ADAPTATIONS IN SKELETAL MUSCLE FOLLOWING SPINAL CORD INJURY AND LOCOMOTOR TRAINING

By

MIN LIU

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

This document is dedicated to my parents, wife, sister and daughter.

ACKNOWLEDGMENTS

The completion of this project would not have been possible without the support and guidance of many people. At the moment when I am finishing my journey of graduate study at UF, I am extremely grateful to all of those involved in my education, research, and life.

I would first like to express my sincere thanks to my advisor, Dr. Krista Vandenborne, who welcomed me as a researcher into her laboratory and permitted me to pursue the area of research most compelling to me. Her excellent guidance and constant encouragement were invaluable throughout my entire doctoral studies. I am very grateful to Dr. Glenn Walter for his remarkable wisdom and invaluable ideas. His passions on science and sharp comments will always be my model in the future. I thank Dr. Floyd Thompson for his broad knowledge. I am blessed to have learned so much about science from him. I thank Dr. Prodip Bose for his immeasurable support and assistance during my doctoral program. I also would like to express my appreciation to Dr. Orit Shechtman and Dr. David Fuller for serving my dissertation committee and for their incredible scientific advice.

It has been a pleasure and honor to work in Dr. Vandenborne's lab. My deepest thanks go to the members in the lab. I am grateful for the assistance of Dr. Jennifer Stevens. This project would not have been completed without her support. I owe great thanks to Ye Li. Her assistance for developing new assay was instrumental in the completion of this project. Special thanks go to Chris, Tiffany, Neeti, Arun, Prithvi,

Gabe, Fan, and Shiv. I am grateful to all for their help and friendship. Due to the presence of all these wonderful people, work is more than enjoyable.

Finally and most importantly, I would like to express my deepest gratitude to my family members. It is with much love that I acknowledge and deeply appreciate the sacrifices and difficulties that my loving wife, Qing Yang, patiently endured. I also thank my lovely daughter, Melodie, who made my graduate study colorful. I am indebted to my father and sister for their relentless support, care and encouragement.

TABLE OF CONTENTS

page

ACKNOWLEDGMENTS iv

LIST OF TABLES x

LIST OF FIGURES xi

ABSTRACT xiii

CHAPTER

1 INTRODUCTION 1

2 REVIEW OF RELATED WORK 3

2.1 Spinal Cord Injury Animal Models 3
2.1.1 Spinal Cord Transection Model 3
2.1.2 Spinal Cord Isolation Model 4
2.1.3 Spinal Cord Hemisection Model 5
2.1.4 Spinal Cord Contusion Model 6
2.1.4.1 Early contusion models 6
2.1.4.2 Rat spinal contusion models 7
2.1.4.3 The New York university impactor 8
2.1.5 Summary 9
2.2 Muscular Adaptations Following Spinal Cord Injury 10
2.2.1 Muscle Atrophic Response Following SCI 10
2.2.2 Force Mechanical Properties Following SCI 10
2.2.3 Myosin Heavy Chain Expression Following SCI 12
2.3 Treadmill Locomotor Training Following Spinal Cord Injury 16
2.3.1 The Recovery of Walking Ability Following SCI and Locomotor Training 17
2.3.1.1 Locomotor recovery following SCI in animals 17
2.3.1.2 The recovery of walking ability in individuals after SCI. 18
2.3.2 The Impact of Locomotor (Treadmill or Cycling) Training on Skeletal Muscle Following SCI 20
2.4 IGF-1 Signaling and Muscle Plasticity 22
2.4.1 IGF-I and Its Related Receptor and Binding Proteins 22
2.4.2 Protein Synthesis Induced by IGF-I/PI3K/Akt Pathway 25
2.4.3 IGF-I/PI3K/Akt Pathway and Protein Degradation 26

2.4.4 Role of IGF-I in Satellite Cell Proliferation. 27

3 OUTLINE OF EXPERIMENTS 29

3.1 Experiment 1 29
3.1.1 Specific Aim 29
3.1.2 Hypotheses 29
3.2 Experiment 2 30
3.2.1 Specific Aim 30
3.2.2 Hypotheses 30
3.3 Experiment 3 31
3.3.1 Specific Aim 31
3.3.2 Hypotheses 31
3.4 Experiment 4 32
3.4.1 Specific Aim 32
3.4.2 Hypotheses 32

4 A LONGITUDINAL STUDY OF SKELETAL MUSCLE FOLLOWING SPINAL CORD INJURY AND LOCOMOTOR TRAINING 33

4.1 Abstract 33
4.2 Introduction 34
4.3 Materials and Methods 36
4.3.1 Experimental Animals 36
4.3.2 Spinal Cord Contusion Injury 36
4.3.3 Treadmill and Cycling Locomotor Training 37
4.3.4 Magnetic Resonance Imaging 38
4.3.5 Open Field Locomotor Function 39
4.3.6 Statistical Procedures 40
4.4 Results 40
4.4.1 Muscle Size After Spinal Cord Contusion Injury 40
4.4.2 Effect of Locomotor Training on Muscle Size. 42
4.4.3 Relationship Between Hindlimb Muscle Size and Locomotor Function. 44
4.5 Discussion 46

5 CHANGES IN SOLEUS MUSCLE FUNCTION AND FIBER MORPHOLOGY WITH ONE WEEK OF LOCOMOTOR TRAINING IN SPINAL CORD CONTUSION INJURED RATS 52

5.1 Abstract 52
5.2 Introduction 53
5.3 Materials and Methods 55
5.3.1 Experimental Animals 55
5.3.2 Open Field Locomotor Function 56
5.3.3 Spinal Cord Contusion Injury 56
5.3.4 Treadmill Locomotor Training 57
5.3.5 In situ Soleus Force Measurements 58

5.3.6 Tissue Harvest 59
5.3.7 Immunohistolochemistry 60
5.3.8 Data Analysis 60
5.4 Results 61
5.4.1 BBB Open Field Locomotor Scores 61
5.4.2 Soleus Contractile Properties 62
5.4.3 Soleus Muscle Fiber Cross Sectional Area 66
5.5 Discussion 67

6 CHANGES IN SOLEUS FIBER TYPE COMPOSITION WITH ONE WEEK OF TREADMILL LOCOMOTOR TRAINING IN SPINAL CORD CONTUSION INJURED RATS 73

6.1 Abstract 73
6.2 Introduction 74
6.3 Materials and Methods 75
6.3.1 Experimental Animals 75
6.3.2 Spinal Cord Contusion Injury 75
6.3.3 Treadmill Locomotor Training 76
6.3.4 Immunohistolochemistry 77
6.3.5 Data Analysis 78
6.4 Results 78
6.4.1 Soleus fiber type composition 78
6.4.2 Soleus fiber cross-sectional area 80
6.5 Discussion 81

7 EFFECTS OF TREADMILL TRAINING ON IGF-I EXPRESSION IN RAT SOLEUS MUSCLE FOLLOWING SPINAL CORD INJURY 85

7.1 Introduction 85
7.2 Materials and Methods 86
7.2.1 Experimental Animals 86
7.2.2 Spinal Cord Contusion Injury 86
7.2.3 Treadmill Locomotor Training 87
7.2.4 Tissue Harvest 88
7.2.5 RT-PCR Measurement 88
7.2.6 Immunohistolochemistry 90
7.2.7 Data Analysis 90
7.3 Results 91
7.3.1 mRNA Expression of IGF-I and Its Receptor and Binding Proteins 91
7.3.2 Embryonic Myosin 94
7.3.3 Central Nuclei 94
7.4 Discussion 95

8 CONCLUSION 99

LIST OF REFERENCES 101

BIOGRAPHICAL SKETCH 122

LIST OF TABLES

Table page

4-1. Maximal cross-sectional area of individual muscles. 51

6-1. Monoclonal antibody specificity 78

LIST OF FIGURES

Figure page

4-1. A representative trans-axial MR image of the rat lower hindlimb. 39

4-2. Relative changes in the CSAmax of the tibialis anterior, triceps surae, extensor digitorum, and flexor digitorum muscles following SCI.. 41

4-3. Relative change in total CSAmax in the no training group, cycling training group and treadmill training group.. 42

4-4. Relative change in the CSAmax of the triceps surae in the no training group, cycling training group and treadmill training group.. 44

4-5. Relative change in the CSAmax of the tibialis anterior in the no training group, cycling training group and treadmill training group.. 45

4-6. Relationship of rat locomotor function and total lower hindlimb CSA 45

5-1. Average BBB locomotor scores for the SCI + TM and SCI no TM group at 1 and 2 weeks post SCI. 61

5-2. Soleus muscle peak tetanic force 62

5-3. Soleus muscle specific force 63

5-4. Peak soleus tetanic force vs BBB score 64

5-5. Soleus muscle fatigue 64

5-6. Soleus muscle force-time integral during a fatiguing protocol 65

5-7. Soleus muscle peak twitch force 65

5-8. Soleus muscle time to peak 66

5-9. Soleus muscle ½ relaxation time 66

5-10. Average soleus muscle fiber CSA 67

6-1. Serial cross sections of a control soleus stained with monoclonal antibodies directly against specific MHC isoforms 79

6-2. Serial cross sections of a SCI no TM soleus stained with monoclonal antibodies directly against specific MHC isoforms. 79

6-3. Serial cross sections of a SCI + TM soleus stained with monoclonal antibodies directly against specific MHC isoforms 80

6-4. MHC based fiber type percentage composition. 81

6-5. Soleus muscle fiber type specific CSA 81

7-1. IGF-I mRNA expression relative to 18s in soleus muscle. 92

7-2. MGF mRNA expression relative to 18s in soleus muscle. 92

7-3. IGF-R mRNA expression relative to 18s in soleus muscle. 93

7-4. IGF-BP4 mRNA expression relative to 18s in soleus muscle. 93

7-5. IGF-BP5 mRNA expression relative to 18s in soleus muscle. 93

7-6. Cross-section of soleus muscle stained with monoclonal antibody against embryonic myosin isoform. 94

7-7. Cross-section (hemotoxilin & eosin stained) of soleus muscle. 95

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ADAPTATIONS IN SKELETAL MUSCLE FOLLOWING SPINAL CORD INJURY AND LOCOMOTOR TRAINING

By

Min Liu

May 2006

Chair: Krista Vandenborne
Major Department: Rehabilitation Science

Spinal cord injury (SCI) is one of the most devastating human afflictions, which leaves its victims paralyzed. Skeletal muscles distal to the injury site experience significant muscle atrophy and loss of function, leading to impaired walking and motor function. Recently, novel intervention therapies, focusing on repetitive locomotor training, have shown great promise in promoting spinal plasticity and recovery in motor function following SCI. Recovery of motor function after SCI likely requires both neural and muscular adaptations. The major goal of this study was to investigate adaptations in skeletal muscles following SCI and locomotor training. A combination of MR imaging, in situ functional measurements, immunohistochemical assays and RT-PCR was performed in an animal model of incomplete SCI. Our findings demonstrate that SCI results in significant atrophy in all rat hindlimb muscles, and that locomotor training halts the atrophic process and accelerates the rate of recovery. Additionally, our data suggest that early therapeutic intervention using treadmill training significantly increases animal

locomotor function and soleus muscle size and function. Compared to untrained SCI animals, one week of treadmill training results in a 32% improvement in BBB scores, a 38% increase in peak soleus tetanic force, a 9% decrease in muscle fatigue, larger muscle fiber CSA (23%), and a reduced shift toward faster fiber types. Finally, our findings demonstrate that treadmill training significantly increases mRNA levels for IGF-I, MGF, IGFR, IGFBP4 and decreases IGFBP5 mRNA expression in the soleus muscle. In addition, immunohistochemical analysis shows the increased presence of small fibers expressing embryonic myosin, a hallmark of muscle regeneration, following treadmill training. Taken together, the findings from the present work demonstrate that early therapeutic intervention promotes muscular plasticity following SCI. We anticipate that this study will provide essential feedback for the development of early rehabilitation interventions in SCI individuals.

CHAPTER 1
INTRODUCTION

Spinal cord injury (SCI) is one of the most devastating human afflictions, which leaves its victims paralyzed or with impaired motor control (57, 67, 107, 132, 245). It is estimated that there are approximately 200,000 persons with SCI living in the United States, with roughly 11,000 new cases occurring each year, making SCI a leading cause of disability (200). Skeletal muscles distal to the injury site experience significant muscle atrophy and loss of function, leading to impaired walking and motor function. The neural responses to spinal cord injury vary from patient to patient, depending on the severity of the injury. As a result of new developments in the acute management of spinal cord injury, the majority of spinal cord injuries sustained are clinically incomplete.

Traditionally, orthotic and assistive devices have been used as compensatory strategies to counter muscle weakness in SCI patients in an attempt to facilitate functional walking. Successful mobility is often dependent on learning a new behavior requiring either a wheelchair and/or bracing with assistive devices. More recently, locomotor training has emerged as an alternative modality for retraining walking after incomplete SCI and has revealed encouraging breakthroughs (27, 62, 64, 65, 105, 106, 234). Improved gait speed, improved balance, less reliance on assistive devices and orthoses, less physical assistance from caregivers, and improved functional performance have all been documented with locomotor training (63, 235).

The purpose of this study was to investigate the impact of locomotor training on skeletal muscle plasticity and muscle recovery as well as growth factors known to play a

role in muscle regeneration in a rat contusion SCI model. In addition, we set out to determine the effect of locomotor training on muscle function in contusion spinal cord injured animals and to perform a preliminary investigation of the relationship between alterations in muscle size/function and locomotor behavior.

CHAPTER 2
REVIEW OF RELATED WORK

2.1 Spinal Cord Injury Animal Models

Animal models of SCI can be used to characterize the lesion development, study the mechanism of recovery, develop therapeutic interventions, and also to study skeletal muscle adaptations with decreased use (7, 18, 44, 59, 72, 73, 76, 79, 91, 98, 117, 118, 137, 138, 152, 155, 161, 170, 194, 200, 222, 248). Currently, the experimental SCI animal models include the transection, isolation, hemisection, and contusion model. Due to differences in limb loading, neural activation and behavioral outcomes resulting from each of these methods, it is important to compare the similarities and differences across these models.

2.1.1 Spinal Cord Transection Model

In the transection spinal cord injury model, the transmission of descending and ascending information between the caudal cord and the brain is mechanically eliminated (83). In this case, SCI is created by an incision into the spinal cord. The spinal cord can be either completely transected and left in place or a small section of the spinal cord can be removed (83). Following transection injury, there is an initial flaccid paraplegia stage in which animals drag their limbs (135, 187). The animals are able to move using their forelimbs, and have no difficulty reaching food and water. At approximately 3 to 4 weeks, the paralyzed hind limbs of the animals change from flaccid to spastic. After spasticity develops, the limbs are almost always held in extension and no recovery of voluntary activity is observed (135, 187). EMG recordings monitored over 24-hour

periods show a 75% decrease in the total integrated EMG and a 66% decrease in the total duration of muscle activity in the SOL muscle, 5 to 6 months after transection when compared to control (7). Thus, in the spinal transection model hind limb muscles experience a significant reduction in both electrical activation and loading.

The complete transection model has been used extensively to evaluate the effectiveness of interventions with regard to both axonal regeneration and functional recovery (83). The advantage of this model is a relative stabilization of pathological changes and subsequent neurological outcomes (210). Therefore, the effectiveness of particular strategies can be readily assessed (201). However, the transected spinal cord model also has some disadvantages. First, due to the natural tension present in spinal cords, the two ends of a cut cord will separate. Such a gap is rarely present in human SCI. In addition, in order to cut the spinal cord, the dura has to be opened, allowing invasion by external cells. Therefore, the spinal cord fails to demonstrate spontaneous spinal regeneration (210). Finally, due in part to the advanced emergency care, the number of spinal cord injuries classified as incomplete has risen dramatically (http://Ref-www.spinalcord.uab.edu/). Thus, the transection model, while valuable for certain applications, may not be the best model to study the potential of rehabilitation interventions to promote neuromuscular plasticity.

2.1.2 Spinal Cord Isolation Model

Spinal cord isolation has been referred to as the classic “silent preparation”, which was first described in dogs by Tower (228). In this preparation, the lumbar region of the spinal cord is functionally isolated via complete spinal cord transections at two sites. In addition, all the dorsal roots are cut bilaterally between the two transection sites (190). Thus, this model eliminates supraspinal, infraspinal, and peripheral afferent input to

motoneurons located in the isolated cord segments while leaving the motoneuron skeletal muscle fiber connections intact. The motoneurons within the islated segment of the spinal cord do not receive sensory input from the dorsal roots, or neural signals originating from either above or below the two transection sites. Based on 48 hours of continuous intramuscular EMG recordings from a representative ankle extensor and a representative ankle flexor, the muscles innervated by these motoneuron pools are essentially electrically silent, even during passive manipulation (190). Acute recordings, during tactile stimulation of the legs or feet, also showed virtually no EMG activity in a variety of hindlimb muscles (75, 76, 209). Moreover, based on observations during cage activity, it can be assumed that minimal forces are generated in these paralyzed muscles (187). Therefore, spinal cord isolation represents one model that has been successfully used to study the effects of a complete elimination of neuromuscular activity on muscle properties (221).

2.1.3 Spinal Cord Hemisection Model

In partial transection models, an attempt is made to cut tracts of the spinal cord selectively. Depending on the severity of the lesion, the resulting neurologic deficit can be relatively mild, thus making the postoperative animal care fairly easy, particularly with regard to bladder function. Partial injury models also may allow for comparison of the regenerative response in a particular tract with its uninjured partner on the contralateral side (92).

Most hemisection injuries are performed on the cervical spinal cord, interrupting the descending respiratory pathways and causing respiratory muscle paresis or paralysis. Thus, this model has long been used to understand the mechanisms related to plasticity and recovery of the respiratory pathways after spinal cord injury. Unfortunately, partial

injury models also suffer from difficulties determining whether observed functional improvement is due to true regeneration of the injured tract or to functional compensation from other systems that are spared. For this reason, this model is not commonly used to study hind limb muscle adaptations.

2.1.4 Spinal Cord Contusion Model

Most human spinal cord injuries result from fracture and dislocation of the spinal cord column (88). Although penetrating wounds of the spinal cord can result from a knife or gunshot, most human spinal cord injuries are caused by transient compression or contusion of the spinal cord (39, 119, 125, 223). Even in the setting of complete paraplegia after blunt injury, the cord rarely is completely transected, but rather leaves some residual, normal-appearing cord parenchyma peripherally at the injury zone (39).

The first observation of human SCI neuropathology begins with an early phase of spreading hemorrhagic necrosis and edema. Then it reaches an intermediate phase of tissue repair and reorganization. Finally it ends up with a chronic phase characterized by the formation of cystic cavities (108). These injury patterns are well simulated by spinal cord contusion injury (37, 99, 100). Therefore, scientists have long used animal spinal cord contusion models to study the pathophysiology of spinal cord injury and regeneration (23, 35, 37, 44, 87, 99-101, 121, 131, 147, 166, 167, 182, 190, 235, 253). In our investigation we utilize the contusion injury model and in the section below a brief overview of the history of this model is provided.

2.1.4.1 Early contusion models

In 1911, Reginald Allen described a spinal cord injury model where he dropped a weight onto dog cords exposed by laminectomy (9). In 1914 (199), he reported that midline myelotomy reduced progressive tissue damage in the contused spinal cord.

Unfortunately, Allen died in World War I and his work was discontinued for nearly 50 years.

In 1968, Albin and colleagues (8) revived the contusion model when they used a primate spinal cord contusion model to assess the efficacy of hypothermic therapy following SCI. After that, several investigators started using the canine spinal cord contusion model again. Parker and colleagues (168, 169) assessed the effects of dexamethasone and chlorpromazine on edema in contused dog spinal cords. At the same time, Koozekanani and colleagues (128) examined the causes of variability in this model, while Collmans and others (52) measured edema, blood flow and histopathological changes in the contused dog spinal cord. In 1971, Osterholm and Mathews proposed that catecholamine accumulation explains the progressive central hemorrhagic necrosis in the contused cat spinal cord (165). Although subsequent studies did not confirm the predicted spinal cord catecholamine changes, this was the first excitotoxic theory of neural injury (109).

2.1.4.2 Rat spinal contusion models

In 1985, the Wrathall group (85, 163, 242) described morphological and behavioral changes in a rat weight-drop contusion model. The weight-drop device was similar to that used for the feline spinal cord contusion model, i.e., a weight dropped dorsally onto thoracic spinal cord exposed by laminectomy. In addition, Wrathall and colleagues developed a combined behavioral score to assess motor, sensory, and reflex recovery in spinal injured rats, correlating these scores with quantitative axonal counts, neuronal and glial loss, and evoked potentials (175). In 1987, Somerson and stokes described a feedback controlled electromechanical device that indented the spinal cord at a defined force, duration, and extent (206). This device has two levels of indentations that cause

mild or moderate injury, producing consistent 3D morphological and locomotor changes. The device was subsequently used to assess the neuroprotective effects of MP and other drugs (84).

2.1.4.3 The New York university impactor

The most commonly used rodent spinal cord contusion model is the New York University (NYU) impactor, developed at the NYU Neurosurgery Laboratory and first described by Gruner (98) in 1992. This model uses a weight-drop device that differs from previous devices in several respects. First, the impactor dropped a steel rod directly onto the spinal cord exposed by a laminectomy, achieving more consistent contusions. Second, the device used digital optical potentiometers to measure the trajectory of the falling rod with a precision of ± 20 um and ± 20 us, providing accurate measurements of the delivered trauma. Third, the impactor measured movement of the spinal column at the impact site, subtracting this movement from the cord movement. Fourth, the device was designed to deliver three different levels of injury to rat spinal cord by dropping a 10-g rod 12.5, 25.0, or 50.0 mm onto the spinal cord, respectively producing mild, moderate, and severe injuries (98).

In 1993, NIH funded a Multicenter Animal Spinal Cord Injury trial (MASCIS), in which a group of eight spinal cord injury laboratories validated and standardized the Impact model (246). The group demonstrated that the impactor produced consistent spinal cord injuries, reflected in a variety of measurements. This device allows for the precise measurement of a number of biomechanical parameters including the impact velocity of the rod, the distance of cord compression, the cord compression rate, and the dynamic force applied to the cord (23). In addition, the MASCIS group standardized the ages of the rats (77 ± 1 day), anesthesia, and injury timing (60 ± 5 min after anesthesia).

Using these guidelines, this device apparently can produce extremely consistent injuries in terms of the resulting neuropathology. Finally, MASCIS validated the Basso-Beattie-Bresnahan (BBB) locomotor score, a 21-point ordinal behavioral scale that linearly predicts spinal cord histological changes (246).

The BBB locomotor rating scale was described by Basso, Beattie, and Bresnahan in 1995 (24) as a measure of motor performance in rats. With this 21-point scale, animals achieving scores in the lower third (1 to 8) are capable of hindlimb joint movements without weight support. Those rating in the middle part of the scale (9 to 13) demonstrate varying degrees of hindlimb weight support and forelimb–hindlimb coordination, and those achieving scores in the upper one third (14 to 21) show improvements in paw and tail position, toe clearance, and trunk stability during a fully supported and coordinated gait. The BBB scale has been shown to be a relatively reliable measure of locomotor function and a sensitive reflection of the degree of tissue injury after spinal cord contusion (23). Its fairly widespread use has been valuable for allowing the communication and standardized comparison of results from different institutions (43, 142, 145).

2.1.5 Summary

Animal models have proved to be invaluable for the development of experimental therapies, and undoubtedly will continue to play an essential role in the field of spinal cord injury research. Models in which the spinal cord is sharply transected, either completely or partially, are useful for studying the anatomic regeneration of axons, whereas the contusion models better simulate the biomechanics and neuropathology of human spinal cord injury. In order to study the effect of locomotor training on muscle

plasticity and recovery we selected to use a moderate mid-thoracic rat contusion injury model.

2.2 Muscular Adaptations Following Spinal Cord Injury

2.2.1 Muscle Atrophy Following SCI

Following SCI, due to the reduced muscle activity and limb unloading, skeletal muscles show a significant reduction in their size and mass (45, 46, 59, 65, 70, 72-74, 118, 137, 141, 150, 155, 174, 178, 194, 200, 201, 210, 212, 218, 248). In addition, muscle atrophy is more pronounced in paralyzed muscles that normally bear weight, especially those that cross single joints (148, 186, 237). These muscles often contain a large proportion of slow fatigue-resistant muscle fibers and are largely responsible for maintaining posture and bearing weight (112, 186). For example, the soleus muscle, a postural muscle that extends the ankle, undergoes significant muscle atrophy following SCI. In contrast, the tibialis anterior muscle, which flexes the ankle and does not normally contract against a high load, atrophies considerably less in a number of species, including humans (148). The medial gastrocnemius muscle, which crosses both the knee and ankle joints, also undergoes less atrophy than the soleus muscle even though it serves as a synergist to the soleus muscle during plantar flexion (186).

2.2.2 Force Mechanical Properties Following SCI

Neural activity is very important to determine the mechanical properties of a skeletal muscle. In 1960, Buller et al. (38) first reported that cross-reinnervation of the cat slow soleus muscle with the nerve of the fast flexor digitorum longus resulted in incomplete conversion of the isometric twitch properties from slow to fast. Similarly, cross-reinnervation of the fast flexor digitorum longus muscle with the nerve of the slow soleus muscle led to incomplete conversion of the isometric twitch properties from fast to

slow (38). These findings demonstrate that the pattern or amount of motoneuron activation strongly influences the muscle properties.

Following SCI, animal hindlimb muscles show pronounced decrease in maximal force-generating capacity and the acquisition of faster mechanical properties (185, 191-193, 215, 217, 220, 221). In these studies, muscle force mechanical properties were determined using an in situ set up. Briefly, the animal leg was securely positioned in an apparatus such that the muscle was in a near-horizontal plane. The muscle distal tendon was cut and then attached to a force transducer. The muscles were stimulated via bipolar silver electrodes placed on the transected tibial nerve. They found that the maximum isometric twitch and tetanic tension of the cat soleus muscle were reduced by 38 and 39%, respectively, ~10 mo after spinal cord transection (185, 191). In these same cats, the time to peak tension and half-relaxation times were 41 and 50% shorter, when compared to control cats (185). The changes in force generating capacity and contractile speed were accompanied by a rightward shift in the force-frequency relationship and a small, but significant, decrease in fatigue resistance (193). The adaptations in the mechanical properties of the cat medial gastrocnemius, a fast ankle extensor, were less pronounced than that in the soleus, in both young and adult animals (185, 191).

Presently, rat is the most prevalent animal model used to assess the effects of SCI and to determine the potential beneficial influence of rehabilitative procedures in the recovery of function after SCI (60, 69-71, 115). In general, the adaptations in the mechanical properties of the rat soleus after a complete spinal transection are relatively similar to those reported for cat muscles, although the magnitude of the effects appears somewhat greater in cats than rats (194, 214). Talmadge and his colleagues (214) showed

that in rats maximal tetanic force is reduced by ~44% at 3 months post spinal cord transection. In addition, spinal cord transection resulted in faster twitch properties as evidenced by a shorter time to peak tension (~45%) and half-relaxation time (~55%). In addition, a significant reduction in fatigue resistance of the soleus was observed (214).

The adaptations in the mechanical properties of muscle following SCI are similar to those observed in other models of reduced neuromuscular activity. For example, chronic muscle unloading, via either hindlimb suspension or actual space flight, results in significant reductions in tetanic force, and twitch contraction times of the rat soleus (41, 42, 219). Most unloading and spaceflight studies have also reported a decrease in half-relaxation time and an increase in *V*max (41, 42, 239). The similarity in the adaptations in SCI and chronic unloading models further indicates the role of loading and neuromuscular activity in mediating muscle adaptations following spinal cord injury.

