SKELETAL MUSCLE ADAPTATIONS FOLLOWING INCOMPLETE SPINAL CORD INJURY AND EXERCISE TRAINING

By

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To my mother, wife and, the almighty

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Recovery of function after incomplete spinal cord injury (incomplete-SCI) is in an exciting phase of research. Paralysis and paresis of lower extremity muscles following incomplete-SCI result in persistent motor dysfunction and impaired walking. Advances in research have led to promising exercise-training strategies in both humans and animals following SCI. However, the mechanisms that explain the functional improvements reported following incomplete- SCI and exercise training are not clearly understood and could possibly result from musculoskeletal changes, neural adaptations, or a combination thereof. The primary purpose of this dissertation was to explore the adaptations in lower extremity skeletal muscle following incomplete-SCI and exercise training in both humans and animals. Ours findings indicate a significant loss of both peak isometric and explosive strength in lower extremities after incomplete-SCI in humans. Additionally, this loss in strength was attributed to a severe loss in voluntary activation of the paretic muscles. Locomotor training and resistance training were two exercise interventions that were tested in our study, and our findings suggest that both locomotor training and resistance training helped in significantly improving both voluntary and explosive strength, and voluntary activation in the lower extremity muscles of persons with incomplete-SCI. In the rat model,

incomplete-SCI resulted in significant atrophy in all four lower extremity muscles. In addition, SCI resulted in a shift in fiber type composition measured using myosin heavy chain (MHC) composition towards faster isoforms in all four lower extremity muscles. Locomotor training in the rats resulted in significantly reducing the atrophy in all lower extremity muscles. In addition, there was also a significant shift in fiber types in all hind limb muscles towards slower isoforms. In addition, our results indicate that recovery in muscle size following SCI and locomotor training was due to the activation of satellite cells which went to form multinucleated myotubes which repaired or replaced damaged or lost muscle fibers. The overall findings from the present work will provide essential feedback on deficits in muscle function following SCI and also effects of exercise training interventions towards reducing the musculoskeletal deficits and promoting muscle plasticity following incomplete- SCI. These findings might provide feedback for the development and integration of these exercise interventions into the community.

CHAPTER 1
BACKGROUND

1.1 Introduction

Spinal cord injury (SCI) causes a host of physical and psychosocial problems that interferes with an individual's personal health, feeling of well being and societal interaction. The goal of rehabilitation after spinal cord injury is to enable the person to resume a life style which is physically and functionally healthy and also helps the person integrate with his family, community and society. The central role of rehabilitation requires a comprehensive understanding of all different physiological and functional adaptations that occur with SCI. The main focus of this dissertation is to identify adaptations in muscle function after SCI and its response to different exercise interventions. Background literature pertaining to all chapters is briefly discussed in chapter-1.

1.2 Demographics of Spinal Cord Injury (SCI)

It is estimated that the annual incidence of spinal cord injury (SCI), is approximately 40 cases per million population in the U. S. or approximately 11,000 new cases each year.[1] The number of people in the United States who are alive as of June 2006 who have had a SCI has been estimated to be approximately 253,000, with a range of 225,000 to 296,000 persons.[1]

SCI primarily affects young adults. From 1973 to 1979, the average age at injury was 28.7 years, and most injuries occurred between the ages of 16 and 30. Since 2000, the average age at injury is 38.0 years since the median age of the general population of the United States has increased by approximately 8 years since the mid-1970s, the average age at injury has also steadily increased over time. Moreover, the percentage of persons older than 60 years of age who had a SCI has increased from 4.7% prior to 1980 to 11.5% among injuries occurring since 2000. [1]Prior to 1980, 81.8% of new spinal cord injuries occurred among males. Since 2000, 77.8% of

SCI reported to the national database have occurred among males.[1]Over the history of the database, there has been a slight trend toward a decreasing percentage of males sustaining SCI.[1] Among those injured since 2000, 63.0% are Caucasian, 22.7% are African American, 11.8% are Hispanic, and 2.4% are from other racial/ethnic groups.[1, 2]

Looking at the etiology of SCI, it is plausible that this injury by itself causes dramatic changes in one's lifestyle and occupational status. However, by ten years post-injury, 32.4% of persons with paraplegia become employed, while only 24.2% of those with tetraplegia are employed during the same year.[1,2] The average yearly health care and living expenses and the estimated lifetime costs that are directly attributable to SCI vary greatly according to severity of injury (Fig.1-1).

The last section of the facts following SCI pertains to the causes of the death after SCI and level and extent of the lesion. The most common cause of death in persons with SCI is respiratory ailment, whereas, in the past it was renal failure. An increasing number of people with SCI are dying of unrelated causes such as cancer or cardiovascular disease, similar to that of the general population. Mortality rates are significantly higher during the first year after injury than during subsequent years. Since 2000, the most frequent neurological category is incomplete tetraplegia (34.1%) and incomplete paraplegia (23.1%), followed by complete paraplegia (23.0%), and complete tetraplegia (18.3%). This makes the incidence of incomplete injuries equals to ~57% of total SCI as of 2000. Over the past few years, the percentage of persons with incomplete tetraplegia has increased slightly while both complete paraplegia and complete tetraplegia have decreased slightly (Fig.1-2).[1, 2]

In Summary, in this section the statistics and demographics pertaining to SCI were briefly described. A summary of the etiology, age, gender, causes of death and common types of SCI were discussed.

1.3 SCI in Humans-Pathophysiology and Classification

The information in this section mainly pertains to the medical definition and classification of SCI. This includes the anatomy, physiology, diagnosis and classification of SCI. This information will help us better understand the diagnosis, extent of injury and recovery levels of the subjects with SCI described in following chapters.

1.3.1 Definition of SCI

SCI can be categorized into traumatic or non-traumatic injuries. The spinal cord is often violently displaced or compressed momentarily during the injury with forceful flexion, extension, and rotation of the spine. The vertebral body can burst and cause pressure or scatter bone fragments into the spinal cord. SCIs are classified as concussion, contusion, laceration, or transection. A **concussion** is an injury caused by a blow or violent shake and results in temporary loss of function.[2,3] In **contusion** injury, the glial tissue and spinal cord surface remain intact. There is loss of central gray matter and white matter, which creates a cavity that, is surrounded by a rim of intact white matter at the periphery of the spinal cord.[3] **Laceration** of the cord occurs with more severe injuries in which the glia is disrupted, and the spinal cord tissue may get torn. Occasionally this can result in complete dissection of the spinal cord known as **transection** injuries. [3] Gun shot wounds, knife wounds, and puncture wounds fall into this category. Hemorrhages caused by contusion or laceration injuries can cause further compression of the cord.[2,3]

SCIs can also be classified as primary or secondary based on the modus of injury. Primary SCIs arise from mechanical disruption, transection, or distraction of neural elements. This injury

usually occurs with fracture and/or dislocation of the spine. However, primary SCI may occur in the absence of spinal fracture or dislocation. Penetrating injuries due to bullets or weapons may also cause primary SCI. More commonly, displaced bony fragments cause penetrating spinal cord and/or segmental spinal nerve injuries. Extradural pathology may also cause primary SCI. Spinal epidural hematomas or abscesses cause acute cord compression and injury. Spinal cord compression from metastatic disease is a common oncologic emergency. Longitudinal distraction with or without flexion and/or extension of the vertebral column may result in primary SCI without spinal fracture or dislocation.[1-3]

Major causes of secondary SCI are vascular injury to the spinal cord caused by arterial disruption, arterial thrombosis, and hypoperfusion due to shock. Anoxic or hypoxic effects compound the extent of secondary SCI. In summary SCI can vary in nature, hence the disability associated with it is also extremely varied based on the type, level, and extent of injury.

1.3.2 Spinal Cord Neuro-anatomy

The spinal cord is divided into 31 segments, each with a pair of anterior (motor) and dorsal (sensory) spinal nerve roots. On each side, the anterior and dorsal nerve roots combine to form the spinal nerve as it exits from the vertebral column through the neuro-foramina. The spinal cord extends from the base of the skull and terminates near the lower margin of the L1 vertebral body. Thereafter, the spinal canal contains the lumbar, sacral, and coccygeal spinal nerves that comprise the cauda equina.[4] Therefore, injuries below L1 are not considered SCIs because they involve the segmental spinal nerves and/or cauda equina. Spinal injuries proximal to L1, above the termination of the spinal cord, often involve a combination of spinal cord lesions and segmental root or spinal nerve injuries.[1-5]

Spinal tracts: The spinal cord itself is organized into a series of tracts or neuro-pathways that carry motor (descending) and sensory (ascending) information. These tracts are organized

anatomically within the spinal cord. The corticospinal tracts are descending motor pathways located anteriorly within the spinal cord. Axons extend from the cerebral cortex in the brain as far as the corresponding segment, where they form synapses with motor neurons in the anterior (ventral) horn. They decussate (cross over) in the medulla prior to entering the spinal cord. The dorsal columns are ascending sensory tracts that transmit light touch, proprioception, and vibration information to the sensory cortex. They do not decussate until they reach the medulla. The lateral spinothalamic tracts transmit pain and temperature sensation. These tracts usually decussate within 3 segments of their origin as they ascend. The anterior spinothalamic tract transmits light touch. Autonomic function traverses within the anterior interomedial tract. Sympathetic nervous system fibers exit the spinal cord between C7 and L1, while parasympathetic system pathways exit between S2 and S4.[4]

1.3.3 SCI Pathophysiology

Trauma to the spinal cord results in primary destruction of neurons at the level of the injury by disruption of the membrane, hemorrhage, and vascular damage. Secondary neural damage to the spinal cord extends beyond the initial contusion. The spread of the damage is thought to be due to the activation of biochemical events leading to necrosis and excitotoxic damage and can continue for hours, days or weeks. Injury to the corticospinal tract or dorsal columns, respectively, results in ipsilateral paralysis or loss of sensation of light touch, proprioception, and vibration.[1-3] Unlike injuries of the other tracts, injury to the lateral spinothalamic tract causes contralateral loss of pain and temperature sensation. Because the anterior spinothalamic tract also transmits light touch information, injury to the dorsal columns may result in complete loss of vibration sensation and proprioception but only partial losses of light touch sensation. Anterior cord injury causes paralysis and incomplete loss of light touch sensation.[1-5]

Autonomic function is transmitted in the anterior interomedial tract. The sympathetic nervous system fibers exit from the spinal cord between C7 and L1. The parasympathetic system nerves exit between S2 and S4. Therefore progressively higher spinal cord lesions or injury causes increasing degrees of autonomic dysfunction.

1.3.4 Classification of SCI

ASIA Impairment Scale: Clinicians have long used a clinical scale to grade severity of neurological loss. First devised at Stokes Manville before World War II and popularized by Frankel in the 1970's, the original scoring approach segregated patients into five categories, i.e. no function (A), sensory only (B), some sensory and motor preservation (C), useful motor function (D), and normal (E). The ASIA Impairment Scale is follows the Frankel scale but differs from the older scale in several important respects.

The mechanism of the injury influences the type and degree of spinal cord lesion. The SCI injuries are often classified as complete and incomplete. The difference between a complete and incomplete injury depends on the survival of a small fractions of axons in the spinal cord. According to the American Spinal Injury Association (ASIA), a person is a "complete" if they do not have motor and sensory function in the anal and perineal region representing the lowest sacral cord (S4-S5).

ASIA A is defined as a person with no motor or sensory function preserved in the sacral segments S4-S5. This definition is clear and unambiguous. **ASIA B** is essentially is the preservation of sacral S4-S5 function. It should be noted that ASIA A and B classification depend entirely on a single observation, i.e. the preservation of motor and sensory function of S4-5.A patient would be an **ASIA C** if more than half of the muscles evaluated had a grade of less than 3/5 on a manual muscle test. If not, the person was assigned to **ASIA D**. **ASIA E** is of interest because it implies that somebody can have spinal cord injury without having any

neurological deficits at least detectable on a neurological examination of this type. Also, the ASIA motor and sensory scoring may not be sensitive to subtle weakness, presence of spasticity, pain, and certain forms of dyesthesia that could be a result of spinal cord injury. Note that such a person would be categorized as an ASIA E.

The ASIA committee has identified five types of ***incomplete*** spinal cord injury syndromes. A central cord syndrome is associated with greater loss of upper limb function compared to the lower limbs. The Brown-Sequard syndrome results from a hemisection lesion of the spinal cord. Anterior cord syndrome occurs when the injury affects the anterior spinal tracts, including the vestibulospinal tract. Conus medullaris and cauda equina syndromes occur with damage to the conus or spinal roots of the cord. Measures of ambulatory function are another commonly used method to classify people with SCI. Information can be obtained from the following references.[6-11]

In summary, in this section we briefly discussed the definition of the different types of SCI. This was followed by a summary of the neuroanatomy and physiology of SCI. Finally we summarized the most common classification of SCI; the ASIA scale.

1.4 SCI in the Animal Model

To obtain the necessary experimental evidence to begin clinical trials, compelling evidence for benefit must be demonstrated in reproducible animal models of SCI. Although no single experimental SCI animal model exactly mimics the clinical condition, animal models allow for the rigorous study of the pathophysiology and mechanism of injury and recovery.

Appropriate cat and rodent models that are being currently investigated include the compression, hemisection, transection, isolation, and contusion.[12-14] With each model, injury severity and areas of damage to the spinal cord can be varied so that a spectrum of histopathological, behavior and functional deficits can be reproduced.[12-16] Rat models of SCI are

the most commonly studied because of their low cost, size factor, ease in handling and care, and well-established SCI methods.[17-20] Recently, mouse models of SCI have been developed. These models give us the ability to enhance or delete specific genes by transgenic mechanisms. Non-human primate models of SCI are also important in testing experimental therapeutic strategies. In addition to various neuroanatomical considerations, the primate spinal cord more closely resembles that of a human spinal cord and this becomes important when therapeutic and pharmaceutical interventions are focused directly towards the injured spinal cord.[11-20]

1.4.1 Spinal Cord Isolation Model

Spinal cord isolation referred to as the classic silent preparation was attempted in dogs by Tower in 1937.[4] In this model; the lumbar region of the spinal cord is functionally isolated via complete spinal cord transections at two levels and bilateral dorsal rhizotomy between the two transection sites. 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. Electromyographic recordings (EMG) and/or reflex testing after spinal isolation have shown the hindlimb muscles to be virtually silent for prolonged periods.[21-23]

1.4.2 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. In this model, SCI is created by an incision into the spinal cord is completely transected. Following transection injury, there is an initial flaccid paraplegia stage in which the limbs of the animals are totally paralyzed.[12] The animals are only able to move using their forelimbs to reach for food and water. At approximately 3 to 4 weeks following SCI, 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. There exists a 75% decrease in the total

integrated EMG and a 66% decrease in the total duration of muscle activity in the soleus muscle, 5 to 6 months after transection when compared to normal controls.[24] 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. The advantage of this model is a relative stabilization of pathological changes and subsequent neurological outcomes.[24-26]Therefore, the effectiveness of particular strategies can be readily assessed. Models in which the spinal cord is fully transected ensure the absolute completeness of the injury, making it somewhat easier to evaluate the effectiveness of interventions with regard to both axonal regeneration and functional recovery. The implication in studies using transection models is that with the ensured completeness of the lesion, anterogradely labeled axons observed distal to the lesion have indeed regenerated from above and are responsible for the functional recovery of the animal. While this is largely accepted, and hence remains the main advantage of full transection models, there is a mounting body of literature from animal studies that describes considerable native locomotive abilities of the completely transected spinal cord (the so-called "spinalized" animal).[24-26] However, the transected spinal cord model also has some disadvantages. First, due to the natural tension present in spinal cord, 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 leading to higher chances of infection.[24-26]

1.4.3 Spinal Cord Hemisection Model

In hemisection 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.[27] Hemisection models also may allow for comparison of the regenerative response in a particular tract with its uninjured partner on the contralateral side. The rat rubrospinal system is a useful model in this regard because the tract emerges from the red nucleus in the brain stem, crosses over nearly completely, and descends in the dorsolateral aspect of the spinal cord, where its lateral position makes it relatively easy to cut in a unilateral fashion while leaving the contralateral tract uninjured. In the rat, the rubrospinal system is thought to be important for the control of skilled limb movement, particularly of the forelimbs.[18,27,28] Most of the corticospinal tract in rats descends in the ventral aspect of the dorsal columns, just dorsally to the central canal. In dorsal hemisection models, the lesion transects the rubrospinal and corticospinal tracts bilaterally. In general, partial transection models inherently raise the possibility that axons of the particular tract in question might have escaped injury. Retrograde tracers are useful in identifying such spared axons.[27,28,18] If a tracer is applied distally to the site of partial injury, its histologic appearance proximally in the cell body of a neuron implies that this neuron's axon was not cut during the injury. Conversely, the absence of tracer confirms the injury's completeness. 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.[27, 28, 18]

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, a limitation of partial injury models is the difficulty in determining whether observed functional improvement is due to true regeneration of the injured tract or to functional compensation from other systems that are

spared. This is one the reasons this model is not commonly used to study adaptations of the locomotor muscles of the hind limb.

