradual motor recovery, such as hindlimb joints movement and sweeping. Therefore, moderate contusion SCI animals may experience a certain degree of limb loading induced by self training and may show less atrophy and larger effects of exercise compared to other types of SCI models, such as transection. Finally, it is noteworthy that the soleus muscle studied in this experiment is a very important postural muscle. It contains a large proportion of slow fatigue-resistant muscle fibers. The soleus muscle is highly recruited during treadmill training and always shows the greatest response to exercise intervention. Therefore, similar effects of exercise training may not be expected in other hindlimb muscles.

In summary, the present results indicate that treadmill locomotor training ameliorated muscle atrophy in moderate contusion SCI rats. Training-induced muscle regeneration and fiber hypertrophy following SCI was associated with increased IGF1 and MGF levels, and coordinated regulation of IGFBP4 and IGFBP5 expression. Accelerated muscle regeneration following SCI is likely to involve increased satellite cell activation/recruitment and the upregulation of important MRFs.

Acknowledgments Data were presented in part at the ACSM 54th annual meeting New Orleans May 2007. This work was supported by the Paralyzed Veterans Association, PVA Research Foundation Grant # 2347 and the National Institutes of Health (P01HD05975 and R01HD042955). Funding for Min Liu was provided through the Craig H. Neilsen foundation #84004.

References

Adams GR (1998) Role of insulin-like growth factor-I in the regulation of skeletal muscle adaptation to increased loading. Exerc Sport Sci Rev 26:31–60

Adams GR, Haddad F (1996) The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. J Appl Physiol 81:2509–2516

Adams GR, Haddad F, Baldwin KM (1999) Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. J Appl Physiol 87:1705–1712

Allen DL, Monke SR, Talmadge RJ, Roy RR, Edgerton VR (1995) Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibers. J Appl Physiol 78:1969–1976

Allouh MZ, Yablonka-Reuveni Z, Rosser BW (2008) Pax7 reveals a greater frequency and concentration of satellite cells at the ends of growing skeletal muscle fibers. J Histochem Cytochem 56:77–87

Awede B, Thissen J, Gailly P, Lebacq J (1999) Regulation of IGF-I, IGFBP-4 and IGFBP-5 gene expression by loading in mouse skeletal muscle. FEBS Lett 461:263–267

Barton ER (2006) The ABCs of IGF-I isoforms: impact on muscle hypertrophy and implications for repair. Appl Physiol Nutr Metab 31:791–797

Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL (1998) Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. Proc Natl Acad Sci USA 95:15603–15607

Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12:1–21

Basso DM, Beattie MS, Bresnahan JC (1996) Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp Neurol 139:244–256

Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA, Adams GR (2005) Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. J Appl Physiol 98:482–488

Carson JA, Booth FW (1998) Myogenin mRNA is elevated during rapid, slow, and maintenance phases of stretch-induced hypertrophy in chicken slow-tonic muscle. Pflugers Arch 435:850–858

Chambers RL, McDermott JC (1996) Molecular basis of skeletal muscle regeneration. Can J Appl Physiol 21:155–184

Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C, Schwartz RJ (1995) Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. J Biol Chem 270:12109–12116

Day K, Shefer G, Richardson JB, Enikolopov G, Yablonka-Reuveni Z (2007) Nestin-GFP reporter expression defines the quiescent state of skeletal muscle satellite cells. Dev Biol 304:246–259

Dupont-Versteegden EE, Houle JD, Gurley CM, Peterson CA (1998) Early changes in muscle fiber size and gene expression in response to spinal cord transection and exercise. Am J Physiol 275:C1124–C1133

Edgerton VR, Leon RD, Harkema SJ, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, Tobin A (2001) Retraining the injured spinal cord. J Physiol 533:15–22

Engert JC, Berglund EB, Rosenthal N (1996) Proliferation precedes differentiation in IGF-I-stimulated myogenesis. J Cell Biol 135:431–440

Ewton DZ, Coolican SA, Mohan S, Chernausek SD, Florini JR (1998) Modulation of insulin-like growth factor actions in L6A1 myoblasts by insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5: a dual role for IGFBP-5. J Cell Physiol 177:47–57

Faulkner JA, Brooks SV, Opiteck JA (1993) Injury to skeletal muscle fibers during contractions: conditions of occurrence and prevention. Phys Ther 73:911–921

Florini JR, Ewton DZ, Coolican SA (1996) Growth hormone and the insulin-like growth factor system in myogenesis. Endocr Rev 17:481–517

Gayraud-Morel B, Chretien F, Flamant P, Gomes D, Zammit PS, Tajbakhsh S (2007) A role for the myogenic determination gene Myf5 in adult regenerative myogenesis. Dev Biol 312:13–28

Glass DJ (2005) Skeletal muscle hypertrophy and atrophy signaling pathways. Int J Biochem Cell Biol 37:1974–1984

Goldspink G (1999) Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. J Anat 194(3):323–334

Goldspink G (2005) Mechanical signals, IGF-I gene splicing, and muscle adaptation. Physiology (Bethesda, MD) 20:232–238

Goldspink DF, Cox VM, Smith SK, Eaves LA, Osbaldeston NJ, Lee DM, Mantle D (1995) Muscle growth in response to mechanical stimuli. Am J Physiol 268:E288–E297