2.2.3 Myosin Heavy Chain Expression Following SCI

Myosin is one of the molecules that regulates contractile speed in mammalian skeletal muscle and is highly correlated with the myofibrillar ATPase histochemical identification of specific muscle fiber types (216, 217). It is a hexamer composed of two heavy and four light chains (197). To date, four different myosin heavy chain (MHC) isoforms have been identified in varying proportions in the hindlimb muscles of rats. These have been identified as a slow isoform called MHC-I and three fast isoforms called MHC-IIa, MHC-IIx, and MHC-IIb (224). A number of studies have closely linked the MHC isoform composition of the individual muscle fibers with their velocities of unloaded shortening, such that there is a gradation in the contractile speed of fibers containing a given isoform in the order of (fastest to slowest) IIb > IIx > IIa > I (36).

The complement of MHC isoforms expressed by mammalian hindlimb muscles can be regulated by conditions that alter the levels of neuromuscular activation (defined as electrical activation and load-bearing) of a muscle (97, 170, 187, 193, 217). Interventions that result in increased neuromuscular activation, such as chronic electrical stimulation, functional overload and endurance exercise, result in MHC shifts towards the "slower" isoforms. In contrast, factors that reduce neuromuscular activation, including space flight, hindlimb suspension, and spinal cord injury, result in MHC shifts towards the "faster" isoforms (222).

With a spinal cord injury there is no direct damage to the muscle, and innervation of muscles is not physically disrupted; therefore, the interruption of transfer of electrical activity through the motoneurons likely stimulates the changes that are observed (136, 171). Spinal motoneurons caudal to a lesion site lose neural input from descending brain stem pathways, which influences their tonic and phasic firing patterns and consequently affects muscle contractile and metabolic activity. Studies involving cross-reinnervation of muscles have further defined the influence of motoneuron input on the structural and functional characteristics of a muscle (188).

Myosin type adaptations following spinal cord transection. Almost all muscles studied show an increase in the percentage of fast fibers and a decrease in the percentage of slow fibers following spinal cord transection (15, 114, 121, 137, 147, 166, 213, 217, 222). In cat soleus muscle, as many as 50% of the fibers react with only a fast MHC, and a small percentage of the fibers react with both a fast and a slow MHC antibody following transection (121, 122). In contrast, control adult soleus muscle does not appear to express the fast MHC (121, 122, 215). The fast fibers post-SCI tended to be found

disproportionately at the boundaries of the fascicles, suggesting that myogenic spatial factors had influenced the ATPase conversion of the fibers. Roy et al (186, 192) also showed that cat fast muscle (tibialis anterior) also shows an increase in the fast fiber proportion and MHC-IIx expression after 6 months of spinal cord transection, but the increases are less prominent than in the soleus.

Rodents show a higher degree of MHC isoform transformation after spinal transection than cats. The proportion of MHC-I in the rat soleus is reduced from ~90% in controls to ~25% only 3 months following a complete mid-thoracic spinal cord transection (222). The MHC-IIx, which is normally not found in the rat soleus, increased to nearly 50% and that of MHC-IIa to ~30% 6 months after spinal transection (217, 222).

Myosin type adaptations following spinal cord isolation. In cats, spinal isolation also has been shown to result in an increase in the percentage of fibers labeled by antibodies specific for fast MHC in the cats soleus and tibialis anterior (93). Based on mATPase staining at an alkaline preincubation, Graham (93) found 64% fast fibers in regions sampled in the soleus of control cats and 100% in similar regions in the spinal-isolated cats. In addition, essentially all fibers in the MG of the spinal isolated cats reacted exclusively with a fast MHC antibody (93). These fiber type data are consistent with the observation that the myosin ATPase activities in spinal isolated cats are 85% and 30% higher in the SOL and MG, respectively, 6 months after spinal isolation (123). In rats, spinal isolation also resulted in a slow-to-fast shift in the myosin isozyme pattern (97). Similar to spinal transection, the MHC isoform that is primarily up-regulated at the expense of MHC-I is the MHC-IIx isoform (97).

In addition, the reduction in the proportion of fibers containing MHC-I after spinal isolation has been shown to be greater than that observed for spinal transection alone. For example, 6 months following spinal transection the cat soleus contains only 67% MHC-I fibers (215) compared to 48% after spinal isolation (93). Thus, the magnitude of the adaptations observed following spinal isolation is greater than that observed after spinal transection. This suggests that the residual amount of electrical activation in the cat soleus after spinal transection plays a role in maintaining the levels of MHC-I expression.

Myosin type adaptation following spinal cord contusion injury. Hutchinson (116) found that there was no change in the relative percent of MHC expression at 1 week post spinal cord contusion injury. However, at the 3 week time point, there was an upregulation of the transitional IIx heavy chain (116). Although the increased expression of IIx MHC after SCI was not statistically significant, it appears to have been sufficient to induce effects of biological significance (speeding up of ½ RT). That such a modest increase in IIx MHC profiles could produce significant physiological differences indicates the tight regulatory control MHC phenotype imparts on contractile speed.

Skeletal muscle MHC expression in individuals with SCI. A few studies have directly assessed the effects of SCI on MHC isoform expression. In 1999, Castro (45) reported that there is very little adaptation in MHC isofrom expression in the vastus lateralis muscle during the first several months after complete SCI in patients. This study investigated vastus lateralis biopsy samples in 12 patients as soon as they were clinically stable (45). No significant slow-to-fast changes in MHC isoform composition of the vastus lateralis were found at any time point (6, 11, 24 weeks post SCI), and the mean proportions of each of the MHC isoforms were comparable to non-disabled control

subjects. However, there was an apparent conversion among type II fibers, with a significant MHC-IIa to MHC-IIx shift, in the vastus lateralis at 24 weeks post SCI. Thus, at 24-week after injury there was an elevation in the proportion of MHC-IIx, the fastest of the human limb MHC isoforms. These data are consistent with a previous study showing that in long term SCI subjects (3-20 years post SCI), the vastus lateralis muscle contained a higher percentage of MHC-IIx or in combination with MHC-IIa (11).

Histochemical data support the hypothesis that slow to fast MHC isoform transformations occur in human muscle after SCI, even though to less of an extent than observed in animal models. For instance, the proportion of type IIb fibers was elevated and type I fibers decreased in the tibialis anterior and VL of SCI subjects (95, 148). The decrease in the proportion of histochemically identified type I fibers appeared to be related to the duration of time following the SCI injury. In fact, Round (184) found that there was no decrease in the proportion of type I fibers in 2 individuals who had suffered a SCI less than 15 months prior to biopsy of the VL. In contrast, in those individuals that had suffered a SCI 3 years or more prior to biopsy, the proportion of type I fibers decreased relative to non-injured control values (184). These data also highlight that the change in fiber type composition in human skeletal muscle occurs slower, a phenomina that has also been observed in other models of unloading (184).

2.3 Treadmill Locomotor Training Following Spinal Cord Injury

Motor recovery following spinal cord injury can be enhanced or accelerated by repetitive locomotor training (26, 27, 64, 86, 174, 233-235, 240, 241). The most frequently used locomotor training is treadmill training. Treadmill training is performed using a body weight support and manual assistance for stepping. The underlying premise of locomotor training relates to the hypothesis that rhythmic loading of the limbs and

force feedback from the hindlimb muscles induces task appropriate activity-dependent plasticity. The following several paragraphs review the effects of locomotor training on functional recovery in both SCI animals and subjects.

2.3.1 The Recovery of Walking Ability Following SCI and Locomotor Training

2.3.1.1 Locomotor recovery following SCI in animals

Experimental studies have shown that locomotor training of SCI animals can effectively improve the ability to step on a treadmill in cats following complete SCI (18, 54, 58, 59, 113, 140). Over the last ten years or so, the Edgerton group has published several papers to address this issue. For example, de leon (58) characterized the effects of training by comparing groups of untrained with trained spinal cats and reported that step-trained spinal cord cats can reach considerably higher speeds and make more consecutive steps. Treadmill training also improved interlimb coupling, overall limb excursion and joint excursion significantly (59). In addition, Edgerton's group demonstrated a certain task-specificity in the improvement of locomotor function. Therefore, spinal cats trained to stand were not able to walk. More importantly, they claimed that the spinal cord can 'learn' or 'store' memories of simple motor responses that are acquired through conditioning (59). For instance, the acquired motor function can last up to six weeks followed by a graduate decay of locomotor ability (59). This reduction can be reinstated within one week of training.

However, studies using rats with incomplete SCI are scarcer and have provided contradictory results even if it appears clinically and pathophysiologically relevant. Fouad et al. (81) found no beneficial effect on free locomotor activity after a very partial lesion of the cord, whereas Thota et al. (227) reported improved functional recovery after 7 weeks treadmill training in rats with partial spinal cord lesion. However, Fouad (81) proposed a

possible explanation for the absence of observed benefits in their study. They noted that animals in their study had a high level of motor function and were very mobile after SCI, limiting the potential beneficial effects of the treadmill training. Recently, a group in Belgium (158) studied the effect of treadmill training on motor recovery in adult rats after severe contusion injury induced by balloon inflation. Multon et al. (158) reported that early treadmill training improved locomotor recovery and showed that the beneficial effect was observable after only 2 week, with a maximal effect at 4 weeks. As a result, severely contused injured rats could support their body weight after 12 weeks of treadmill training, while non-trained rats could not (158).

2.3.1.2 The recovery of walking ability in individuals after SCI.

Based on animal research, the interactive approach using treadmill and body weight support system for locomotor training has been extended to human individuals and several studies have yielded positive reports (18, 26, 27, 64, 80, 89, 110, 233-235). In 1987, Barbeau and colleagues (18) first reported suspending a human over a treadmill to assess the feasibility for walking retraining. During locomotor training, SCI individuals were suspended over a treadmill in a harness and an overhead support system. In addition, Behrman (27) emphasized the following training principles: 1) generating stepping speeds approximating normal walking speeds (0.75-1.25 m/s); 2) providing the maximum sustainable load on the stance limb; 3) maintaining an upright and extended trunk and head; 4) approximating normal hip, knee, and ankle kinematics for walking; 5) synchronizing timing of extension of the hip in stance and unloading of limb with simultaneous loading of the contralateral limb; 6) avoiding weight bearing on the arms and facilitating reciprocal arm swing; 7) facilitating symmetrical interlimb coordination; and 8) minimizing sensory stimulation that would conflict with sensory information

associated with locomotion. Each of these principles is designed to maximize sensory stimulation that matches the kinetic and kinematic properties associated with phases of stepping. Therefore, this system provides an environment in which one can facilitate balance control and manually assist trunk and leg movement during weight-bearing stepping.

In 1992, Wernig and Muller reported that patients with varying degrees of paralysis increased their overground speed following treadmill training with BWS (233). In addition, subjects with unilateral limb paralysis were able to walk a short distance without using knee-stabilizing braces and could negotiate stairs with the help of cane and handrails. In 1993, Barbeau and colleagues (17) reported an increase of overground walking speed and endurance in nine subjects with incomplete SCI after walking on the treadmill with BWS. Moreover, subjects walked with a more normal gait pattern and lower limb muscle EMG profiles (17).

Subsequently, Wernig (234) did a comprehensive study of the efficacy of locomotor training in 44 chronic and 45 acute clinically incomplete SCI subjects. In addition, those subjects were compared with 64 patients (24 chronic and 40 acute) treated conventionally. Following locomotor training, 25 subjects learned to walk independently and 7 patients could walk with assistance. In addition, those patients who could already walk before therapy improved their walking speed and endurance. By comparison, there were much fewer people improving their walking ability in the conventionally treated group. In a follow-up study, it was reported that all subjects retained and in some cases further improved their ability to walk over ground (236). Since then, this intervention has

been widely applied in the incomplete SCI subjects (18, 26, 27, 64, 80, 89, 110, 233-235).

Unfortunately, to date, there has not been a similar training effect on overground walking in humans with clinically complete SCI. However, treadmill training can improve several aspects of walking on a treadmill. For example, Dietz et al. (36, 62) reported that, after several weeks of treadmill training, the levels of weight bearing significantly increased during training. In addition, when stepping on a treadmill with BWS, rhythmic leg muscle activation patterns can be elicited in clinically complete subjects who are otherwise unable to voluntarily produce muscle activity in their legs (144). Another study has demonstrated that the levels of leg extensor muscle activity recorded in clinically complete SCI subjects significantly improved over the course of several weeks of step training (240). Thus clinically complete SCI subjects can improve their stepping ability by locomotor training, but the level of improvement has not reached a level that allows them to walk without assistance.

2.3.2 The Impact of Locomotor (Treadmill or Cycling) Training on Skeletal Muscle Following SCI

Recovery of locomotor function after spinal cord injury likely requires neural plasticity as well as the maintenance or restoration of skeletal muscle. Some of the adaptations observed in the muscles following SCI are effectively ameliorated by inducing full weight-bearing in spinalized animals while they are stepping on a treadmill (112, 173, 189). In spinal cats trained to step, the atrophic response of the muscles with a high proportion of slow fibers, i.e., the SOL, is significantly reduced (186). The largest effect has been shown in the soleus muscle, the extensors at the ankle and knee which is expected to be highly recruited during treadmill exercise (112, 173, 189).

Compared with treadmill training, however, cycling exercise is less frequently used and studied. The cycling training consists of a circular pedaling motion, which flexes one limb while extending the other. The animal is suspended in the harness, and the hindlimb feet are strapped onto the pedals of the motorized cycle, which is controlled by a speed controller (115, 203). Houle (115) studied the hind limbs of spinalized rats by daily cycling exercise for relatively long duration (60 min) at low intensity (45 rotations per minutes, 30 minutes per trials, two trials per day, 5 days per week); that is, there was no apparent load on the muscle. He found that 3 months cycling exercise diminished the extent of atrophy that was observed without exercise (115).

In addition, treadmill training has been demonstrated to prevent conversion of SOL myofibers from slow oxidative to fast glycolytic properties in spinalized cats. For example, Roy et al. (192) trained the adult spinal cord transected cats on a treadmill for around 5 months and found that step trained cats showed 100% type I MHC in soleus. In contrast, cycling did not show a similar effect. Houle et al. (115) reported that cycling exercise did not prevent or reverse the observed changes in muscle fiber type following SCI. The expression of primarily fast MHC isoforms persisted in soleus muscle, and in fact, there was an exaggerated decrease in the expression of type I MHC following 3 months of cycling exercise.

Treadmill training has also been shown to have a positive effect on muscle function. Lovely and his colleagues (141) found that 5 months treadmill training significantly improve hindlimb muscle tetanic force and specific tension. The magnitude of the forces produced at the soleus tendon while stepping on the treadmill during the last 2 weeks of experiment were close to normal levels (141). In addition, Roy et al. (192) studied

the mechanical and biochemical response of a slow extensor (SOL) and fast extensor muscle (MG) in the adult cat hindlimb following a complete low-thoracic spinal cord transection, with and without a daily short bout of stepping training on treadmill. He found that step training had no effect on the adaptations in the contractile properties of the MG associated with chronic spinal transection. However, step training had a positive effect in maintaining force in the soleus muscle.

2.4 IGF-1 Signaling and Muscle Plasticity

IGF-I has been shown to play an important role in muscle regeneration following injury (4, 5, 16, 127, 154, 195, 204), muscle hypertrophy (51, 90, 160). Systemic release of IGF-I may contribute to an increase in protein content and a reduction in protein degradation in skeletal muscle (247). Incubation of muscle cells with IGF-I (1 MicroM) for 3-7 day stimulates both cell hyperplasia and myofiber hypertrophy (230). In addition, over-expression of IGF-I using a muscle specific promoter has been associated with myofiber hypertrophy in transgenic mice (51), and local infusion of IGF-I has been shown to contribute to skeletal muscle hypertrophy (1), as well as block the aging-related loss of muscle mass in mice (19). Moreover, numerous in vivo activity models, such as increased loading, stretch and eccentric contraction are known to result in increases of IGF-I peptide and IGF-I mRNA expression in skeletal muscle (4, 5, 16, 127, 195, 204). Resistance training also has been associated with increased IGF-I mRNA expression in both animal models (3) and individuals with complete spinal cord injuries (29).

2.4.1 IGF-I and Its Related Receptor and Binding Proteins

IGF-I: mature IGF-I is a 70-amino acid single-chained polypeptide with many three-dimensional structural similarities to the proinsulin (see Review (202)). IGF-I is synthesized in the liver as a consequence of growth hormone. Systemic IGF-I produced

in the liver promotes cell division and is generally responsible for normal growth and development (157). More recently, it has been recognized that IGF-I is also produced locally by skeletal muscle in a growth hormone independent manner (244). There is significant evidence that locally produced, autocrine and/or paracrine IGF-I, is important for muscle regeneration and hypertrophy (4, 5, 16, 127, 195, 204). Specifically, Jennische (120) showed IGF-I immunoreactvity in the cytoplasm of myoblasts and myotubes and in satellite cells during muscle regeneration. LeFaucheur (134) further showed that antibodies that neutralized IGF-I reduced the number and size of regenerating myofibers after muscle damage. Experimental manipulation of muscle of IGF-1 levels, in the absence of changes in loading state, also has been shown to induce muscle hypertrophy. For example, transgenic mice in which IGF-I is over-expressed using a muscle specific promoter undergo hypertrophy (51, 160). In addition, direct infusion (1) of IGF-I in muscle results in hypertrophy, whereas inhibition of IGF-I function can prevent this response (32). Over-expression of IGF-I in muscle has also been shown to prevent some of the age-related decline in muscle mass (19).

Muscle specific isoforms and mechano-growth factor (MGF): IGF-I has different isoforms by alternative splicing. One of them is only detectable following injury and/or mechanical activity. Yang (244) cloned the cDNA of a splice variant of IGF-I that is produced by active muscle and that seems to be the factor that controls local repair, maintenance, and remodeling. This protein has been called mechano growth factor (MGF). Because of a reading-frame shift, MGF has a different 3' sequence and a different mode of action compared with systemic or liver IGF-I. Although MGF has been called a growth factor, it may be regulated as a local repair factor (244). In addition,

MGF has been shown to be significantly upregulated in response to stretch and increased loading (151, 167). Yang (244) showed that MGF was markedly upregulated in rabbit extensor digitorum longus muscle, which had been subjected to acute stretch by immobilizing the hindlimb in the extended position. This work was further supported in a study in which mRNA expression of the muscle-specific isoforms of IGF-I was up-regulated in rabbit muscle after electrical stimulation at 10 Hz for 4 days (151). It appears that the expression of both systemic and autocrine IGF-I in muscle provides an interesting link between the mechanical signal and tissue remodeling and repair.

IGF-I receptor (IGF-IR): the cellular effects of IGF-I are mediated by the activation of a specific receptor, which has two binding alpha subunits and two transmembrane beta subunits (101). It is known that the biological activity of growth factor depends on the concentration of the receptor and the affinity of its interaction (238). It has been shown that there is a change in IGF-IR mRNA expression during muscle inactivity (102, 103) and resistance exercise (101). Willis (238) also reported an increase of IGF-IR density in aged skeletal muscle, suggesting that animals retain plasticity for IGF-IR.

IGF binding protein (IGFBP): the interaction between IGFs and their binding proteins represents prereceptor regulation. So far, six IGFBP were identified. Their functions are: 1) stabilize and transport IGFs from the circulation to peripheral tissues, 2) maintain a reservoir of IGFs in the circulation, 3) potentiate or inhibit IGF function, and 4) mediate IGF-independent biological effects. Of the six systemic-type binding proteins, IGFBP-3 is primarily responsible for maintaining IGF-1 levels in the circulation in conjunction with another protein called acid labile subunit (82, 207). In contrast, IGFBP-4 and IGFBP-5 are located in skeletal muscle (207). There is some evidence that

alterations in IGFBP expression occur during unloading or overloading and that IGFBPs play a role in skeletal muscle adaptation (14, 101-103).

After binding with the receptor, the IGFR subsequently recruits the insulin receptor substrate, which results in the activation of two signaling pathways: the Ras-Raf-MEK-ERK pathway and the PI3K-Akt pathway (53). The Ras-Raf-MEK-ERK pathway is crucial for cell proliferation and cell survial in mitosis-competent cells (152). However, in adult skeletal muscle, the function of the Ras-Raf-MEK-ERK pathway is less clear. By contrast, activation of PI3K is thought to play an important role in inducing skeletal muscle hypertrophy (159).

2.4.2 Protein Synthesis Induced by IGF-I/PI3K/Akt Pathway

The binding of IGF-I to its receptor induces a conformational change in the IGF-I receptor tyrosine kinase, resulting in the activation of several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K) (180). PI3K is a lipid kinase, which phosphorylates phosphatidylinositol-4-5-bisphosphate to phosphatidylinositol -3-4-5-trisphosphate (PtdIns(3,4,5)P3). PtdIns(3,4,5)P3 provides a binding site for serine/threonine kinase Akt. After translocation to the membrane, Akt is phosphorylated and activated by phosphoinositide-dependent protein kinase (PDK).

Akt plays a very important role in mediating muscle hypertrophy. Recently, it has been demonstrated that activation of Akt is sufficient to induce hypertrophy in vivo (131). Lai (131) showed that acute activation of Akt induces dramatic increases (> 2 folds) in the size of skeletal muscle in a transgenic mouse in which a constitutively active form of Akt can be inducibly expressed in adult skeletal muscle. In addition, skeletal muscle atrophy is coupled with a decreased ability to activate the Akt pathway in burned

rats (211) and expression of active form of Akt in skeletal muscle cells is sufficient to cause hypertrophy in normal mouse gastrocnemius muscle (212).

Akt can phosphorylate mammalian target of rapamycin (mTOR) directly and indirectly by inhibition of Tsc1-Tsc2 complex (117). In turn, activation of mTOR results in an increase in protein translation by two mechanisms: first, mTOR activates p70S6k (153, 164), a positive regulator of protein translation; second, mTOR inhibits the activation of PHAS-1, a negative regulator of the protein initiation factor eIF-4E. In addition, glycogen synthesis kinase (GSK) is another substrate of Akt that has been shown to play an important role in mediating hypertrophy. GSK is inhibited following phosphorylation of Akt and inhibition of GSK is sufficient to stimulate myogenic differentiation (56). In addition, expression of a kinase-inactive form of GSK induces dramatic hypertrophy in skeletal myotubes (180) and blocks protein translation initiated by the eIF2B protein (104).

2.4.3 IGF-I/PI3K/Akt Pathway and Protein Degradation

Even though it is difficult to identify the muscle specific mediators of atrophy, a search for markers of the atrophy suggests that two genes are up-regulated in multiple models of skeletal muscle atrophy. They are called MuRF1 (32) and MAFbx (91). Both genes encode ubiquitin ligases (E3), proteins that mediate ubiquitination of specific substrates. MuRF1 has recently been shown to be able to induce the ubiquitination of the cardiac form of TroponinI and possibly the myofibrillar protein titin at the M line (126). MAFbx is suggested to bind to substrates including MyoD and calcineurin (91).

Recently, studies have shown that the upregulation of MAFbx and MuRF1 is antagonized by treatment of IGF-I (196). The mechanism is focused on the function of FOXO. The phosphorylation of Akt can inhibit the FOXO (133), whose activation is

required for the upregulation of MuRF1 and MAFbx (196). Thus, the activation of Akt can block the upregulation of MuRF1 and MAFbx by inhibition of FOXO.

2.4.4 Role of IGF-I in Satellite Cell Proliferation.

Satellite cells are quiescent muscle precursor cell in adult skeletal muscle. They are located under the basal lamina of the muscle fiber, but separate from the muscle fiber itself. Satellite cells are the main, if not only, cell type that contributes to muscle regeneration (30, 31).

During muscle hypertrophy, there appears to be a myogenic response where satellite cell-derived myoblasts are thought to fuse with existing myofibers (49, 205). The importance of this response stems from the observations that mature mammalian skeletal muscle fibers appear to maintain a relatively finite relationship between the size of the myofiber and the number of myonuclei present in a given myofiber (10, 111, 150, 219). However, mammalian myofibers become permanently differentiated shortly after birth and cannot undergo mitotic division or directly increase their myonuclear number (47). The requirement for additional nuclei to support hypertrophy appears to be met via the proliferation, differentiation, and finally the fusion of muscle satellite cells or their progeny with the enlarging myofibers, providing the new myonuclei needed to support the hypertrophy process (10, 172, 181, 182). Among the well-characterized growth factors, IGF-I is the only one that has been consistently reported to facilitate each of these processes.

Increasing evidence indicates that IGF-I stimulates both myoblast proliferation and differentiation (78). IGF-I acts via the mitogen-activated protein (MAP) kinase pathway, activating the expression of the cell cycle progression markers, such as cycling D, cdk4, c-fos, and c-jun (78, 124). After withdrawal of the Akt pathway, IGF-I subsequently

modulates expression of terminal muscle differentiation markers, such as p21, MyoD, myocyte enhancer factor 2 (MEF2) and myogenin (161).

However, although it is not clear what role the PI3K pathway plays in differentiation, recent evidence demonstrates a key role for the PI3K pathway in primary satellite cell proliferation (46). Chakravarthy et al. (46) demonstrated that IGF-I-stimulated proliferation of primary satellite cells isolated from transgenic mice overexpressing IGF-1 is associated with the activation of the PI3K/Akt signalling pathway and the downregulation of the cell-cycle inhibitor p27Kip1 (46). In addition, ectopic expression of p27Kip1 has been shown to block the IGF-I-induced increase in satellite cell proliferation (46). Furthermore, Machida et al. (143) recently reported that IGF-I represses p27Kip1 transcriptional activity through phosphorylation of Akt. Thus, p27Kip1 has been proposed to be a key regulatory factor, particularly in its ability to regulate satellite cell cycle progression.

CHAPTER 3
OUTLINE OF EXPERIMENTS

The purpose of this study was to investigate locomotor training impacts on muscular adaptations following incomplete SCI. An outline of the experiments performed is provided in the section below:

3.1 Experiment 1

3.1.1 Specific Aim

To quantify changes in rat hindlimb muscle cross-sectional area following moderate T8 spinal cord contusion injury and 3 months of locomotor training (treadmill vs. cycling).

Animals (n=8/group) were assigned to either a treadmill training group, cycle training group or a SCI (no training) group. Moderate spinal cord contusion injuries were produced using a standard NYU (New York University) impactor. Animals assigned to either training group were trained continuously for 3 months (5 days/week, 2 trials/day, 20 minutes/trial), starting on post-operative day 8. Non-invasive 3D magnetic resonance (MR) images were collected from the lower hindlimb muscle at pre-injury as well as at 1, 2, 4, 8, and 12 weeks post injury. Based on the MR images, the in vivo maximal muscle cross-sectional area of the tibialis anterior, triceps surae, extensor digitorums and flexor digitorums were determined.

3.1.2 Hypotheses

a) Following moderate T8 spinal cord contusion injury, rat hindlimb muscles show an acute decrease in maximal CSA, followed by spontaneous recovery.

b) Following moderate T8 spinal cord contusion injury, hindlimb extensor muscles show a greater decrease in maximal CSA than hindlimb flexor muscles.

c) Both cycling and treadmill locomotor training attenuate the decrease of muscle CSA following T8 spinal cord contusion injury and facilitate the recovery of muscle CSA.

3.2 Experiment 2

3.2.1 Specific Aim

a) To determine the impact of moderate T8 spinal cord contusion injury on the rat soleus morphology (fiber CSA) and in situ contractile properties. b) To determine the effect of 1-week treadmill locomotor training on the rat soleus morphology (fiber CSA) and in situ contractile properties following T8 spinal cord contusion injury.

Adult female rats (n=8 rats; 16 muscles/group) were assigned to either a SCI-treadmill training group, a SCI-no training group, or a control group. Animals assigned to the training group were trained continuously for 1week (5 days/week, 2 trials/day, 20 minutes/trial), starting on post-operative day 8. Morphological and contractile properties of the predominantly slow twitch soleus muscle were assessed at 2 weeks post-injury. Specifically, we measured the soleus fiber CSA, in situ isometric force, twitch properties and fatigability.

3.2.2 Hypotheses

a) Two weeks after moderate T8 spinal cord contusion injury, rat soleus muscle experiences a significant decrease in muscle fiber CSA and in situ isometric force production, compared to the normal control rat soleus. In addition, the rat soleus muscle is more fatigable compared to the normal control soleus.

b) One-week treadmill locomotor training attenuates the decrease in rat soleus fiber CSA and loss in in situ isometric force production observed following moderate T8 spinal cord contusion injury. In addition, the rat soleus muscle is less fatigable following 1-week treadmill training, compared to the soleus muscle in the SCI-no training group.