1.4.4 Spinal Cord Contusion Model

In 1911, Reginald Allen described a spinal cord injury model where he dropped a weight onto the spinal cords of dogs exposed by laminectomy. In 1914, he reported that midline myelotomy reduced progressive tissue damage in the contused spinal cord.[29] Unfortunately, Allen died in World War I and his work was discontinued for nearly 50 years. In 1968, Albin and colleagues revived the contusion model when they used a primate spinal cord contusion model to assess the efficacy of hypothermic therapy following SCI.[29] After that, several investigators started using the canine spinal cord contusion model again. Parker and colleagues assessed the effects of dexamethasone and chlorpromazine on edema in contused dog spinal cords. At the same time, Koozekanani and colleagues examined the causes of variability in this model, while Collmans and others measured edema, blood flow and histopathological changes in the contused dog spinal cord.[30]

Beginning with a crude weight drop model by Reginald Allen in 1911, many models in various animals have been developed to deliver a blunt contusive force to the spinal cord, which is more representative of what occurs in most human injuries.[31] Two important aspects of human injury warrant discussion because they are particularly relevant to injury models. The first is observed evolution of neuropathology over time, beginning with an early phase of spreading hemorrhagic necrosis and edema, progressing to an intermediate phase of partial repair and tissue reorganization, and reaching a chronic phase characterized by the establishment of central cystic cavities within atrophic parenchyma and glial scar.[30-36] This temporal pattern of injury maturation appears to be reasonably well simulated in the spinal cords of animals after a contusion injury, thereby providing a setting for evaluating neuroprotective strategies in the

acute phase of injury.[30-36] The second important observation in humans is that even in the setting of complete paraplegia after blunt injury; the spinal cord rarely is completely transected, but rather leaves some residual, normal-appearing cord parenchyma peripherally at the injury zone. Contusion injury models produce a similar lesion, in which neuronal tissue remains intact along a peripheral rim, the quantity of which is correlated with residual locomotor function.[30-36]

In general, contusion injury models appear to induce reproducible and consistent neurologic injuries, thereby providing a good setting for the functional and histologic evaluation of SCI and new treatment interventions. However, due to the incomplete nature of injury and the complexity of the tracts, it is very difficult to verify exact changes in pathophysiology in these models.

Common devices used to create contusion injuries: The Georgetown University device Wrathall, is a free falling weight down a guide tube onto a footplate resting on the cord. The NYU or MASCIS device was developed at the NYU Neurosurgery Laboratory and first described by Gruner in 1992. In this model, a 10-g rod is dropped from different heights onto the exposed dorsal surface of the spinal cord producing more severe neurologic injuries with increasing height. The ESCID device (Ohio State University device displacement driven) is somewhat different rat cord contusion model that use a computer feedback-controlled electromechanical impactor rather than a weight drop. The Infinite Horizon device (University of Kentucky device-force driven) is an instrument that enables the application of standard-force injuries to the spinal cords of mice and rats. Force levels are user-selectable between 30 and 200 kDynes. A “clip compression” model of spinal cord injury in rats was introduced by Rivlin and Tator in 1978, in which the spinal cord was compressed for variable durations between the arms of a modified aneurysmal clip. The devices commonly used currently are the NYU impactor and

the Infinite Horizon impactor device. Overall, all these devices provide consistent, reliable spinal cord injuries. However, based on the experimental requirement or type of injury, one device might be more suitable than the other.

To summarize, in this section the different types of SCI in animals was briefly described with emphasis given to the contusion SCI which is the model of SCI pertaining to this dissertation. In the last section we saw the different types of injury devices pertaining to the contusion injury. In the following sections the NYU impactor device will be used extensively to cause moderate contusion SCI in the rat model. The moderate contusion injury was used in all the animal experiments as it closely resembles the histopathologic sequela and mechanism of an incomplete SCI in the humans, helping us to relate our animal experiments to our human studies.

1.5 Skeletal Muscle Adaptations in Human Following SCI

1.5.1 Muscle Size

Numerous studies have been conducted to study muscle atrophy after SCI.[5] Of the various techniques used to measure muscle size, measures of whole muscle cross-sectional area (CSA) have been identified to be the most accurate and reliable.[37] Initially, muscle CSA was calculated either by measuring the limb girth by a tape measure or by in-vitro measurements such as fiber CSA. Gregory *et al. 2003,* quantified both human and rat fiber CSA after 11 weeks-SCI. Both the rat and human vastus lateralis muscle showed significant atrophy (~50%) with chronic SCI. [17] Adams *et al.* 2006 and Stewart *et al.* 2004 reported significant atrophy in the vastus lateralis muscle using muscle fiber size measures following chronic SCI.[38,39]

Recently, muscle crossectional area (CSA) has been extensively measured by means of Magnetic Resonance Imaging (MRI) and other non-invasive measuring tools like ultrasonography and computed tomography.[14,16,18,40-47] Not only is MRI non-invasive, it is without harmful radiation, and has a unique ability to visualize non-muscle tissue like fat,

connective tissue and bone. It has greater tissue sensitivity and contrast resolution with multi-planar and 3D capabilities than ultrasonography and CT. [40-47] Moreover, MRI has the advantage of visualizing the entire length of a muscle compared to a biopsy or ultrasound. Numerous studies have utilized MRI to study CSA after SCI in both animals and humans. Castro *et al.* 1999 used MRI to show that the average maximal CSA of gastrocnemius and soleus decreased by 24% and 12% within six months of SCI, while the tibialis anterior CSA showed no change.[47] The average CSA of the quadriceps femoris, the hamstring muscle group and the adductor muscle group decreased by 16%, 14% and 16%, respectively. [47] The average CSA of atrophied skeletal muscle in the patients was 45-80% of that of age- and weight-matched able-bodied controls 24 weeks after the injury. The incomplete-SCI model in humans also showed significant skeletal muscle atrophy measured using MRI.[37] Individuals with chronic incomplete-SCI showed a ~28%-33% change in their muscle size as compared to able bodied controls. Maximum difference was seen in the plantarflexor muscles (32%) followed by knee extensors (31%), dorsiflexors (28%) and the knee flexors (22%).[37] Skeletal muscle atrophy following SCI is a result of injury to motor neurons in the spinal cord and concurrent inactivation of affected skeletal muscle along with subsequent changes in muscle length and mechanical loading conditions**.** Fractional presence of neural inputs to the muscle allows for variable activation of lower limb musculature after an incomplete-SCI, thus resulting in more modest atrophy in this population after injury and also better changes for positive prognosis compared complete-SCI group. [37-47]

1.5.2 Fiber Type Composition

The type of MHC expressed in human skeletal muscle also determines the characteristics of the muscle. Generally, muscle fibers in humans do not express more than one distinct MHC type.[5] However; the atrophic response in skeletal muscle following spinal cord injury

demonstrates a number of hybrid fibers co-expressing different MHC types. In general, MHC type transforms towards a faster type by the first year of injury with significant increases in MHC-IIx.[5] Histochemical fiber-typing studies also support the fact that there are dramatic increases in faster (type II) fibers after SCI. Talmadge *et al. (*2002), measured the effects of SCI on the expression of sarcoplasmic reticulum, calcium-ATPase (SERCA) and MHC isoforms in the vastus lateralis (VL) muscle.[48] SCI resulted in significant increases in fibers with MHC IIx with ~14% and ~16% increases at six weeks and 24 weeks after SCI.[48] In addition, SCI resulted in high proportions of MHC I and MHC IIa fibers with both SERCA isoforms (~29% and ~16% at six weeks and ~54% and ~28% at 24 weeks for MHC I and MHC-IIa fibers respectively).[48] The appearance of faster isoforms of MHC after SCI suggests that the muscle will have faster contractile properties, ultimately making it highly fatigable. These changes seen in the muscle are anticipated to contribute towards the functional limitations observed in this patient population.[48]

Studies have used different muscle fiber classification schemes such as myofibrillar ATPase activity, and activity (or concentration) of specific enzymes including succinate dehydrogenase (SDH), and alpha-glycerol-phosphate dehydrogenase (GPDH), to identify and quantify skeletal muscle adaptations after SCI.[5, 17] These measurement techniques visualize the activity of enzymes which are specific to each fiber type. When the enzyme reacts with an energy source a reactive product is formed. Thus the product from the assay is used to determine if the muscle fibers are fast or slow (mATPase), oxidative or non-oxidative (SDH), or generate ATP aerobically or anaerobically (GPDH).[5, 17] Gregory *et al.* (2003), quantified both human and rat VL fiber adaptations 11 weeks following SCI. The VL was sectioned and fibers were analyzed for type (I, IIa, IIb/x), SDH, GPDH, and actomyosin adenosine triphosphatase

(qATPase) activities.[17] The IIa to IIB shift was the major phenotypic adaptation that occurred in VL after SCI in both humans and rats. Rat fibers had 1.5- to 2-fold greater SDH and GPDH activity compared to humans.[17] The most striking differences, however, were the absence of slow fibers in the rat and its four-fold greater proportion of IIb/x fibers compared to humans which could be viewed as the rat's ability to counter the greater decline in SDH activity with regard to resistance to fatigue. SCI decreased SDH activity more in rats whereas IIa to IIb/x fiber shift occurred to a greater extent in humans.[17] Thus fiber type adaptations are species specific and each species has their own mechanism of countering an insult to its neuromuscular framework.

1.5.3 Electrically Elicited Contractile Properties

Contractile properties in the humans have been studied in muscles, like the quadriceps and soleus muscles after SCI. [42,49-53] Gerrits *et al*. 1999 indicated that muscles after SCI demonstrated faster rates of contraction and relaxation than normal control muscles and also had extremely large force oscillation amplitudes at the 10-Hz signal frequency (~65 % in SCI versus ~23% in controls).[50,51] In addition, force loss and slowing of relaxation following repeated fatiguing contractions were greater in SCI muscles compared with controls. The faster contractile properties and greater fatigability of the SCI muscles are in agreement with a characteristic predominance of fast glycolytic muscle fibers.[50,51] Within the SCI population, the chronically paralyzed soleus on average has a 20ms shorter time to peak twitch torque and a 25% shorter twitch half-relaxation time when compared to individuals with acute paralysis. This indicates that the muscle functioning gets faster with the progression of the diseased state. Fast fatigable motor units show progressive slowing during fatigue induced by repetitive activation.[52] Consistent with properties of faster muscle and motor units, the soleus after SCI demonstrates a near doubling of the half-relaxation time during fatigue.[52] This indicates that as the muscle fatigues, the calcium uptake becomes compromised or the cross-bridge cycling rate is impaired.

Normally, as the frequency of an electrical stimulus increases, muscle contraction becomes progressively more fused and the muscle generates greater torque. A muscle that has a slower contractile speed will fuse at a lower frequency when compared to a faster contractile speed. [52] The torque-frequency curve for a slow muscle will be shifted to the left of the torque-frequency curve of a fast muscle. Thus a torque-frequency curve for a muscle after SCI is shifted to the right of the torque-frequency curve for a normal muscle. Another measure that is specific to adaptations after SCI is low-frequency fatigue, which refers to repetitive activation of a chronically paralyzed muscle at low frequencies. [52] The preferential loss of force at low frequency can be recovered at higher frequencies. Impairments in excitation-contraction coupling (E-C coupling) is associated with low-frequency fatigue and likely represents an internal safety mechanism in skeletal muscle to prevent ATP depletion.[52] Low-frequency fatigue is characterized by being delayed in onset as well as being long lasting. This type of fatigue is found to be most prominent in fast-intermediate or fast fatigable motor units.[52]

1.5.4 Voluntary Contractile Measurements

Paralysis of the voluntary musculature is the most obvious effect of SCI in humans. Damage to the descending motor tracts, anterior horn cells, and/or nerve roots leads to an impaired capacity to voluntarily contract the skeletal muscles innervated at or below the level of the lesion.[49-52] In patients with SCI, the maximal voluntary contractions of the affected muscles are extremely weak compared to the range of absolute forces typically produced by non-injured individuals.[49-52]This may relate to reduced voluntary activation of the muscle, failure of neuromuscular transmission, problems within the muscle itself, or some combination of these possibilities. For example, if voluntary drive does not recruit all of the motoneurons that supply a muscle, the voluntary force produced will be reduced. Failure to activate each motor unit at its maximal firing frequency will also reduce force production. Similarly, the force contributed by

each motor unit will be lower if fiber size decreases (muscle fiber atrophy) from altered use of muscle. The sensory deficits that accompany these injuries may also exacerbate the ability of subjects to contract their muscles maximally.[49-52]

Few studies have measured voluntary muscle strength objectively after human SCI [54-56] or have delineated the factors that contribute to the weakness. The manual muscle test (MMT) has been used to measure strength historically in the field of physical therapy. The face and content validity of MMT in SCI is high, however manual muscle tests are subject to a ceiling effect, lack sensitivity to change and have a relatively poor inter-rater reliability, especially at scores greater than 3.[54-56] Studies have compared different methods to assess strength after SCI (the manual muscle test (MMT), the hand-held myometry and isokinetic dynamometry (Cybex, KinCom, Biodex). These studies suggest that the MMT method does not seem to be sufficiently sensitive to assess muscle strength, at least for grade 3 and higher and to detect small or moderate increases of strength over the course of rehabilitation. Further, it has been concluded that myometry and dynamometry measurements detect increases in strength over time, which are not reflected by changes in MMT scores.[55] Thus, dynamometry is currently considered a more sensitive measure of voluntary strength in human SCI population.

In summary, following SCI, there is significant atrophy quantified using muscle and fiber crossectional, a slow to fast muscle fiber transformation. Changes in contractile properties are more dramatic in fibers which have a larger proportion of slow fibers. There exists a reduction is muscle strength and voluntary muscle control. However, depending on the type of injury being incomplete or complete, the neuromuscular architecture and function are not necessarily compromised.

1.6 Skeletal Muscle Adaptations in the Animal Models Following SCI

A decrease in neuromuscular activity as a result of spinal cord injury (SCI) results in significant changes in morphological, mechanical and metabolic properties of skeletal muscles below the level of injury. However, the relationship between the injury and the muscle adaptations is confounded by the variability among injuries, and the type of injury. Below is a description of various adaptations that occur in skeletal muscle following SCI.

1.6.1 Muscle Size

This section covers atrophy measured using muscle wet weight and fiber size. Reduced muscle activity and loading or inactivity results in a significant reduction of skeletal muscle mass and muscle fiber size following SCI.[26,57] Specifically, muscle atrophy is more pronounced in single joint muscles which are involved in weight bearing and postural control.[26, 57] For example, the soleus muscle, a postural muscle crossing over the ankle joint, undergoes significant muscle atrophy following SCI.[5,26] In contrast, the TA or EDL are known to show relatively less atrophy compared to the soleus. 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.[5,26] Degree of atrophy is fiber-type specific, with the slow twitch muscles being more affected than the fast twitch muscles, and extensors atrophy more than flexors. For example, following spinal transection injury in adult cats, the morphological adaptations in the medial gastrocnemius (slow muscle) are higher than that seen in the tibialis anterior (fast muscle).[5,26,58] Similar to fiber size, absolute wet weight also decreases with SCI. Hutchinson *et al.* 2001 reported a 20-25% significant decrease in absolute wet weight in the soleus, while there was a 6% decrease in EDL wet weight when compared to matched controls.[20] While it is clear that atrophied muscles produce less contractile force, there appears to

be dissociation between the percent loss of muscle mass and percent decline in contractile tension indicating a loss in muscle specific force.[20]

1.6.2 Fiber Type Composition

Numerous methods have been used to understand the differences between fiber types. In early 1800s fibers were grossly differentiated to red and white based on their appearance.[5] With sophistication of experimental techniques different classification of fiber type have come to existence. The histochemical assay of myofibrillar ATPase activity is one of the few experimental techniques used to distinguish between fast and slow-contracting muscle fibers.[5] Myosin ATPase activity is positively correlated with muscle contraction velocity. Basically, fast contracting fibers hydrolyze ATP faster than slow-contracting fibers.[5] For example, cross-sections of a normal soleus stained for myofibrillar ATPase show a composition with a minimal number of fast fibers, where as a transection SCI-soleus is composed entirely of fast fibers.[5] The transection SCI-soleus represents a dramatic slow to fast muscle fiber type transformation. In addition, the average area of slow fibers in the soleus decreased by about 50% following SCI. There were no changes in fiber area for the EDL after SCI.[1] The soleus however generated the same absolute force in spite of its smaller muscle fibers, indicating an increase in its specific tension, and a significant conversion of its slow fibers to the fast type.[5,24,59] So, the first adaptation indicated after SCI is the reduction in fiber area and fiber type transformation from slow to fast muscle. This methodology was however used starting about 20 years ago and now more sensitive measures have been developed to substantiate this fiber type conversion after SCI.[24,59]

Myosin, the molecular motor of the skeletal muscle, is a protein comprised of two myosin heavy chains (MHC). The heavy chains determine the rate of cross-bridge reactions with actin filaments and hence help determine the speed of muscle contraction.[59] 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.[59] 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. Antibodies specific to these proteins identify fiber types based on these MHC's. Animal soleus muscles stained after transection SCI for MHC composition analysis indicate differences in the distributions of fiber types with a greater percentage of hybrid muscle fibers which coexpress different MHCs in SCI animals and a greater shift in MHC composition towards faster isoforms.[60,61] The control normal soleus primarily contains fibers reacting exclusively with type I myosin antibody (slow, 86.1 %) and a small percentage of fibers reacting exclusively with type IIa myosin antibody (fast, 13.9%).[60] One-week after SCI transection (ST), the proportion of pure type I fibers decreased to ~75%.[9] The remaining difference in the MHC composition in SCI animals was accounted for by an increase in hybrid fibers, with ~15% of fibers reacting to I & IIa myosin antibody and ~10% reacting to type IIa & IIx myosin antibody.[60]

Interestingly, the reduction in the proportion of fibers containing MHC-I after spinal isolation (SI) is greater than that observed for spinal transection. Talmadge *et al.* (1996) with MHC-specific antibodies demonstrated that the soleus from control cats contained 99% type I, 1% IIa. Following ST 67% of the fibers were positive for type I, 17% IIa, 3% IIb, and 13% hybrid fibers. After SI, 48% of the fibers were positive for type I, 11% were IIa, 1% was IIb, 25% were hybrid, and 15% contained embryonic MHC.[62] Roy *et al.* (1999) also showed that cat fast muscle (tibialis anterior) shows an ~4% increase in the fast fiber proportion and MHC-IIx

expression after 6 months of ST, while there is ~4% decrease in MHC-I fibers. Overall compared to control values, the percent composition of MHCs in the TA was unaffected by ST with or without training. Talmadge (1995) demonstrated that ST results in dramatic shifts in the expression of MHC isoforms of the rat soleus (normally approx. 90% MHC-I, approx. 10% MHC-IIa), such that 1 month after ST approx. 33% of the total MHC was MHC-IIx.[48,59,61,62]

Rodents show a higher degree of MHC isoform transformation after ST 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 ST. 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 ST. Immunohistochemical analyses revealed that MHC-I was progressively decreased after ST, to only approx. 12% 1 year after ST. The reductions in the proportion of MHC-I were countered by increases in MHC-IIa and MHC-IIx with the increase in MHC-IIx preceding the increase in MHC-IIa. Curiously, MHC-IIb was expressed only at very low levels. Thus, a complete transformation from predominantly MHC-I to MHC-IIb did not occur. Many fibers (up to approx. 80%) contained multiple MHCs (hybrid fibers) after ST. The proportion of hybrid fibers was maintained at a high level (approx. 50%) 1 year after ST.