Gomez-Pinilla F, Ying Z, Opazo P, Roy RR, Edgerton VR (2001) Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle. Eur J Neurosci 13:1078–1084

Gregory CM, Bowden MG, Jayaraman A, Shah P, Behrman A, Kautz SA, Vandenborne K (2007) Resistance training and locomotor recovery after incomplete spinal cord injury: a case series. Spinal Cord 45:522–530

Haddad F, Adams GR (2002) Selected contribution: acute cellular and molecular responses to resistance exercise. J Appl Physiol 93:394–403

Hutchinson KJ, Linderman JK, Basso DM (2001) Skeletal muscle adaptations following spinal cord contusion injury in rat and the relationship to locomotor function: a time course study. J Neurotrauma 18:1075–1089

Hyatt JP, Roy RR, Baldwin KM, Edgerton VR (2003) Nerve activity-independent regulation of skeletal muscle atrophy: role of MyoD and myogenin in satellite cells and myonuclei. Am J Physiol 285:C1161–C1173

Kadi F, Eriksson A, Holmner S, Butler-Browne GS, Thornell LE (1999) Cellular adaptation of the trapezius muscle in strength-trained athletes. Histochem Cell Biol 111:189–195

Kim SJ, Roy RR, Kim JA, Zhong H, Haddad F, Baldwin KM, Edgerton VR (2008) Gene expression during inactivity-induced muscle atrophy: effects of brief bouts of a forceful contraction countermeasure. J Appl Physiol 105:1246–1254

Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM (2006) Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol 101:531–544

Kunkel-Bagden E, Dai HN, Bregman BS (1993) Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. Exp Neurol 119:153–164

Legerlotz K, Smith HK (2008) Role of MyoD in denervated, disused, and exercised muscle. Muscle Nerve 38:1087–1100

Levinovitz A, Jennische E, Oldfors A, Edwall D, Norstedt G (1992) Activation of insulin-like growth factor II expression during skeletal muscle regeneration in the rat: correlation with myotube formation. Mol Endocrinol 6:1227–1234

Li Y, Zhao Z, Song J, Feng Y, Wang Y, Li X, Liu Y, Yang P (2009) Cyclic force upregulates mechano-growth factor and elevates cell proliferation in 3D cultured skeletal myoblasts. Arch Biochem Biophys 490:171–176

Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59–72

Liu M, Bose P, Walter GA, Anderson DK, Thompson FJ, Vandenborne K (2006) Changes in muscle T2 relaxation properties following spinal cord injury and locomotor training. Eur J Appl Physiol 97:355–361

Liu M, Bose P, Walter GA, Thompson FJ, Vandenborne K (2008) A longitudinal study of skeletal muscle following spinal cord injury and locomotor training. Spinal Cord 46(7):488–493

Moelling K, Schad K, Bosse M, Zimmermann S, Schweneker M (2002) Regulation of Raf-Akt cross-talk. J Biol Chem 277:31099–31106

Murgia M, Serrano AL, Calabria E, Pallafacchina G, Lomo T, Schiaffino S (2000) Ras is involved in nerve-activity-dependent regulation of muscle genes. Nat Cell Biol 2:142–147

Psilander N, Damsgaard R, Pilegaard H (2003) Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle. J Appl Physiol 95:1038–1044

Reardon KA, Davis J, Kapsa RM, Choong P, Byrne E (2001) Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. Muscle Nerve 24:893–899

Reese NB, Houle JD, Peterson CA (1994) Effects of fetal spinal cord (FSC) implants and exercise on muscle atrophy in chronic spinal rats. Soc Neurosci Abstr 20:1706

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953

Roy RR, Talmadge RJ, Hodgson JA, Zhong H, Baldwin KM, Edgerton VR (1998) Training effects on soleus of cats spinal cord transected (T12–13) as adults. Muscle Nerve 21:63–71

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA (2000) Pax7 is required for the specification of myogenic satellite cells. Cell 102:777–786

Shefer G, Van de Mark DP, Richardson JB, Yablonka-Reuveni Z (2006) Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle. Dev Biol 294:50–66

Stevens JE, Liu M, Bose P, O'Steen WA, Thompson FJ, Anderson DK, Vandenborne K (2006) Changes in soleus muscle function and fiber morphology with one week of locomotor training in spinal cord contusion injured rats. J Neurotrauma 23:1671–1681

Yang SY, Goldspink G (2002) Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. FEBS Lett 522:156–160

Yang S, Alnaqeeb M, Simpson H, Goldspink G (1996) Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch. J Muscle Res Cell Motil 17:487–495

Yoshida N, Yoshida S, Koishi K, Masuda K, Nabeshima Y (1998) Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells'. J Cell Sci 111(Pt 6):769–779

Zammit PS (2008) All muscle satellite cells are equal, but are some more equal than others? J Cell Sci 121:2975–2982

Zdanowicz MM, Moyse J, Wingertzahn MA, O'Connor M, Teichberg S, Slonim AE (1995) Effect of insulin-like growth factor I in murine muscular dystrophy. Endocrinology 136:4880–4886

Zeman RJ, Zhao J, Zhang Y, Zhao W, Wen X, Wu Y, Pan J, Bauman WA, Cardozo C (2009) Differential skeletal muscle gene expression after upper or lower motor neuron transection. Pflugers Arch 458:525–535