3.3 Experiment 3

3.3.1 Specific Aim

a) To determine the impact of moderate T8 spinal cord contusion injury on the rat soleus fiber type composition. b) To determine the effect of 1-week treadmill locomotor training on rat soleus fiber type composition following moderate T8 spinal cord contusion injury.

Adult female rats (n=12 rats; 24 muscles/group) were assigned to either a SCI-treadmill training group, a SCI-no training group, or a control group. Animals assigned to the training group were trained continuously for 1week (5 days/week, 2 trials/day, 20 minutes/trial), starting on post-operative day 8. Fiber type composition of the predominantly slow twitch soleus muscle was assessed at 2 weeks post-injury. Specifically, the soleus fiber type composition was determined using immunofluorescence techniques with monoclonal antibodies (BA-D5, SC-71, BF-F3, and BF-35).

3.3.2 Hypotheses

a) Two weeks after moderate T8 spinal cord contusion injury, the rat soleus muscle will show a fiber type shift from MHC-I towards MHC-II compared to the normal control soleus.

b) One-week treadmill locomotor training will attenuate the rat soleus fiber type shift observed following T8 spinal cord contusion injury.

3.4 Experiment 4

3.4.1 Specific Aim

a) To determine the impact of moderate T8 spinal cord contusion injury on mRNA expression of IGF-I and its related receptor and binding proteins in rat skeletal muscle.

b) To determine the impact of 1-week treadmill locomotor training on mRNA expression of IGF-I and its related receptor and binding proteins in rat skeletal muscle following T8 spinal cord contusion injury.

Adult female rats (n=6 animals, 12 muscles/group/time point) were assigned to either a SCI-treadmill training group, a SCI-no training group, or a control group. Treadmill training started on post-operative day 8. mRNA expression levels of IGF-I were assessed at both 36 hours (4-6 hrs after last (3^{rd}) bout of training) and 1 week post-treadmill training. Specifically, a semi-quantitative RT-PCR was used to quantify mRNA expression of IGF-I, MGF, IGF-R, IGFBP4, and IGFBP5.

3.4.2 Hypotheses

a) Two weeks after moderate T8 spinal cord contusion injury, rat soleus IGF-I and MGF mRNA expression levels will not be significantly different than that of control animals. However, mRNA expression of IGF-R, IGFBP4, and IGFBP5 will be significantly altered after spinal cord contusion injury.

b) One week of locomotor training increases rat soleus IGF-I and MGF mRNA expression levels in the spinal cord contused injured rat, compared to non-trained SCI animals. In addition, 1-week treadmill locomotor training attenuates the alterations observed in soleus mRNA expression of IGF-R, IGFBP4, and IGFBP5 following moderated thoracic contusion injury.

CHAPTER 4
A LONGITUDINAL STUDY OF SKELETAL MUSCLE FOLLOWING SPINAL CORD INJURY AND LOCOMOTOR TRAINING

4.1 Abstract

Spinal cord injury (SCI) results in loss of muscle mass and motor function. Recently, novel intervention therapies, focusing on repetitive locomotor training, have shown great promise in promoting spinal plasticity and recovery in motor function following SCI. Recovery of motor function after spinal cord injury likely requires both neural and muscular adaptations. The objective of this study was to implement magnetic resonance imaging to characterize the longitudinal changes in rat lower hindlimb muscle morphology following contusion SCI and to determine the therapeutic potential of two modes of locomotor training. After moderate midthoracic contusion SCI, Sprague Dawley rats were assigned to either treadmill training, cycle training or an untrained group. Lower hindlimb muscle size was examined at pre, 1-, 2-, 4-, 8-, and 12-weeks post injury. Following SCI, we observed significant atrophy in all rat hindlimb muscles. The greatest amount of atrophy (11.1 -26.3%) was measured at 2-week post-injury and spontaneous recovery in muscle size was observed by 4 weeks post SCI. Both cycling and treadmill training halted the atrophic process and accelerated the rate of recovery. The therapeutic influence of both training interventions was observed within 1 week of training. Finally, a significant positive correlation was found between locomotor functional scores and hindlimb muscle size following SCI.

4.2 Introduction

Spinal cord injury (SCI) is one of the most devastating human afflictions, leaving its victims paralyzed or with impaired motor control (107, 132). Animal models have long been used to characterize spinal cord lesions, study the mechanisms of recovery, and develop effective therapeutic interventions. Among SCI animal models, contusion injury is the most clinically relevant since it reproduces the histpathological features observed in approximately 40% of human trauma cases (39). Mid-thoracic spinal cord contusion injury of moderate severity disrupts connectivity in the lumbar spinal cord, but allows some communication between the supraspinal centers and caudal regions of the spinal cord(24).

Novel intervention therapies, focusing on repetitive locomotor training, have shown promise in promoting spinal plasticity and recovery in motor function after spinal cord injury in animal models (18, 58, 73, 74, 140). Clinical research has also shown encouraging results for treadmill locomotor training in incomplete paraplegic patients(27, 62, 106). The underlying premise of locomotor treadmill training relates to the hypothesis that rhythmic loading of the limbs and force feedback from the hindlimb muscles induces task appropriate activity-dependent plasticity (63, 74, 105). Depending upon the requirements of each subject, treadmill training benefits from the use of partial body weight support and a team of experienced gait facilitators (27). A less equipment and personnel intensive alternative training intervention is cycling locomotor training. Cycle locomotor training is accomplished by the simple circular movement of the hind limbs on a bicycle-type device, driven by a motorized belt (203). Since there are significant practical differences in the equipment and personnel requirements for the performance of

treadmill versus cycle locomotor training, there are important questions to be addressed regarding the relative efficacy of each type of training modality.

Recovery of locomotor function after spinal cord injury likely requires neural plasticity as well as the maintenance or restoration of skeletal muscle. Patients with chronic complete spinal cord injury display extensive muscle atrophy and associated secondary health related complications (44, 45). In addition, a large number of studies have shown a rapid loss in muscle mass following spinal cord transection and spinal isolation in animals (70, 137, 149, 178, 186). Both treadmill locomotor training and cycling training have demonstrated a positive influence on muscle size after spinal cord transection. Spinal cord transected cats showed a significant reduction in the atrophic response of muscles with a high proportion of slow twitch muscle fibers after about 6 months of treadmill training. The largest effect of treadmill training was observed in the soleus, a muscle containing approximately 90% slow fibers (112). A similar benefit has been reported in spinalized rats following cycling training. Interestingly, the impact of either cycling or treadmill locomotor training on skeletal muscle following contusion SCI has not been explored, even though the remaining communication between the supraspinal input and peripheral skeletal muscles may create a better target for activity dependent plasticity. In addition, only a limited amount of studies have investigated the atrophic response in skeletal muscle following contusion injury (116).

The purpose of this study was to utilize magnetic resonance (MR) imaging to investigate the longitudinal changes in muscle morphology in the rat lower hindlimb muscles following midthoracic (T8) contusion SCI. A second aim of this study was to

determine the potential of treadmill versus cycling locomotor training to ameliorate muscle atrophy and to induce muscle plasticity following spinal cord contusion injury.

4.3 Materials and Methods

4.3.1 Experimental Animals

Twenty-four Sprague Dawley rats (12 week, 228-260g; Charles River, NJ) were studied before SCI, and at early (1, 2 weeks), intermediate (4 weeks), and late (8, 12 weeks) time points following contusion SCI. The rats were housed in a temperature-controlled room at 21 ^{0}C with a 12:12 hours light:dark cycle and were provided rodent chow and water ad libitum. All procedures were performed in accordance with the U.S. Government Principle for the Utilization and Care of Vertebrate Animals and were approved by the Institutional Animal Care & Use Committee at the University of Florida.

4.3.2 Spinal Cord Contusion Injury

Spinal cord contusion injuries were produced using a NYU (New York University) impactor device. A 10g weight was dropped from a 2.5-cm height onto the T8 segment of the spinal cord exposed by laminectomy under sterile conditions. Animals received two doses of Ampicillin per day for 5 days, starting at the day of surgery. Procedures were performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia (details in (179, 226)). Subcutaneous lactated Ringer's solution (5 ml) and antibiotic spray were administered after completion of the surgery. 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, as required, 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 (24) and animals that did not fall within a preset range (0-7) were excluded from the study. Animals were subsequently divided (randomly) into three groups (n=8/group). Two groups were assigned to either treadmill or cycling locomotor training, and the third group did not receive training.

4.3.3 Treadmill and Cycling Locomotor Training

In both training paradigms animals were trained continuously for 3 months (5 days/week, 2 trials/day, 20 minutes/trial), starting on post-operative day 8. Animals assigned to the treadmill training group were given five minutes to explore the treadmill on the first training day and then encouraged to walk on the moving treadmill (11 mpm) (130) for a series of four, five-minute bouts. A minimum of five minutes rest was provided between bouts. On the second day of training, animals completed two bouts of ten minutes each, twice a day. Starting on day 3, animals trained continuously for 20 minutes with a minimum interval between trials of 2 hours. Training consisted of quadrapedal treadmill stepping. Body weight support was provided manually by the trainer. The level of body weight support was adjusted to make sure that rats could bear their weight and there was no collapse of their hindlimbs. Typically, the rats started stepping when they experienced some small load on their hindlimbs. In addition, during the first week of training, when all rats had profound paraplegia, assistance was provided to place the rat hind paws in plantar stepping position during training. In general, following 3-4 weeks of training, rats were independent in stepping with occasional assistance.

The design of the cycle trainer used in these studies was adapted from the one developed by Houle and Skinner (115). The rat bicycle is composed of a direct drive gear box, adjustable foot pedals, and a support harness, as described previously (34). The

animal was suspended in the harness, and the hindlimb feet were strapped onto the pedals of the motorized cycle, which is controlled by a speed controller and an on/off switch. The exercise consisted of a circular pedaling motion, which flexes one limb while extending the other. Care was taken not to overstretch either one of the limbs. The pedaling rate was set at 31 rpm as it approximates the self-selected cadence at which animals walk on the treadmill. During the first week of training, the rat tail was attached to an aluminum support beam with surgical tape to maintain the trunk stability during exercise. Gradually the load was increased by positioning the body harness towards the chest, so that the hind portion of the body falls over the pedal. A similar exercise method has been used in spinalized rabbits (231). The timing and duration of bicycle training was equivalent to the treadmill training protocol.

4.3.4 Magnetic Resonance Imaging

All imaging procedures were performed in a horizontal, 4.7 Tesla magnet with Paravision 2.1.1 software (Bruker Medical, Ettlingen, Germany). The spectrometer was equipped with the Bruker S116 actively shielded gradient coil and a custom-built, 5-cm long, 3.3-cm inner diameter birdcage extremity coil. Rats were initially anesthetized with 4% gaseous isoflourane in oxygen (1 L/min flow rate) and maintained with 1-2% isoflourane in oxygen for the duration of the MR experiment. Animals were placed on a cradle in the prone position with the right leg secured in the center of the coil covering the region from mid-thigh to ankle. Pulse rate, blood oxygen saturation, respiration rate, and body temperature were monitored continuously.

3D proton MR images were obtained at pre-injury as well as at 1, 2, 4, 8, and 12 weeks post injury, using a fast gradient echo imaging sequence. The data were acquired with an encoding matrix of 516 x 256 x 64, field of view of 2.5 x 2.5 x 4 cm, pulse

repetition time of 100 ms, and an echo time of 6.4 ms. The total region imaged extended from the mid-thigh to the calcaneus. Chemically selective fat suppression was used to enhance the definition between muscle groups. The CSA of the tibialis anterior, triceps surae, extensor digitorums and flexor digitorums was determined for each slice and the maximal CSA (CSA_{max}) was recorded (Fig 1). Image analysis was performed using a custom-designed interactive computer program, EXTRACTOR (77). The total CSA_{max} was defined as the sum of the individual CSA_{max}s.

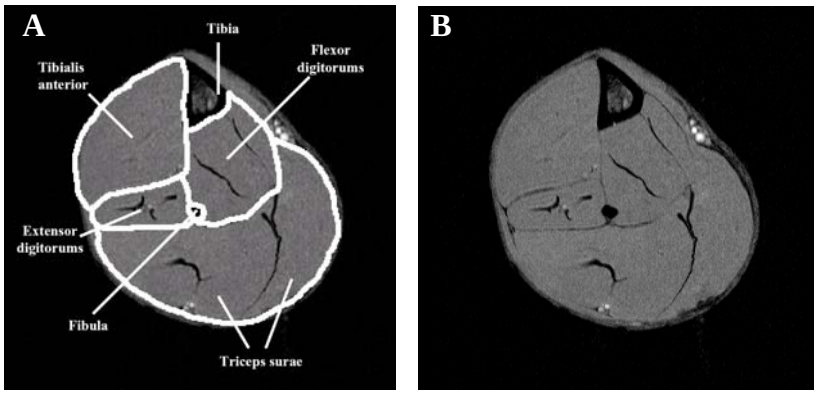

Figure 4-1. Representative trans-axial MR image of the rat lower hindlimb. A) Data were acquired with a slice thickness of 1 mm and field of view of 2.5 x 2.5 cm. B) Outline of tibialis anterior, triceps surae, extensor digitorum, and flexor digitorum muscles.

4.3.5 Open Field Locomotor Function

In order to determine the relationship between changes in muscle size and locomotor function, a standardized test for open field locomotion known as the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale was implemented (24). Rat locomotor function was evaluated at pre-injury as well as at 1, 4, 8, and 12 weeks post injury. The animal was placed in a test apparatus, observed for 4-minute, and scored in real time by

2-blinded observers (24). All open field locomotor testing was video-taped for further analysis and review.

4.3.6 Statistical Procedures

All statistical analysis was performed using SPSS for Windows (Version 10.0). Results were expressed as mean ± standard error of mean (S.E.M). Research hypotheses were tested at an alpha level of 0.05. One-way analysis of variance (ANOVA) for repeated measurements was performed to test CSA_{max} changes following contusion SCI. In addition, one-way ANOVA was used to compare individual muscles at each experimental time point. Training effects on the muscle CSA_{max} of the hindlimb muscles were assessed using two-way repeated measures ANOVA (group x time). Post hoc tests were performed using Bonferroni-Dunn procedure for multiple pairwise comparisons. In addition, Regression analysis was performed to assess the correlation between BBB locomotor rating scale and hindlimb total CSA_{max} in all rats studied.

4.4 Results

4.4.1 Muscle Size After Spinal Cord Contusion Injury.

MR images acquired before and after spinal cord contusion injury showed significant atrophy in all hindlimb muscles studied (Table 4-1). The amount of atrophy was both muscle- and time-dependent. The rate of atrophy was greatest during the first week after SCI across all muscles. At 1w-SCI, the CSA_{max} of the extensor digitorums, flexor digitorums, tibialis anterior, and triceps surae was reduced to 90.6 ± 1.9%, 86.8 ± 2.4%, 88.3 ± 1.9%, and 83.4 ± 2.2% of pre-injury values, respectively (Figure 4-2). The most extensive amount of atrophy was observed at 2w-SCI, and varied across muscles. The triceps surae demonstrated the greatest amount of atrophy (73.7 ± 4.1% of pre-injury

values), the extensor digitorums the least (88.9 ± 2.6%), followed by the tibialis anterior (79.5 ± 3.2%) and flexor digitorums (84.6 ± 7.5%) muscles (Figure 4-2).

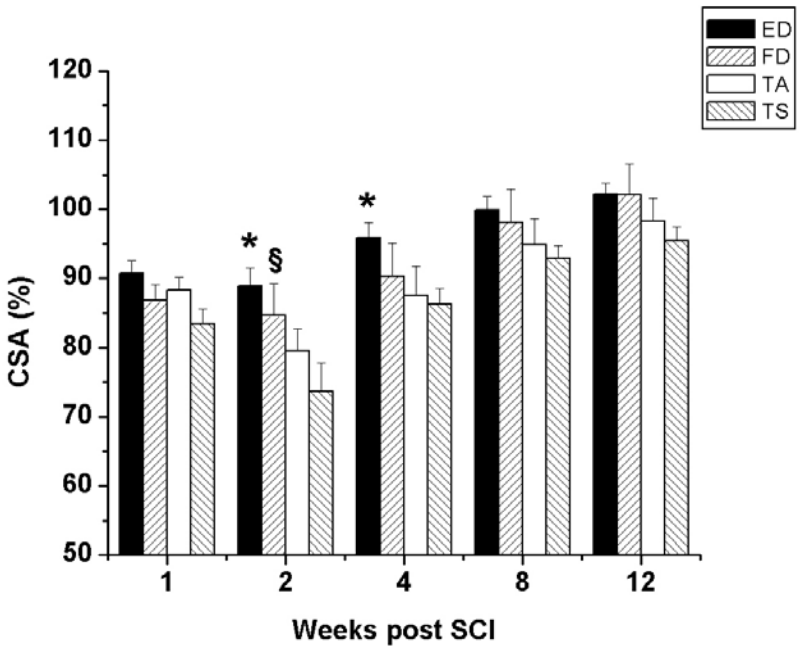

Figure 4-2. Relative changes in the CSAmax of the tibialis anterior (TA), triceps surae (TS), extensor digitorum (ED), and flexor digitorum (FD) muscles at 1, 2, 4, 8, and12 weeks following SCI. Data are expressed as a percentage of the CSAmax measured at pre-injury. * Statistically significant differences between ED and TS ($P < 0.05$). § Statistically significant differences between FD and TS ($P < 0.05$).

Starting at the fourth week post SCI, a steady increase in the muscle CSA_{max} was observed. The rate of increase in muscle CSA_{max} ranged from 0.48 to 6.26 mm^2/week. By 12 weeks post-SCI, the CSA_{max} of all muscles studied had recovered to values that were no longer statistically different from pre-injury control values. At 12w-SCI, the CSA_{max} was 102.2 ± 1.6% in the extensor digitorums, 102.1 ± 4.4% in the flexor digitorums, 98.3 ± 3.4% in the tibialis anterior and 95.4 ± 2.1% in the triceps surae. As a result, the total CSA_{max} for all muscles combined was 98.3 ± 1.6% of pre injury values.

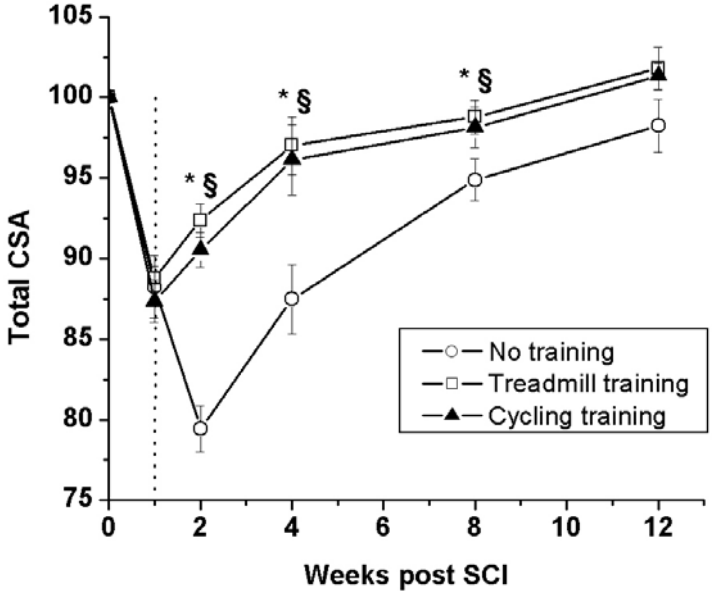

Figure 4-3. Relative change in total CSAmax in the no training group, cycling training group and treadmill training group. Data are expressed as a percentage of the CSAmax measured at pre-injury. Training started at 1 week post injury (dashed line). * Statistically significant differences between no training group and treadmill training group ($P < 0.05$). § Statistically significant differences between no training group and cycling training group ($P < 0.05$).

4.4.2 Effect of Locomotor Training on Muscle Size.

Both cycling and treadmill training decreased the rate and magnitude of atrophy in the lower hindlimb muscles following spinal cord contusion injury and accelerated the rate of recovery in muscle CSA_{max}. The influence of locomotor bicycle and treadmill training on CSA_{max} was observed as early as 1 week after the onset of training (Figure 4-3). The control untrained triceps surae muscles showed a significant decrease (-10.6 ± 4.0 mm^2/week) in muscle CSA_{max} from 1 week to 2 weeks post-SCI. In contrast, the triceps surae muscle in both training groups revealed significant muscle hypertrophy, $+3.6 \pm 1.4$ mm^2/week in the bicycle training and $+4.7 \pm 0.9$ mm^2/week in the treadmill training group. As a result, at 2 weeks post-SCI the CSA_{max} of the triceps surae was 73.4

± 4.1% of pre-injury values in the non-training SCI group and 88.8 ± 1.4% and 91.2 ± 0.9% in the cycling and treadmill training groups, respectively (Figure 4-4). As shown in Figure 4-4, a similar acute response to training was observed in the tibialis anterior muscle as well as the extensor digitorum and flexor digitorum muscles (data not shown). Consequently, at 2 weeks post-SCI the total CSA_{max} of the lower hindlimb muscles was on average 10.5% and 12.4% higher in the cycling trained and treadmill trained animals, respectively, compared to the non-trained group (Figure 4-3).

The therapeutic effect of locomotor training was observed not only at the early time point but also throughout the remaining training period (Figure 4-3). The total CSA_{max} in both cycling and treadmill locomotor training animals was significantly greater than that of non-trained SCI animals. By 12w-SCI, both cycle and locomotor training groups demonstrated a full recovery in the total CSA_{max}, with values of 101.3 ± 0.8% and 101.8 ± 1.3%, respectively compared to pre-injury control values. In addition, a direct comparison between the cycling and treadmill trained animals showed no significant difference between the two locomotor training interventions for all muscles studied, except the tibialis anterior muscle. The CSA_{max} of the tibialis anterior muscle was fully recovered at 4-week post SCI in the treadmill training group, whereas in the cycling training group pre injury values were only reached at 8-week post SCI (Figure 4-4).

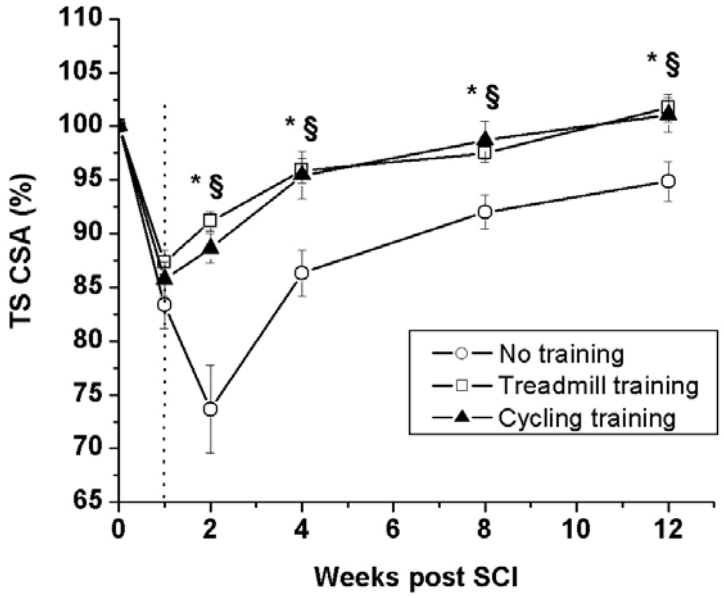

Figure 4-4. Relative change in the CSAmax of the Triceps surae (TS) in the no training group, cycling training group and treadmill training group. Data are expressed as a percentage of the CSAmax measured at pre-injury. Training started at 1 week post injury (dashed line). * Statistically significant differences between no training group and treadmill training group ($P < 0.05$). § Statistically significant differences between no training group and cycling training group ($P < 0.05$).

4.4.3 Relationship Between Hindlimb Muscle Size and Locomotor Function.

Our animals participated in a parallel study evaluating the influence of locomotor training on several functional measures after spinal cord injury (34). In the present study, we examined the relationship between the hindlimb muscle CSA and locomotor function following SCI. Our data demonstrated a significant positive correlation between scores on the BBB locomotor rating scale and hindlimb total CSA_{max} (r =0.71, $P < 0.001$). Figure 4-5 provides the results of the regression analysis.

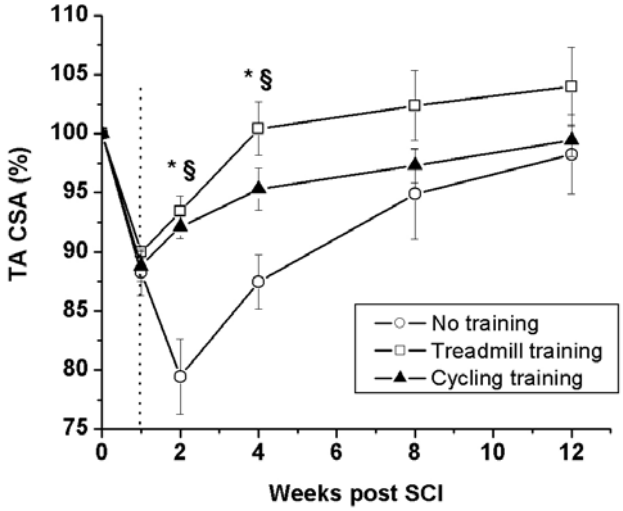

Figure 4-5. Relative change in the CSAmax of the Tibialis anterior (TA) in the no training group, cycling training group and treadmill training group. Data are expressed as a percentage of the CSAmax measured at pre-injury. Training started at 1 week post injury (dashed line). * Statistically significant differences between no training group and treadmill training group ($P < 0.05$). § Statistically significant differences between no training group and cycling training group ($P < 0.05$).

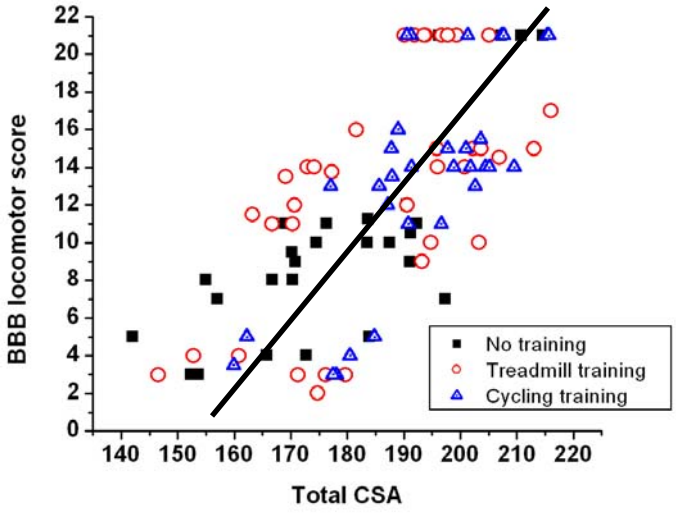

Figure 4-6. Relationship of rat locomotor function (BBB scale) and total lower hindlimb CSAmax ($r = 0.71$, $P < 0.001$). Data of all the rats at each time point were pooled together. Note that the line is drawn for visual purposes only and not to indicate a linear function.

4.5 Discussion

Following midthoracic spinal cord contusion injury, we observed significant atrophy in the rat hindlimb muscles. The degree of atrophy appeared to be muscle-specific, with the anti-gravity extensor muscles showing greater atrophy than the flexor muscles. In the non-trained animals, the greatest amount of atrophy (11.1 -26.3%) was measured at two weeks post-injury and spontaneous recovery in muscle size was observed by 4 weeks post SCI. Both cycling and treadmill training halted the atrophic process and significantly accelerated the rate of recovery. The therapeutic influence of both treadmill and bicycle training was observed within 1 week of training. Finally, a direct comparison between the cycling and treadmill trained animals showed no significant difference between the two locomotor training interventions for all muscles studied, except the tibialis anterior muscle.