Zhong and colleagues (2005) studied the effects of short-term (4 days) and long-term (60 days) SI on the rat soleus.[63] The control and SI-4d groups were ~90% pure type I and ~0.5 to 5% types I+IIa, I+IIa+IIx and IIa fibers in both groups. The SI-60d rats showed seven MHC combinations: pure type I (37%), I+IIa (32%), I+IIa+IIx (10%), I+IIx (16%), IIa (2%), IIa+IIx (~1%), and IIx (~2%) fibers. Thus the most dramatic adaptations in the SI-60d soleus muscles were a marked decrease in pure type I fibers, an increase in I+IIa, and appearance of fibers containing only IIx MHC. All of the hybrid fibers (fibers co expressing type I and II MHC isoforms) in

control and SI-4d rats contained >50% type I MHC. In the SI-60d group, however, 21% of the hybrid fibers contained <50% type I MHC. Similarly in the medial gastrocnemius (MG) and tibialis anterior (TA) muscles were also studied after short-term (4 days) and long-term (60 days) spinal isolation. Pure type I fibers were rare: 3%, 5%, and 0% in the control, SI-4d, and SI-60-d rats, respectively.[63] Approximately 90% of the fibers in all groups contained only types IIx and/or IIb MHC. Fibers containing type I plus some type II MHCs were more prevalent in the SI than control rats. There was a significant shift towards the fastest MHC isoform with inactivity: pure IIb fibers comprised 13%, 38%, and 41% of the population of the control, 4-day, and 60-day SI rats, respectively. In addition, there was a concomitant decrease in fibers containing only type IIx+IIb after 4 (trend) and 60 days of SI. Thus, it appears that type IIb MHC was the default MHC isoform in the inactive MG. TA muscles from control and 4-day SI rats contained ~5% pure type I fibers and ~15% pure type IIa fibers. In contrast, there were no pure type I or pure type IIa fibers in the 60-day SI rats. Approximately 70%, 80%, and 95% of the fibers expressed only types IIx and/or IIb MHC in the control, 4-day, and 60-day SI rats, respectively. Compared to control and SI-4d rats, there was a significant decrease in type IIb fibers and increases in type IIx and IIx+IIb fibers in the SI-60d rats. Thus, it appears that type IIx MHC was the default MHC isoform in the inactive TA. Thus, the magnitude of the adaptations observed following spinal isolation is more severe than 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.[63]

Hutchinson and colleagues (2001) measured adaptations in muscle size using muscle wet weight and MHC composition in the soleus (85% slow) and EDL (90% fast) muscles following moderate contusion SCI. They reported a 20-25% decrease in soleus wet weight after 1-week of

contusion, while the EDL showed a non-significant 6% decrease in wet weight.[20] Three weeks post contusion both the soleus and EDL wet weights returned to normal levels. Analysis of the MHC composition showed no change in fiber type composition at 1-week after spinal contusion in either muscle, while after three-weeks there was an upregulation of IIx MHC in both the soleus and EDL. [20] Interestingly, the soleus muscle showed a downward trend in IIa fibers, while the EDL demonstrated an increase in IIb fibers. Preliminary results in our lab indicate ~13% decrease in Type I fibers in the soleus and EDL two-weeks following moderate contusion SCI. The soleus also shows a ~ 9% increases in hybrid fibers of both myosin type I and IIa. The EDL shows a ~20% decrease in type IIx fibers and ~9% increase in IIb fibers following contusion SCI. [24,25,60-62] In the current study, we propose to use immunohistochemistry for MHC staining to study fiber type transformation in four important locomotor muscles with different fiber type composition and functional roles, specifically the soleus, gastrocnemius, EDL and the TA.

1.6.3 Electrically Elicited Contractile Properties

The physiological measurements such as muscle contractile speed, and force potentiation, delayed onset of fatigue, force frequency relationship, doublet potentiation, sarcolemmal membrane properties, and motoneuronal pool suppression are useful methodologies in assessing the mechanical adaptations in skeletal muscle after SCI. [20,48,62,64]

SCI results in faster twitch properties as evidenced by shorter time to peak tension and half-relaxation time; however the maximal isometric force generated is significantly reduced.[1] Maximum shortening velocity is significantly increased in SCI rats whether measured by extrapolation from the force-velocity curve or by slack-test measurements.[20,48,62,64] At a minimum 10Hz of electrical-stim, the soleus of the SCI animal develops greater force and is less fused than the normal soleus, implying faster contraction and relaxation times. [5] Unfused tetani of the EDL stimulated at different frequencies did not show significant difference between the

normal EDL and SCI-EDL.[5] Time to peak tension was decreased by ~50% in the SCI-soleus. In the cat soleus muscle there was ~38% reduction in isometric tetanic force 10-months post-SCI, while their time to peak tension was ~41% and half-relaxation time ~50% shorter than control cats.[2, 3] These changes in twitch and tetanic properties suggest a change in the properties of the sarcoplasmic reticulum (SR). The change in time to peak tension suggests an increase in the calcium transportability of the SR.[26,58]

In the spinal transection model, reduction in maximal tetanic force is significant. Talmadge *et al*. 2002 found ~44% reduction is maximal tetanic force three months post- spinal transection. In addition, the time to peak tension and half-relaxation time were ~45% and ~55% shorter respectively. [20,48,62,64] In the contusion model, our model of interest, one week post-SCI showed a ~20% decrease in both peak twitch and tetanic tension compared to controls and by three weeks they further decreased to ~41-51% respectively.[20,65] However, no significant changes existed between the controls and injured rats for the time to peak tension and half-relaxation times. Overall, the decline in contractile force seen in most animal models of SCI is related to the decrease in mechanical load and neural activation associated with the injury.

In summary, with SCI 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 can stimulate the skeletal muscle changes that were observed in the above sessions.

1.7 Rehabilitation Training Strategies Following SCI

1.7.1 Locomotor Training

1.7.1.1 Locomotor training in humans

Studies about locomotor training in people with SCI were first reported by Barbeau and colleagues (1987, 1993) where they assessed the feasibility of locomotor training on a treadmill

using body-weight support.[66,67] Currently, there is a significant increase in the use of locomotor training to retrain people to walk following numerous neurological conditions in the clinical setting. As described previously, this therapeutic intervention was derived from the elaborate models of locomotor training in animals' models of SCI which showed consistent positive findings.[14,68-74] Several studies in people with SCI have suggested that locomotor training may increase the likelihood that persons with upper motor neuron injuries will learn to walk over ground independently.[75-78]

Locomotor training guidelines compiled by Behrman and Harkema (2000)[79] were derived from basic and applied science findings and include the following principles: a) maximize weight-bearing through the legs and minimize or eliminate weight-bearing through the arms, b) provide sensory input consistent with the motor task; specifically standing or walking, c) promote postural control and optimize the trunk, upper and lower extremities, and hip kinematics for walking and associated motor tasks, and d) maximize the recovery and use of normal walking patterns and minimize the use of compensatory movement strategies. These strategies can be applied both in the clinical setting and in community settings.

Locomotor training also focuses on achieving independent community ambulation at normal walking speeds without assistive devices, bracing, or use of compensatory movements. Locomotor training consists of training people on a treadmill with their body-weight partially supported. Therapists manually assist in step training at joint angles and timing of stance and swing phases typical of normal gait. This is followed by overground step training which consists of evaluating factors which are limiting this individual from walking independently in the community at normal walking speeds without an assistive device, brace, or compensatory movements. Once these are evaluated then the person is trained to ambulate in the community.

Thus locomotor training is the combination of different gait training techniques to help get a person with incomplete-SCI to ambulate in the community independent of assistive devices.

However, the largest clinical trial comparing the efficacy of locomotor training with over-ground practice to defined over-ground mobility therapy in persons with SCI reported that physical therapy strategies of body weight support on a treadmill and defined overground mobility therapy did not produce different outcomes. It was suggested that the finding was partly due to the unexpectedly high percentage of ASIA C subjects who achieved functional walking speeds, irrespective of treatment.[80] Interestingly, there is still a lot of controversy surrounding the methodology and implementation of this clinical trial.[80]

1.7.1.2 Locomotor training in the animal model

SCI results in the loss of motor function due to the lack of supraspinal input. The concept that locomotor movements can be initiated even in the absence of supraspinal input was studied as early as the end of the 19th century (Freusberg 1874; Philippson 1905; Sherrington 1899, 1910; Naunyn, Dentan, and Eichorst 1874). During the 1940s and 1950s, several researchers suggested that spinally injured animals (i.e., cats and dogs) could not only produce stepping as described in earlier work, but these animals could use all four of their limbs for walking overground (Freeman1952; Kellogg *et al*. 1946; Shurrager 1955; Shurrager and Dykman 1951; Ten Cate 1939,1962). In 1951, Shurrager and Dykman first reported that training could restore locomotion after spinal cord transection in cats.[81] Later, Sten Grillner's laboratory in the 1980's (Forssberg 1979; Forssberg and Grillner 1973; Forssberg *et al*. 1974, 1976, 1980a Grillner 1973) clearly showed that thoracic SCI cats could walk with their hind limbs on the treadmill while the forelimbs stood on a fixed platform.[81] These animals had good coordination between their hind limbs, placed their fore paws properly on the plantar surface during the stance phase, and

supported the weight of the hindquarters. Not only did the kinematics of the spinal cat resemble those found in the normal cat, but so did the muscular activity.[81]

However, it is only in the past 20 years that this phenomenon of locomotor training has been vigorously explored, in concert with the growing recognition of the spinal cord's considerable capacities for plasticity and of other new possibilities for restoring function after spinal cord injury.[68-71] Today several studies have shown that recovery of motor function following spinal cord injury can be enhanced or accelerated by repetitive locomotor treadmill training. The underlying principle of locomotor training relates to rhythmic loading and repetitive motor training that provides sufficient stimulation of specific neural pathways to facilitate functional reorganization within the spinal cord leading to improved motor output. Furthermore, appropriate sensory input provided during training helps to achieve the optimal motor output of the spinal neuronal circuitry. [68-80]

1.7.2 Functional Electrical Stimulation (FES)

Functional electrical stimulation (FES) has been used as a therapeutic resistance exercise strategy to assist patients in strengthening as well as executing functional movements after SCI. [82-84] FES has been used in both the complete and incomplete SCI population to help reverse atrophic changes, reduce muscle fatigability and increase bone density after SCI. [85] FES used in combination with treadmill walking, cycling, external bracing holds considerable promise in assisting persons with SCI execute functional movements.[82-85] In the SCI population FES and FES in combination with other exercise interventions have been the main resistance or strength training protocols used in people with both complete and incomplete-SCI. In the following sections, we will review some the muscle adaptations following FES and FES in combination with other exercise interventions. In the current study, we will propose to investigate the effect of resistance training on muscle function following incomplete-SCI. However, our resistance

training protocol will be non-FES based and will include regular gym based resistance exercise training.

FES consists of a variety of stimulation parameters. It can be used at contraction times ranging from approximately 1 to 20 seconds, frequencies of 10Hz to 80Hz and voltages from 30V- 135V. [82-85] Gerrits *et al.* 2002 compared the effects of two types of FES (high-frequency and low-frequency) on neuromuscular activity after a motor complete SCI. Twelve weeks of FES resulted in ~20% increase in quadriceps tetanic force with no differences between the two stimulation frequencies. Neither training intervention had a significant effect on the contractile properties (maximal isometric force, maximal rate of force rise, half-relaxation time, and force-frequency amplitude) of SCI muscles.[86] Crameri *et al.* 2000 looked at effects of 16-weeks of FES (35Hz, 70V, 60min/day) after acute SCI. They found that FES helped in controlling the phenotype expression of the VL towards faster isoforms and prevented fiber atrophy after acute-SCI. [87] Dudley *et al*. 1999 in the sub-acute SCI population, showed that 8-weeks of FES resulted in substantial increases in the quadriceps cross-sectional area. In a similar study, 24 weeks of FES resulted in a significant strength gain with increased bone density in the quadriceps muscle of persons with chronic SCI when compared to untrained controls.[45] Long-term FES (two years) has also proven to yield significant differences in torque, fatigue index, bone mineral density and twitch properties in persons with SCI. when compared to their untrained leg.[88] FES has been the predominant means of resistance training people with SCI. [88,89] It would be interesting to identify the effects of regular exercise based resistance training on people with SCI.

FES is generally not a very favorable therapeutic intervention with the incomplete-SCI population as they have the ability to voluntarily activate their muscles to a certain extent. Bajd *et al.* 2000 conducted a two month FES training study on persons with incomplete-SCI. He

concluded that long term FES resulted in a significant improvement in knee extensor strength and also improved the ability to activate the dorsi and plantar flexors muscle groups in the incomplete-SCI group.[90] Modlin *et al.* 2005 performed a FES clinical trial on 40 persons with either a conus medullaris or cauda equina lesion. One year of FES resulted in significant increases in quadriceps muscle CSA compared to pre-training CSA levels. [91] Overall, FES has shown considerable promise in improving muscle function in all different models of SCI ranging from complete injuries to cauda equina injuries. Hence, FES on its own can be used as a resistance training therapeutic modality in the SCI population. However, the point of interest is that FES in the incomplete takes longer periods of time to cause significant improvements in the incomplete-SCI population. [90-91] This can be attributed to higher levels of function and voluntary control in this population. FES stimulated cycle ergometer training (FES-CE) has been used to improve whole muscle girth and muscle mass with persons with chronic SCI.[92,93] Baldi *et al.* 1998 examined if FES-CE was able to prevent atrophy after acute SCI. The study concluded that FES-CE prevents lower extremity muscle atrophy in acute SCI after 3 months of training, and also causes significant hypertrophy after 6 months.[93] In a similar study by Crameri *et al.* (2002), 10weeks of FES-CE resulted in significant increases in muscle fiber cross-sectional area, reduction in percentage of IIx fibers and increase in the citrate synthase activity, indicating a greater oxidative capacity of muscle, in persons with chronic SCI.[94]

Interestingly, numerous other studies have reported significant improvements after FES-CE on muscle morphometric and histochemical characteristics in the chronic complete SCI population.[95-99] These changes include increases in whole muscle and fiber cross-sectional area, muscle to adipose tissue ratio, fatigue resistance, maximal rate of force rise and speed of relaxation, and switch in MHC from fast to slower isoforms, doubling of enzymatic activity of

citrate synthase, and finally an increase in over-ground walking speed and endurance.[95-99] Overall, FES-CE has been able to provide a resistance exercise program without the potential of over-use injury in the complete-SCI population. The actual benefit of this training intervention in improving the functional capabilities in the complete-SCI population remains speculative. Similarly, the functional implications of FES-CE on the incomplete SCI population are yet to be studied.

1.7.3 Resistance Training

There is always curiosity regarding the effect of exercise on the functional well being of people with SCI. [100-102] In the above section we saw the effect of electrically stimulated resistance training either using weights or using CE. However, various impediments exist in the SCI population to complete successful regular resistance exercise training protocols. Specifically, only the incomplete-SCI population with limited voluntary muscle control can perform regular non-electrically induced resistance training. Although they have certain degree of voluntary control, they are still structurally and functionally ill-suited for strong propulsive and weight-bearing exercises. One has to be aware of inducing overuse bone and muscle injury, nociceptive and neuropathic pain, reflex sympathetic dystrophy, and some cases, cardiovascular complications.[103,104] Few studies have looked at the effects of resistance or strength training protocols on the SCI population. Nilsson *et al.* 1975 was the first to report significant improvement in the triceps muscles in persons with incomplete-SCI following resistance training. Cooney *et al.* 1986 used a hydraulic device in a nine-week training program which improved upper extremity power output in the chronic SCI population.[105,106]

Persons with SCI as we know exhibit deficits in voluntary control and sensation that limit not only the performance of daily tasks but also the overall functional and social activity. This leads to extremely sedentary lifestyle with an increased incidence of secondary complications

including diabetes mellitus, hypertension and lipid profiles. As the daily lifestyle of the average person with SCI is without adequate activity, structured exercise activities must be added if the individual is to reduce the likelihood of secondary complications and/or to enhance their physical capacity. The acute exercise responses and the capacity for exercise conditioning are related to the level and completeness of the SCI. Appropriate exercise testing and training of persons with SCI should be based on the individual's exercise capacity as determined by accurate assessment of the spinal lesion. Other issues that need to be taken into consideration before resistance training can be incorporated as a therapeutic activity. For example, the scientific basis for the exercises needs to be identified, training parameters; like dosage refinement, safety instructions, and inclusion-exclusion criteria need to be postulated. Overall, clinicians involved in SCI rehabilitation need to consider resistance training as a therapeutic intervention rather than concentrate on compensation as their modus operandi. Wheelchair strength training has shown considerable promise in improving muscle power and strength in the SCI population. However, these studies are either limited to the upper extremity or are for wheelchair athletes only.[107,108] To conclude, important strides need to be taken in the research field on studying the effects of resistance training on improving skeletal muscle function after SCI. In current study, we will examine the effect of gym based resistance exercise training on muscle function on people with chronic incomplete-SCI. The current study will be one of the first studies which will look at strength training lower extremity locomotor muscles in persons with incomplete-SCI.