Previous studies have examined the atrophic response of skeletal muscle after complete and incomplete SCI in humans (44, 45, 96) as well as in a variety of animal models (70, 136, 149, 178, 186). A large number of studies have shown a rapid loss of muscle mass in spinal cord transected and spinal isolated animals, with more extensive atrophy in slow twitch muscles compared to fast twitch muscles. However, only a limited amount of studies have investigated the effect of contusion SCI on skeletal muscle morphology. Specifically, Hutchinson et al (116) found a significant decrease in muscle wet weight (20-25%) in all lower hindlimb muscles, except the EDL, compared to age-matched controls at 1week following moderate contusion injury. They also reported that muscle atrophy occurred in flexor as well as extensor muscle groups and that the severity was similar in fast and slow muscles. In contrast, a pattern of differential decrease was noted following contusion injury in the present study. At 2 weeks post spinal cord

contusion injury, when muscle CSA measures revealed the greatest degree of atrophy, MR quantitative assessments showed the following atrophic hierarchy: triceps surae > tibialis anterior > flexor digitorums > extensor digitorums. This differential muscle response may be attributed to the higher neuromuscular activity in extensor muscles relative to flexor muscles (173). The greater degree of atrophy in the extensor vs. flexor muscles may also be influenced by the ankle joint position post-SCI. The paralyzed hindlimbs of spinalized or contusion-injured rats are maintained in an extended position and dragged by the forelimbs during cage walking (23, 187, 213). Larger muscle loss has also been observed when muscles are immobilized in a shortened position, compared to a neutral or stretched position (13).

Muscle atrophy following midthoracic contusion injury of moderate severity was transient and restoration of muscle morphology mirrored the spontaneous recovery in locomotor function. In the non-trained SCI-animals increases in CSA_{max} (or hypertrophy) were first noted at the 4 week post-SCI time point. The rate of spontaneous hypertrophy ranged from 0.1 to 6.3 mm^2/week, with the largest gains in muscle size at the intermediate time point (4 weeks). As a result, by 12 weeks post-SCI, the total CSA_{max} for all muscles combined was no longer statistically different from pre-injury control values. In comparison, Hutchinson and colleagues (116) reported that muscle atrophy, assessed by wet weights, was attenuated starting at 3-week post spinal cord contusion injury. By 10 weeks post-injury muscle wet weights were no longer different from control values, except for the medial and lateral gastrocnemius. In contrast, spinal transection animal models, in which communication between the supraspinal centers and

caudal region of the spinal cord is eliminated, have shown little spontaneous recovery in muscle size (97).

The restoration in muscle size in the moderate contusion injury model appears to mirror the spontaneous improvement in locomotor function. Basso et al. (23) showed that after a moderate thoracic spinal cord contusion injury, animals demonstrate hindlimb paralysis until 7 days post injury, which is followed by a progressive recovery in locomotor function over the next 5 weeks. In the present study, we observed a significant positive correlation between scores on the BBB locomotor rating scale and hindlimb total CSA_{max}. The consistency between the muscle morphometry and behavioral data supports the contention that neuromuscular activity is an important determinant of skeletal muscle size and *vice versa.*

One of the primary goals of this study was to determine the effect of both treadmill and cycle training on the posterior and anterior hindlimb muscles of moderate contusion spinal cord injured animals. The impact of treadmill training on skeletal muscle has been studied in a variety of animal models. Roy et al. (186) demonstrated that in spinal cats trained to step, the atrophic response of the muscles with a high proportion of slow fibers is significantly reduced. The largest effect of training was observed in the soleus muscle, followed by the extensors at the ankle and knee; muscles which are expected to be highly recruited during treadmill exercise (112). In contrast, treadmill stepping appeared to have little effect on a large number of muscles with a high proportion of fast twitch fibers, such as the tibialis anterior (186). In this study we found that treadmill training effectively facilitated muscle hypertrophy in both the tibialis anterior and triceps surae muscles of contusion injured animals. Throughout the 12 weeks of training, the tibialis

anterior and triceps muscles were significantly larger than the corresponding muscles in the untrained animals. The largest difference between the treadmill trained and untrained animals was noted at the early and intermediate time points post-SCI.

Depending upon the access and availability of treadmill locomotor training, cycle training may provide a practical alternative training strategy, contingent upon the continued demonstration of safety, feasibility, and efficacy of this approach. In the present study, we performed a direct of comparison between cycle and treadmill locomotor training and showed that both locomotor interventions induce significant muscle plasticity in all hindlimb muscles, as early as 1 week after initiation of training. While the treadmill training protocol used in this study was designed to maximize loading on the hindlimb muscles, cycling exercise uses a different strategy and is accomplished by simple circular movements of the hindlimbs on a bicycle-type device, driven by a motorized belt (34, 203). EMG data acquired in previous studies show that during cycling locomotor training the left and right hind limb muscles are stretched in an alternating pattern, which results in alternating bursts of muscle activity (115). Thus, cycling training also initiates sensory input to the spinal cord and subsequently influences the firing pattern of the motoneurons that innervate the hind-limb muscles. Houle (115) studied the hind limbs of spinalized rats following cycling training (45 rotations per minutes, 30 minutes per trials, two trials per day, 5 days per week) and found that cycling locomotor training ameliorates muscles atrophy in spinalized rats. Similarly, in this study we found that in contusion-injured rats cycling training effectively halts the atrophic process and accelerates muscle recovery. While untrained triceps surae muscles demonstrated a significant loss in muscle size from 1 week to 2 weeks post-SCI (–10.6 ±

4.0 mm^2/week), 1 week of bicycle training induced significant muscle hypertrophy (+3.6 ± 1.4 mm^2/week). However, a direct comparison between cycling and treadmill training showed that while cycling training was equally effective in promoting muscle hypertrophy in the triceps surae muscles throughout the 12 weeks of training, treadmill training induced a larger hypertrophic response in the tibialis anterior muscle compared to cycling at the intermediate and late time points. One explanation for this discrepancy may be that during cycling revolutions the ankle was kept in a dorsiflexed (shortened) position on the pedal, minimizing the stretch reflex in the tibialis anterior muscle.

A large number of investigators are studying the mechanisms responsible for neural repair, neuroplasticity and muscle hypertrophy (69). Based on our data and the well-established role of loading for muscle growth, we speculate that the training itself is not of sufficient duration to effectively induce muscle hypertrophy. Consequently we propose that repetitive muscle activation, even if induced by passive motion (e.g. during motor-driven bicycling), promotes the restoration of the neuromuscular interface and the recovery of "functional" motor units. The activation of these functional motor units under loaded conditions, such as is observed during cage reambulation, may provide the appropriate stimulus to induce muscle fiber regeneration and hypertrophy. This in turn may result in improvements in motor function, increased ambulatory activity and use-dependent neuroplasticity and muscle hypertrophy.

In conclusion, this study demonstrates that rats after spinal cord contusion injury suffer significant atrophy in the lower hindlimb muscles, with the greatest amount of atrophy noted 2 weeks following injury. Starting at 4 weeks post-SCI, rat hindlimb muscles show spontaneous recovery resulting in near normal values at 12 weeks post-

SCI. This study also demonstrates that both treadmill and cycle training diminish the extent of atrophy and facilitate muscle plasticity after contusion injury. The therapeutic influence of training on skeletal muscle was observed within the first week of training. Since muscle atrophy is associated with a myriad of secondary health problems, the potential of maintaining muscle mass with repetitive locomotor training is exciting and warrants further research. In addition, the recovery of locomotor function after spinal cord injury likely requires both neural and muscle plasticity, as well as recovery of the neuromuscular interface.

Table 4-1. Maximal cross-sectional area (mm^2) of individual muscle. Values are means ± SME. * Significantly different with pre injury value ($p < 0.05$)

	pre injury	1 week	2 week	4 week	8 week	12 week
Extensor digitorums	**14.5±0.1**	**13.2±0.3***	**12.9±0.4***	**13.9±0.4**	**14.5±0.3**	**14.8±0.3**
Flexor digitorums	**32.1±0.7**	**27.8±0.7***	**26.9±1.1***	**28.6±1.1**	**31.1±0.1**	**32.3±0.9**
Tibialis anterior	**41.8±0.9**	**36.9±1.4***	**33.3±1.7***	**35.9±1.8***	**38.8±1.4**	**40.3±1.5**
Triceps surae	**106.4±3.3**	**88.7±3.5***	**78.1±4.4***	**91.5±2.6***	**98.5±3.3***	**101.3±3.5**

CHAPTER 5
CHANGES IN SOLEUS MUSCLE FUNCTION AND FIBER MORPHOLOGY WITH ONE WEEK OF LOCOMOTOR TRAINING IN SPINAL CORD CONTUSION INJURED RATS

5.1 Abstract

Recently, a new approach in the treatment of individuals with SCI involves rehabilitation to maximize residual function using locomotor training. The purpose of this study was to examine the influence of midthoracic contusion SCI on skeletal muscle function and to evaluate the therapeutic influence of early treadmill locomotor training on soleus muscle function (peak force, fatigability, contractile properties), size (fiber area), as well as overall locomotor function (BBB) after SCI in rats. Thirty five adult Sprague Dawley rats (female, 16-20 weeks, weighing 250-290g) were studied. Histological measurements of muscle fiber size were made on all animals. Twenty four animals were designated for muscle contractile measurements (8 controls and 16 receiving a moderate T8 spinal cord contusion injury). Eight of the SCI rats received treadmill locomotor training (TM) starting 1 week after SCI for 5 consecutive days, 20 minutes/trial, 2 trials/day. The additional eight injured rats received no exercise intervention (no TM). Locomotor training resulted in a significant improvement in BBB scores (32%), muscle fiber size and function. Compared to untrained injured animals, injured animals that trained for one week exhibited 38% greater peak soleus tetanic forces, a 9% decrease in muscle fatigue, and 23% larger muscle fiber CSA. In addition, there was a strong correlation between BBB scores of injured animals and peak soleus muscle force ($r=0.704$). Collectively, these results indicate that early therapeutic intervention using

treadmill locomotor training can significantly improve functional locomotor recovery following SCI. The magnitude of these changes is remarkable considering the relatively short training interval, and clearly illustrates the potential that early exercise intervention has for countering some of the early functional deficits resulting from SCI.

5.2 Introduction

Spinal cord injury (SCI) is a devastating human condition that results in paralysis or impairments in motor control that lead to significant disability (62, 73, 229). Currently, a major therapeutic problem centers around the profound dysfunction of the primary locomotor skeletal muscles following SCI (176). Recently, a new approach in the treatment of individuals with SCI involves rehabilitation to maximize residual function using locomotor training (63, 176). Such locomotor training uses principles derived from animal and human studies showing that stepping can be generated by virtue of the neuromuscular system's responsiveness to phasic, peripheral sensory information associated with locomotion (18, 27, 58, 63, 106, 140). Although these locomotor training programs may promote functional recovery, the particular contributions of this therapy for addressing primary locomotor skeletal muscle dysfunctions are not well understood and investigation regarding these important issues is needed.

Animal models of SCI can offer a practical approach to efficiently evaluating the safety, feasibility, and efficacy of therapeutic procedures on skeletal muscle adaptations during recovery (21, 74). Current animal models of SCI include transection, compression, and contusion (24, 183). The transection model has been widely used to study limb disuse because it allows for a reproducible complete SCI (23, 69, 70, 166, 218), yet the contusion model most closely parallels the mechanism of injury of the majority of human SCIs (incomplete injuries) (116, 183) and has been shown to reproduce the

histopathologic sequence of SCI observed in about 40% of human trauma cases (39). In contrast to the transection model where animals experience a complete loss of locomotor capabilities (23, 87), animals with a moderate contusion injury regain some locomotor function without specific training (2, 22, 23, 28, 183, 225). Although contusion injuries have been performed in a variety of animal species, electrophysiologic, behavioral, and imaging studies have indicated that SCI in rats can reasonably model events occurring after human SCI (94, 146).

While much attention has been focused on neural damage and recovery after SCI (35, 72, 74, 87, 155), less attention has focused on skeletal muscle adaptations after injury, especially following a contusion injury. One of the few studies to closely examine muscle adaptations after a contusion injury in rats found that, soleus and extensor digitorum longus muscle phenotype and contractile properties were significantly affected within the first 1-3 weeks after SCI (116). With the recovery of weight supported hindlimb stepping over the course of 10 weeks, there was a concurrent return to baseline levels for contractile properties and muscle morphology, which suggests that weight bearing plays a critical role in maintaining normal muscle physiology. In addition, since changes occur within weeks after SCI, early exercise interventions may offer the greatest potential to counter these muscle adaptations and restore normal function. In Chapter 4 we showed that exercise training (bicycle or treadmill) after a contusion SCI results in faster recovery of muscle size (anterior and posterior compartment muscles) and preservation of normal muscle morphology after 3 months of training. In this investigation, we also found that the greatest differences in muscle size between trained and untrained rats occurred within the first weeks of training (chapter 4). The time course

of muscle adaptations after SCI in the aforementioned study are consistent with those of Hutchinson et al. (2001). Therefore, we were interested in investigating the short term benefits of early locomotor training in reducing muscle atrophy and improving muscle function. It has been reported that the greater disuse adaptations occur in slow twitch than in fast twitch muscle fibers and in postural (extensor) muscles more than flexor muscle groups (2, 28, 225). Accordingly, we chose to study adaptations of the soleus muscle because it is composed of slow twitch muscle fibers and functions as a postural muscle, which makes it more susceptible to muscle atrophy.

The purpose of this study was to examine the influence of SCI on skeletal muscle function and to evaluate the therapeutic influence of early treadmill locomotor training on soleus muscle function (peak force, fatigability, contractile properties), size (fiber area), as well as overall locomotor function (BBB) after SCI in rats. Our hypothesis was that early locomotor training would attenuate some of the functional changes in muscle seen early after injury by improving muscle force and contractile properties, decreasing muscle fatigue, and preserving normal muscle morphology.

5.3 Materials and Methods

5.3.1 Experimental Animals

Thirty five adult Sprague Dawley rats (female, 16-20 weeks, weighing 250-290g) were studied. Histological measurements were made on 35 animals (11 controls, 12 treadmill-SCI, and 12 no treadmill-SCI). Twenty four animals were designated for muscle contractile measurements, with eight serving as controls and sixteen receiving a moderate T_8 spinal cord contusion injury using a standard NYU impactor (23). Eight of the injured rats received treadmill locomotor training (TM) starting 1 week after SCI for 5 consecutive days, 20 minutes/trial, 2 trials/day. The additional eight injured rats

received no exercise intervention (no TM). All rats were housed in a temperature-controlled room at 21°C with a 12:12 hours light:dark cycle and were provided unrestricted access to food and water. All procedures were performed in accordance with the U.S. Government Principle for the Utilization and Care of Vertebrate Animals and were approved by the Institutional Animal Care & Use Committee at the University of Florida.

5.3.2 Open Field Locomotor Function

One of the most common and reproducible measures of locomotor function following SCI contusion injury employs a standardized test for open field locomotion known as the BBB Locomotor Rating Scale (24, 25). The BBB rates behavior ranging from individual joint movements of the hindlimb to plantar stepping and coordinated walking, progressing further to finer elements of locomotion, such as trunk stability, paw position, and tail position. Rats were evaluated for locomotion prior to surgery, 1 week after SCI, and 2 weeks after SCI. Movement was evaluated for 4 minutes by 2 examiners using the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale (24, 25).

5.3.3 Spinal Cord Contusion Injury

Spinal cord contusion injuries were produced using a MASCIS (Multicenter Animal Spinal Cord Injury Study) impactor and protocol (23). Briefly, a 10g weight was dropped from a 2.5-cm height onto the T8 segment of the spinal cord exposed by laminectomy under sterile conditions. Animals received two doses of Baytril (10mg/kg) per day for 5 days, starting the day of surgery. Procedures were performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia (179, 226). Subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given Buprenorphine (0.05mg/Kg IM) and Ketoprofen (5.0 mg/Kg SC) for pain and

inflammation over the first 36hrs 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, as required, and animals were monitored for the possibility of urinary tract infection. After SCI, animals were housed individually. On post-operative day 7, open field locomotion was assessed using the Basso-Beattie-Bresnahan (BBB) locomotor scale (24, 25) and animals that did not fall within a preset range (BBB, 4-8) were excluded from the study. Injured animals were subsequently divided into two groups (n=12/group) and were randomly assigned to either the treadmill locomotor training or no training groups. The third group consisted of non-injured control rats (n=12/group).

5.3.4 Treadmill Locomotor Training

Animals that received locomotor treadmill training were trained for 5 consecutive days (hereafter defined as 1 week of training), 2 trials/day, 20 minutes/trial, starting on post-operative day 8. Training consisted of a quadrapedal treadmill stepping. On the first day of training, animals were given five minutes to explore the treadmill and then encouraged to walk on the moving treadmill (11 mpm) (130) for a series of four, five-minute bouts. A minimum of five minutes rest was provided between bouts. Body weight support was provided manually by the trainer. The level of body weight support was adjusted to make sure that rats could bear their weight and there was no collapse of their hindlimbs. Typically, the rats started stepping when they experienced some small load on their hindlimbs. In addition, during the first week of training, when all rats had profound paraplegia, assistance was provided to place rat hindlimbs appropriately for plantar stepping during training. On the second day of training, animals completed two 10 minute bouts, twice a day. Starting on day 3, animals trained continuously for 20 minutes

with a minimum interval between trials of 6 hours. Bodyweight support through the trunk and the base of the tail was provided as necessary and gradually removed as locomotor capability improved.

5.3.5 In situ Soleus Force Measurements

In situ soleus force measurements were performed in control animals and 2 weeks after SCI (following 1 week of training). Animals were anesthetized with isoflurane (4% for induction, 1-2% for maintenance). A small dorsal, midline incision was made along the posterior lower hindlimb to expose the gastrocnemius-soleus complex. The gastrocnemius and soleus muscles were dissected free from each other and surrounding connective tissue with care not to disrupt the blood supply. A non-compliant steel wire suture (5/0 AESCULAP Inc.) was attached to the tendon at the distal end of the soleus muscle. A stimulating bipolar electrode cuff was placed around the tibial nerve, proximal to its innervation of the soleus muscle. The sciatic nerve was crushed proximal to the site of the bipolar electrode cuff and the cutaneus tibial branch was severed to ensure that all electrical stimuli were transmitted directly to the soleus muscle. The animal was then placed in a supine position in a specially fabricated experimental set up that allowed the animal to be secured in a reproducible position over a circulating warm water bath to maintain body temperature (37^{o}C). The left leg was secured in place by a pair of screw-driven pins at the condyles of the femur, while the foot was securely clamped such that soleus muscle was oriented in the horizontal plane. The distal end of the soleus tendon was attached to the variable range force transducer (Biopac Systems Inc, TSD105A) via the wire suture. The room temperature was set at 28°C and monitored throughout testing. A mineral oil drip (30^{o}C) was used to maintain a consistent muscle temperature and prevent drying of the muscle during testing.

Testing began by stimulating the soleus muscle using supramaximal (~7V, 0.2ms duration) unidirectional square-wave pulses via an S88 Grass stimulator (Grass-Telefactor, RI). The transducer force output was amplified and digitized using a Biopac MP100 System (BIOPAC Systems, Inc. Goleta CA) and Acqknowledge 3.7 computer software. The physiological tests were performed on the soleus adjusted to the isometric optimum length, determined by measuring maximal isometric forces generated at graded muscle lengths. Supramaximal stimulus intensity was verified for each animal by progressively increasing the intensity until a plateau in twitch force was achieved. A force frequency relationship was generated for each animal using supramaximal 1500ms stimulating trains ranging from 1-120Hz to determine the optimal frequency for maximal tetanic force production. This optimal frequency (70-80Hz) was used for all subsequent testing. Using the optimal stimulation frequency, maximal isometric tetanic force was repeated 3 separate times using 1500ms trains with a 5 minute rest interval between each stimulation. The maximum tetanic force of 3 attempts was recorded. In addition, three twitch stimulations were evoked for maximal twitch tension and contractile property measurements. Soleus specific tension was calculated by maximal titanic force / muscle weight (N/g). A fatigue test was also performed and consisted of a modified Burke's fatigue test with 300ms trains delivered every second for 2 minutes at the predetermined optimal frequency. Force time integral was calculated as the area under the force-time curve during muscle contraction as a measure of muscle isometric work.

5.3.6 Tissue Harvest

At the conclusion of the contractile measurements, the soleus muscles were removed from both hindlimbs of the animal. The muscles were subsequently rapidly

frozen in isopentane precooled in liquid nitrogen (storage at -80˚C) for the following histological measurements.

5.3.7 Immunohistolochemistry

Cryostat sections (10 μm) in a transverse plane were prepared from the central portion of each muscle taken from the both legs and mounted serially on gelatin-coated glass slides. Immunocytochemical reactions were performed on each cryostat section with anti-laminin to outline the muscle fibers for cross sectional area quantification. The soleus fiber CSAs were analyzed using The NIH image program (version 1.62). The pixels setting used for conversion of pixels to micrometer were 1.50 pixels- 1 um^2 for a 10 X objective. The average fiber CSA of all the fibers in each fiber type was determined.

5.3.8 Data Analysis

One way ANOVAs with Bonferroni-Dunn post hoc testing were used to compare results across groups (controls, trained and untrained animals) for peak soleus tetanic and twitch forces, peak fatigue, average fiber CSA, time to peak tension, ½ relaxation time. A p value of <0.05 was considered significant. The force decrease during fatigue was calculated as (initial-final force)/initial force. Independent t-tests were used to compare differences in BBB scores between groups before training (1 week post SCI) to ensure that there were no differences between groups prior to training. Independent t-tests were also used to compare differences in BBB scores after 1 week of training/no training (2 weeks post SCI). A Pearson's correlation was used to examine the relationship between the soleus peak force and BBB scores for the left leg across both injured groups 2 weeks after SCI.

5.4 Results

5.4.1 BBB Open Field Locomotor Scores

Intact control animals consistently demonstrated normal locomotor behavior on the BBB scale (21 point scale). One week post SCI, injured animals displayed no significant differences in BBB scores between groups designated for TM training (5.44 ± 1.61) vs no training (5.79 ± 1.98) ($p>0.05$) (Figure 5-1). At two weeks after SCI, and after one week of training for the TM group, BBB scoring was repeated and scores were significantly different between untrained (6.31 ± 2.8) and trained (10.63 ± 1.67) animals ($p<0.05$) (Figure 5-1). The significant difference between these scores indicated that one week of training had significantly improved open field locomotion scores in the trained compared with the untrained animals.

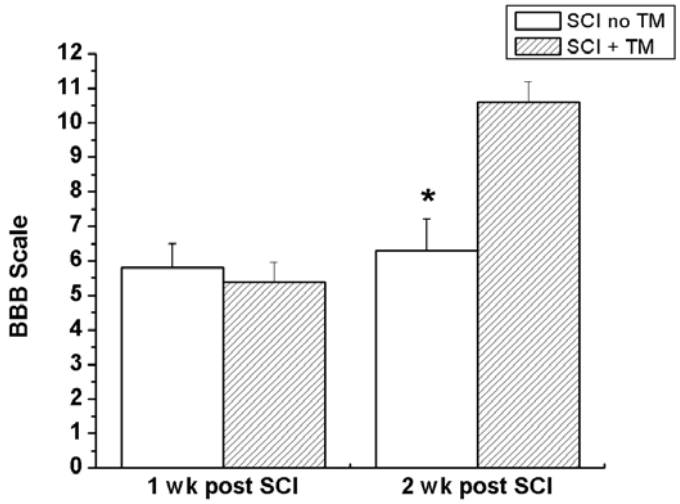

Figure 5-1. Average BBB locomotor scores for the SCI + TM and SCI no TM group at 1 and 2 weeks post SCI (n=8/group). *Significantly different between SCI no TM and SCI + TM at 2wks post SCI ($p<0.05$). SCI + TM received 5 days of TM locomotor training beginning 1 wk post SCI (mean+SEM).

5.4.2 Soleus Contractile Properties

At two weeks following SCI, the mean peak soleus muscle force measured in the untrained injured animals was 117 ± 29mN. However, the mean peak soleus muscle force measured in the injured TM group was 178 ± 19mN. This difference was significantly greater ($p<0.05$) than that recorded in the untrained injured group, but not significantly different from the mean peak force of 201 ± 14mN recorded in the intact control group (Figure 5-2). Specific tension was significantly lower in untrained injured animals compared control rats ($p<0.05$). Although a trend existed, no statistically significant differences were measured between muscle specific tension in the treadmill trained SCI rats and untrained SCI rats ($p>0.05$) (Figure 5-3).

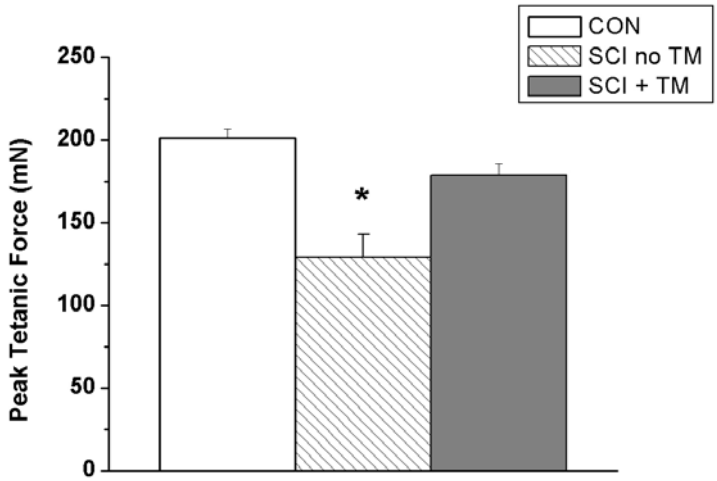

Figure 5-2. Soleus muscle peak tetanic force for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group). *Significantly lower force for SCI no TM compared to SCI + TM and CON, $p<0.05$.

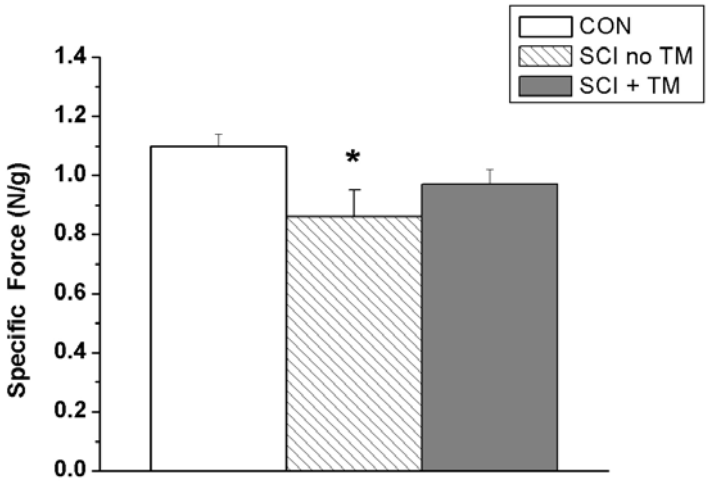

Figure 5-3. Soleus muscle specific force for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group). *Significantly lower specific force for SCI no TM compared to CON, $p<0.05$.

The possibility of a relationship between open field locomotor score and force production in skeletal muscle was tested by plotting the peak soleus muscle forces for all animals vs. their respective BBB scores (Figure 5-4). Least squares linear regression of these points revealed a correlation coefficient of $r=0.704$, suggesting a predictable relationship between BBB scores and peak soleus muscle force following SCI.

In addition, injured rats that received training demonstrated comparable fatigue to control animals ($27 \pm 0.04\%$ and $26 \pm 0.09\%$ respectively) while SCI animals without training were more fatigable ($36 \pm 0.1\%$) ($p<0.05$) (Figure 5-5). Accordingly, the isometric work (force time integral) generated during fatigue contractions was significantly lower in the untrained injured rats than control animals and treadmill trained rats (Figure 5-6). However, no significant differences were found in peak twitch force, as well as time to peak tension and half relaxation time for controls, trained SCI, and untrained SCI ($p<0.05$) (Figure 5-7, 5-8, 5-9).