1.8 Skeletal Muscle Adaptations Following SCI and Locomotor training

1.8.1 Impact on Humans

Current rehabilitation research has described loss of skeletal muscle function as one of the significant problems impacting the health care and quality of life of persons after SCI.[1,2] A significant portion of the SCI related costs can be attributed to degradation of the

musculoskeletal system resulting in decreased skeletal muscle function.[1,2] Even though locomotor training is not considered a therapeutic intervention designed to induce muscle hypertrophy; previous studies have shown that in the incomplete-SCI population the training stimulus and loading can be of sufficient magnitude to induce muscle plasticity. Giangregorio *et al.* 2006 reported increases in whole-body lean mass, from ~45.kg to ~47kg and increases in muscle CSAs by an average of 4.9% and 8.2% at the thigh and lower leg after 144 sessions of locomotor training in persons with chronic incomplete-SCI.[109] In a similar study performed in the acute-SCI population, 48 sessions of locomotor training resulted in increases in muscle CSAs ranging between ~4% to ~58%.[110] The study concluded that twice-weekly locomotor training appeared to partially reverse muscle atrophy after SCI, but failed to prevent bone loss.[109,110]. These findings are supported by research examining changes at the muscle fiber level. Stewart *et al.* 2004 reported a 25% increase in the mean muscle fiber area of type I and IIa fibers in the vastus lateralis following 6 months of body weight supported treadmill training in chronic incomplete-SCI subjects.[39] Adams *et al.* 2006 in a single case study (chronic ASIA B) reported that the vastus lateralis mean fiber area increased by 27.1% and type I fiber % distribution increased to 24.6%, whereas type IIa and type IIx fiber % distributions both decreased following 48 sessions of locomotor training.[38]

1.8.2 Impact on the Animal Model

The effects of locomotor training on SCI-induced muscle adaptations have been studied to a limited extent over the past two decades. Roy *et al.* as early as 1986[26] identified that spinalized adult cats who exercised on a treadmill for a week showed less atrophy and fiber type adaptations, especially in the postural muscles (slow extensors).[26] In a similar study, the same group identified that only 30 min of daily step training emphasizing weight support on a treadmill ameliorated, and in some cases prevented, the contractile and morphological

adaptations in the soleus muscle associated with a complete low thoracic spinal cord transection in adult cats.[111]

Similarly, a few studies have also been conducted in the rodent model, identifying muscle adaptations after SCI and locomotor training. Versteegden *et al*. (1999, 2000) reported that locomotor training resulted in an increase in muscle fiber size, myonuclear number, satellite cell count and a decrease in the apoptotic nuclei in the soleus muscle after spinal transection in the rat model.[112,113] A unique finding in these studies was that satellite cell fusion and restoration of myofiber nuclear number contributed to increased muscle size in the soleus after locomotor training.[113] Stevens *et al.* 2006 reported that locomotor training following contusion SCI resulted in a significant improvement in overall locomotor function (32% improvement in BBB scores) when compared to no training group. Also, the injured animals that trained for one week had 38% greater peak soleus tetanic forces, a 9% decrease in muscle fatigue, 23% larger muscle fiber CSA, and decreased expression of fast myosin heavy chain fiber types compared to rats receiving no training.[65,114] Overall, locomotor training has shown significant promise in attenuating the adaptations in skeletal muscle seen after SCI. This includes prevention of atrophy following SCI, reduced fatigability, improved muscle force production and transformation of fiber type towards slower isoforms. However, further investigation is required to identify the training effects on specific models of SCI.

In conclusion, locomotor training has shown to induce positive alterations in skeletal muscle function in both humans and animals. However, most of the current data still revolve around the complete SCI model. Further investigation in the incomplete SCI model in both humans and animals is warranted. In this dissertation we will to answer some of the questions

regarding skeletal muscle adaptations following locomotor training in both the animal and human model.

1.9 Mechanisms Involved in Training Induced Muscle Plasticity and Recovery

The primary functions of skeletal muscle are production of movement, posture control, and respiration. Interestingly, skeletal muscle is susceptible to injury from direct trauma (e.g., intensive activity, stab wounds, gun shots etc.) or resulting from indirect causes such as neurological disease or genetic complications.[115] Direct or indirect injuries may lead to loss of muscle mass and strength leading to a functional limitation. The maintenance of a working skeletal muscle is conferred by its remarkable ability to regenerate.[115] Indeed, upon muscle injury a finely orchestrated set of cellular and molecular responses is activated, resulting in the regeneration of a well-innervated, fully vascularized muscle apparatus.[115]

Muscle fibers are the single cells that form skeletal muscles. They are individually surrounded by a connective tissue layer (endomysium) and grouped into bundles surrounded by the perimysium, and these bundles are surrounded by the epimysium to form a skeletal muscle.[115] As the muscle fiber or myofiber matures, it is contacted by a single motor neuron and expresses molecules for contractile function, principally different MHC isoforms and metabolic enzymes. Both the origin of the myoblast and the motor neuron play an important role in specifying the contractile properties of their myofiber. Nevertheless, adult skeletal muscles are composed of a mixture of myofibers with different physiological properties, ranging from a slow/fatigue-resistant type to a fast-/non-fatigue-resistant type. The proportion of each fiber type within a muscle determines its overall contractile property.[115]

1.9.1 Plasticity of Skeletal Muscle

Adult skeletal muscle is a very stable tissue with little turnover of nuclei. Minimal damage inflicted by daily wear and tear elicits only a slow turnover of its multinucleated muscle fibers. It

is estimated that in an adult rat muscle, no more than 1–2% of myonuclei are replaced every week.[116] Nonetheless, mammalian skeletal muscle has the ability to complete rapid and extensive regeneration in response to severe injury or damage. The majority of this regeneration is carried out by the activation, proliferation and differentiation of a resident population of myogenic cells called satellite cells. Under normal conditions, satellite cells are quiescent but become activated in response to injury giving rise to proliferating myogenic precursor cells that eventually differentiate and fuse to form multinucleated myotubes. Quiescent satellite cells and their descendant myogenic precursors are the key effectors of muscle regeneration.[116-118]

The early phase of muscle injury is usually accompanied by the activation of mononucleated cells, principally inflammatory cells and myogenic cells. The factors released by the injured muscle activate inflammatory cells within the muscle. Neutrophils are the first inflammatory cells to invade the injured muscle. After neutrophil infiltration, macrophages infiltrate the injured site to phagocytose cellular debris and initiate muscle regeneration by activating myogenic cells. Thus muscle fiber necrosis and/or increased number of non-muscle mononucleated cells within the damaged site are the main histopathological characteristics of the initial activity following muscle injury.[117-119]

Muscle degeneration after injury is followed by the activation of a muscle repair process. The myogenic cells provide an ample source of new myonuclei for muscle repair. On cross-section, classic characteristics of muscle regeneration are small newly formed myofibers with centrally located myonuclei. [120,121] Newly formed myofibers are often basophilic and express embryonic/developmental forms of MHC which reflect new fiber formation. [120,121] Fiber splitting or branching is also a characteristic feature of muscle regeneration and is likely due to the incomplete fusion of fibers regenerating within the same basal lamina. Once fusion of myogenic

cells is completed, newly formed myofibers increase in size, and myonuclei move to the periphery of the muscle fiber. Under normal conditions, the regenerated muscle is morphologically and functionally indistinguishable from undamaged muscle.[120,121]

1.9.2 Markers of Muscle Recovery and Regeneration

1.9.2.1 Adult muscle satellite cells

Muscle satellite cells are a population of undifferentiated, mononuclear myogenic cells found in skeletal muscles including muscle spindles. Even though the temporal appearance of satellite cells follows the appearance of both embryonic and fetal myoblasts, satellite cells display specific characteristics in culture allowing their distinction from embryonic and fetal myoblasts. [116,122,123] Satellite cells are situated between the plasma membrane and the basal lamina of the muscle fiber. These cells are further identifiable by their relatively minute amount of cytoplasm, sparse organelles, and high ratio of heterochromatin to euchromatin, indicative of the inactive state of these cells. Satellite cells are present in different types of skeletal muscles and are associated with all fiber types, although the distribution might be unequal. For instance, the percentage of satellite cells in adult slow soleus muscle is two- to threefold higher than in the adult fast tibialis anterior or extensor digitorum longus muscle.[124] Similarly, high numbers of satellite cells are found associated with slow muscle fibers compared with fast fibers within the same muscle.[116-122-124] Increased density of satellite cells have been observed at the motor neuron junctions and adjacent to capillaries, suggesting that some factors associated with these structures may play a role in homing satellite cells to specific locations or in regulating the satellite cell pool by other means. The regulation of satellite cell density at the single fiber level is also suggestive of a role for the muscle fiber in regulating the satellite cell pool (Fig.1-3).[116,124]

Satellite cells are activated upon muscle injury, resulting from mechanical stress, direct injury to the muscle or in course of a disease to help in muscle regeneration. In the initiation of

muscle regeneration, satellite cells first change from their quiescent state to a highly proliferating stage. After proliferating several times, the majority of satellite cells fuse to form new myofibers or join and repair the damaged one. During proliferation, a certain percentage of satellite cells are restored underneath the basal lamina for subsequent rounds of regeneration (Fig.1-4).[122]

The gene or the marker responsible for specification of muscle progenitor cells to the satellite cell lineage is pax-7.[125] The Pax7 gene is a member of the paired box containing gene family of transcription factors implicated in development of the skeletal muscle of the trunk and limbs, as well as elements of the central nervous system. [125,126] The number of Pax7 expressing cells corresponds well with the expected number of satellite cells. Pax7 expression is upregulated in proliferating satellite cell-derived myoblasts and a rapid down regulation of Pax7 transcripts is seen upon myogenic differentiation. Pax7 is not expressed at detectable levels in a variety of non-muscle cell lines. In addition, analysis of RNA from selected mouse tissues revealed only a low level expression of Pax7 in adult skeletal muscles. [125] Normally Pax7 mRNA and protein are found in less than 5% of satellite cells in undamaged skeletal muscle. However, the number of Pax7-positive cells increases in muscles undergoing regeneration such as in MyoD$^{-/-}$, mdx, and mdx: MyoD$^{-/-}$ skeletal muscles. [125,126] Centrally located nuclei within newly regenerated muscle fibers are also associated with Pax7 expression, suggesting that recently activated and fusing satellite cells express Pax7. Together, these data demonstrate the specific expression of Pax7 in quiescent and activated muscle satellite cells.[124,125,126]

The analysis of Pax7$^{-/-}$ skeletal muscles demonstrates the important role this gene has in satellite cell development.[125-128] Pax7$^{-/-}$ mice appear normal at birth but fail to grow post-natally, leading to a 50% decrease in body weight by 7 days of age compared with wild-type littermates. Pax7 mutant animals fail to thrive and usually die within 2 weeks after birth. These animals are

also characterized by a decreased skeletal muscle mass resulting from a fiber size decrease rather than a decrease in fiber number.[125-128] $Pax7^{-/-}$ skeletal muscles have a striking absence of satellite cells. Overall, the data suggest a key role for Pax7 in lineage determination, especially in the specification of myogenic progenitors to the satellite cell lineage. Pax7 is unequivocally required for satellite cell development (Fig.1-5).[125-128]

In the next stage, proliferating satellite cells are referred to as myogenic precursor cells (mpc). At the molecular level, activation of mpcs are characterized by the upregulation of two muscle regulatory factors (MRF), Myf5 or MyoD. MRFs are part of a super family of basic helix-loop-helix (bHLH) transcription factors. The MRF subfamily consists of MyoD (Myf-3), Myf-5, myogenin (Myf-1), and MRF4 (Myf-6/Herculin).[123,129] In general, quiescent satellite cells do not have any detectable levels of MRFs. Upon satellite cell activation, MyoD upregulation appears the earliest within 12 hrs of activation. Activation of MyoD and Myf5 expression following muscle injury has also been observed in various in vivo models for muscle regeneration and in varying muscle types.[129,123] A study by Megeney *et al*. indicated that $MyoD^{-/-}$ mice show increase in mpc population compared to normals, however they have a decrease in the number of regenerated myotubes. Furthermore, $MyoD^{-/-}$ muscles display an increased occurrence of branched myofibers suggestive of chronic or inefficient muscle regeneration.[129-135] In vitro cultures of $MyoD^{-/-}$ satellite cells demonstrate a myogenic cell population with abnormal morphology characterized by a stellate, flattened appearance in contrast to the compact rounded appearance displayed by normal myoblasts. Overall, these data suggest an important role for MyoD in the process of satellite cell differentiation during muscle regeneration.[129-135]

Myf5-deficient mice display a delayed epaxial (back muscle) embryonic myogenesis and a normal hypaxial (trunk and limb muscles) embryonic myogenesis.[129-131] These data combined

with the reciprocal delay in hypaxial myogenesis in MyoD-deficient mice and the mutually exclusive expression of Myf5 and MyoD in early stages of embryonic muscle precursor cells have led to the hypothesis that Myf5 and MyoD support distinct myogenic lineages during embryonic muscle development. [129-131] Myf5 promotes satellite cell self-renewal, whereas MyoD promotes satellite cell progression to terminal differentiation. There is new compelling evidence that the satellite cell population is composed of hierarchal subpopulations of stem cells: the $Pax7^{+}$/ $Myf5^{+}$ satellite cells preferentially differentiate and become committed myogenic progenitors, while the $Pax7^{+}$/ $Myf5^{-}$ satellite cells extensively contribute to the satellite cell compartment.[129-131]

After the mpc proliferation phase, expression of Myogenin and MRF4 is upregulated in cells, beginning their terminal differentiation program. This is followed by cell cycle arrest and permanent exit from the cell cycle. The differentiation program is then completed with the activation of muscle-specific proteins, such as MHC, and the fusion of mpc to repair damaged muscle or form their own fibers. Overall, Myf5, MyoD, and Myogenin possibly play distinct roles in myofiber maturation.[122] Gross defects in embryonic muscle development of mutant mice for Myogenin and MRF4 have impeded further study of these genes in muscle regeneration. Mice lacking myogenin display a normal number of myoblasts but die at birth because of an absence of myofibers. It has also been suggested that Myogenin helps in the conversion of myoblasts to myotubes and helps in the maturation of myotubes.[122]

1.9.2.2 Other stem cells

Mammalian skeletal muscle regeneration involves the activation of the quiescent muscle satellite cell population to proliferate, differentiate, and fuse to provide new myonuclei for muscle repair. Pax7 is required for muscle satellite cell specification/survival, whereas MRFs are essential in satellite cell proliferation and differentiation. Multipotential stem cells in adult

muscles (adult muscle-derived stem cells) are also capable of myogenic commitment. [122,123] Adult muscle-derived stem cells contribute to both muscle satellite cell pool and myonuclei. Similarly, stem cells capable of myogenic commitment can be isolated from other adult tissues (bone marrow stem cells, neuronal stem cells, and various mesenchymal stem cells)can be used for repair following muscle damage or towards new fiber formation.[122,123]

1.8.2.3 Growth factors and muscle regeneration

Muscle regeneration is a complex process in which growth factors play an important role. Mechanisms that are controlled or altered by growth factors include satellite cell activation, migration to the injury site, proliferation of satellite cell-derived mpcs and differentiation to myotubes and myofibers. Insulin-like growth factors (IGFs) I and II are involved in almost all stages of muscle regeneration; they promote satellite cell activation and proliferation, are upregulated in regenerating muscle and may protect cells from apoptosis. Both IGF-I and hepatocyte growth factor/scatter factor (HGF) are upregulated during muscle regeneration, HGF being crucial during the initial stages and IGF during the initial to mid stages of regeneration. A significant increase in muscle regeneration was observed when human mpcs were cultured with IGF-I prior to their implantation into damaged muscle.[122] IGF-I has a significant effect on proliferate arrest and hypertrophy of myotubes derived from human fetal mpcs in culture and causes an increase in myosin heavy chain content. HGF is known to increase the chemotaxis of mpcs.[122,132-134]

C2C12 myoblasts treated with HGF reorganized their actin cytoskeleton and developed a polarized cell shape.[136] HGF appears to increase the mpc population by means of mitogenic and chemotactic activities, possibly resulting in an optimal myoblast density. IGFs most certainly promote muscle repair by signaling to both the satellite cells and the myofibers. Whether distinct

roles are played by different IGFs is possible, since IGF-II appears to be upregulated later during the process of muscle regeneration.[122,132-134]

Fibroblast growth factors (FGF) are also involved with satellite cell activation, proliferation and differentiation. FGF-2 acts as a regulator of satellite cell activity and FGF-6 expression is upregulated during muscle regeneration. Expression of FGF-6 in C2C12 cells induces morphological changes; reduces cell adhesion and differentiation.[137] A greater proportion of the cells which expresses FGF-6 were side population cells, suggesting that FGF-6 may be involved in the maintenance of the reserve pool of progenitor cells in skeletal muscle. Also the role of FGF in muscle regeneration may reside in the revascularization process during regeneration through their recognized angiogenic properties. [122,132-134]

In summary, mammalian skeletal muscle has little turn over of satellite cells under normal conditions. However, upon injury, skeletal muscle activates satellite cells to both repair and regenerate muscle fibers to prevent atrophy and damage. There are certain key phases in the regeneration process and these processes are supported by different growth factors.

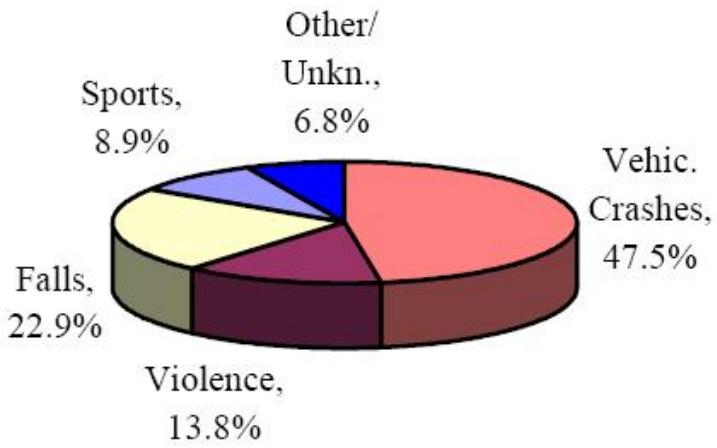

Figure 1-1. Etiology of SCI since 2000 modified from www.spinalcord.uab.edu.

Severity of Injury	25 years old	50 years old
High Tetraplegia (C1-C4)	$2,924,513	$1,721,677
Low Tetraplegia (C5-C8)	$1,653,607	$1,047,189
Paraplegia	$977,142	$666,473
Incomplete Motor Functional at any Level	$651,827	$472,392

Figure 1-2. Estimated lifetime costs by age at injury modified from www.spinalcord.uab.edu.

Animal Model	Muscle	Age, mo	Satellite Cell Nuclei, %
Mouse cross-sections (291)	EDL	5–7	1.2
	Soleus	5–7	4.1
Rat cross-sections (118)	EDL	1	7
		12	2.9
		24	1.9
	Soleus	1	9.6
		12	6.6
		24	4.7
Rat cross-sections (265)	TA	2	4
	Soleus	2	11

Figure 1-3. Satellite cell number in skeletal muscle of different ages and type modified from Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev*. 2004; **84:**209.

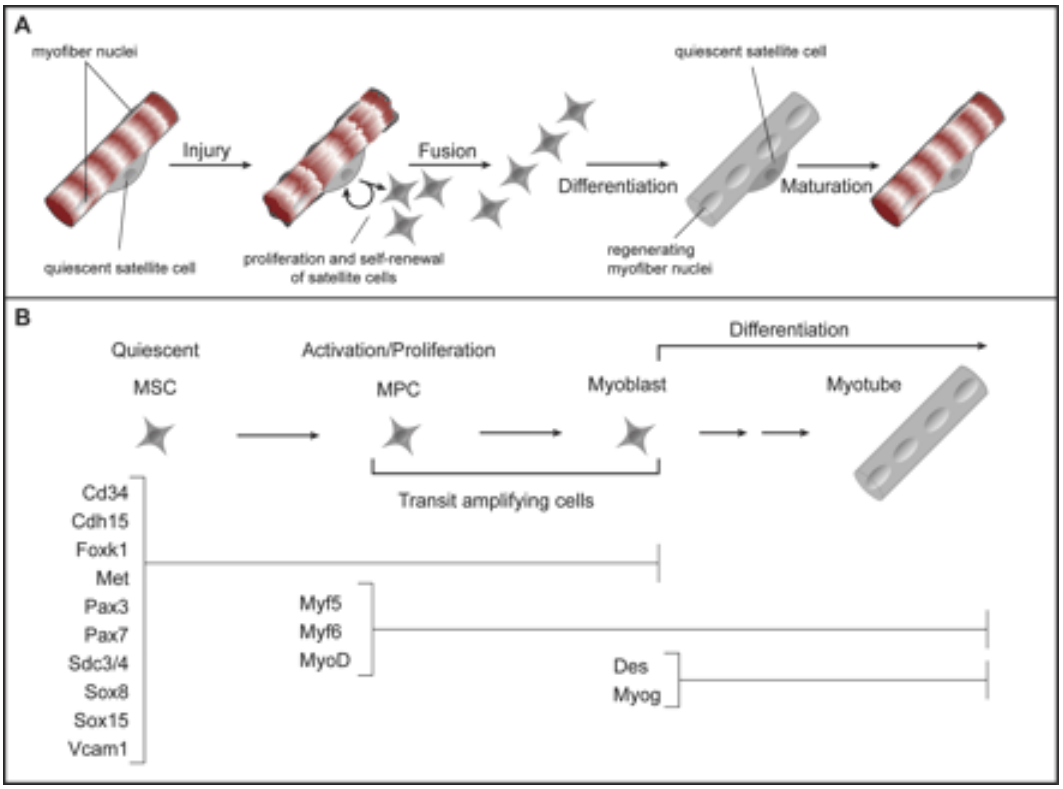

Figure 1-4. Satellite cell activity modified from Shi X and Garry DJ. Muscle stem cells in development, regeneration, and disease. *Genes Dev*. 2006; **20:**1692. A) Schematic outline of satellite cell activity in muscle regeneration, B) gene expression of satellite cells and myogenic precursor cells.

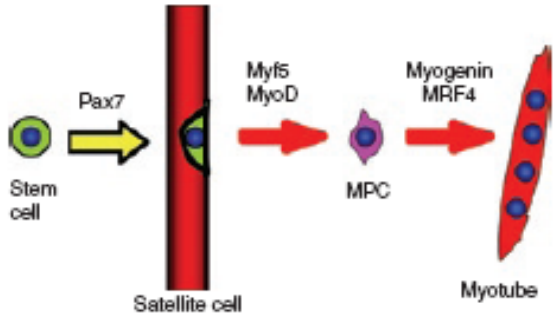

Figure 1-5. Schematic outline of a stem cell passing through the stages of muscle regeneration modified from Péault B, Rudnicki M, Torrente Y, Cossu G, Tremblay JP, Partridge T, Gussoni E, Kunkel LM, Huard J. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol Ther*. 2007; **15:**867.

CHAPTER 2
OUTLINE OF EXPERIMENTS

2.1 Experiment 1

2.1.1 Specific Aim

To characterize and specifically quantify impairments in lower extremity skeletal muscle function after chronic incomplete spinal cord injury (SCI).

Peak isometric torque, torque developed within the initial 200 ms of contraction ($torque_{200}$), average rate of torque development (ARTD), and voluntary activation deficits will be calculated as indices of muscle function. Measures of muscle function and electrically elicited contractile measurements will be quantitatively performed in the knee extensor (KE) and ankle plantar flexor (PF) muscle groups in ten individuals with chronic incomplete SCI.

2.1.2 Hypothesis

Reduced peak and instantaneous torque production, as well as greater voluntary activation deficits of the lower extremity muscles will be found to be characteristic of individuals with chronic incomplete SCI compared to healthy controls. In addition, significant bilateral asymmetries will exist between limbs, with one limb being more affected than the other.

2.2 Experiment 2

2.2.1 Specific Aim

To determine the impact of nine weeks of locomotor training on lower extremity skeletal muscle function in persons with chronic incomplete SCI.

Five individuals with chronic incomplete SCI will undergo nine weeks (five sessions per week) of locomotor training (LMT). Indices of muscle function will be determined for the KE and PF muscle groups before and after LMT.

2.2.2 Hypothesis

Nine weeks of locomotor training will result in positive alterations in the lower extremity muscles that include improved voluntary activation as well as an improved ability to generate both peak and explosive torque about the knee and ankle joints in persons with chronic incomplete SCI.

2.3 Experiment 3

2.3.1 Specific Aim

a) To determine the impact of a 12-week resistance and plyometric training on lower extremity skeletal muscle function in persons with chronic incomplete SCI.

b) To determine the effect of 12-weeks of resistance and plyometric training on gait speed in persons with chronic incomplete SCI.

Three ambulatory individuals with chronic motor incomplete SCI (18.7±2.2 months post injury) will complete 12-weeks of lower extremity resistance training combined with plyometric training (RPT). Indices of muscle function will be determined for the KE and PF muscle groups before and after RPT. Maximal, as well as self-selected gait speeds will also be determined pre- and post-RPT.

2.3.2 Hypotheses

a) Twelve weeks of RPT will result in improved ability to generate peak and instantaneous torque as well as improved voluntary activation and reduced time to peak torque in the lower extremities of persons with chronic incomplete SCI. In addition, the magnitude of improvements in these outcomes will be most pronounced in the PF versus the KE muscle group.

b) Twelve weeks of RPT will result in significant improvements in both self-selected and maximal gait speed in persons with chronic incomplete SCI.

2.4 Experiment 4

2.4.1 Specific Aim

a) To determine the effect of moderate T8 contusion spinal cord injury (rat model of incomplete SCI) on muscle fiber cross-sectional area (CSA) and fiber type composition in four lower extremity muscles (soleus, gastrocnemius, tibialis anterior, and extensor digitorum longus) with different fiber type compositions and functional roles.

b) To compare the effect of 1-week of locomotor training on fiber CSA and fiber type composition in lower extremity muscles with different fiber type composition and functional roles in rats following moderate T8 contusion spinal cord injury.

c) To determine the effect of one week of locomotor training on fiber crossectional area and fiber type composition in four lower extremity muscles (soleus, gastrocnemius, tibialis anterior, and extensor digitorum longus) in healthy controls.

Rats (n=6 per group) will be assigned to four groups; a SCI-treadmill training group, a SCI-no training group, control-treadmill training group, control-no training group. Moderate spinal cord contusion injuries will be produced using a standard NYU (New York University) impactor. Animals assigned to the training groups will be trained continuously for 1week (5 days/week, 2 trials/day, 20minutes/trial), starting on post-operative day eight for the SCI training group. Fiber CSA will be assessed at two weeks post-injury for the slow-twitch, plantarflexor muscle, soleus, fast-twitch plantarflexor, gastrocnemius and fast-twitch dorsiflexor muscles (tibialis anterior [TA] and extensor digitorum longus [EDL]).

2.4.2 Hypotheses

a) Two weeks following moderate T8 spinal contusion injury, the injured rats will experience the maximum decrease in fiber CSA in the slow-twitch plantarflexor (extensor) soleus when compared to the non-injured control rats. The next largest decline in muscle fiber

CSA will be seen in the gastrocnemius, followed by the fast-twitch dorsiflexors, TA and EDL. Similarly, following moderate T8 spinal contusion injury, all muscles in the injured rats will show a shift in fiber type towards faster myosin isoforms. Specifically, the soleus from the injured rats will show a fiber type shift from a slower isoform to a faster isoform (MHC-I to MHC-IIa) compared to the soleus of controls. Similarly, the gastrocnemius, TA and EDL of the injured rats will show a fiber type shift towards faster isoforms (MHC-IIa to MHC-IIx and MHC-IIx- MHC-IIb) when compared to controls.

b) One week of treadmill training will attenuate the decrease in fiber CSA of the injured rats in all the muscles. Specifically, the soleus will experience maximum gains in fiber CSA, followed by the gastrocnemius, and then the dorsiflexors. In addition, treadmill training will attenuate the fiber type shift observed following a moderate T8 contusion spinal cord injury. Specifically, the soleus will show fiber type transformation towards slower isoforms (MHC- IIa to MHC-I), while the gastrocnemius, TA and EDL will show a transformation from MHC- IIb towards IIx or IIa.

c) Healthy control rats trained for one week will show increases in fiber CSA compared to untrained control rats. However, we anticipate that there will be no difference in the CSA values between the trained and the untrained group.

2.5 Experiment 5

2.5.1 Specific Aim

a) To determine the impact of moderate T8 contusion SCI on satellite cell activity on the slow-twitch (soleus) and fast-twitch (TA) rat muscles.

b) To determine the influence of one week of locomotor training on satellite cell activity on the slow (soleus) and fast twitch (TA) muscles on spinal cord-injured rats.

c) To determine the impact of one week of locomotor training on satellite cell activity on the slow (soleus) and fast twitch (TA) muscles on control rats.

Rats (n=6 per group) will be assigned to either a SCI-treadmill training group, a SCI-no training group, control-treadmill training group or control-no training group. Expression of markers of muscle regeneration will be assessed for all four training groups. Specifically, immunofluorescence techniques will be used on the soleus and TA to quantify for Pax-7 and EM-MHC expression and Western blot analysis will be used to quantify for MyoD, Myf5, and Myogenin expression.

2.5.2 Hypotheses

a) Two weeks following moderate T8 spinal contusion injury, both the slow twitch (soleus) and the fast twitch (TA) muscles will show increase in the regulation of muscle regeneration markers compared to controls. Specifically, the levels of the markers will be higher in the slow twitch muscles when compared to the fast twitch muscles.

b) One week of locomotor training, will result in increased regulation of muscle regeneration markers in both the muscles types, compared to untrained SCI rats. Specifically, the slow twitch (soleus) will show significant elevations in regeneration markers after the training in comparison to the fast twitch (TA) following moderate T8 contusion spinal cord injury.

c) SCI rats trained for one week will show increased regulation of regeneration markers compared to trained control rats.

CHAPTER 3
METHODOLOGY

3.1 Studies in People with Incomplete-SCI

3.1.1 Subjects Description

Subjects who participate in the first three experiments are persons with chronic upper motor neuron lesions and motor incomplete-SCI. Criteria for inclusion include: 1) age 18-70; 2) first time SCI (C5-T10); 3) medically stable and asymptomatic for bladder infection, decubitis, cardiopulmonary disease or other significant medical complications prohibiting testing and/or training; 4) if using antispasticity medication, agreement to maintain current levels throughout study; Exclusion criteria will be: 1) participation in a rehabilitation or research protocol that could influence outcomes of this study; 2) history of congenital SCI or other disorders that may confound treatment, study, and/or evaluation procedures; Prior to participation, written informed consent will be obtained from all subjects, as approved by the Institutional Review Board at the University of Florida.

3.1.2 Locomotor Training

The locomotor training intervention consists of 45 training sessions (5x/ week) spread over nine weeks, with each session consisting of 30 minutes of step training on the treadmill with body weight support (BWS) immediately followed by 20 minutes of level overground walking and community ambulation training. Including pre-training stretching, donning/doffing the harness, and additional time spent on the treadmill for stand training and standing rest breaks, the total session duration will be approximately 75 to 90 minutes per day. Each subject is expected to complete all of the training sessions. With the aid of the body weight support, treadmill and manual trainers, the treadmill training environment will facilitate delivery of locomotor specific practice. Trunk, lower limb, and upper limb kinematics will be consistently assisted and/ or

monitored by trainers to assure appropriateness in relation to normal walking. Speed of treadmill stepping will be kept in a range consistent with normal walking (2.2-2.8 miles/hr). Progression of training will be achieved by decreasing BWS, altering speed, increasing trunk control, decreasing manual assistance for limb control and increasing the time spent walking on the treadmill per bout. A more detailed description of the training principles, parameters and progression has been provided by Behrman & Harkema *et al.* 2000. Overground training will consist of an immediate assessment of the participant's ability to stand and/or walk independently overground and an evaluation of the deficits limiting achievement of this goal. These deficits became the focus for goal setting in the next day's training session. Additionally, overground training addressed translation of the skills from the treadmill to the home and community identifying practical ways for the participants to incorporate new skills into everyday activities (Figure 3-1).

3.1.3 Resistance and Plyometric Training

3.1.3.1 Resistance training

Lower extremity progressive resistance training will be 12 weeks in duration and subjects will complete 2 to 3 sessions/week for a total of 30 sessions. Resistance exercises will include unilateral leg press, knee extension/flexion, hip extension/flexion and ankle plantar flexion exercises performed on adjustable load weight machines. During the initial training session a predicted one-repetition maximum (1-RM) will be calculated for each subject and for each exercise. 1-RM will be determined using a prediction table based on a single set to volitional failure with load that allowed between 6 and 12 repetitions. During subsequent training sessions, subjects will perform 2-3 sets of 6-12 repetitions at a relative intensity of ~70-85% of predicted 1-RM. Maximal strength will be evaluated weekly to assess for training-related improvements and exercise loads will be adjusted accordingly. Specifically, if the subject achieved the target

number of repetitions for all prescribed sets of a given exercise, a new predicted 1-RM will be prescribed and resistance will be increased for subsequent training sessions.

3.1.3.2 Plyometric training

Unilateral plyometric jump-training exercises will be performed in both limbs in a supine position on a ballistic jump-training device (ShuttlePro MVP ®, Contemporary Design Group, Figure3-2). Session intensity for this exercise will be modified by changing either the resistance or the number of ground contacts and progressed over the training period, accordingly. Briefly, after familiarization with the training device, subjects will complete a total of 20 unilateral ground contacts (e.g. jumps) with each limb at a resistance of ~25% of body mass. Thereafter, upon successful completion of at least 20 ground contacts per limb (e.g. complete clearance from the foot plate), resistance will be increased in increments of 10 lbs. When a new resistance is set, repetition goal will be set at 10 ground contacts per limb for the initial session. Subsequent sessions allowed for up to 20 contacts per limb. Thus, a minimum of two sessions at a given resistance will be required before load is increased. Resistance will be held constant between limbs throughout the training program.