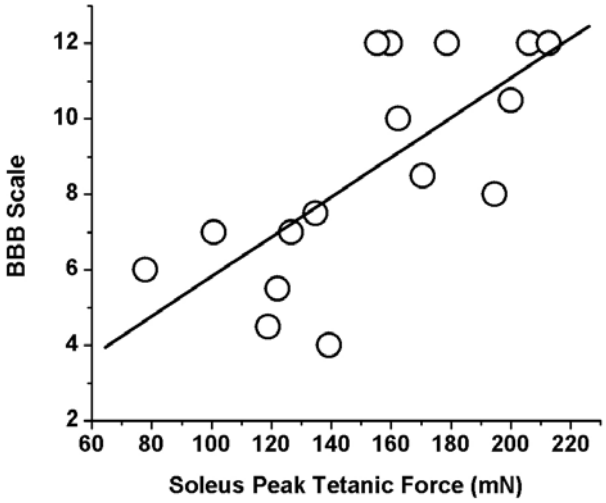

Figure 5-4. Peak soleus tetanic force vs BBB score for the left legs of SCI + TM and SCI no TM combined (n=16). Pearson correlation coefficient=0.704 ($p<0.05$). Note: controls are not included in this figure.

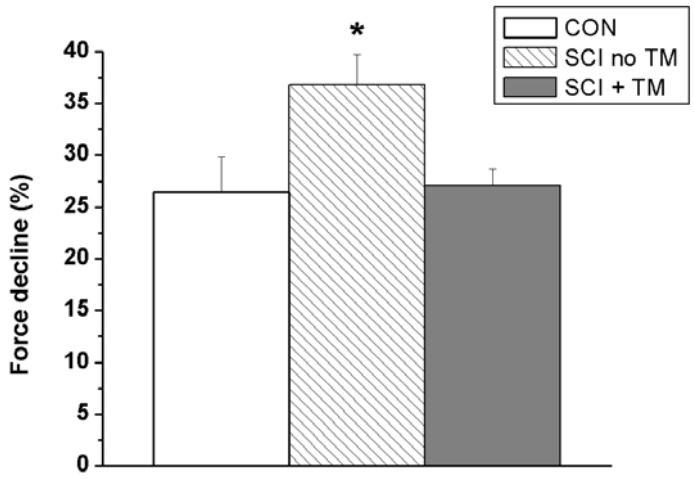

Figure 5-5. Soleus muscle fatigue (Initial-Final Force/Initial Force) (n=8/group). *Significantly greater fatigue in SCI no TM compared to CON and SCI + TM groups, $p<0.05$.

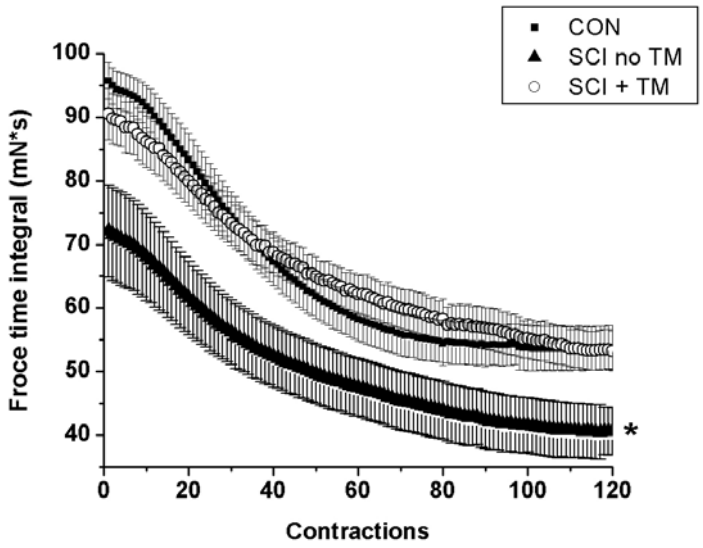

Figure 5-6. Soleus muscle force-time integral during a fatiguing protocol (n=8/group). *Significantly lower force-time integral in SCI no TM compared to CON and SCI + TM groups, $p<0.05$.

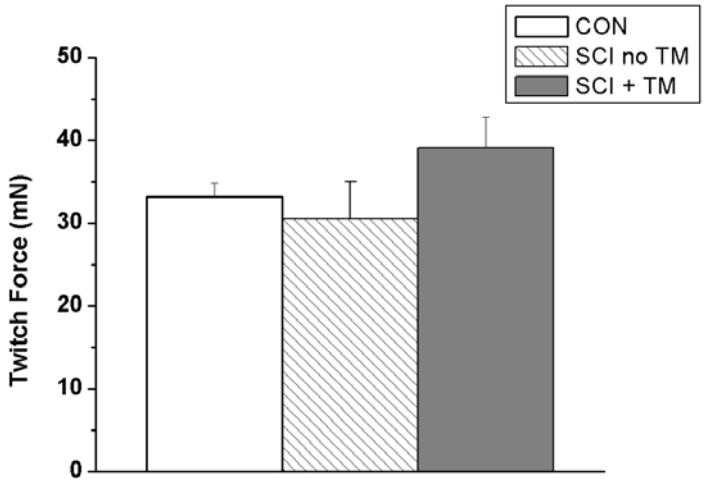

Figure 5-7. Soleus muscle peak twitch force for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group).

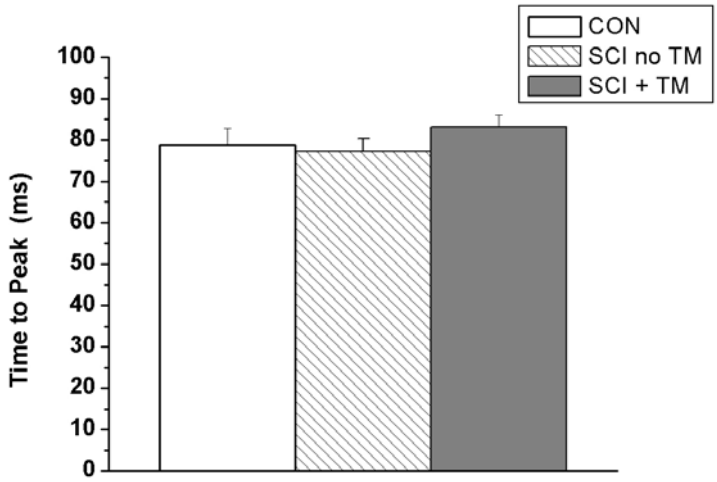

Figure 5-8. Soleus muscle time to peak for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group).

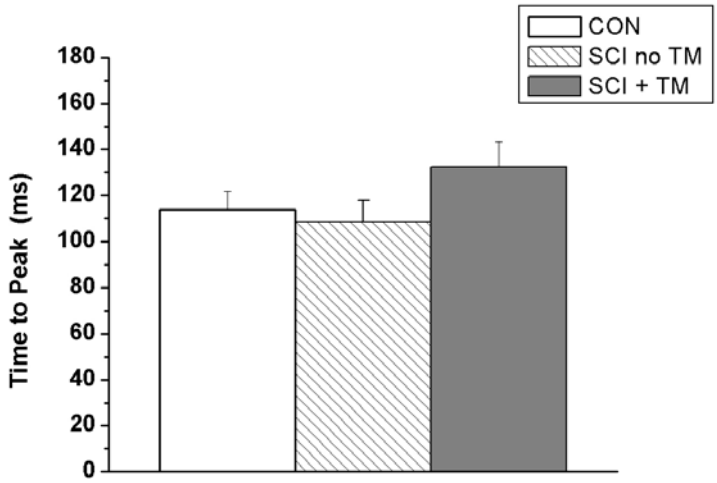

Figure 5-9. Soleus muscle ½ relaxation time for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group).

5.4.3 Soleus Muscle Fiber Cross Sectional Area

The mean soleus muscle fiber cross sectional area (CSA) observed in the untrained animals at two weeks following SCI was 1999 ± 541μm^2. By comparison, the mean CSA observed in the trained injured animals was 2654 ± 359μm^2, which was significantly

greater ($p<0.05$) than that of the untrained injured group, but not significantly different from the mean peak fiber CSA of uninjured control animals ($2467 \pm 497 \mu m^2$) ($p>0.05$) (Figure 5-10).

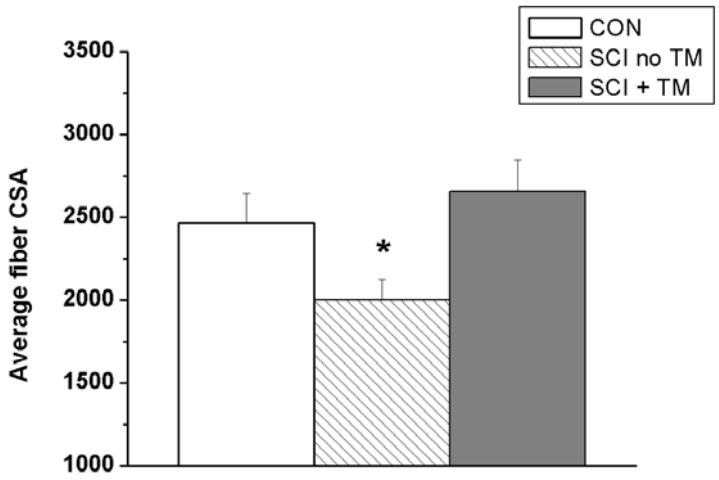

Figure 5-10. Average soleus muscle fiber CSA for CON, SCI no TM, and SCI + TM groups at 2 weeks post SCI (n=8/group). *Significantly smaller average muscle fiber CSA in SCI no TM compared to CON and SCI + TM groups, $p<0.05$.

5.5 Discussion

Collectively, these results indicate that early therapeutic intervention using treadmill locomotor training significantly increases locomotor recovery and soleus muscle size and strength following SCI. Compared to untrained SCI animals, one week of training resulted in a 32% improvement in BBB scores, a 38% greater improvement in peak soleus tetanic force, and larger muscle fiber CSA (23%). The magnitude of some of these changes is remarkable considering the relatively short training interval, and clearly illustrates the potential that early exercise intervention may have on countering some of the early functional deficits resulting from SCI.

Changes in limb loading and neural activation produced by repetitive motor training early after SCI provide critical activity in remaining spinal cord circuits that facilitate neuroplasticity and muscular plasticity for better functional recovery. The specific mechanisms by which locomotor training facilitates plasticity are poorly understood, although it has been proposed that plasticity is guided in an activity-dependent manner via biochemical changes at the cellular level of the nervous system including altered synaptic connections, altered sensitivities of neurotransmitter receptors, and altered production of neurotransmitters and growth factors (69, 74, 156). One theory that is receiving increasing attention is the role that exercise may play in increasing the release of myotrophic and neurotrophic factors in the spinal cord as well as skeletal muscle (69). Another possible explanation to account for improvements with locomotor training after SCI is that training may facilitate reorganization of undamaged neural pathways (58). It has been suggested that moving paralyzed limbs activates muscle, joint, and skin afferents which provides critical guidance to existing circuits to form new patterns of motor output (87, 106)

The results of the present study indicate that muscular plasticity occurs via a combination of changes in muscle morphology and physiological contractile properties. Very limited information exists regarding muscular plasticity early after a contusion SCI (116, 198), or early adaptive changes in muscle after locomotor training following a contusion SCI. Hutchinson et al. (2001) explored muscle adaptations in rats over 10 weeks of recovery from a moderate contusion SCI without locomotor training. They found that 3 weeks after injury, there were significant changes in muscle morphology (wet weight) and phenotype (MHC composition) that corresponded to changes in

contractile properties. Even as early as 1 week after SCI, muscle wet weights were different compared to controls even if no significant differences in contractile properties were noted. Our present study examined soleus muscle properties at an intermediate time point to that of Huchinson et al. (2 weeks post SCI) and found that there were significant differences in soleus tetanic muscle force between injured and control animals. These findings are consistent with those of our untrained SCI rats at 2 weeks after injury, and going one step further, we have found that even as little as one week of locomotor training prevents the phenotypic changes towards faster muscle seen within weeks after injury.

Using magnetic resonance imaging to longitudinally quantify changes in muscle size after SCI, we have recently found that the rate of hindlimb muscle atrophy without locomotor training was greatest during the first week after a moderate contusion SCI in rats, although the greatest absolute amount of atrophy in untrained animals occurred 2 weeks after SCI (chapter 4). Of the hindlimb muscles studied, the triceps surae (soleus and gastrocnemius muscles combined) demonstrated the greatest amount of atrophy (26.3%), which is consistent with other studies of muscle disuse that have described greater atrophy of the antigravity extensor muscles than the flexor muscles across a variety of animal species. In addition, the aforementioned study (chapter 4) found that one week of locomotor training reduced atrophy of the triceps surae by over 15% compared to untrained, injured animals. The present study provides additional evidence for the merits of early intervention in the form of not only whole muscle cross sectional area measurements, but via measurements of muscle contractile function combined with muscle fiber size measurements.

Additional studies that have investigated the impact of early locomotor training after a contusion SCI have not included muscle-specific measures of adaptations, yet they provide additional support for functional improvements with early exercise intervention (79, 158). Engesser-Cesar et al. (2005) found that mice with a contusion SCI that participated in one week of flat-surface wheel running starting one week after injury had significantly better BBB scores than injured mice without training. Multon et al. (2003) began a 12 week locomotor training study with weekly BBB measurements after inducing a spinal cord compression injury in rats with a subdurally inflated microballoon. Training began within a week of injury and resulted in differences in BBB scores by 2 weeks post injury, consistent with the locomotor behavior results of the present study.

In the present study, we found a good relationship between BBB scores and soleus tetanic force production, even though the soleus only represents one of many lower extremity muscles that are involved in hindlimb function. Nevertheless, these results suggest that while the soleus may be more susceptible to disuse with injury (2, 28, 225), its force production appears to be fairly representative of collective hindlimb functional deficits as measured by the BBB Locomotor Rating Scale. The BBB scale has been shown to be a valid and predictive measure of locomotor recovery and allows for comparisons across different injuries to evaluate treatment efficacy after SCI (24, 25). In fact, our BBB scores 1 week after injury were similar to those reported by Hutchinson et. al. (2001) and therefore allow for more direct comparisons across these 2 investigations of muscle adaptations after a moderate contusion SCI.

As little as five days of locomotor treadmill training appeared to decrease soleus muscle fiber atrophy, with no differences in fiber CSA between control and trained SCI

animals. On the other hand, SCI animals without training responded similarly to other models of limb disuse (70, 116), such that fiber CSA without training was significantly different from control and trained animals after only two weeks after SCI. In addition, the transformation towards faster, more fatigable fiber types in the untrained, SCI group was evident from our electrically elicited fatigue test with the soleus muscle of untrained animals demonstrating ~27% more fatigue than trained, SCI animals or control animals. At the same time, twitch contraction properties (peak force, time to peak tension, ½ relaxation time) were not different across groups, despite evidence for initial fiber type transformations in injured animals. Quite possibly, measurements of twitch contractile properties were not sensitive enough to detect small changes in fiber type MHC composition with a single electrically elicited contraction early after injury. In contrast to single isolated twitches, fatigue testing consisted of a series of consecutive contractions delivered over 2 minutes, the cumulative effects of which might allow for better detection of early differences in phenotypic transformations of fiber types such as increased fatigability.

It is not clear from the present study whether locomotor training early after injury prevents changes in contractile function and maintains normal muscle morphology or actually reverses any changes that have occurred within the first week after injury. But it is clear that early exercise intervention with force hindlimb loading counters the disuse atrophy present in the limbs of injured animals, which are flaccid and unloaded. A study by Burnham et al. (1997) supports our findings that early changes in MHC after SCI are present in the absence of training and suggests that interventions aimed at preventing or minimizing the transformation would need to be implemented within weeks after SCI to

prevent changes (40). Timing appears to be critical because waiting longer after SCI injury to initiate an exercise training program has been shown to have less benefit on the soleus muscle of rats injured by transection (71). Although comparisons across different SCI methods are not always generalizable, this study suggests that waiting as long as 4 weeks after injury may not offer the protective benefits that early exercise interventions may offer.

Although there are limitations in using animal models to understand human SCI recovery with locomotor training, animal models allow for a better understanding of the functional properties of complex systems. The results of the present suggest that starting some form of exercise training after SCI as early as medical stabilization of patients allows may offer the greatest protective benefits for maximizing functional recovery.

CHAPTER 6
CHANGES IN SOLEUS FIBER TYPE COMPOSITION WITH ONE WEEK OF TREADMILL LOCOMOTOR TRAINING IN SPINAL CORD CONTUSION INJURED RATS

6.1 Abstract

Neuromuscular activity plays a very important role in specifying muscle phenotypic properties. The objective of this study was to determine the impact of one week of treadmill training on the soleus fiber type composition following spinal cord contusion injury. Thirty five adult Sprague Dawley rats (female, 16-20 weeks, weighing 250-290g) were studied. Twenty four animals received a moderate T8 spinal cord contusion injury. Twelve of the SCI rats received treadmill locomotor training (TM), starting 1 week after SCI for 5 consecutive days, 20 minutes/trial, 2 trials/day. The additional twelve injured rats received no exercise intervention (no TM). The soleus muscle fiber type composition was determined using immunofluorescence techniques with monoclonal antibodies (BA-D5, SC-71, BF-F3, and BF-35). Two weeks following spinal cord contusion injury, the proportion of type I fibers decreased to 75.1 ± 3.1% (vs 86.1% in the uninjured animal). In addition, the soleus contained 8.8 ± 2.0% of fibers that were co-labeled for MHC-I and MHC-IIa and 1.4 ±1.1% of fibers that reacted with both IIa and IIx. Compared to the SCI soleus, 1-week of locomotor training resulted in a significant increase in the soleus muscle type I fibers (81.3 ± 1.7%) and a reduction in the proportion of fibers that were stained positively with both type I and IIa (3.4 ± 1.2%). There were no fibers that reacted with both type IIa and IIx in the SCI-TM soleus. In addition, training paradigms significantly increased the soleus fiber cross-sectional area

(CSA) across the different fiber types, with an average type I fiber CSA of 2170μm^2 and type IIa of 1402μm^2 in the no TM group, 2884μm^2 and 1781μm^2 in the TM group, respectively. Collectively, these results indicate that early therapeutic intervention using treadmill locomotor training can effectively reverse both the shift in fiber type composition and decrease in fiber type specific CSA following SCI.

6.2 Introduction

Neuromuscular activity (including electrical activation and limb loading) plays a very important role in specifying muscle phenotypic properties (see review (213)). Following SCI, skeletal muscle shows an increase in the percentage of fast fibers and a decrease in the percentage of slow fibers in both human subjects and animal models (15, 114, 121, 122, 137). Unfortunately, to date, very little data exists describing muscle fiber type changes after incomplete SCI. Interestingly, the innate plasticity associated with incomplete SCI furnishes the potential to possess greater plasticity than complete SCI (162, 232). In a pilot study, we found that 3 months after spinal cord contusion injury the rat soleus muscle displays a much higher proportion of fibers expressing the fast MHC isoforms compared to control soleus muscles, with 22% of the total number of fibers being MHC-I compared to ~10% in control muscles (138). However, limited studies have investigated the fiber type composition of skeletal muscle early after contusion spinal cord injury. In addition, little information is available in regard to the fiber type specific changes in fiber CSA.

Interventions that result in increased neuromuscular activation, such as electrical stimulation (170), functional overload and endurance exercise, have been shown to induce shifts in MHC towards the slower isoforms (6). For example, treadmill training of spinalized cats has been demonstrated to prevent conversion of soleus myofibers from

slow oxidative to fast glycolytic (193). Roy et al. (193) trained adult spinal cord transected cats on a treadmill for around 5 months and found that step trained cats showed 100% type I MHC in the soleus, compared to 82% untrained cast. Despite these promising findings, less attention has focused on how skeletal muscle responds to acute training interventions of shorter duration.

Therefore the purpose of this study was to determine the impact of moderate T8 spinal cord contusion injury on acute changes in rat soleus fiber type composition. A second aim of this study was to determine the effect of 1-week treadmill locomotor training on rat soleus fiber type composition following moderate T8 spinal cord contusion injury.

6.3 Materials and Methods

6.3.1 Experimental Animals

Thirty five adult Sprague Dawley rats (female, 16-20 weeks, weighing 250-290g) were studied. The rats were housed in a temperature-controlled room at 21 ^{0}C with a 12:12 hours light:dark cycle and were provided rodent chow and water ad libitum. All procedures were performed in accordance with the U.S. Government Principle for the Utilization and Care of Vertebrate Animals and were approved by the Institutional Animal Care & Use Committee at the University of Florida.

6.3.2 Spinal Cord Contusion Injury

Spinal cord contusion injuries were produced in all rats except controls using a NYU (New York University) impactor. Briefly, a 10g weight was dropped from a 2.5-cm height onto the T8 segment of the spinal cord exposed by laminectomy under sterile conditions. Animals received two doses of Baytril (10mg/kg) per day for 5 days, starting the day of surgery. Procedures were performed under ketamine (100mg/kg)-xylazine

(6.7mg/kg) anesthesia (details in (179, 226)). Subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given Buprenorphine (0.05mg/Kg IM) and Ketoprofen (5.0 mg/Kg SC) for pain and inflammation over the first 36hrs 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, as required, and animals were monitored for the possibility of urinary tract infection. After SCI, animals were housed individually. On post-operative day 7, open field locomotion was assessed using the Basso-Beattie-Bresnahan (BBB) locomotor scale (24) and animals that did not fall within a preset cutoff (BBB<8) were excluded from the study. Animals were subsequently randomly assigned to either SCI-treadmill training group (SCI-TM, n=12) and SCI no training control group (SCI, n=12). In addition, the rats that did not receive SCI were served as control group (CON, n=11).

6.3.3 Treadmill Locomotor Training

Animals in training paradigms animals were trained continuously for 5 Day (2 trials/day, 20 minutes/trial), starting on post-operative day 8. Animals assigned to the treadmill training group were given five minutes to explore the treadmill on the first training day and then encouraged to walk on the moving treadmill (11 mpm) (130) for a series of four, five-minute bouts. A minimum of five minutes rest was provided between bouts. On the second day of training, animals completed two bouts of ten minutes each, twice a day. Starting on day 3, animals trained continuously for 20 minutes with a minimum interval between trials of 2 hours. Body weight support was provided manually by the trainer. The level of body weight support was adjusted to make sure that rats could bear their weight and there was no collapse of their hindlimbs. Typically, the rats started

stepping when they experienced some small load on their hindlimbs. In addition, during the first week of training, when all rats had profound paraplegia, assistance was provided to place rat hindlimbs appropriately for plantar stepping position during training.

6.3.4 Immunohistolochemistry

At the end of the experiment, the soleus muscles were removed from both hindlimbs. The muscles were subsequently rapidly frozen at resting length in isopentane, precooled in liquid nitrogen (storage at -80˚C). Tranverse cryostat sections (10 μm) 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 quantification. Four anti-MHC Mabs (BA-D5, SC-71, BF-F3, and BF-35) were selected on the basis of their reactivity toward adult MHC (Table 6-1). Sections were incubated with rabbit anti-laminin and one of the anti-MHC antibodies (4^0C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and Fitc-conjugated anti-mouse IgG (Nordic Immunological Laboratories). Stained sections were mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4^0C to diminish fading. Stained cross sections were photographed (10X magnification) by using a Leica fluorescence microscope with a digital camera. A region of the stained serial sections from each muscle were randomly selected for MHC composition analysis. The proportions of each fiber type were determined from a sample of 150-250 fiber across the entire section of each muscle. In addition, the soleus fiber CSAs were analyzed using NIH image (version 1.62). The pixels setting used for

conversion of pixels to micrometer were 1.50 pixels- 1 μm^2 for a 10 X objective. The average fiber CSA of all the fibers in each fiber type was determined.

Table 6-1. Monoclonal antibody specificity

MAb	MHC isoforms			
	I	IIa	IIx	IIb
BA-D5	+	-	-	-
SC-71	-	+	-	-
BF-F3	-	-	-	+
BF-35	+	+	-	+

6.3.5 Data Analysis

One way ANOVAs with Bonferroni-Dunn post hoc testing were used to compare results across groups (controls, trained and untrained animals) for soleus muscle fiber type composition and average CSA. A p value of <0.05 was considered significant.

6.4 Results

6.4.1 Soleus fiber type composition

As shown in Figure 6-1 & 6-4, the control soleus muscleprimarily contained fibers reacting exclusively with type I mAb (86.1 ± 2.2%) and a small percentage of fibers (13.9 ± 2.2%) reacting with type IIa mAb exclusively. Two weeks following spinal cord contusion injury, the proportion of type I fibers decreased to 75.1 ± 3.1%. In addition, the soleus contained 8.8 ± 2.0% of fibers that were co-labeled for MHC-I and MHC-IIa and 1.4 ±1.1% of fibers that reacted with both IIa and IIx (Figure 6-2, 6-4). Compared to the SCI soleus, 1-week of locomotor training resulted in a significant increase in the soleus muscle type I fibers (81.3 ± 1.7%). There was no significant difference in fibers that were stained only with type IIa mAb among different groups. In addition, the proportion of fibers that were stained positively with both type I and IIa deceased to 3.4 ± 1.2% during

the first week of treadmill training. There were no fibers that reacted with both type IIa and IIx in the SCI-TM soleus (Figure 6-3, 6-4).

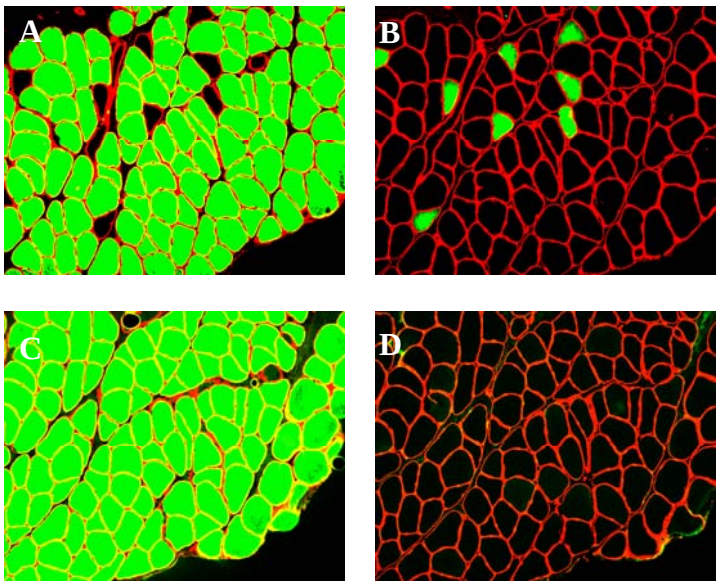

Figure 6-1. Serial cross sections of a control soleus stained with monoclonal antibodies directly against specific MHC isoforms. A) BA-D5 (anti-MHC-I). B) SC-71(anti-MHC-IIa). C) BF-35(anti-all MHCs except IIx). D) BF-F3 (anti-MHC-IIb).

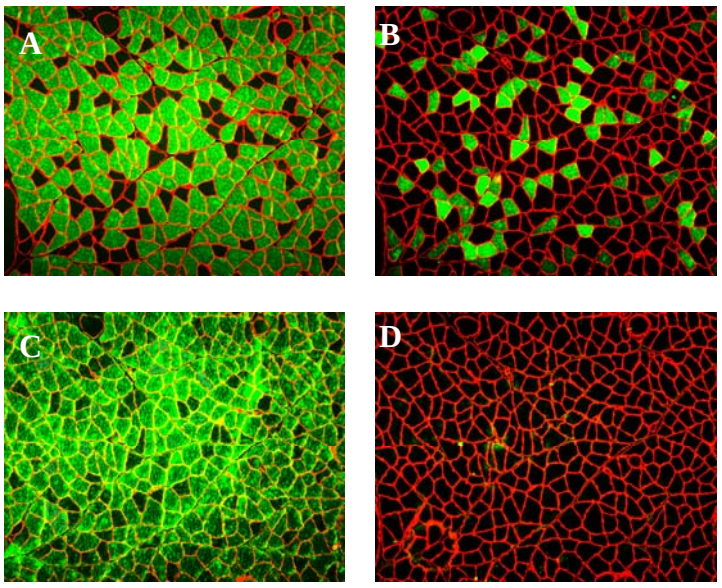

Figure 6-2. Serial cross sections of a SCI no TM soleus stained with monoclonal antibodies directly against specific MHC isoforms. A) BA-D5 (anti-MHC-I). B) SC-71(anti-MHC-IIa). C) BF-35(anti-all MHCs except IIx). D) BF-F3 (anti-MHC-IIb).