3.1.4 Muscle Function Assessment

3.1.4.1 Experimental set-up

Voluntary and electrically elicited contractile measurements are performed in the self-reported more-involved and less-involved limbs for the knee extensor and plantarflexor muscle groups, using a Biodex System 3 Dynamometer. Knee extensor testing will be performed with subjects seated in an upright position with hips flexed to ~85° and knees flexed to ~90°. The axis of rotation of the dynamometer will be aligned with the axis of the knee joint and the lever arm secured against the anterior aspect of the leg, proximal to the lateral malleolus. Testing of the

plantar flexor muscle group will be performed with the hips flexed at 90- 100°, the knee flexed at ~10° and the ankle at ~0° plantar flexion. The anatomical axis of the ankle will be aligned with the axis of the dynamometer, while the foot was secured to the footplate with straps placed at the forefoot and ankle. Proximal stabilization was achieved with straps across the chest, hips and thigh (Figure3-3).

3.1.4.2 Voluntary contractile measurements

Prior to testing, subjects perform three warm-up contractions to get familiarized with the testing procedures. This was followed by three maximal voluntary isometric contractions (~5 second each with 1 minute rest intervals) while being given verbal encouragement. Peak torque will be defined as the highest value obtained during the 3 maximal isometric contractions. In the event that the peak torque values differed by more than 10%, additional contractions will be performed.

In addition to peak torque we also will determine the average rate of torque development (ARTD) and the $torque_{200}$, as indices of explosive muscle strength. The ARTD will be defined as the average increase in torque generated in unit time, and will be calculated in the time interval corresponding to 20% to 80% of peak amplitude, starting from muscle perturbation. This time interval was selected to reduce the effect of errors in calculating peak amplitude. Hence ARTD was calculated through numerical differentiation as:

$$ARTD = \frac{1}{N}\sum_{i=1}^{N}\frac{\delta f_i}{\delta t}$$

Where, N is the total number of time slots for numerical differentiation, δf_i is the change in torque in the time slot i and δt is the unit time duration for a slot. Torque200 will be defined as the absolute torque reached at 200ms during a maximal voluntary contraction (Nm).

3.1.4.3 Electrically elicited contractile measurements

Peak twitch torque, time to peak twitch and twitch half-relaxation time will be determined by delivering a supra-maximal electrical stimulus (600μs pulse duration) to the muscles at rest. Supramaximal intensity will be determined by increasing the current voltage until twitch torque production plateaued. Time to peak twitch and twitch half-relaxation time will be calculated from the peak twitch contractions.

Voluntary activation deficits. are performed using self-adhesive electrodes covering the width of the muscles with sizes ranging from 3.8 6.35 cm to 7.6×12.7 cm. For the KE, electrodes will be placed across the width of the distal portion of the thigh (quadriceps muscles), just above the knee joint and across the proximal portion of the thigh, near the origin of the muscle group. For the PF, electrodes will be placed across the width of the proximal portion of the calf (triceps surae muscles) just below the knee joint line and across the distal portion of the soleus superior to the Achillies tendon (Figure3-3).

Voluntary activation deficits will be determined using the twitch interpolation method. A Grass S8800 stimulator with a Grass Model SIU8T stimulus isolation unit (Grass Instruments, West Warwick, RI) will be used to briefly deliver a single biphasic, and supra-maximal pulse was delivered at rest and during maximal voluntary isometric contraction. The stimulator and the dynamometer will be interfaced with a personal computer through a commercially available hardware system (MP150 system). The data will be sampled at 400Hz and analyzed with commercially available software (AcqKnowledge 3.7.1). The voluntary activation deficit will be calculated based on the ratio between the torques produced by the superimposition of a supramaximal twitch on a peak isometric contraction (a) and the torque produced by the same stimulus in the potentiated, resting muscle (b).

Voluntary activation deficit (%) = (a/b)*100

3.1.5 Measures of Ambulatory Function

Lower extremity motor scores (LEMS). The voluntary muscle strength of 5 key muscles (hip flexors, knee extensors, ankle dorsiflexors, toe extensors, ankle plantar flexors) of both lower extremities is tested in accordance with the standard neurologic assessment developed by ASIA. Each muscle will be given a value between 0 and 5 according to the strength of voluntary muscle contraction. Maximum and minimum LEMS are 50 and 0, respectively.

Walking index for spinal cord injury (WISCI II). Physical limitation for walking secondary to impairment is defined at the person level and indicates the ability of a person to walk after spinal cord injury. The development of this assessment index required a rank ordering along a dimension of impairment, from the level of most severe impairment (0) to least severe impairment (20) based on the use of devices, braces and physical assistance of one or more persons. The order of the levels suggests each successive level is a less impaired level than the former. The ranking of severity is based on the severity of the impairment and not on functional independence in the environment.

Level description.

0. Unable to stand and/or participate in assisted walking.
1. Ambulates in parallel bars, with braces and physical assistance of two persons, less than 10meters
2. Ambulates in parallel bars, with braces and physical assistance of two persons, 10 meters.
3. Ambulates in parallel bars, with braces and physical assistance of one person, 10 meters.
4. Ambulates in parallel bars, no braces and physical assistance of one person, 10 meters.
5. Ambulates in parallel bars, with braces and no physical assistance, 10 meters.
6. Ambulates with walker, with braces and physical assistance of one person, 10 meters.
7. Ambulates with two crutches, with braces and physical assistance of one person, 10

meters.

8. Ambulates with walker, no braces and physical assistance of one person, 10 meters.

9. Ambulates with walker, with braces and no physical assistance, 10 meters.

10. Ambulates with one cane/crutch, with braces and physical assistance of one person, 10 meters.

11. Ambulates with two crutches, no braces and physical assistance of one person, 10 meters.

12. Ambulates with two crutches, with braces and no physical assistance, 10 meters.

13. Ambulates with walker, no braces and no physical assistance, 10 meters.

14. Ambulates with one cane/crutch, no braces and physical assistance of one person, 10 meters.

15. Ambulates with one cane/crutch, with braces and no physical assistance, 10 meters.

16. Ambulates with two crutches, no braces and no physical assistance, 10 meters.

17. Ambulates with no devices, no braces and physical assistance of one person, 10 meters.

18. Ambulates with no devices, with braces and no physical assistance, 10 meters.

19. Ambulates with one cane/crutch, no braces and no physical assistance, 10 meters.

20. Ambulates with no devices, no braces and no physical assistance, 10 meters.

3.2 Experiments in Contusion Spinal Cord Injured Animals

3.2.1 Animals

The animal model will consist of young adult, female Sprague Dawley rats (16-20 weeks, weighing 250-290gms). The animals will be housed in an AALAC accredited animal facility in a temperature (22±1°C), humidity (50±10%) and light controlled room (12:12 hours light: dark cycle), and will be provided rodent chow and water ad libitum. The rats will be acclimatized for a week prior to the start of experiments. All procedures will be performed in accordance with the

US Government Principle for the Utilization and Care of Vertebrate Animals and will be approved by the Institutional Animal Care & Use Committee at the University of Florida.

3.2.2 Contusion Injury

Spinal cord contusion injuries will be produced using a NYU (New York University) impactor device (Figure3-4). A 10g weight will be dropped from a 2.5-cm height onto the T8 segment of the spinal cord exposed by laminectomy under sterile conditions. Procedures will be performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia. Subcutaneous lactated Ringer's solution (5 ml) and antibiotic spray will be administered after completion of the surgery. Animals will receive two doses of Ampicillin per day for 5 days, starting at the day of surgery. Animals will also be 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 will be kept under vigilant postoperative care, including daily examination for signs of distress, weight loss, dehydration, and bladder dysfunction. Manual expression of bladders will be performed 2 to 3 times daily, as required, and animals will be monitored for the possibility of urinary tract infection. Animals will be housed in pairs with the exception of the first few hours following surgery. At post-operative day 7, open field locomotion will be assessed using the Basso-Beattie-Bresnahan (BBB) locomotor scale and animals that do not fall within a preset range (1-4) will be excluded from the locomotor training study.

3.2.3 Locomotor Training

Animals that will receive locomotor treadmill training will be trained for five consecutive days (1 week of training), 2 trials/day, 20 minutes/trial, starting on post-operative day eight. Training will consist of a quadrapedal treadmill stepping (Figure3-5). On the first day of training, animals will be given five minutes to explore the treadmill and then encouraged to walk on the moving treadmill at a speed of 11 meters per minute, for a series of four, five-minute bouts. A

minimum of five minutes rest will be provided between bouts. Body weight support will be provided manually by the trainer. The level of body weight support will be adjusted to make sure that the rats can bear their weight and there will be no collapse of their hind limbs. Typically, the rats will start stepping when they have experienced some small load on their hind limbs. In addition, during the first week of training, when all rats have profound hind limb paralysis, assistance will be provided to place rat hind limbs appropriately for plantar stepping during training. On the second day of training, animals will complete two 10 minute bouts, twice a day. Starting on day three, animals will be trained continuously for 20 minutes with a minimum interval between trials. Bodyweight support through the trunk and the base of the tail will be provided as necessary and gradually removed as locomotor capability improved.

3.2.4 In-Vitro Assay of Muscle Composition and Regeneration

3.2.4.1 Immunohistochemical analysis

The muscles required for analysis will removed from one of the hind limbs of the animal. The muscles were subsequently rapidly frozen in isopentane pre-cooled in liquid nitrogen (storage at -80°C) for the following immunohistological measurements.

Fiber CSA measures: Cryostat sections (10 μm) in a transverse plane will be prepared from the central portion of each muscle taken from the both legs and mounted serially on gelatin-coated glass slides. Immunocytochemical reactions will be performed on each cryostat section with anti-laminin to outline the muscle fibers for cross-sectional area (CSA) quantification. The fiber CSAs will be analyzed using the SCION image program. The pixels setting used for conversion of pixels to micrometer is 1.50 pixels- 1 μm^2 for a 10 X objective. The average maximal CSA for the soleus, gastrocnemius, TA and EDL will be quantified.

MHC Measures: Immunocytochemical reactions for quantifying fiber type transformation will be performed on serial cryostat sections with anti-laminin and anti-MHC antibody at various

dilutions. Rabbit anti-laminin (Neomarker, Labvision, Fremont, CA) will be used to outline the muscle fibers for cross-sectional area quantification. Four anti-MHC antibodies (BA-D5, SC-71, BF-F3, and BF-35) will be selected on the basis of their reactivity toward adult MHC (figure3-6). Sections will be 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 will be mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4^0C to diminish fading. Stained cross sections will be photographed (10X magnification) by using a Leica fluorescence microscope with a digital camera. A region of the stained serial sections from each muscle will be randomly selected for MHC composition analysis. The proportions of each fiber type will be determined from a sample of 150-250 fiber across the entire section of each muscle.

Immunohistochemical measures for Pax-7 and embryonic myosin will be performed using similar methodologies.

3.2.4.2 Western blot analysis

Quantification and expression of MyoD, Myf5 and Myogenin will be measured using Western blot analysis. Muscles will be homogenized in a lysis buffer with Fast-Prep homogenizer machine at 13,000 RPM at 4^0C for five minutes. The supernatant will be preserved for protein assay. Protein will be denatured by heating samples to 95-100 ^{0}C for 5 minutes. Protein will be measured using BCA protein assay kit from Pierce. Electrophoresis will be performed by mixing 40-50 μg protein with 5X loading buffer and loading it to 4-15% SDS page gel from Bio-Rad. Protein will then be transferred from gel to nitrocellulose membrane. Blocking will be conducting using 5% non fat dry milk in TBS/T (Tris Buffer Saline, Tween-

20). Blot with be incubated with primary antibody overnight at 4^0C according to manufacturer's instruction. Blot will then be incubated with HRP-conjugate secondary antibody for 40 minutes to one hour at room temperature. Finally protein will be detected using Western Blotting Luminal Reagent from Santa Cruz.

Figure 3-1. Set-up for locomotor training.

Figure 3-2. Plyometric training set-up.

Figure 3-3. Experimental set-up on a Biodex System 3 Dynamometer.

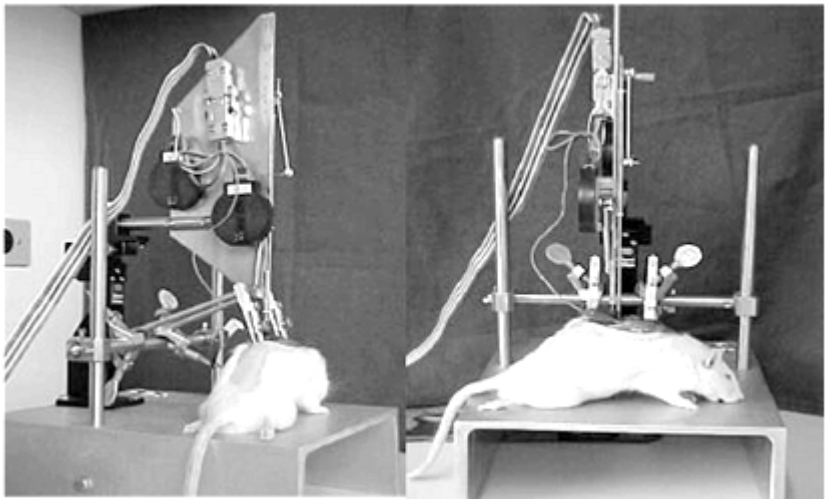

Figure 3-4. Contusion injury set-up modified from Meyer *et al.*2003.

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

Figure 3-5. anti-MHC antibodies.

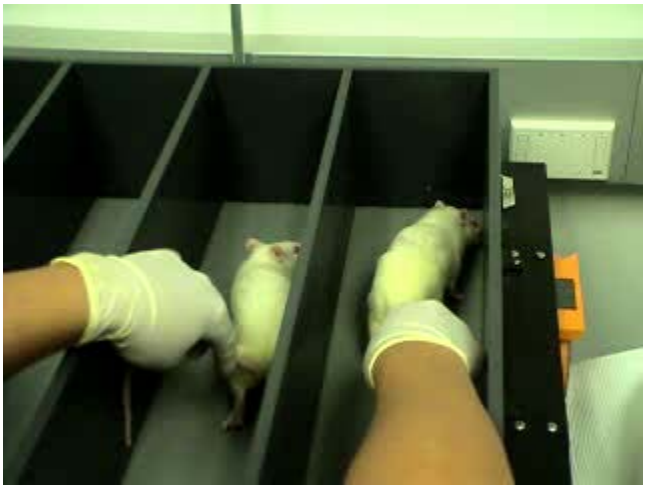

Figure 3-6. Locomotor training in the rat model.

CHAPTER 4
LOWER EXTREMITY SKELETAL MUSCLE FUNCTION IN PERSONS WITH INCOMPLETE SPINAL CORD INJURY

4.1 Introduction

Approximately 200,000 persons with spinal cord injury (SCI) live in the United States alone; with roughly 11,000 new injuries occurring each year.[1] In addition, the relative number of new injuries is rising and expected to have increased by approximately 20% by 2010, compared to the 1994 prevalence. The reported costs associated with the care and treatment of persons after SCI is estimated to range between $15,000- $125,000 annually, with an approximate lifetime total of $435,000-$1,590,000.[1] Interestingly, a significant proportion of these costs can be related to the loss of skeletal muscle mass and associated secondary health-related complications [52], (i.e. non-insulin dependent diabetes mellitus, cardio-vascular disease, osteoporosis). A decrease in skeletal muscle function has been described as one of the most significant problems impacting the health care and quality of life of persons after SCI.[52, 39] Consequently, the potential to decrease costs and improve quality of life by maintaining or partially restoring skeletal muscle size and function seems high.

Due in part to advancements in the quality of emergency care, the relative number of injuries classified as *incomplete* has risen dramatically over the past 20 years.[2] In fact, the majority of new injuries occurring annually are now classified as incomplete.[1] Despite the rise in the proportion of persons with incomplete-SCI; the preponderance of scientific literature describing the effects of SCI on skeletal muscle involves persons with complete injuries.[49,138-140] To date very little data exits describing muscle function in persons with chronic incomplete-SCI. Interestingly, the innate plasticity associated with incomplete- SCI furnishes these persons with the potential to progress functionally to a greater extent than the complete SCI population.[141,142] In addition, novel intervention therapies have shown promise in promoting spinal plasticity and

motor function after spinal cord injury. However, improvements in functional capacity in persons with I-SCI with rehabilitation vary greatly and the incidence of disability still remains high.[143,144]

In order to provide a foundation for the development of rehabilitation strategies targeting neuromuscular deficits in persons with incomplete-SCI, a need exists to characterize and objectively quantify existing impairments. Therefore, the purpose of this study was to quantify lower extremity muscle function in persons with chronic incomplete-SCI compared to age-, gender-, height- and body weight matched healthy controls. Specifically, we measured isometric peak torque and performed measures of explosive or instantaneous muscle strength in the knee extensor and ankle plantar flexor muscle groups and quantified voluntary activations deficits using a combination of voluntary contractile measurements and superimposed electrical stimulation.

4.2 Methods

4.2.1 Subjects

Ten persons with chronic, upper motor neuron lesions and motor incomplete-SCI participated. Characteristics of the persons with incomplete-SCI are provided in Table 4-1. Average age, height and body mass ± standard deviation (SD) at the time of the study enrollment were 45.4 ± 14.8yrs, 155.9 ± 9.4cm, and 79.9 ± 12.2kg. Eight of the subjects were classified ASIA D and two as ASIA C. Four subjects were able to ambulate over ground, while six used a wheelchair as their primary mode of locomotion. The incomplete-SCI subjects were matched on the basis of age, gender, height and weight with ten recreationally active controls (45.1 ± 14.9yrs, 159.1 ± 9.0cm, and 78.0± 11.7kg). Prior to participating in the study, written informed consent was obtained from all subjects, as approved by the Institutional Review Board at the University of Florida, Gainesville.