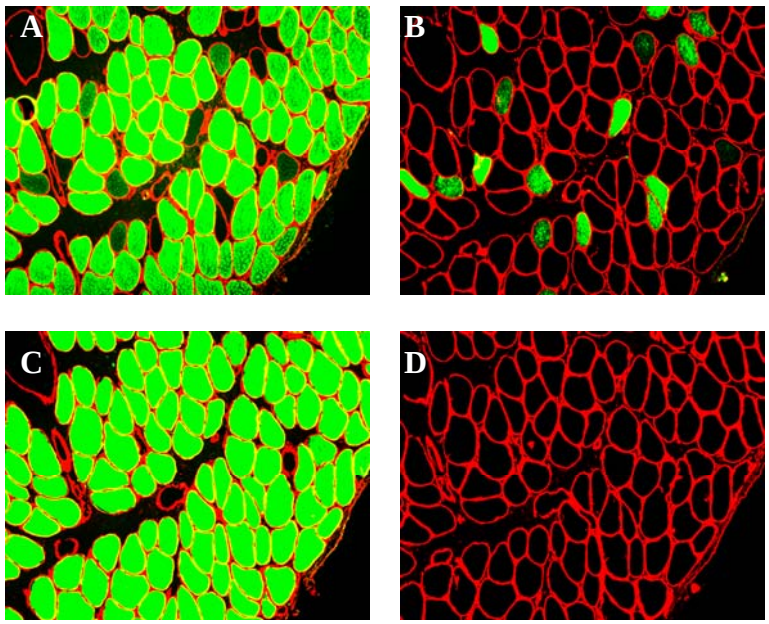

Figure 6-3. Serial cross sections of a SCI + TM soleus stained with monoclonal antibodies directly against specific MHC isoforms. A) BA-D5 (anti-MHC-I). B) SC-71(anti-MHC-IIa). C) BF-35(anti-all MHCs except IIx). D) BF-F3 (anti-MHC-IIb).

6.4.2 Soleus fiber cross-sectional area

Compared with the control soleus muscle, spinal cord contusion injury resulted in a significant decrease in soleus fiber CSA, across the different fiber types. At 2w-SCI, the CSA of type I and IIa fiber was reduced by 20.5% and 17.0% respectively (Figure 6-5). 1-week treadmill training effectively prevented the atrophic response of skeletal muscle observed following SCI. There were no significant differences in type I and IIa fiber areas between control and treadmill trained soleus. In addition, the fiber CSA of hybrid fibers (type I + type IIa) was significantly higher when compared with SCI soleus (Figure 6-5).

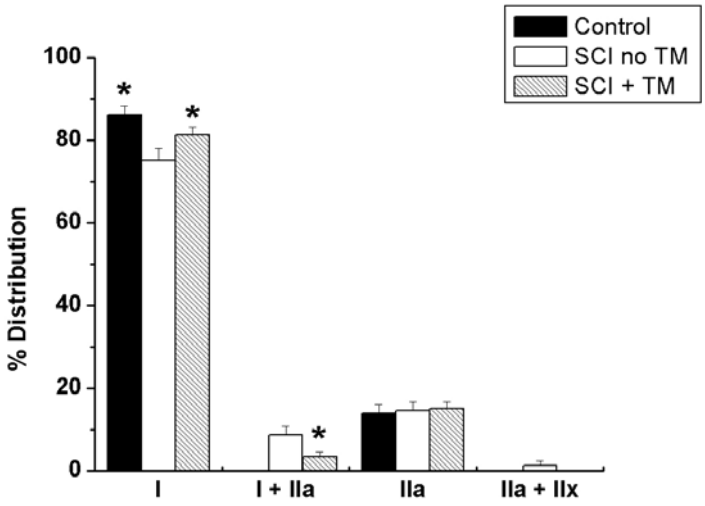

Figure 6-4. MHC based fiber type percentage composition, as determined by immunohistochemistry, of rat soleus muscle form control, SCI no TM and SCI + TM group. * Significant difference compared with the SCI no training group.

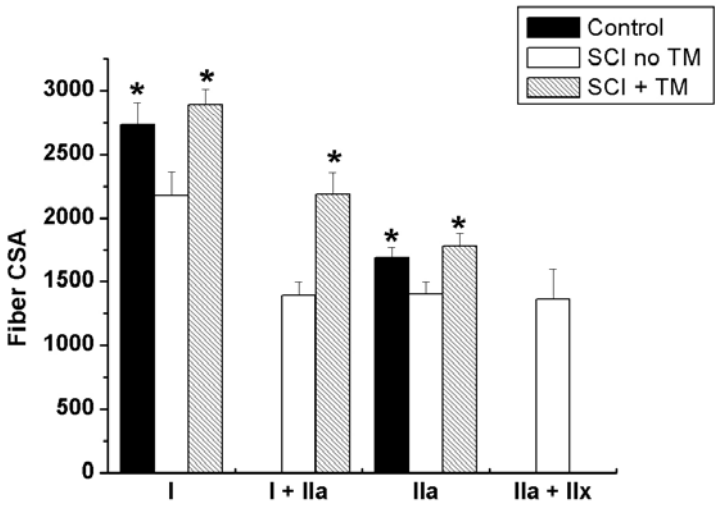

Figure 6-5. Soleus muscle fiber type specific CSA in control, SCI no TM, and SCI + TM groups at 2 weeks post SCI. * Significantly less average muscle fiber CSA in SCI no TM compared to control and SCI + TM groups.

6.5 Discussion

Two weeks following midthoracic spinal cord contusion injury, we observed a significant shift in fiber type composition from slow to fast in the rat soleus muscle, as

well as a significant decrease in fiber type specific soleus fiber CSA. However, 1-week treadmill training effectively attenuated the soleus fiber type shift as well as the fiber type specific decrease in fiber CSA.

SCI results in measurable reductions in the amount of electrical activation in mammalian muscles. For example, in cats, a complete SCI results in a 75% reduction in daily integrated electromyographic activity of the soleus muscle (7). As a result, complete spinal cord transection has been shown to induce a large increase in the expression of fast MHC isoforms (121, 122, 215, 217, 222). Talmadge et al. (222) showed that the proportion of MHC-I in the rat soleus is reduced from ~90% in control to ~25% only 3 months following a complete mid-thoracic SCI. One of the few studies to closely examine muscle adaptations after a contusion injury in rats showed an upregulation of the transitional type IIx in the soleus and extensor digitorum longus muscle within the first 3 weeks after SCI (116). In addition, we previously showed in a pilot study that the soleus MHC isoforms were shifted from MHC-I towards MHC-II 3 months after SCI, with approx. 77.9% (vs 85.1% in the uninjured animal), 19.9% (vs 15.0% in the uninjured animal), and 4.3% (vs 0% in the uninjured animal) of the total fibers containing MHC-I, MHC-IIa and MHC-IIx, respectively (138). In the present study, we observed a shift in the fiber type composition towards type II as early as 2 week post SCI, providing additional evidence to support the role of neuromuscular activity in specifying muscle phenotypic properties.

In this study we also found an increase in the presence of hybrid fibers following contusion SCI. 2 weeks after contusion SCI nearly 8.2% of the soleus muscle fibers in the untrained SCI rats were hybrid fibers. Our findings are consistent with observations

previously reported (222). For example, Talmadge (222) found that there were high proportions of type I/IIa hybrids in the soleus muscle at 15 days after spinal cord transection. The mechanism behind the presence of hybrid fiber is not clear. Originally, it was thought that muscle was in a transitional state of fiber transformation. However, studies have found that hybrid fibers could exist for up to one year after SCI, suggesting that hybrid fibers could be a stable phenotype under a particular condition (222). In the present study, we found there were no pure type IIx fibers. All fibers that contained IIx were mixed with IIa.

The unique findings of the present study were that only 1 week of daily step training emphasizing weight support on a treadmill could effectively ameliorate, although not reverse the shift in muscle fiber type composition observed following mid-thoracic contusion injury. The soleus muscle of the 1-week trained SCI animals showed 81.3% type I fibers, whereas the soleus muscle in the untrained SCI animals displayed 75% type I fibers. Numerous studies have shown an increased proportion of type I fibers in skeletal muscle after different exercise and training programs (6, 61, 193). For example, Demirel et al. (61) found that treadmill training at all different durations (30, 60, or 90 min/day) resulted in a reduction in the percentage of MHCIIb and an increase in the percentage of MHCIIa in the plantaris muscle. Unfortunately, to date, the regulatory mechanism for changes in muscle fiber type composition is still under debate. Recent studies suggested that calcineurin plays a very important role in the regulation of fiber phenotypic transformations (48, 208, 243). It has been hypothesized that calcineurin acts as a calcium server to transform mechanical stimulis to muscle signaling pathways (208). Thus increased muscle activation and limb loading during treadmill training may elevate

cytosolic calcium, and as such facilitate the fiber type transformation mediated by calcineurin (see review (129)).

Collectively, the data presented here clearly demonstrate that 2 weeks of SCI result in a fiber type shift in the rat soleus muscle towards faster fiber types. In addition, the increased coexpression of MHC isoforms in all injured animals may point to a dynamic process of fiber type transformation in the early weeks following contusion SCI. More importantly, as little as five days of locomotor treadmill training appeared to attenuate the shift in MHC composition towards faster isoforms and ameliorate fiber type specific changes in fiber CSA. Future work should be directed towards explaining mechanisms by which physical activity changes skeletal muscle phenotype and influences muscle plasticity after SCI.

CHAPTER 7
EFFECTS OF TREADMILL TRAINING ON IGF-I EXPRESSION IN RAT SOLEUS MUSCLE FOLLOWING SPINAL CORD INJURY

7.1 Introduction

One of the primary consequences of spinal cord injury (SCI) is pronounced muscle atrophy and loss of muscle function distal to the site of injury (116, 136, 137, 178). Recently, locomotor training programs have shown to help maintain muscle mass and strength and thereby promote functional recovery (63, 176). In a previous study, we showed that locomotor treadmill training ameliorates the loss in muscle size in the rat hind limb muscles following midthoracic spinal cord contusion injury (139). Although the effect of training was prevalent for up to 3 months, the largest therapeutic impact of locomotor training was observed within the first week of training. In addition, we showed that following 1 week of locomotor training muscle strength and fiber specific muscle CSA in the postural slow twitch soleus muscle of trained spinal cord injured rats was significantly higher than that of non-trained SCI rats (Chapter 5, 6).

Although the exact mechanisms for how locomotor training potentially confers its benefits are not well understood, a number of signaling pathways have been proposed to potentially regulate cellular and molecular processes involved in skeletal muscle remodeling (33, 53, 68, 118). Insulin-like growth factor I (IGF-I) has been shown to play a particularly important role in mediating protein synthesis, protein degradation and satellite cell mediated repair (1). Systemic release of IGF-I may contribute to an increase in protein content and a reduction in protein degradation in skeletal muscle (247). In

addition, overexpression of IGF-I has been associated with myofiber hypertrophy in transgenic mice (51), and local infusion of IGF-I has been shown to contribute to skeletal muscle hypertrophy (1), as well as block the aging-related loss of muscle mass in mice (19). Moreover, resistance training has been associated with increased IGF-I mRNA expression in both animal models (3) and individuals with complete spinal cord injuries (29). Although, the effect of treadmill locomotor training on the muscle IGF-I signaling pathway has not been studied, such information may help explain some of the positive therapeutic benefits of locomotor training.

The purpose of this study was to determine the impact of treadmill training on mRNA expression of IGF-I and its related receptor (R) and binding proteins (BP) in rat soleus muscle following moderate T_8 spinal cord contusion injury.

7.2 Materials and Methods

7.2.1 Experimental Animals

Thirty adult Sprague Dawley rats (female, 16-20 weeks, weighing 250-290g) were studied. The rats were housed in a temperature-controlled room at 21^0C with a 12:12 hours light:dark cycle and were provided rodent chow and water ad libitum. All procedures were performed in accordance with the U.S. Government Principle for the Utilization and Care of Vertebrate Animals and were approved by the Institutional Animal Care & Use Committee at the University of Florida.

7.2.2 Spinal Cord Contusion Injury

Spinal cord contusion injuries were produced in all rats except controls using a NYU (New York University) impactor. Briefly, a 10g weight was dropped from a 2.5-cm height onto the T_8 segment of the spinal cord exposed by laminectomy under sterile conditions. Animals received two doses of Baytril (10mg/kg) per day for 5 days, starting

the day of surgery. Procedures were performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia (details in (179, 226)). Subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given Buprenorphine (0.05mg/kg) and Ketoprofen (5.0 mg/kg) for pain and inflammation over the first 36hrs 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, as necessary, and animals were monitored for the possibility of a urinary tract infection. After SCI, animals were housed individually. On post-operative day 7, open field locomotion was assessed using the Basso-Beattie-Bresnahan (BBB) locomotor scale (24) and animals that did not fall within a preset range (BBB: 4-8) were excluded from the study.

Animals were subsequently assigned to one of four groups (n=6 rats/group): (1) SCI-8D and (2) SCI-2W represented untrained spinal cord injured animals, sacrificed 8 days and 2 weeks after SCI respectively; (3) SCI-8DTM represented animals that were sacrificed on day 8 post SCI within 36hrs of initiating three 20 minute training bouts; (4) SCI-2WTM represented animals that were trained for 5 consecutive days and sacrificed at week 2 following SCI. In addition, the rats that did not receive SCI served as a control group (CON).

7.2.3 Treadmill Locomotor Training

Animals in training paradigms animals were trained continuously for 5 days (2 trials/day, 20 minutes/trial), starting on post-operative day 8. Animals were given five minutes to explore the treadmill on the first training day and then encouraged to walk on the moving treadmill (11 mpm) (130) for a series of four, five-minute bouts. A minimum of five minutes rest was provided between bouts. On the second day of training, animals

completed two bouts of ten minutes each, twice a day. Starting on day 3, animals trained continuously for 20 minutes with a minimum interval between trials of 6 hours. Body weight support was provided manually by the trainer. The level of body weight support was adjusted to make sure that rats could bear as much weight as possible without collapse of their hindlimbs. Typically, the rats started stepping when they experienced a small load on their hindlimbs. In addition, during the first week of training, when all rats had profound paraplegia, assistance was provided to place rat hindlimbs appropriately for plantar stepping position during training.

7.2.4 Tissue Harvest

At the time points indicated above, the soleus of both legs were dissected and weighted. Half of the muscle was embedded in freezing medium and the whole muscle was snap frozen at resting length in isopentane, pre-cooled in liquid nitrogen and stored at –80 ^{0}C. The half that was embedded in freezing medium was used for immunohistochemistry. The other portion of muscle was used for RT-PCR measurements.

7.2.5 RT-PCR Measurement

RNA extraction: Total RNA was extracted from frozen muscle samples (pre weighed) by using the TRIzol Reagent (Invitrogen life technologies, Carlsbad, CA) according to the company's protocol. Briefly, around 40 mg of muscle was homogenized with a blade homogenizer in 1ml of TRIzol Reagent. Extracted RNA was precipitated from the aqueous phase with isopropanol and, after being washed with 75% ethanol, was dried and suspended in a known volume of DEPC treated water. The RNA concentration was determined by optical density at 260 nm. The muscle total RNA concentration was calculated on the basis of total RNA yield and the weight of the extracted muscle piece.

The RNA samples were stored at –80 ^{0}C to be used subsequently in determining the specific mRNA expression via relative RT-PCR procedures.

Reverse transcription: A relative RT-PCR method using 18S as internal standard (Ambion, Austin, TX) was employed to study the expression of specific mRNAs of interest. The primers used for PCR were purchased from fisher Scientific and previously used by Haddad et al (2002). In each PCR reaction, 18S ribosomal RNA was co-amplified with the target cDNA (mRNA) to serve as an internal standard and to allow for correction of differences in starting amounts of total RNA. For the 18S amplification, we used the alternate 18S internal standard (Ambion), which yields a 324-bp product. The 18S primers were mixed with competimers at an optimized ratio that ranged from 1:8 to 1:10, depending on the abundance of the target mRNA. Inclusion of the 18S competimers was necessary to decrease the 18S signal, which allowed its linear amplificaiton in the same range as the coamplified target mRNA according to Ambion's relative RT-PCR kit protocol. For each specific target mRNA, the RT-PCR reactions were carried out under identical conditions by using the same reagent premix for all the samples to be compared in the study. To validate the consistency of the analysis procedures, at least one representative from each group was included in each RT-PCR run.

Polymerase chain reaction: One microliter of each RT reaction was used for the PCR amplification. The PCR reactions were carried out in the presence of 2 mM $MgCL_2$ by using standard PCR buffer, 0,2 mM dNTP, 2 μM specific primer set, 1 μM 18S primer/competimer mix, and 0.75 unit Taq DNA polymerase in 50 ul total volume. Amplifications were carried out in a Biometra cycle with an initial denaturing step of 3 min at 94 ^{0}C, followed by 25 cycles of 45 sec 94 ^{0}C, 30 sec at 55 ^{0}C – 60 ^{0}C, 1.5 min at

72 ^{0}C, and a final step of 3 min at 72 ^{0}C. PCR products were separated on a 2% agarose gel by electrophoresis and stained with ethidium bromide. The signal quantification was conducted by scanning densitometry. In each approach, each specific mRNA signal was normalized to its corresponding 18S.

7.2.6 Immunohistolochemistry

Tranverse cryostat sections (10 μm) were prepared from the central portion of each muscle and mounted on gelatin-coated glass slides. Rabbit anti-laminin (Neomarker, Labvision, Fremont, CA) was used to outline the muscle fibers. Sections were incubated with rabbit anti-laminin and anti-Embryonic myosin (1:10) (4^{0}C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and Fitc-conjugated anti-mouse IgG (Nordic Immunological Laboratories). Stained sections were mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4^{0}C to diminish fading. Stained cross sections were photographed (10X magnification) by using a Leica fluorescence microscope with a digital camera. The proportions of positive fiber type were determined from a sample of 150-250 fiber across the entire section of each muscle.

In addition, the proportion of central nuclei in the soleus fibers was determined by H & E staining, as an indicator of muscle fiber regeneration and satellite cell activation.

7.2.7 Data Analysis

All statistical analysis was performed using SPSS for Windows (Version 10.0). 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 (ANOVA) was performed to test gene expression between groups. Post hoc tests were performed using the Bonferroni-Dunn procedure for multiple pair-wise comparisons.

7.3 Results

7.3.1 mRNA Expression of IGF-I and Its Receptor and Binding Proteins

Eight days following SCI, IGF-I mRNA levels in the soleus muscle relative to 18S were not different in injured animals compared to control animals, but IGF-I mRNA levels were increased 2.5 fold at day 14 in untrained, injured animals (Figure 7-1). mRNAs encoding the IGF-IR and IGF-I BP5 were elevated 1.2 to 1.75 fold at either 8 days or 2 weeks following SCI (Figure 7-3 and 7-5). In contrast, the IGF-I BP4 mRNA level did not change significantly throughout the experimental period in all untrained animals, although there was a trend towards a decrease at 2 weeks (Figure 7-4).

Compared with the control and untrained soleus muscles, the expression of the mRNAs for IGF-I and the loading-sensitive IGF-I isoform, MGF, were significantly increased (range of 3.8-4.7 fold) after three trials of treadmill training (Figure 7-1 and 7-2). One week of training further increased MGF and IGF-I expression (range of 4.5-6.2 fold). The magnitude of these changes was significantly higher than that seen with only three trials of training. Both three bouts and 1-week treadmill training resulted in significant increases in IGF-BP4 and IGF-R mRNA expression (Figure 7-3 and 7-4). However, there were no significant differences in the levels of mRNA for IGF-R between the training and no training groups. In addition, mRNA expression of IGF-BP5 was decreased significantly in the trained animals compared with the untrained animals at both time points (Figure 7-5).

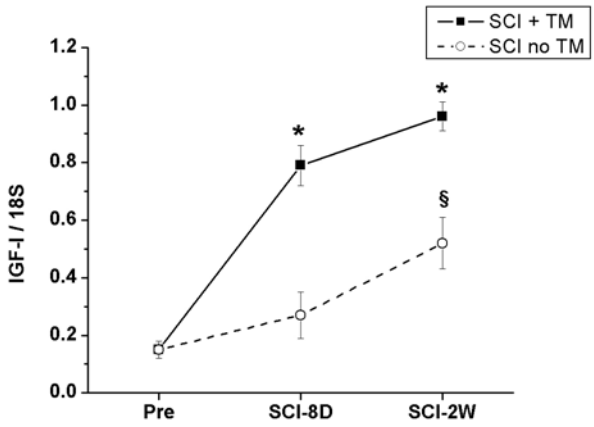

Figure 7-1. IGF-I mRNA expression relative to 18s in soleus muscle. * Significant differences compared with both control and SCI no training group. § Significant differences compared with control group.

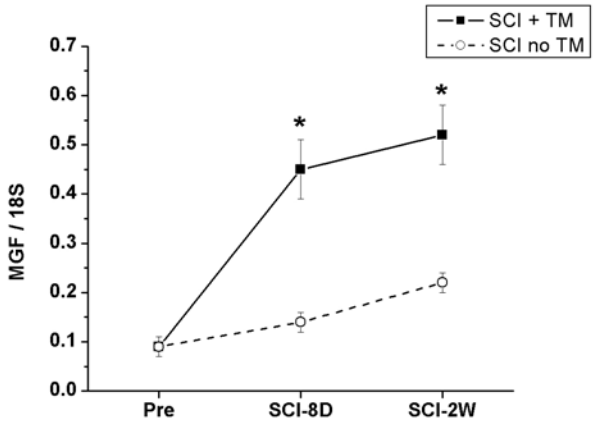

Figure 7-2. MGF mRNA expression relative to 18s in soleus muscle. * Significant differences compared with both control and SCI no training group.

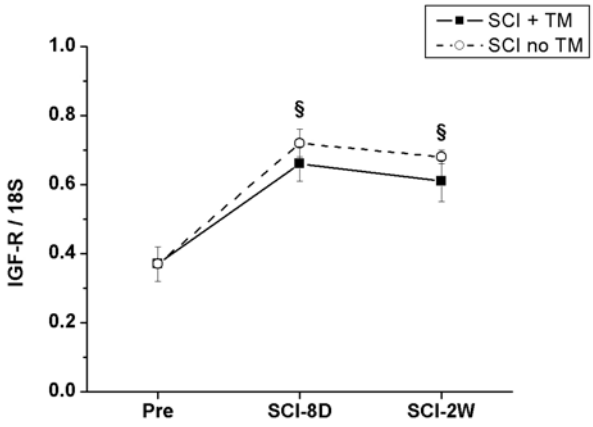

Figure 7-3. IGF-R mRNA expression relative to 18s in soleus muscle. § Significant differences compared with control group.

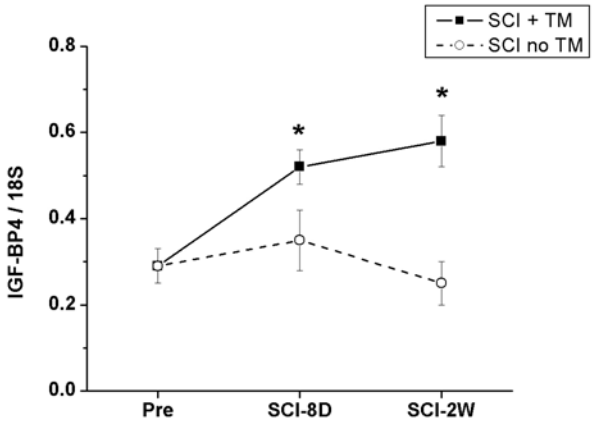

Figure 7-4. IGF-BP4 mRNA expression relative to 18s in soleus muscle. * Significant differences compared with both control and SCI no training group.

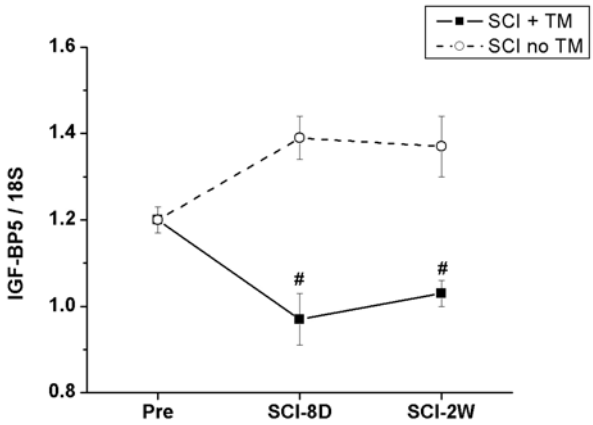

Figure 7-5. IGF-BP5 mRNA expression relative to 18s in soleus muscle. # Significant differences compared with SCI no training group.

7.3.2 Embryonic Myosin

Immunohistochemical analysis of the soleus muscle revealed the presence of small fibers expressing embryonic myosin in both SCI groups (Figure 7-6). However, the proportion of small fibers expressing embryonic myosin was 6.4 ± 2.0% and 1.6 ± 0.9% in the trained SCI and untrained SCI animals, respectively (p=0.001). We did not observe any expression of embryonic myosin in the control adult soleus muscle.

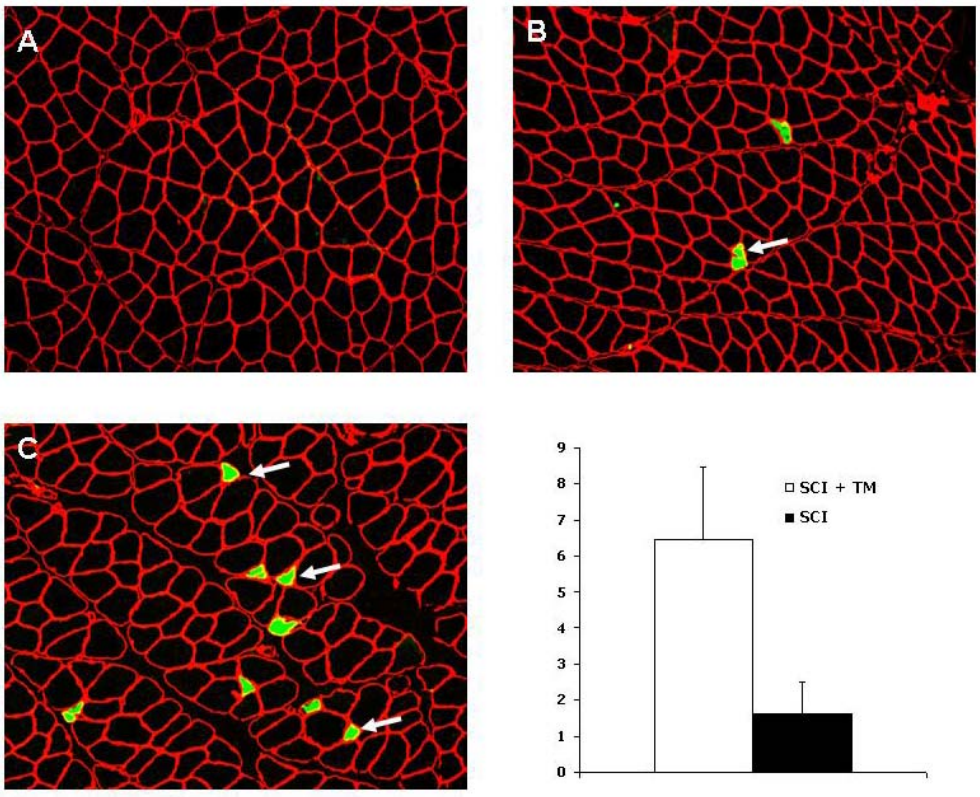

Figure 7-6. Cross-section of soleus muscle stained with monoclonal antibody against embryonic myosin isoform. (A) Control soleus; (B) SCI-2W soleus; (C) SCI-2WTM soleus.

7.3.3 Central Nuclei

Hemotoxilin and eosin staining was utilized to analyze the proportion of central nuclei in the soleus fibers as an indicator of muscle fiber regeneration (Figure 7-7). Our

data showed the presence of a small number of fibers containing central nuclei (~1% of the fibers) in the soleus muscle following 1-week of treadmill training. However, central nuclei were not visible in the control and SCI untrained soleus.

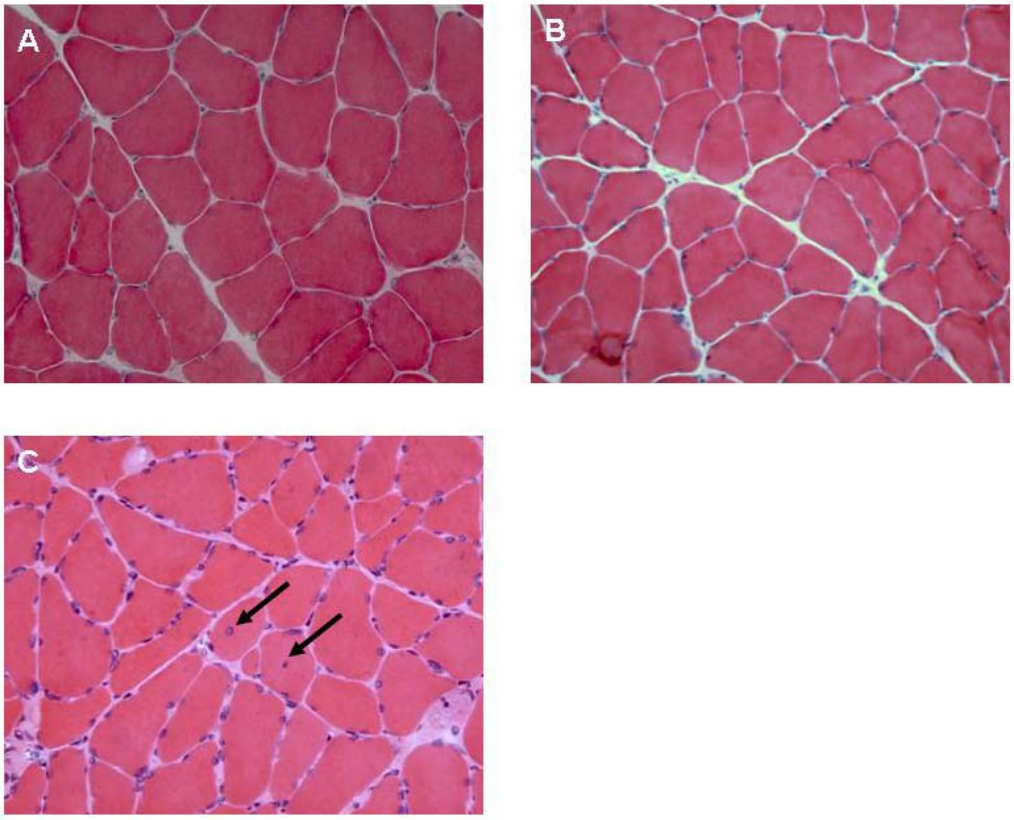

Figure 7-7. Cross-section (hemotoxilin & eosin stained) of soleus muscle showing the presence of central nuclei (arrow marks a central nucleus) after 1 week treadmill training (C), which were absent in control (A) and SCI untrained soleus (B).

7.4 Discussion

Following SCI, the soleus muscle experiences significant muscle. In chapter 6 we reported a 20.5 % and 17.0 % decrease in the CSA of the type I and IIa fibers, respectively. However, one week of treadmill training significantly ameliorated the loss in fiber size. To better understand the mechanisms involved in mediating muscle plasticity in the paralyzed muscle following contusion SCI, we measured IGF-I and its

associated receptor and binding protein mRNA expression in the soleus, an important postural muscle.

IGF-I acts as an autocrine and paracrine growth factor that has skeletal muscle anabolic properties. Therefore, we hypothesized that the IGF-I system might be downregulated in response to SCI. However, our results suggest that the IGF-I system components were either unchanged (8 days) or upregulated (2 weeks) in the paralyzed muscles following contusion SCI. Other studies have also reported contradictory findings regarding IGF-I expression in atrophic muscle in various disuse models (13, 55, 177). For example, Awede (13) reported that unloading by hindlimb suspension resulted in atrophy of soleus muscle (20%) associated with a 30% decrease of IGF-I mRNA. However, Haddad et al (103) reported that IGF-I mRNA in soleus muscle increased by 40% at 15 days after SCI, despite the muscle experiencing severe atrophy. In addition, Criswell et al. also showed that overexpression of IGF-I in skeletal muscle does not prevent atrophy following limb unloading (55).

Unlike the ambiguous results of studies examining the role of IGF-I in muscle atrophy, numerous studies have consistently shown that IGF-I plays a critical role in mediating muscle mass after a variety of exercises and training programs (1, 19, 51). A number of studies have shown that increased load on muscle can significantly increase expression of IGF-I and the loading sensitive IGF-I isoform, MGF (1, 4, 19, 20). In addition, a number of studies demonstrated that IGF-I mRNA and peptide levels increased during compensatory hypertrophy in both animal models and humans (1, 5, 13, 29). In the present study, we observed that treadmill training significantly increased soleus IGF-I and MGF expression in the soleus muscle. In specific, mRNA expression

for IGF-I and MGF was significantly increased (range of 3.8 – 4.7 folds) after only three bouts of training. One week of training resulted in a further increase of MGF and IGF-I expression. The most important feature of the treadmill is the rhythmic loading of limbs and force feedback from the hindlimb muscles. Thus, our study provides further evidence to suggest that limb loading may play an important role in skeletal muscle IGF-I expression.

The cellular effects of IGF-I may be mediated by its associated receptor and binding proteins. After binding with IGF-I, IGF-IR activates two signaling pathways: the Ras-Raf-MEK-ERK pathway (53) and the PI3K-Akt pathway (152), which are important in regulating muscle mass (159). These processes can be mediated by IGF-I binding proteins. Among six known IGF binding protein, IGFBP-4 and IGFBP-5 may play a role in skeletal muscle adaptations (14). Both IGF-BP4 and BP5 are suggested to inhibit IGF-I function, whereas IGFBP-5 also has been shown to promote IGF-I action (50, 66). In the present study, the expressions of IGFBP-4 and IGF-R were increased significantly after either 3 bouts or 5 days of treadmill training. In contrast, mRNA expression of IGFBP-5 was decreased significantly following locomotor training. These results were similar to observations previously reported using resistance exercise where Haddad (101) reported an increase of IGFBP-4 and no changes of IGFBP-5. Unfortunately, to date, the function of IGF binding proteins in muscle hypertrophy is still not clear (50, 66) .

IGF-I has been shown to promote satellite cell proliferation and differentiation. As a result of satellite differentiation, new myoblasts are formed, which subsequently fuse to form small cells known as myotubes. In this study, we observed the presence of small-sized muscle fibers expressing embryonic isoforms following treadmill training to a

larger extent than without training following SCI. The expression of embryonic myosin isoforms in the adult muscle suggests that new muscle fibers are forming (12). In addition, the presence of central nuclei after treadmill training in the soleus also suggests ongoing muscle regeneration.

In summary, 1 week of treadmill training significantly augmented IGF-I and MGF mRNA expression. Treadmill training also modulated mRNA expression of IGFR and IGFBPs. We speculate that the IGF-I signaling pathway plays an important role in mediating muscle plasticity following contusion SCI. Future studies utilizing viral-mediated gene delivery of IGF-I may have the potential to induce greater muscular plasticity following SCI.

CHAPTER 8
CONCLUSION

In this dissertation, we used a set of in vivo, in situ, and in vitro measures to examine changes in muscle characteristics following locomotor training in a rat model of incomplete SCI.

The objective of chapter 4 was to implement magnetic resonance imaging to characterize the changes in rat lower hindlimb muscle morphology following contusion SCI and to determine the therapeutic potential of two modes of locomotor training. The non-invasive nature of MR imaging allowed us to quantify skeletal muscle size longitudinally. Following SCI, we observed significant atrophy in all rat hindlimb muscles. The greatest amount of atrophy (11.1 -26.3%) was measured at 2-week post-injury, and spontaneous recovery in muscle size was observed in the untrained SCI rats by 4 weeks post SCI. Both cycling and treadmill training halted the atrophic process and accelerated the rate of recovery. The therapeutic influence of both training interventions was observed within 1 week of training, and a significant positive correlation was found between locomotor functional scores and total hindlimb muscle CSA.

Based on the findings of chapter 4, the next two chapters (5 and 6) systematically studied the adaptations in soleus muscle at a very critical time point, one week of training. Functional and immunohistochemical measurements were used to characterize soleus muscle function (peak force, fatigability, contractile properties, fiber types), muscle size, as well as overall locomotor function (BBB) after SCI in rats. Locomotor training resulted in a significant improvement in BBB scores (32%), muscle fiber size

and function. Compared to untrained injured animals, injured animals that trained for one week exhibited 38% greater peak soleus tetanic forces, a 9% decrease in muscle fatigue, and 23% larger muscle fiber CSA. In addition, there was a strong correlation between BBB scores of injured animals and peak soleus muscle force (r=0.704).

Insulin-like growth factor I (IGF-I) is a potent myogenic and neurotrophic factor and has been shown to play a critical role in muscle regeneration, muscle hypertrophy, inhibition of neuronal cell death and motor neuron regeneration. Finally, in chapter 7, we determined the impact of 1-week treadmill training on mRNA expression of IGF-I and its related receptor and binding proteins in rat skeletal muscle following contusion SCI. Our findings demonstrated that training significantly increased the expression of mRNAs for IGF-I, MGF (the loading-sensitive IGF-I isoform), IGFR, and IGFBP4. Treadmill training also significantly decreased IGFBP5 mRNA expression. In addition, immunohistochemical analysis showed an increased presence of small fibers expressing embryonic myosin following treadmill training, an indication that new muscle fibers are being formed.

Taken together, the findings from the present work demonstrate that early therapeutic intervention induces significant muscular plasticity in the rat hindlimb muscles following contusion midthoracic SCI. We hope that this study will provide essential feedback for the development of early rehabilitation interventions in SCI individuals.

LIST OF REFERENCES

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

2. **Adams GR, Caiozzo VJ, and Baldwin KM**. Skeletal muscle unweighting: spaceflight and ground-based models. *J Appl Physiol* 95: 2185-2201, 2003.

3. **Adams GR, Cheng DC, Haddad F, and Baldwin KM**. Skeletal muscle hypertrophy in response to isometric, lengthening, and shortening training bouts of equivalent duration. *J Appl Physiol* 96: 1613-1618, 2004.

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

5. **Adams GR, Haddad F, and Baldwin KM**. Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. *J Appl Physiol* 87: 1705-1712, 1999.

6. **Adams GR, Hather BM, Baldwin KM, and Dudley GA**. Skeletal muscle myosin heavy chain composition and resistance training. *J Appl Physiol* 74: 911-915, 1993.

7. **Alaimo MA, Smith JL, Roy RR, and Edgerton VR**. EMG activity of slow and fast ankle extensors following spinal cord transection. *J Appl Physiol* 56: 1608-1613, 1984.

8. **Albin MS, White RJ, Acosta-Rua G, and Yashon D**. Study of functional recovery produced by delayed localized cooling after spinal cord injury in primates. *J Neurosurg* 29: 113-120, 1968.

9. **Allen AR**. Surgery of experimental lesion of spinal cord euivalent to crush unjury of fracture dislocation of spinal column. *JAMA* 57: 878-880, 1911.

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

11. **Andersen JL, Mohr T, Biering-Sorensen F, Galbo H, and Kjaer M**. Myosin heavy chain isoform transformation in single fibres from m. vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES). *Pflugers Arch* 431: 513-518, 1996.

12. **Antonio J, and Gonyea WJ**. Skeletal muscle fiber hyperplasia. *Med Sci Sports Exerc* 25: 1333-1345, 1993.

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

14. **Balagopal P, Rooyackers OE, Adey DB, Ades PA, and Nair KS**. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 273: E790-800, 1997.

15. **Baldwin KM, Roy RR, Sacks RD, Blanco C, and Edgerton VR**. Relative independence of metabolic enzymes and neuromuscular activity. *J Appl Physiol* 56: 1602-1607, 1984.

16. **Bamman MM, Shipp JR, Jiang J, Gower BA, Hunter GR, Goodman A, McLafferty CL, Jr., and Urban RJ**. Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *Am J Physiol Endocrinol Metab* 280: E383-390, 2001.

17. **Barbeau H, Chau C, and Rossignol S**. Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats. *Brain Res Bull* 30: 387-393, 1993.

18. **Barbeau H, and Rossignol S**. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res* 412: 84-95, 1987.

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

20. **Barton-Davis ER, Shoturma DI, and Sweeney HL**. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand* 167: 301-305, 1999.

21. **Basso DM**. Neuroanatomical substrates of functional recovery after experimental spinal cord injury: implications of basic science research for human spinal cord injury. *Phys Ther* 80: 808-817, 2000.

22. **Basso DM, Beattie MS, and Bresnahan JC**. Descending systems contributing to locomotor recovery after mild or moderate spinal cord injury in rats: experimental evidence and a review of literature. *Restor Neurol Neurosci* 20: 189-218, 2002.

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

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

25. **Basso DM, Beattie MS, Bresnahan JC, Anderson DK, Faden AI, Gruner JA, Holford TR, Hsu CY, Noble LJ, Nockels R, Perot PL, Salzman SK, and Young W**. MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multicenter Animal Spinal Cord Injury Study. *J Neurotrauma* 13: 343-359, 1996.

26. **Behrman AK, Lawless-Dixon AR, Davis SB, Bowden MG, Nair P, Phadke C, Hannold EM, Plummer P, and Harkema SJ**. Locomotor training progression and outcomes after incomplete spinal cord injury. *Phys Ther* 85: 1356-1371, 2005.

27. **Behrman AL, and Harkema SJ**. Locomotor training after human spinal cord injury: a series of case studies. *Phys Ther* 80: 688-700, 2000.

28. **Berry P, Berry I, and Manelfe C**. Magnetic resonance imaging evaluation of lower limb muscles during bed rest--a microgravity simulation model. *Aviat Space Environ Med* 64: 212-218, 1993.

29. **Bickel CS, Slade JM, and Dudley GA**. Long-term spinal cord injury increases susceptibility to isometric contraction-induced muscle injury. *Eur J Appl Physiol* 91: 308-313, 2004.

30. **Bischoff R**. Cell cycle commitment of rat muscle satellite cells. *J Cell Biol* 111: 201-207, 1990.

31. **Bischoff R**. Control of satellite cell proliferation. *Adv Exp Med Biol* 280: 147-157; discussion 157-148, 1990.

32. **Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, and Glass DJ**. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704-1708, 2001.

33. **Booth FW, Tseng BS, Fluck M, and Carson JA**. Molecular and cellular adaptation of muscle in response to physical training. *Acta Physiol Scand* 162: 343-350, 1998.

34. **Bose P, Li XY, Parmer R, Andersen DK, Reier PJ, and Thompson FJ**. Bicycle locomotor training showed equally effective to more labor-intensive treadmill training in reducing spasticity and improving locomotion following spinal cord injury in a rat model. *Submitted J of Neuroscience* 2005.

35. **Bose P, Parmer R, and Thompson FJ**. Velocity-dependent ankle torque in rats after contusion injury of the midthoracic spinal cord: time course. *J Neurotrauma* 19: 1231-1249, 2002.

36. **Bottinelli R, Schiaffino S, and Reggiani C**. Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. *J Physiol* 437: 655-672, 1991.

37. **Bresnahan JC, Beattie MS, Stokes BT, and Conway KM**. Three-dimensional computer-assisted analysis of graded contusion lesions in the spinal cord of the rat. *J Neurotrauma* 8: 91-101, 1991.

38. **Buller AJ, Eccles JC, and Eccles RM**. Differentiation of fast and slow muscles in the cat hind limb. *J Physiol* 150: 399-416, 1960.

39. **Bunge RP, Puckett WR, Becerra JL, Marcillo A, and Quencer RM**. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Adv Neurol* 59: 75-89, 1993.

40. **Burnham R, Martin T, Stein R, Bell G, MacLean I, and Steadward R**. Skeletal muscle fibre type transformation following spinal cord injury. *Spinal Cord* 35: 86-91, 1997.

41. **Caiozzo VJ, Baker MJ, Herrick RE, Tao M, and Baldwin KM**. Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle. *J Appl Physiol* 76: 1764-1773, 1994.

42. **Caiozzo VJ, Haddad F, Baker MJ, and Baldwin KM**. Influence of mechanical loading on myosin heavy-chain protein and mRNA isoform expression. *J Appl Physiol* 80: 1503-1512, 1996.

43. **Cao Q, Zhang YP, Iannotti C, DeVries WH, Xu XM, Shields CB, and Whittemore SR**. Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. *Exp Neurol* 191 Suppl 1: S3-S16, 2005.

44. **Castro MJ, Apple DF, Jr., Hillegass EA, and Dudley GA**. Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury. *Eur J Appl Physiol Occup Physiol* 80: 373-378, 1999.

45. **Castro MJ, Apple DF, Jr., Staron RS, Campos GE, and Dudley GA**. Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. *J Appl Physiol* 86: 350-358, 1999.

46. **Chakravarthy MV, Abraha TW, Schwartz RJ, Fiorotto ML, and Booth FW**. Insulin-like growth factor-I extends in vitro replicative life span of skeletal muscle satellite cells by enhancing G1/S cell cycle progression via the activation of phosphatidylinositol 3'-kinase/Akt signaling pathway. *J Biol Chem* 275: 35942-35952, 2000.

47. **Chambers RL, and McDermott JC**. Molecular basis of skeletal muscle regeneration. *Can J Appl Physiol* 21: 155-184, 1996.

48. **Chin ER, Olson EN, Richardson JA, Yang Q, Humphries C, Shelton JM, Wu H, Zhu W, Bassel-Duby R, and Williams RS**. A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. *Genes Dev* 12: 2499-2509, 1998.

49. **Clark KR, Sferra TJ, and Johnson PR**. Recombinant adeno-associated viral vectors mediate long-term transgene expression in muscle. *Hum Gene Ther* 8: 659-669, 1997.

50. **Clemmons DR**. Role of insulin-like growth factor binding proteins in controlling IGF actions. *Mol Cell Endocrinol* 140: 19-24, 1998.

51. **Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C, and Schwartz RJ**. 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, 1995.

52. **Collmann H, Wullenweber R, Sprung C, and Duisberg R**. Spinal cord blood flow after experimental trauma in the dog. II. Early changes of spinal cord blood flow in the surrounding area of a traumatic lesion. *Adv Neurol* 20: 443-450, 1978.

53. **Coolican SA, Samuel DS, Ewton DZ, McWade FJ, and Florini JR**. The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. *J Biol Chem* 272: 6653-6662, 1997.

54. **Cote MP, Menard A, and Gossard JP**. Spinal cats on the treadmill: changes in load pathways. *J Neurosci* 23: 2789-2796, 2003.

55. **Criswell DS, Booth FW, DeMayo F, Schwartz RJ, Gordon SE, and Fiorotto ML**. Overexpression of IGF-I in skeletal muscle of transgenic mice does not prevent unloading-induced atrophy. *Am J Physiol* 275: E373-379, 1998.

56. **Cross DA, Alessi DR, Cohen P, Andjelkovich M, and Hemmings BA**. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378: 785-789, 1995.

57. **Daverat P, Sibrac MC, Dartigues JF, Mazaux JM, Marit E, Debelleix X, and Barat M**. Early prognostic factors for walking in spinal cord injuries. *Paraplegia* 26: 255-261, 1988.

58. **de Leon RD, Hodgson JA, Roy RR, and Edgerton VR**. Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. *J Neurophysiol* 79: 1329-1340, 1998.

59. **de Leon RD, London NJ, Roy RR, and Edgerton VR**. Failure analysis of stepping in adult spinal cats. *Prog Brain Res* 123: 341-348, 1999.

60. **de Leon RD, Reinkensmeyer DJ, Timoszyk WK, London NJ, Roy RR, and Edgerton VR**. Use of robotics in assessing the adaptive capacity of the rat lumbar spinal cord. *Prog Brain Res* 137: 141-149, 2002.

61. **Demirel HA, Powers SK, Naito H, Hughes M, and Coombes JS**. Exercise-induced alterations in skeletal muscle myosin heavy chain phenotype: dose-response relationship. *J Appl Physiol* 86: 1002-1008, 1999.

62. **Dietz V, Colombo G, Jensen L, and Baumgartner L**. Locomotor capacity of spinal cord in paraplegic patients. *Ann Neurol* 37: 574-582, 1995.

63. **Dietz V, and Harkema SJ**. Locomotor activity in spinal cord-injured persons. *J Appl Physiol* 96: 1954-1960, 2004.

64. **Dietz V, Wirz M, Colombo G, and Curt A**. Locomotor capacity and recovery of spinal cord function in paraplegic patients: a clinical and electrophysiological evaluation. *Electroencephalogr Clin Neurophysiol* 109: 140-153, 1998.

65. **Dietz V, Wirz M, and Jensen L**. Locomotion in patients with spinal cord injuries. *Phys Ther* 77: 508-516, 1997.

66. **Duan C**. Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. *J Endocrinol* 175: 41-54, 2002.

67. **Ducker TB, Lucas JT, and Wallace CA**. Recovery from spinal cord injury. *Clin Neurosurg* 30: 495-513, 1983.

68. **Dunn SE, Burns JL, and Michel RN**. Calcineurin is required for skeletal muscle hypertrophy. *J Biol Chem* 274: 21908-21912, 1999.

69. **Dupont-Versteegden EE, Houle JD, Dennis RA, Zhang J, Knox M, Wagoner G, and Peterson CA**. Exercise-induced gene expression in soleus muscle is dependent on time after spinal cord injury in rats. *Muscle Nerve* 29: 73-81, 2004.

70. **Dupont-Versteegden EE, Houle JD, Gurley CM, and Peterson CA**. Early changes in muscle fiber size and gene expression in response to spinal cord transection and exercise. *Am J Physiol* 275: C1124-1133, 1998.

71. **Dupont-Versteegden EE, Murphy RJ, Houle JD, Gurley CM, and Peterson CA**. Mechanisms leading to restoration of muscle size with exercise and transplantation after spinal cord injury. *Am J Physiol Cell Physiol* 279: C1677-1684, 2000.

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

73. **Edgerton VR, Roy RR, Hodgson JA, Prober RJ, de Guzman CP, and de Leon R**. Potential of adult mammalian lumbosacral spinal cord to execute and acquire improved locomotion in the absence of supraspinal input. *J Neurotrauma* 9 Suppl 1: S119-128, 1992.

74. **Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, and Roy RR**. Plasticity of the spinal neural circuitry after injury. *Annu Rev Neurosci* 27: 145-167, 2004.

75. **Eldridge L**. Ability of electrical silent nerves to specify fast and slow muscle characteristics. *Plasticity of Muscle* 325-337, 1981.

76. **Eldridge L, Dhoot GK, and Mommaerts WF**. Neural influences on the distribution of troponin I isotypes in the cat. *Exp Neurol* 83: 328-346, 1984.

77. **Elliott MA, Walter GA, Gulish H, Sadi AS, Lawson DD, Jaffe W, Insko EK, Leigh JS, and Vandenborne K**. Volumetric measurement of human calf muscle from magnetic resonance imaging. *Magma* 5: 93-98, 1997.

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

79. **Engesser-Cesar C, Anderson AJ, Basso DM, Edgerton VR, and Cotman CW**. Voluntary wheel running improves recovery from a moderate spinal cord injury. *J Neurotrauma* 22: 157-171, 2005.

80. **Field-Fote EC, Lindley SD, and Sherman AL**. Locomotor training approaches for individuals with spinal cord injury: a preliminary report of walking-related outcomes. *J Neurol Phys Ther* 29: 127-137, 2005.

81. **Fouad K, Metz GA, Merkler D, Dietz V, and Schwab ME**. Treadmill training in incomplete spinal cord injured rats. *Behav Brain Res* 115: 107-113, 2000.

82. **Foulstone EJ, Savage PB, Crown AL, Holly JM, and Stewart CE**. Role of insulin-like growth factor binding protein-3 (IGFBP-3) in the differentiation of primary human adult skeletal myoblasts. *J Cell Physiol* 195: 70-79, 2003.

83. **Friedman JA, Windebank AJ, Moore MJ, Spinner RJ, Currier BL, and Yaszemski MJ**. Biodegradable polymer grafts for surgical repair of the injured spinal cord. *Neurosurgery* 51: 742-751; discussion 751-742, 2002.

84. **Fu ES, and Saporta S**. Methylprednisolone inhibits production of interleukin-1beta and interleukin-6 in the spinal cord following compression injury in rats. *J Neurosurg Anesthesiol* 17: 82-85, 2005.

85. **Gale K, Kerasidis H, and Wrathall JR**. Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. *Exp Neurol* 88: 123-134, 1985.

86. **Gardner MB, Holden MK, Leikauskas JM, and Richard RL**. Partial body weight support with treadmill locomotion to improve gait after incomplete spinal cord injury: a single-subject experimental design. *Phys Ther* 78: 361-374, 1998.

87. **Gazula VR, Roberts M, Luzzio C, Jawad AF, and Kalb RG**. Effects of limb exercise after spinal cord injury on motor neuron dendrite structure. *J Comp Neurol* 476: 130-145, 2004.

88. **Geisler FH, Dorsey FC, and Coleman WP**. Recovery of motor function after spinal-cord injury--a randomized, placebo-controlled trial with GM-1 ganglioside. *N Engl J Med* 324: 1829-1838, 1991.

89. **Giangregorio LM, Hicks AL, Webber CE, Phillips SM, Craven BC, Bugaresti JM, and McCartney N**. Body weight supported treadmill training in acute spinal cord injury: impact on muscle and bone. *Spinal Cord* 43: 649-657, 2005.

90. **Glass DJ**. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* 37: 1974-1984, 2005.

91. **Gomes MD, Lecker SH, Jagoe RT, Navon A, and Goldberg AL**. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A* 98: 14440-14445, 2001.

92. **Goshgarian HG**. The crossed phrenic phenomenon: a model for plasticity in the respiratory pathways following spinal cord injury. *J Appl Physiol* 94: 795-810, 2003.

93. **Graham SC, Roy RR, Navarro C, Jiang B, Pierotti D, Bodine-Fowler S, and Edgerton VR**. Enzyme and size profiles in chronically inactive cat soleus muscle fibers. *Muscle Nerve* 15: 27-36, 1992.

94. **Gregory CM, Vandenborne K, Castro MJ, and Dudley GA**. Human and rat skeletal muscle adaptations to spinal cord injury. *Can J Appl Physiol* 28: 491-500, 2003.

95. **Greve JM, Muszkat R, Schmidt B, Chiovatto J, Barros Filho TE, and Batisttella LR**. Functional electrical stimulation (FES): muscle histochemical analysis. *Paraplegia* 31: 764-770, 1993.

96. **Grimby G, Broberg C, Krotkiewska I, and Krotkiewski M**. Muscle fiber composition in patients with traumatic cord lesion. *Scand J Rehabil Med* 8: 37-42, 1976.

97. **Grossman EJ, Roy RR, Talmadge RJ, Zhong H, and Edgerton VR**. Effects of inactivity on myosin heavy chain composition and size of rat soleus fibers. *Muscle Nerve* 21: 375-389, 1998.

98. **Gruner JA**. A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma* 9: 123-126; discussion 126-128, 1992.