4.2.2 Experimental Set-Up

Voluntary and electrically elicited contractile measurements were performed in the self-reported more-involved and less-involved limbs for the knee extensor and plantar flexor muscle groups, using a Biodex System 3 Dynamometer[†]. Knee extensor testing was performed with subjects seated in an upright position with hips flexed to ~85° and knees flexed to ~90°. The axis of rotation of the dynamometer was aligned with the axis of the knee joint and the lever arm secured against the anterior aspect of the leg, proximal to the lateral malleolus. Testing of the plantar flexor muscle group was performed with the hips flexed at 90- 100°, the knee flexed at ~10° and the ankle at ~0° plantar flexion, as previously described.[13, 14] The anatomical axis of the ankle was aligned with the axis of the dynamometer, while the foot was secured to the footplate with straps placed at the forefoot and ankle. Proximal stabilization was achieved with straps across the chest, hips and thigh. Electrical stimulation was performed using a Grass S8800 [1]stimulator with a Grass Model SIU8T stimulus isolation unit[*]. Electrically induced contractions were delivered through two 3.0" by 5.0" self-adhesive neuromuscular stimulation electrodes placed over the proximal and distal portions of the muscle group being tested. The stimulator and the dynamometer were interfaced with a personal computer through a commercially available hardware system (MP150 system)[£]. The data were sampled at 400Hz and analyzed with commercially available software (AcqKnowledge 3.7.1).

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1 Grass Instruments, West Warwick, Rhode Island, USA.

†Biodex Medical Systems, Inc., 20 Ramsay Road, Shirley, New York 11967

£ BIOPAC systems Inc., Goleta, CA

4.2.3 Voluntary Contractile Measurements

Prior to testing, subjects performed three warm-up contractions to get familiarized with the testing procedures. Subjects then performed three maximal voluntary isometric contractions (~5 second each with 1 minute rest intervals) while being given verbal encouragement. Peak torque was defined as the highest value obtained during the 3 maximal isometric contractions. In the event that the peak torque values differed by more than 10%, additional contractions were performed.

In addition to peak torque we also determined the average rate of torque development (ARTD) and the $torque_{200}$, as indices of explosive muscle strength. The ARTD was defined as the average increase in torque generated in unit time, and was calculated in the time interval corresponding to 20% to 80% of peak amplitude, starting from muscle perturbation. This time interval was selected to reduce the effect of errors in calculating peak amplitude. Hence ARTD was calculated through numerical differentiation as

$$ARTD = \frac{1}{N}\sum_{i=1}^{N}\frac{\delta f_i}{\delta t}$$

where N is the total number of time slots for numerical differentiation, δf_i is the change in torque in the time slot i and δt is the unit time duration for a slot. Torque200 was defined as the absolute torque reached at 200ms during a maximal voluntary contraction (Nm).

4.2.4 Electrically Elicited Contractile Measurements

Peak twitch torque, time to peak twitch and twitch half-relaxation time were determined by delivering a supra-maximal electrical stimulus (600μs pulse duration) to the muscles at rest. Supra-maximal intensity was determined by increasing the current voltage until twitch torque production plateaued. Time to peak twitch and twitch half-relaxation time were calculated from the peak twitch contractions.

4.2.5Voluntary Activation Deficits

Voluntary activation deficits were determined using the twitch interpolation method.[15] Briefly, a single biphasic, and supra-maximal pulse was delivered at rest and during maximal voluntary isometric contraction. The voluntary activation deficit was calculated based on the ratio between the torques produced by the superimposition of a supra-maximal twitch on a peak isometric contraction (a) and the torque produced by the same stimulus in the potentiated, resting muscle (b).

Voluntary activation deficit (%) = (a/b)*100.

4.2.6 Statistical Analyses

Independent sample T-tests were used to determine if differences existed between the groups. Comparisons were made between the self-reported dominant side of the controls and both the self-reported more involved side and less involved side of the incomplete-SCI group. For all analyses, significance was established when $P< 0.05$. Data are presented as means± standard error of mean. All statistical analyses were performed using SPSS for Windows, Version 11.0.1.

4.3 Results

4.3.1 Voluntary Contractile Measurements

Individuals after incomplete-SCI demonstrated significant deficits in their ability to generate peak isometric torque relative to non-injured controls in both the knee extensor and plantar flexor muscle groups ($p<0.05$). A representative ankle plantar flexor torque trace acquired during a peak isometric contraction of both incomplete-SCI and control subject is provided in Figure 4-1. The peak torque deficit measured in both muscle groups was of similar magnitude (Figure. 4-2). Specifically, persons after incomplete-SCI were able to produce 36%

and 24% of the knee extensor torque and 38% and 26% of the plantar flexor torque generated by non-injured controls in the less-involved and more-involved limbs, respectively ($p<0.01$). Significant bilateral asymmetries were noted in peak torque production between the self-reported more-involved versus the less-involved limb in both the knee extensor (57±18 vs. 85±20 Nm) and plantar flexor muscle groups (26±6 vs. 39±7 Nm; $p<0.01$).

Both indices of explosive muscle strength, ARTD and $torque_{200}$, were significantly lower in persons with incomplete-SCI relative to controls in both muscle groups tested ($P<0.01$; Figure4-3, 4-4). Bilateral asymmetries in $torque_{200}$ and ARTD were specific to the ankle plantar flexor muscles. Of interest to note is that both indices of explosive muscle strength, showed more pronounced deficits in the ankle plantar flexor muscles compared to the knee extensor muscles. In particular large deficits were noted in the $torque_{200}$ of the ankle plantar flexor muscles with an 11.7 fold difference between the $torque_{200}$ measured in the self-reported more involved limb and a 5 fold difference in the less-involved limb compared to control muscles (Figure4-1&4-3). The $torque_{200}$ was 4.2±1.6 Nm in the plantar flexor muscles of the more-involved limb, 9.2±1.6 Nm in the less-involved limb and 47.2±9.2 Nm in the non-injured controls, respectively. In contrast, a 5.5 fold difference and 3.7 fold difference was noted in the $torque_{200}$ measured in the knee extensor muscles of the self-reported more involved (27.0±13.2 Nm) and less involved limb (39.9±12.3 Nm) of incomplete-SCI persons compared to non-injured controls (148.6±18.3 Nm). $Torque_{200}$ and ARTD data are summarized in Figures 4-3 & 4-4.

4.3.2 Electrically Elicited Contractile Measurements

No significant differences were found either within or between subject groups for measures of peak twitch torque, time to peak twitch or half-relaxation times in either the knee extensor or plantar flexor muscle groups (Table 4-2).

4.3.3Voluntary Activation Deficits

A significant injury related effect on the ability to voluntarily activate the plantar flexor and knee extensor muscle groups was noted. Activation deficits in the knee extensors were 42±8 % and 66±9% in the less involved and more involved side, respectively, compared to only a 5±2% deficit in non-injured controls. The incomplete-SCI group also demonstrated a 53±6 % voluntary activation deficit in the less involved side and a 64±8% deficit in the more involved side for the plantar flexor muscle group, compared to a 5±2% deficit in non-injured controls (Figure 4-5). Significant bilateral asymmetries existed for both muscle groups for voluntary activation deficits ($p<0.05$, Figure 4-5). A representative torque trace acquired during a peak isometric voluntary contraction with interpolated twitch is provided in Figure 4-6.

4.4 Discussion

The development of novel intervention therapies to promote the recovery of skeletal muscle function after incomplete-SCI is one of the exciting paths of current rehabilitation research.[87,145-149] However, the translation of these experimental therapies to the SCI population is enormously challenging given the extreme heterogeneity in presentation and response to treatment of this population. As such, a comprehensive examination of skeletal muscle function in this patient population might aid in the development of targeted therapies aimed at the recovery of muscle function after incomplete-SCI. Accordingly, the present study demonstrates that after chronic upper motor lesions and incomplete-SCI, both knee extensor and plantar flexor skeletal muscles 1) generate ~70% less peak torque, 2) demonstrate significant bilateral

asymmetry in peak torque, which matches the hierarchy for self-reported functional deficits, 3) experience voluntary activation deficits ranging between 42% and 66%, and 4) demonstrate large deficits in the rate of torque development and instantaneous muscle strength. While in this study both muscle groups demonstrated significant impairments in ARTD and $torque_{200}$, more pronounced deficits were noted in the ankle plantar flexor muscles and bilateral asymmetries in ARTD and $torque_{200}$ were specific to the ankle plantar flexor muscles. Given the role of the ankle plantar flexor muscles in propulsion during gait we put forward that the latter impairments should be targeted in rehabilitative interventions aiming to restore or promote locomotion in this population.

The deficits noted between persons after incomplete SCI and controls in their ability to generate peak torque in the plantar flexor and knee extensor muscle groups may appear somewhat intuitive. In addition, the bilateral asymmetries observed may be considered obvious by many after this type of injury. However, no quantitative measurements of muscle function have previously been reported in this population. Moreover, we contend that the methodologies described here are more suitable than traditional evaluative tests in assessing impairments of muscle function in persons with incomplete-SCI. Muscle strength assessments in persons with incomplete-SCI are typically performed using manual muscle tests during ASIA evaluations. The ASIA is used routinely to describe the level of injury and impairment and imply severity of injury.[150,151] However; this evaluative tool may not be adequate to direct targeted rehabilitation interventions in persons with incomplete-SCI. Manual muscle tests are subject to a ceiling effect, lack sensitivity to change and have a relatively poor inter-rater reliability, especially at scores greater than 3.[54,143]

A myriad of physiological changes occur in persons after spinal cord injury. Many of these changes are due to the direct effects of the injury (i.e. neural circuitry disruption) while others are secondary in nature and attributable to a resultant decrease in neuromuscular activity. An inability to voluntarily activate skeletal muscles may be a product of both primary and secondary mechanisms. Twitch interpolation is a commonly used method to estimate the extent to which a person can voluntarily activate a given muscle or muscle group.[152-155] Our findings of small activation deficits (~5%) in the quadriceps and ankle plantar flexor muscles of non-injured controls are consistent with those from other laboratories.[155] The activation deficits measured in persons with incomplete-SCI (42-66%) are larger in magnitude compared to those measured in patients early after surgery or long-term immobilization.[156,157] Accordingly, persons with incomplete-SCI may benefit from rehabilitation strategies that target voluntary activation deficits to maximize skeletal muscle function, i.e. functional electrical stimulation or bio-feedback.[158] While these interventions may not directly impact the primary injury, they may be able to ameliorate the loss of muscle function secondary to disuse or lack of neuromuscular activity.

Perhaps the most functionally relevant characteristics of muscle torque production for persons with incomplete-SCI are the indices of explosive strength. ARTD is reflective of the average rate of contractile torque development during maximum voluntary contraction while $torque_{200}$ is the absolute torque generated within the initial 200ms of contraction and is indicative of the magnitude of instantaneous torque. Both ARTD and $torque_{200}$ were significantly reduced in the ankle plantar flexor and quadriceps muscle groups of persons with incomplete-SCI. However the deficit in instantaneous strength was more pronounced in the ankle plantar flexor muscles. It is our contention that the initial rate of torque development and the instantaneous strength may be most critical for performance of functional tasks (*i.e.* walking). For example,

steady state walking is characterized by repetitive, reciprocal contractions of the plantar flexor muscles (*i.e. propulsion at push off*) that must be accomplished in finite periods of time. A speed commonly deemed necessary for persons to safely ambulate in the community is 1.2 m/s.[145] At this speed, the time it takes to complete one gait cycle (i.e. right heel strike to right heel strike) is ~1.0 seconds. Given that the plantar flexor muscles are reported to be active for ~40% of the gait cycle and approximately ½ of that time is spent generating concentric torque, roughly 200ms is available for torque generation by this muscle group.[159] Given this available time, plantar flexor muscles must generate torque of sufficient magnitude and at precise rates so as to propel the mass of the body forward, translating to movement or walking.[160] We speculate that the large deficits in instantaneous torque in the ankle plantar flexors observed in this study (11.7 and 5 fold difference in $torque_{200}$) may potentially limit locomotor function in persons with incomplete-SCI. Thus, rehabilitative strategies must be employed that result in improved rates of torque production and enhanced instantaneous torque to meet the imposed demands of walking at community ambulating speeds.[161] Although we chose to examine torque generation at 200ms based on our calculations of muscular demands at a functionally minimal gait speed (1.0 m/s), consideration should also be given to the fact that as functional improvements are realized, the contractile demands (i.e. magnitude and rate of force production) will continue to increase and the available time to generate torque will decrease.

An interesting finding in the present study was the lack of difference in the electrically elicited contractile properties between persons after incomplete-SCI and non-injured controls. Previous studies have used these properties as a means to explain molecular and histochemical changes that occur in skeletal muscle.[52] Studies using both animal and human models have provided evidence for faster contractile properties following SCI.[50,52,162,163] However, we

observed no differences in rate of rise or relaxation of electrically elicited contractions in muscles after incomplete-SCI relative to non-injured controls. This is somewhat surprising in that both the knee extensor and plantar flexor muscle groups have been characterized by faster contractile speeds following SCI [50,162]. These findings have been used to support the idea of a fiber type transformation following SCI (slow→fast). However, whether a fiber type transition occurs after incomplete-SCI and the timeline for any potential shift are yet unclear. Thus, further research and tissue sampling is warranted before we can make any suggestions towards the muscle fiber type transformation based on contractile properties in this population.

In conclusion, this study characterizes the impairments in lower extremity skeletal muscle function in persons after incomplete spinal cord injury relative to non-injured controls. The examination of knee extensor and plantar flexor muscle groups in this study is clinically meaningful given the anti-gravity responsibilities of each of these muscle groups and their purported roles in standing and locomotor function.[164,165] Reduced peak torque production, ARTD and $torque_{200}$, as well as increased voluntary activation deficits were found to be characteristic of affected muscles below the level of incomplete spinal cord injury. In addition, a hierarchy of these impairments existed between limbs with significant bilateral asymmetries in the plantar flexor muscle group for all variables tested. This characteristic asymmetry suggests that recovery and response to rehabilitation may be specific to each side, with rate limiting factors to functional performance potentially being limb rather than subject specific. We speculate that the large deficit in the rate of torque development and instantaneous torque in the ankle plantar flexors of persons with incomplete-SCI limits locomotor function.

Table 4-1. Characteristics of incomplete SCI subjects

	Level of injury	ASIA Classification	Duration of injury (mos)	LEMS	WISCI-II	Mobility Status
S1	C6	D	20	35	19	Ambulator
S2	T4	D	7	44	19	Ambulator
S3	C4	D	16	45	13	Wheelchair
S4	C6	C	14	15	8	Wheelchair
S5	C6	D	37	40	16	Wheelchair
S6	C4	D	18	48	20	Ambulator
S7	C8	D	28	37	16	Wheelchair
S8	C4	C	22	26	9	Wheelchair
S9	C5	D	16	34	13	Wheelchair
S10	C6	D	39	38	19	Ambulator

Table 4-2. Electrically elicited contractile measurements

		Incomplete-SCI	
	Controls	**more-involved**	**less-involved**
Knee Extensors			
Peak twitch force (Nm)	29.1 ± 2.3	27.3 ± 2.8	31.6 ± 3.9
Time to peak twitch (ms)	123.3 ± 5.8	135.6 ± 5.3	129.3 ± 5.8
Twitch-half relaxation time (ms)	73.8 ± 5.7	107.1 ± 20.1	95.1 ± 11.0
Plantar Flexors			
Peak twitch force (Nm)	13.8 ± 1.5	14.8 ± 0.8	14.5 ± 0.8
Time to peak twitch (ms)	143.8 ± 7.2	143.9 ± 7.3	144.3 ± 5.2
Twitch-half relaxation time (ms)	116.2 ± 7.2	125.7 ± 9.2	127.3 ± 11.2

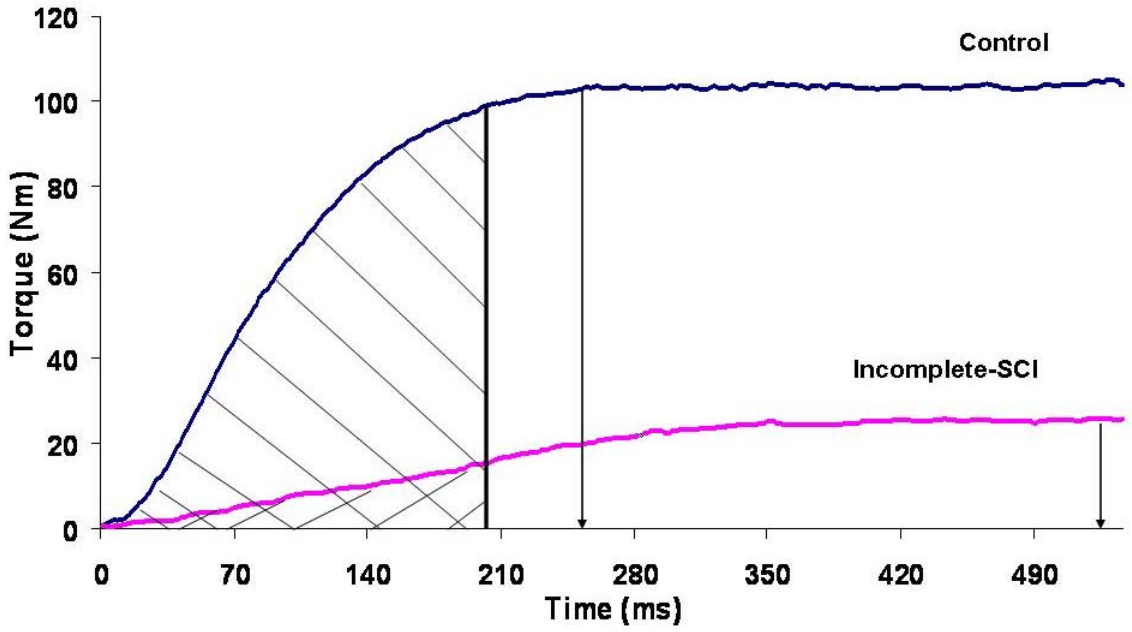


Figure 4-1. Representative torque-time curve. Drop down arrows indicate time points at which peak torque is reached in a representative incomplete-SCI and control subject. Shaded areas indicate $torque_{200}$ in both subjects.