99. **Guizar-Sahagun G, Grijalva I, Madrazo I, Franco-Bourland R, Salgado H, Ibarra A, Oliva E, and Zepeda A**. Development of post-traumatic cysts in the spinal cord of rats-subjected to severe spinal cord contusion. *Surg Neurol* 41: 241-249, 1994.

100. **Guizar-Sahagun G, Rivera F, Babinski E, Berlanga E, Madrazo M, Franco-Bourland R, Grijalva I, Gonzalez J, Contreras B, and Madrazo I**. Magnetic resonance imaging of the normal and chronically injured adult rat spinal cord in vivo. *Neuroradiology* 36: 448-452, 1994.

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

102. **Haddad F, Roy RR, Zhong H, Edgerton VR, and Baldwin KM**. Atrophy responses to muscle inactivity. I. Cellular markers of protein deficits. *J Appl Physiol* 95: 781-790, 2003.

103. **Haddad F, Roy RR, Zhong H, Edgerton VR, and Baldwin KM**. Atrophy responses to muscle inactivity. II. Molecular markers of protein deficits. *J Appl Physiol* 95: 791-802, 2003.

104. **Hardt SE, and Sadoshima J**. Glycogen synthase kinase-3beta: a novel regulator of cardiac hypertrophy and development. *Circ Res* 90: 1055-1063, 2002.

105. **Harkema SJ**. Neural plasticity after human spinal cord injury: application of locomotor training to the rehabilitation of walking. *Neuroscientist* 7: 455-468, 2001.

106. **Harkema SJ, Hurley SL, Patel UK, Requejo PS, Dobkin BH, and Edgerton VR**. Human lumbosacral spinal cord interprets loading during stepping. *J Neurophysiol* 77: 797-811, 1997.

107. **Harvey C, Rothschild BB, Asmann AJ, and Stripling T**. New estimates of traumatic SCI prevalence: a survey-based approach. *Paraplegia* 28: 537-544, 1990.

108. **Hayes KC, and Kakulas BA**. Neuropathology of human spinal cord injury sustained in sports-related activities. *J Neurotrauma* 14: 235-248, 1997.

109. **Hedeman LS, Shellenberger MK, and Gordon JH**. Studies in experimental spinal cord trauma. 1. Alterations in catecholamine levels. *J Neurosurg* 40: 37-43, 1974.

110. **Hicks AL, Adams MM, Martin Ginis K, Giangregorio L, Latimer A, Phillips SM, and McCartney N**. Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. *Spinal Cord* 43: 291-298, 2005.

111. **Hikida RS, Van Nostran S, Murray JD, Staron RS, Gordon SE, and Kraemer WJ**. Myonuclear loss in atrophied soleus muscle fibers. *Anat Rec* 247: 350-354, 1997.

112. **Hodgson JA**. The relationship between soleus and gastrocnemius muscle activity in conscious cats--a model for motor unit recruitment? *J Physiol* 337: 553-562, 1983.

113. **Hodgson JA, Roy RR, de Leon R, Dobkin B, and Edgerton VR**. Can the mammalian lumbar spinal cord learn a motor task? *Med Sci Sports Exerc* 26: 1491-1497, 1994.

114. **Hoffmann SJ, Roy RR, Blanco CE, and Edgerton VR**. Enzyme profiles of single muscle fibers never exposed to normal neuromuscular activity. *J Appl Physiol* 69: 1150-1158, 1990.

115. **Houle JD, Morris K, Skinner RD, Garcia-Rill E, and Peterson CA**. Effects of fetal spinal cord tissue transplants and cycling exercise on the soleus muscle in spinalized rats. *Muscle Nerve* 22: 846-856, 1999.

116. **Hutchinson KJ, Linderman JK, and Basso DM**. 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, 2001.

117. **Inoki K, Li Y, Zhu T, Wu J, and Guan KL**. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4: 648-657, 2002.

118. **Inoue K, Yamasaki S, Fushiki T, Okada Y, and Sugimoto E**. Androgen receptor antagonist suppresses exercise-induced hypertrophy of skeletal muscle. *Eur J Appl Physiol Occup Physiol* 69: 88-91, 1994.

119. **Jakeman LB, Guan Z, Wei P, Ponnappan R, Dzwonczyk R, Popovich PG, and Stokes BT**. Traumatic spinal cord injury produced by controlled contusion in mouse. *J Neurotrauma* 17: 299-319, 2000.

120. **Jennische E, and Hansson HA**. Regenerating skeletal muscle cells express insulin-like growth factor I. *Acta Physiol Scand* 130: 327-332, 1987.

121. **Jiang B, Roy RR, and Edgerton VR**. Enzymatic plasticity of medial gastrocnemius fibers in the adult chronic spinal cat. *Am J Physiol* 259: C507-514, 1990.

122. **Jiang B, Roy RR, and Edgerton VR**. Expression of a fast fiber enzyme profile in the cat soleus after spinalization. *Muscle Nerve* 13: 1037-1049, 1990.

123. **Jiang BA, Roy RR, Navarro C, Nguyen Q, Pierotti D, and Edgerton VR**. Enzymatic responses of cat medial gastrocnemius fibers to chronic inactivity. *J Appl Physiol* 70: 231-239, 1991.

124. **Jones NC, Fedorov YV, Rosenthal RS, and Olwin BB**. ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion. *J Cell Physiol* 186: 104-115, 2001.

125. **Kakulas BA**. Pathology of spinal injuries. *Cent Nerv Syst Trauma* 1: 117-129, 1984.

126. **Kedar V, McDonough H, Arya R, Li HH, Rockman HA, and Patterson C**. Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin I. *Proc Natl Acad Sci U S A* 101: 18135-18140, 2004.

127. **Keller HL, St Pierre Schneider B, Eppihimer LA, and Cannon JG**. Association of IGF-I and IGF-II with myofiber regeneration in vivo. *Muscle Nerve* 22: 347-354, 1999.

128. **Koozekanani SH, Vise WM, Hashemi RM, and McGhee RB**. Possible mechanisms for observed pathophysiological variability in experimental spinal cord injury by the method of Allen. *J Neurosurg* 44: 429-434, 1976.

129. **Koulmann N, and Bigard AX**. Interaction between signalling pathways involved in skeletal muscle responses to endurance exercise. *Pflugers Arch* 1-15, 2006.

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

131. **Lai KM, Gonzalez M, Poueymirou WT, Kline WO, Na E, Zlotchenko E, Stitt TN, Economides AN, Yancopoulos GD, and Glass DJ**. Conditional activation of akt in adult skeletal muscle induces rapid hypertrophy. *Mol Cell Biol* 24: 9295-9304, 2004.

132. **Lazar RB, Yarkony GM, Ortolano D, Heinemann AW, Perlow E, Lovell L, and Meyer PR**. Prediction of functional outcome by motor capability after spinal cord injury. *Arch Phys Med Rehabil* 70: 819-822, 1989.

133. **Lee S, Barton ER, Sweeney HL, and Farrar RP**. Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats. *J Appl Physiol* 96: 1097-1104, 2004.

134. **Lefaucheur JP, Gjata B, Lafont H, and Sebille A**. Angiogenic and inflammatory responses following skeletal muscle injury are altered by immune neutralization of endogenous basic fibroblast growth factor, insulin-like growth factor-1 and transforming growth factor-beta 1. *J Neuroimmunol* 70: 37-44, 1996.

135. **Lieber RL**. Skeletal muscle structure, function, & plasticity. Second edition: 225, 2002.

136. **Lieber RL, Friden JO, Hargens AR, and Feringa ER**. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. II. Morphometric properties. *Exp Neurol* 91: 435-448, 1986.

137. **Lieber RL, Johansson CB, Vahlsing HL, Hargens AR, and Feringa ER**. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Exp Neurol* 91: 423-434, 1986.

138. **Liu M, Bose P, Walter G, Andersen DK, Thompson FJ, and Vandenborne K**. changes in soleus myosin heavy chain expression in response to training following spinal cord injury. *Abstract ACSM 52rd annual meeting* 2005.

139. **Liu M, Bose P, Walter G, Andersen DK, Thompson FJ, and Vandenborne K**. a longitudinal study of skeletal muscle following spinal cord injury and locomotor training. *Submitted to Muscle & Nerve* 2005.

140. **Lovely RG, Gregor RJ, Roy RR, and Edgerton VR**. Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. *Exp Neurol* 92: 421-435, 1986.

141. **Lovely RG, Gregor RJ, Roy RR, and Edgerton VR**. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. *Brain Res* 514: 206-218, 1990.

142. **Loy DN, Talbott JF, Onifer SM, Mills MD, Burke DA, Dennison JB, Fajardo LC, Magnuson DS, and Whittemore SR**. Both dorsal and ventral spinal cord pathways contribute to overground locomotion in the adult rat. *Exp Neurol* 177: 575-580, 2002.

143. **Machida S, Spangenburg EE, and Booth FW**. Forkhead transcription factor FoxO1 transduces insulin-like growth factor's signal to p27Kip1 in primary skeletal muscle satellite cells. *J Cell Physiol* 196: 523-531, 2003.

144. **Maegele M, Muller S, Wernig A, Edgerton VR, and Harkema SJ**. Recruitment of spinal motor pools during voluntary movements versus stepping after human spinal cord injury. *J Neurotrauma* 19: 1217-1229, 2002.

145. **Magnuson DS, Lovett R, Coffee C, Gray R, Han Y, Zhang YP, and Burke DA**. Functional consequences of lumbar spinal cord contusion injuries in the adult rat. *J Neurotrauma* 22: 529-543, 2005.

146. **Marsh DR, Criswell DS, Hamilton MT, and Booth FW**. Association of insulin-like growth factor mRNA expressions with muscle regeneration in young, adult, and old rats. *Am J Physiol* 273: R353-358, 1997.

147. **Martin TP, Bodine-Fowler S, and Edgerton VR**. Coordination of electromechanical and metabolic properties of cat soleus motor units. *Am J Physiol* 255: C684-693, 1988.

148. **Martin TP, Stein RB, Hoeppner PH, and Reid DC**. Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. *J Appl Physiol* 72: 1401-1406, 1992.

149. **Mayer RF, Burke RE, Toop J, Walmsley B, and Hodgson JA**. The effect of spinal cord transection on motor units in cat medial gastrocnemius muscles. *Muscle Nerve* 7: 23-31, 1984.

150. **McCall GE, Allen DL, Linderman JK, Grindeland RE, Roy RR, Mukku VR, and Edgerton VR**. Maintenance of myonuclear domain size in rat soleus after overload and growth hormone/IGF-I treatment. *J Appl Physiol* 84: 1407-1412, 1998.

151. **McKoy G, Ashley W, Mander J, Yang SY, Williams N, Russell B, and Goldspink G**. Expression of insulin growth factor-1 splice variants and structural genes in rabbit skeletal muscle induced by stretch and stimulation. *J Physiol* 516 (Pt 2): 583-592, 1999.

152. **Moelling K, Schad K, Bosse M, Zimmermann S, and Schweneker M**. Regulation of Raf-Akt Cross-talk. *J Biol Chem* 277: 31099-31106, 2002.

153. **Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, and Thomas G**. Drosophila S6 kinase: a regulator of cell size. *Science* 285: 2126-2129, 1999.

154. **Mourkioti F, and Rosenthal N**. IGF-1, inflammation and stem cells: interactions during muscle regeneration. *Trends Immunol* 26: 535-542, 2005.

155. **Muir GD**. Locomotor plasticity after spinal injury in the chick. *J Neurotrauma* 16: 705-711, 1999.

156. **Muir GD, and Steeves JD**. Sensorimotor stimulation to improve locomotor recovery after spinal cord injury. *Trends Neurosci* 20: 72-77, 1997.

157. **Mullis PE**. Genetic control of growth. *Eur J Endocrinol* 152: 11-31, 2005.

158. **Multon S, Franzen R, Poirrier AL, Scholtes F, and Schoenen J**. The effect of treadmill training on motor recovery after a partial spinal cord compression-injury in the adult rat. *J Neurotrauma* 20: 699-706, 2003.

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

160. **Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, and Rosenthal N**. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 27: 195-200, 2001.

161. **Musaro A, and Rosenthal N**. Maturation of the myogenic program is induced by postmitotic expression of insulin-like growth factor I. *Mol Cell Biol* 19: 3115-3124, 1999.

162. **Muslumanoglu L, Aki S, Ozturk Y, Soy D, Filiz M, Karan A, and Berker E**. Motor, sensory and functional recovery in patients with spinal cord lesions. *Spinal Cord* 35: 386-389, 1997.

163. **Noble LJ, and Wrathall JR**. Spinal cord contusion in the rat: morphometric analyses of alterations in the spinal cord. *Exp Neurol* 88: 135-149, 1985.

164. **Ohanna M, Sobering AK, Lapointe T, Lorenzo L, Praud C, Petroulakis E, Sonenberg N, Kelly PA, Sotiropoulos A, and Pende M**. Atrophy of S6K1(-/-) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nat Cell Biol* 7: 286-294, 2005.

165. **Osterholm JL, and Mathews GJ**. Treatment of severe spinal cord injuries by biochemical norepinephrine manipulation. *Surg Forum* 22: 415-417, 1971.

166. **Otis JS, Roy RR, Edgerton VR, and Talmadge RJ**. Adaptations in metabolic capacity of rat soleus after paralysis. *J Appl Physiol* 96: 584-596, 2004.

167. **Owino V, Yang SY, and Goldspink G**. Age-related loss of skeletal muscle function and the inability to express the autocrine form of insulin-like growth factor-1 (MGF) in response to mechanical overload. *FEBS Lett* 505: 259-263, 2001.

168. **Parker AJ, Park RD, and Stowater JL**. Reduction of trauma-induced edema of spinal cord in dogs given mannitol. *Am J Vet Res* 34: 1355-1357, 1973.

169. **Parker AJ, and Smith CW**. Functional recovery from spinal cord trauma following dexamethazone and chlorpromazine therapy in dogs. *Res Vet Sci* 21: 246-247, 1976.

170. **Pette D, and Vrbova G**. Adaptation of mammalian skeletal muscle fibers to chronic electrical stimulation. *Rev Physiol Biochem Pharmacol* 120: 115-202, 1992.

171. **Pette D, and Vrbova G**. Neural control of phenotypic expression in mammalian muscle fibers. *Muscle Nerve* 8: 676-689, 1985.

172. **Phelan JN, and Gonyea WJ**. Effect of radiation on satellite cell activity and protein expression in overloaded mammalian skeletal muscle. *Anat Rec* 247: 179-188, 1997.

173. **Pierotti DJ, Roy RR, Gregor RJ, and Edgerton VR**. Electromyographic activity of cat hindlimb flexors and extensors during locomotion at varying speeds and inclines. *Brain Res* 481: 57-66, 1989.

174. **Protas EJ, Holmes SA, Qureshy H, Johnson A, Lee D, and Sherwood AM**. Supported treadmill ambulation training after spinal cord injury: a pilot study. *Arch Phys Med Rehabil* 82: 825-831, 2001.

175. **Raines A, Dretchen KL, Marx K, and Wrathall JR**. Spinal cord contusion in the rat: somatosensory evoked potentials as a function of graded injury. *J Neurotrauma* 5: 151-160, 1988.

176. **Ramer LM, Ramer MS, and Steeves JD**. Setting the stage for functional repair of spinal cord injuries: a cast of thousands. *Spinal Cord* 43: 134-161, 2005.

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

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

179. **Reier PJ, Anderson DK, Thompson FJ, and Stokes BT**. Neural tissue transplantation and CNS trauma: anatomical and functional repair of the injured spinal cord. *J Neurotrauma* 9 Suppl 1: S223-248, 1992.

180. **Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN, Yancopoulos GD, and Glass DJ**. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol* 3: 1009-1013, 2001.

181. **Rosenblatt JD, and Parry DJ**. Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. *J Appl Physiol* 73: 2538-2543, 1992.

182. **Rosenblatt JD, Yong D, and Parry DJ**. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* 17: 608-613, 1994.

183. **Rosenzweig ES, and McDonald JW**. Rodent models for treatment of spinal cord injury: research trends and progress toward useful repair. *Curr Opin Neurol* 17: 121-131, 2004.

184. **Round JM, Barr FM, Moffat B, and Jones DA**. Fibre areas and histochemical fibre types in the quadriceps muscle of paraplegic subjects. *J Neurol Sci* 116: 207-211, 1993.

185. **Roy RR**. Long-term exercise effects on the mechanical propertis after prolonged inactivation and/or cross-reinnervation of cat soleus. *Soc Neurosci Abstr* 11: 1101, 1985.

186. **Roy RR, and Acosta L, Jr.** Fiber type and fiber size changes in selected thigh muscles six months after low thoracic spinal cord transection in adult cats: exercise effects. *Exp Neurol* 92: 675-685, 1986.

187. **Roy RR, Baldwin KM, and Edgerton VR**. The plasticity of skeletal muscle: effects of neuromuscular activity. *Exerc Sport Sci Rev* 19: 269-312, 1991.

188. **Roy RR, Eldridge L, Baldwin KM, and Edgerton VR**. Neural influence on slow muscle properties: inactivity with and without cross-reinnervation. *Muscle Nerve* 19: 707-714, 1996.

189. **Roy RR, Hutchison DL, Pierotti DJ, Hodgson JA, and Edgerton VR**. EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. *J Appl Physiol* 70: 2522-2529, 1991.

190. **Roy RR, Pierotti, D.J., Baldwin, K.M., Edgerton, V.R.** Effects of cyclical passive stretch in maintaining cat soleus mechanical properties. *Soc Neurosci Abstr* 14: 948, 1988.

191. **Roy RR, Sacks RD, Baldwin KM, Short M, and Edgerton VR**. Interrelationships of contraction time, Vmax, and myosin ATPase after spinal transection. *J Appl Physiol* 56: 1594-1601, 1984.

192. **Roy RR, Talmadge RJ, Hodgson JA, Oishi Y, Baldwin KM, and Edgerton VR**. Differential response of fast hindlimb extensor and flexor muscles to exercise in adult spinalized cats. *Muscle Nerve* 22: 230-241, 1999.

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

194. **Roy RR, Zhong H, Monti RJ, Vallance KA, and Edgerton VR**. Mechanical properties of the electrically silent adult rat soleus muscle. *Muscle Nerve* 26: 404-412, 2002.

195. **Sakuma K, Watanabe K, Totsuka T, Uramoto I, Sano M, and Sakamoto K**. Differential adaptations of insulin-like growth factor-I, basic fibroblast growth factor, and leukemia inhibitory factor in the plantaris muscle of rats by mechanical overloading: an immunohistochemical study. *Acta Neuropathol (Berl)* 95: 123-130, 1998.

196. **Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, and Goldberg AL**. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117: 399-412, 2004.

197. **Schiaffino S, and Reggiani C**. Myosin isoforms in mammalian skeletal muscle. *J Appl Physiol* 77: 493-501, 1994.

198. **Schindler-Ivens S, and Shields RK**. Low frequency depression of H-reflexes in humans with acute and chronic spinal-cord injury. *Exp Brain Res* 133: 233-241, 2000.

199. **Schlesinger EB**. Alfred Reginald Allen: the mythic career of a gifted neuroscientist. *Surg Neurol* 36: 229-233, 1991.

200. **Sekhon LH, and Fehlings MG**. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. *Spine* 26: S2-12, 2001.

201. **Selzer ME**. Promotion of axonal regeneration in the injured CNS. *Lancet Neurol* 2: 157-166, 2003.

202. **Shavlakadze T, Winn N, Rosenthal N, and Grounds MD**. Reconciling data from transgenic mice that overexpress IGF-I specifically in skeletal muscle. *Growth Horm IGF Res* 15: 4-18, 2005.

203. **Skinner RD, Houle JD, Reese NB, Berry CL, and Garcia-Rill E**. Effects of exercise and fetal spinal cord implants on the H-reflex in chronically spinalized adult rats. *Brain Res* 729: 127-131, 1996.

204. **Smith LL**. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc* 32: 317-331, 2000.

205. **Snow MH**. Satellite cell response in rat soleus muscle undergoing hypertrophy due to surgical ablation of synergists. *Anat Rec* 227: 437-446, 1990.

206. **Somerson SK, and Stokes BT**. Functional analysis of an electromechanical spinal cord injury device. *Exp Neurol* 96: 82-96, 1987.

207. **Spangenburg EE, Abraha T, Childs TE, Pattison JS, and Booth FW**. Skeletal muscle IGF-binding protein-3 and -5 expressions are age, muscle, and load dependent. *Am J Physiol Endocrinol Metab* 284: E340-350, 2003.

208. **Spangenburg EE, Williams JH, Roy RR, and Talmadge RJ**. Skeletal muscle calcineurin: influence of phenotype adaptation and atrophy. *Am J Physiol Regul Integr Comp Physiol* 280: R1256-1260, 2001.

209. **Steinbach JH, Schubert D, and Eldridge L**. Changes in cat muscle contractile proteins after prolonged muscle inactivity. *Exp Neurol* 67: 655-669, 1980.

210. **Stokes BT, and Jakeman LB**. Experimental modelling of human spinal cord injury: a model that crosses the species barrier and mimics the spectrum of human cytopathology. *Spinal Cord* 40: 101-109, 2002.

211. **Sugita H, Kaneki M, Sugita M, Yasukawa T, Yasuhara S, and Martyn JA**. Burn injury impairs insulin-stimulated Akt/PKB activation in skeletal muscle. *Am J Physiol Endocrinol Metab* 288: E585-591, 2005.

212. **Takahashi A, Kureishi Y, Yang J, Luo Z, Guo K, Mukhopadhyay D, Ivashchenko Y, Branellec D, and Walsh K**. Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Mol Cell Biol* 22: 4803-4814, 2002.

213. **Talmadge RJ**. Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. *Muscle Nerve* 23: 661-679, 2000.

214. **Talmadge RJ, Castro MJ, Apple DF, Jr., and Dudley GA**. Phenotypic adaptations in human muscle fibers 6 and 24 wk after spinal cord injury. *J Appl Physiol* 92: 147-154, 2002.

215. **Talmadge RJ, Grossman EJ, and Roy RR**. Myosin heavy chain composition of adult feline (Felis catus) limb and diaphragm muscles. *J Exp Zool* 275: 413-420, 1996.

216. **Talmadge RJ, and Roy RR**. Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J Appl Physiol* 75: 2337-2340, 1993.

217. **Talmadge RJ, Roy RR, Bodine-Fowler SC, Pierotti DJ, and Edgerton VR**. Adaptations in myosin heavy chain profile in chronically unloaded muscles. *Basic Appl Myol* 5: 117-137, 1995.

218. **Talmadge RJ, Roy RR, Caiozzo VJ, and Edgerton VR**. Mechanical properties of rat soleus after long-term spinal cord transection. *J Appl Physiol* 93: 1487-1497, 2002.

219. **Talmadge RJ, Roy RR, Chalmers GR, and Edgerton VR**. MHC and sarcoplasmic reticulum protein isoforms in functionally overloaded cat plantaris muscle fibers. *J Appl Physiol* 80: 1296-1303, 1996.

220. **Talmadge RJ, Roy RR, and Edgerton VR**. Distribution of myosin heavy chain isoforms in non-weight-bearing rat soleus muscle fibers. *J Appl Physiol* 81: 2540-2546, 1996.

221. **Talmadge RJ, Roy RR, and Edgerton VR**. Myosin heavy chain profile of cat soleus following chronic reduced activity or inactivity. *Muscle Nerve* 19: 980-988, 1996.

222. **Talmadge RJ, Roy RR, and Edgerton VR**. Persistence of hybrid fibers in rat soleus after spinal cord transection. *Anat Rec* 255: 188-201, 1999.

223. **Tator CH**. Spine-spinal cord relationships in spinal cord trauma. *Clin Neurosurg* 30: 479-494, 1983.

224. **Termin A, Staron RS, and Pette D**. Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle. *Histochemistry* 92: 453-457, 1989.

225. **Thomason DB, and Booth FW**. Atrophy of the soleus muscle by hindlimb unweighting. *J Appl Physiol* 68: 1-12, 1990.

226. **Thompson FJ, Reier PJ, Lucas CC, and Parmer R**. Altered patterns of reflex excitability subsequent to contusion injury of the rat spinal cord. *J Neurophysiol* 68: 1473-1486, 1992.

227. **Thota A, Carlson S, and Jung R**. Recovery of locomotor function after treadmill training of incomplete spinal cord injured rats. *Biomed Sci Instrum* 37: 63-67, 2001.

228. **Tower SS**. Function and structure in the chronically isolated lumbo-sacral spinal cord of the dog. *J Comp Neurol* 67: 109-131, 1937.

229. **van Hedel HJ, Wirth B, and Dietz V**. Limits of locomotor ability in subjects with a spinal cord injury. *Spinal Cord* 43: 593-603, 2005.

230. **Vandenburgh HH, Karlisch P, Shansky J, and Feldstein R**. Insulin and IGF-I induce pronounced hypertrophy of skeletal myofibers in tissue culture. *Am J Physiol* 260: C475-484, 1991.

231. **Viala D, Viala G, and Fayein N**. Plasticity of locomotor organization in infant rabbits spinalized shortly after birth. *Developmental plasticity of the mammalian spinal cord* 301-310, 1986.

232. **Waters RL, Adkins RH, Yakura JS, and Sie I**. Motor and sensory recovery following incomplete paraplegia. *Arch Phys Med Rehabil* 75: 67-72, 1994.

233. **Wernig A, and Muller S**. Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. *Paraplegia* 30: 229-238, 1992.

234. **Wernig A, Muller S, Nanassy A, and Cagol E**. Laufband therapy based on 'rules of spinal locomotion' is effective in spinal cord injured persons. *Eur J Neurosci* 7: 823-829, 1995.

235. **Wernig A, Nanassy A, and Muller S**. Laufband (treadmill) therapy in incomplete paraplegia and tetraplegia. *J Neurotrauma* 16: 719-726, 1999.

236. **Wernig A, Nanassy A, and Muller S**. Maintenance of locomotor abilities following Laufband (treadmill) therapy in para- and tetraplegic persons: follow-up studies. *Spinal Cord* 36: 744-749, 1998.

237. **West SP, Roy RR, and Edgerton VR**. Fiber type and fiber size of cat ankle, knee, and hip extensors and flexors following low thoracic spinal cord transection at an early age. *Exp Neurol* 91: 174-182, 1986.

238. **Willis PE, Chadan SG, Baracos V, and Parkhouse WS**. Restoration of insulin-like growth factor I action in skeletal muscle of old mice. *Am J Physiol* 275: E525-530, 1998.

239. **Winiarski AM, Roy RR, Alford EK, Chiang PC, and Edgerton VR**. Mechanical properties of rat skeletal muscle after hind limb suspension. *Exp Neurol* 96: 650-660, 1987.

240. **Wirz M, Colombo G, and Dietz V**. Long term effects of locomotor training in spinal humans. *J Neurol Neurosurg Psychiatry* 71: 93-96, 2001.

241. **Wirz M, Zemon DH, Rupp R, Scheel A, Colombo G, Dietz V, and Hornby TG**. Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial. *Arch Phys Med Rehabil* 86: 672-680, 2005.

242. **Wrathall JR, Pettegrew RK, and Harvey F**. Spinal cord contusion in the rat: production of graded, reproducible, injury groups. *Exp Neurol* 88: 108-122, 1985.

243. **Wu H, Naya FJ, McKinsey TA, Mercer B, Shelton JM, Chin ER, Simard AR, Michel RN, Bassel-Duby R, Olson EN, and Williams RS**. MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *Embo J* 19: 1963-1973, 2000.

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

245. **Yarkony GM, Roth E, Lovell L, Heinemann A, Katz RT, and Wu Y**. Rehabilitation outcomes in complete C5 quadriplegia. *Am J Phys Med Rehabil* 67: 73-76, 1988.

246. **Young W**. Spinal cord contusion models. *Prog Brain Res* 137: 231-255, 2002.

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

BIOGRAPHICAL SKETCH

Min Liu was born in Tianjin, China, in May 1970. He received his M.D. Degree from Tianjin Medical University in 1993. He worked as an orthopedic and hand surgeon for 7 years before entering the rehabilitation science doctoral program at the University of Florida.