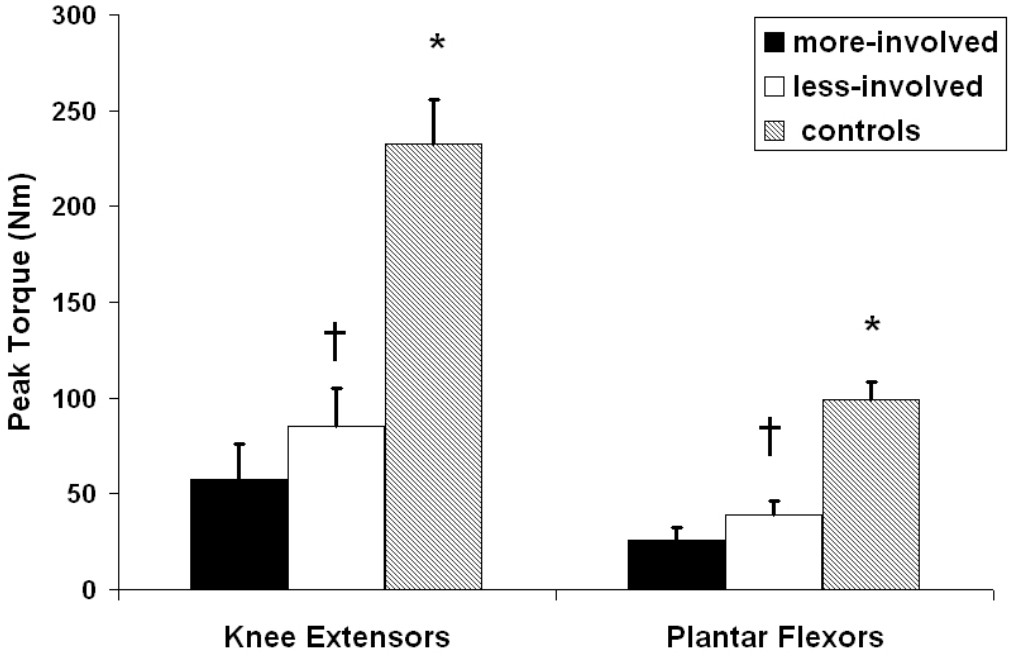
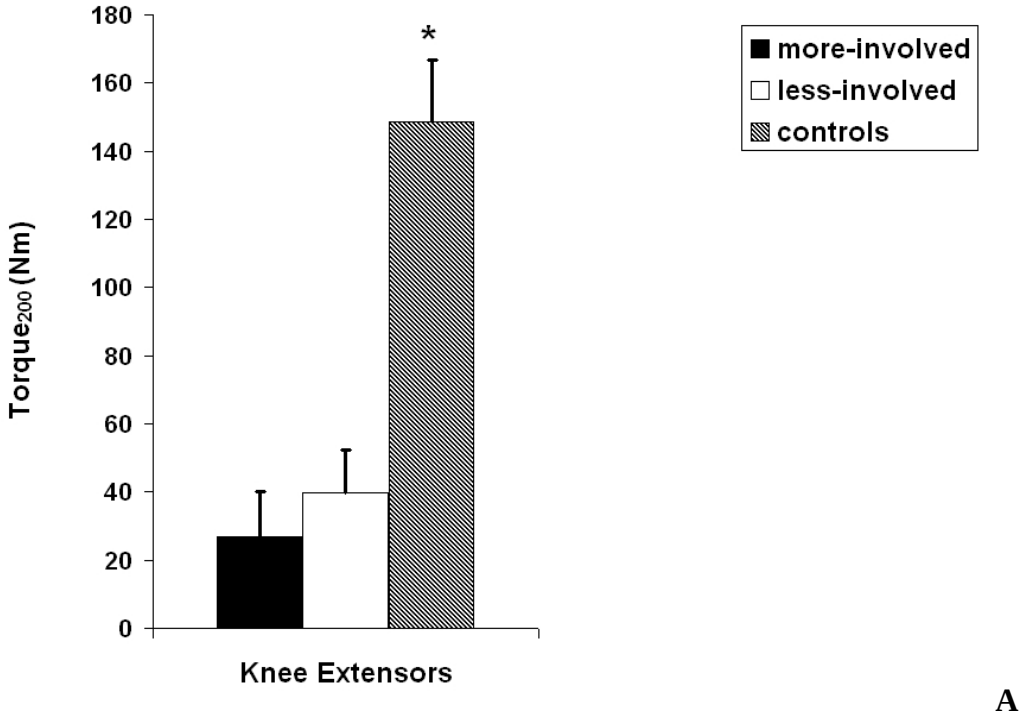
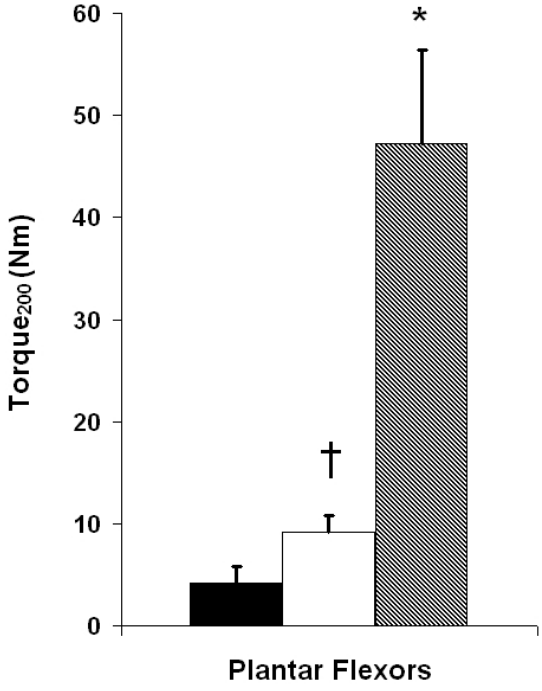


Figure 4-2. Peak torque (Nm) for the knee extensor and plantar flexor muscle groups, comparing the dominant side of the control with the more involved (more-involved) and less involved limb (less-involved) of the incomplete-SCI group. * Significant difference between control group and incomplete-SCI group. † Significant difference between the less-involved versus the more-involved ($p<0.05$).



A



B

Figure 4-3. Torque200 (Nm) (A) knee extensor and (B) plantar flexor muscle groups, comparing the dominant side of the control with the more involved (more-involved) and less involved limb (less-involved) of the incomplete-SCI group. * Significant difference between control group and incomplete-SCI group. . † Significant difference between the less-involved versus the more-involved ($p<0.05$).

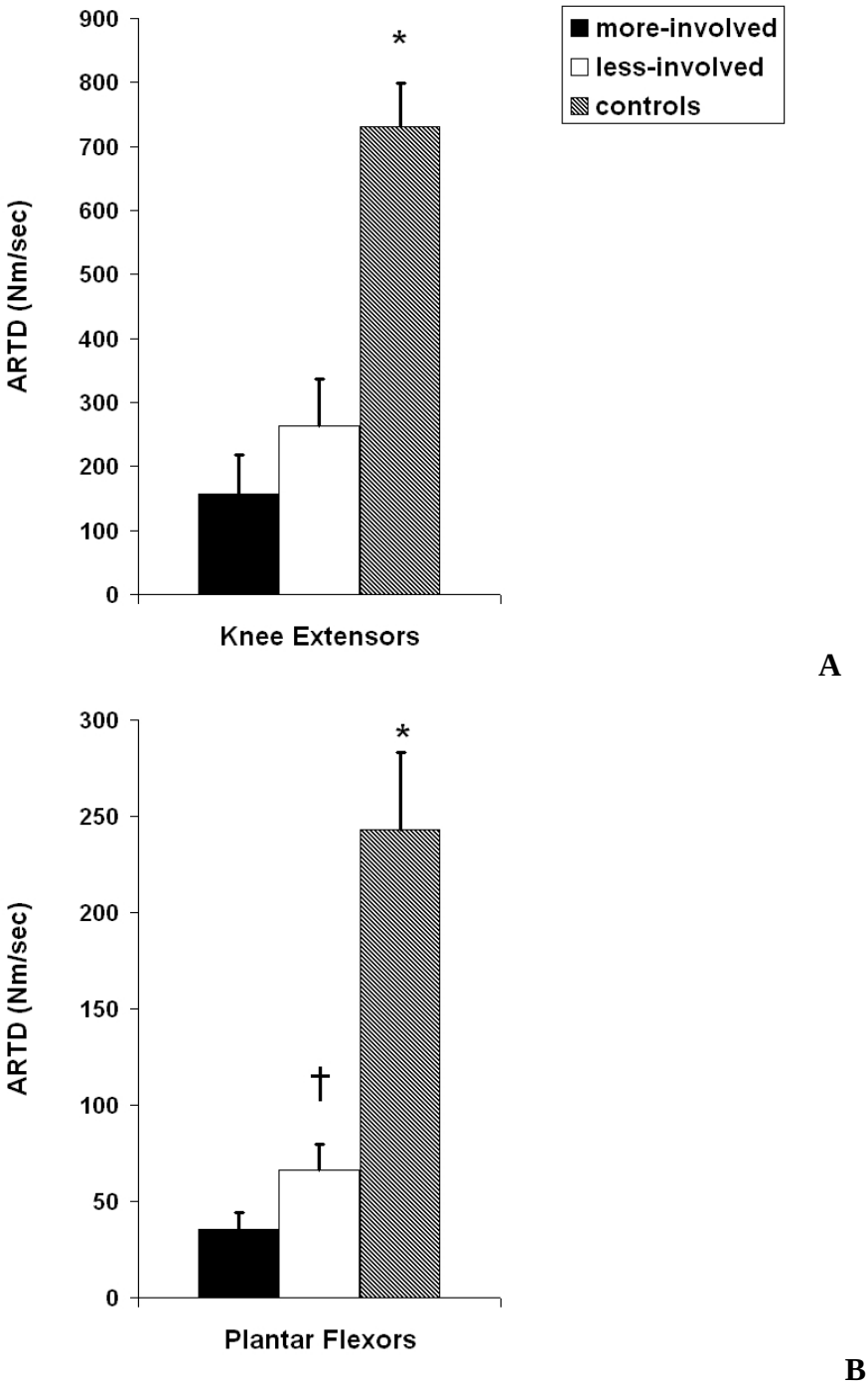


Figure 4-4. Average rate of torque development (ARTD)(Nm/sec) for the (A) knee extensor and (B) plantar flexor muscle groups, comparing the dominant side of the control with the more involved (more-involved) and less involved limb (less-involved) of the incomplete-SCI group. * Significant difference between control group and incomplete-SCI group. † Significant difference between the less-involved versus the more-involved ($p<0.05$).

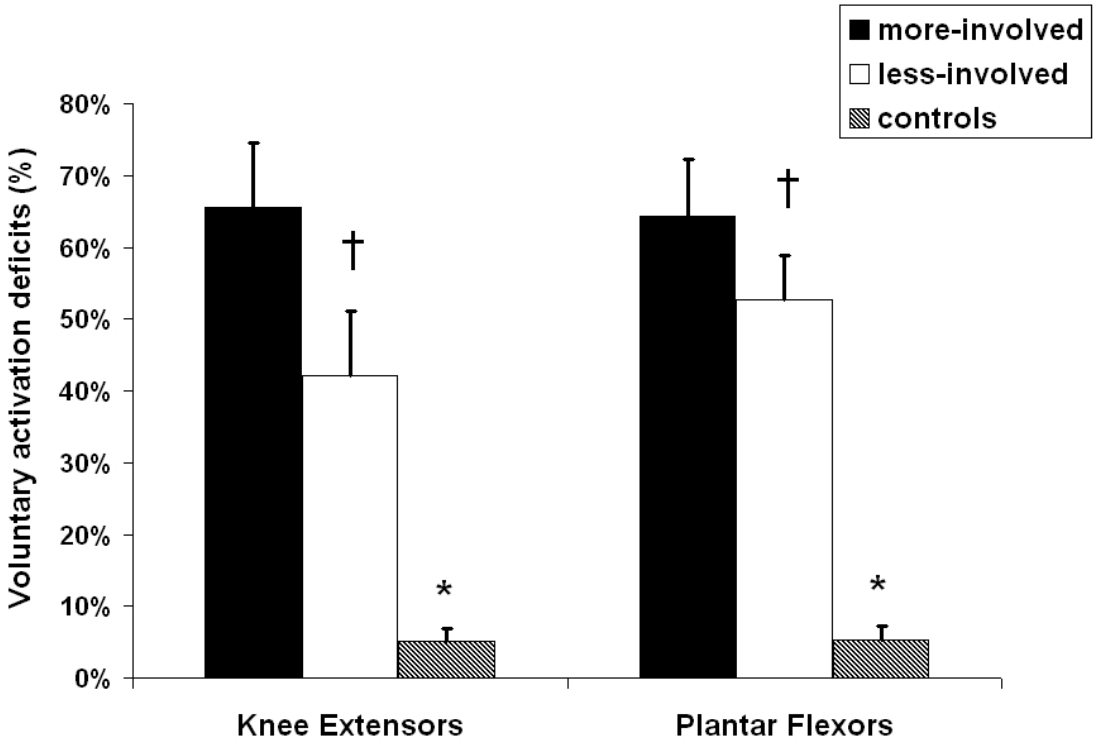


Figure 4-5. Voluntary Activation Deficits (%) for the knee extensor and plantar flexor muscle groups, comparing the dominant side of the control with the more involved (more-involved) and less involved limb (less-involved) of the incomplete-SCI group. * Significant difference between control group and incomplete-SCI group. † Significant difference between the less-involved versus the more-involved ($p<0.05$).

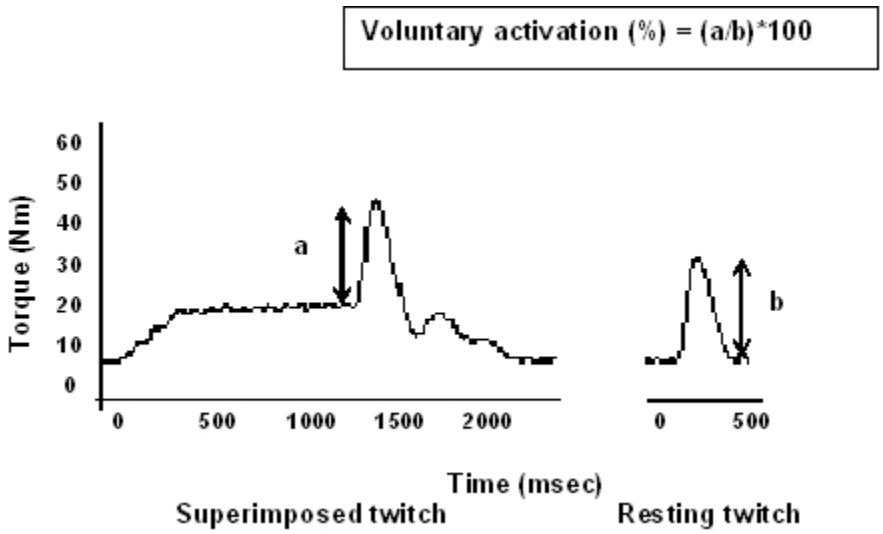


Figure 4-6. Torque trace acquired during MVIC with interpolated twitch to quantify muscle activation deficit. A single supramaximal intensity electrical stimulus was superimposed on a maximal voluntary isometric contraction (a), as well as on a resting, potentiated plantar flexor muscle (b).

CHAPTER 5
LOCOMOTOR TRAINING AND MUSCLE FUNCTION AFTER INCOMPLETE SPINAL CORD INJURY: A CASE SERIES

5.1 Introduction

Traumatic spinal cord injury (SCI) is one of the most disabling health problems facing adults today. Despite advances in treatment interventions individuals with SCI often lose the ability to walk and are at risk to develop secondary health complications. Muscle atrophy and reduced ability to generate force play essential roles in the development of disability after SCI. Individuals with chronic complete spinal cord injury show 42-68% atrophy in the calf and thigh muscles one year after injury, while subjects with incomplete-SCI demonstrate a 25-30% reduction in average lower extremity muscle cross-sectional area (CSA).[46] Few studies have performed a quantitative analysis of skeletal muscle strength after incomplete-SCI.[37] However, we recently demonstrated in persons with chronic upper motor lesions and incomplete-SCI, that both knee extensor and plantar flexor skeletal muscles generate ~70% less peak torque, with even larger reductions in measures of instantaneous or explosive peak torque.[53]

Repetitive locomotor training with body weight support has emerged as a potential promising therapeutic intervention to promote motor recovery and ambulation following incomplete-SCI. [166-168] Locomotor training has been suggested to have a positive impact on walking ability, [168,169] functional independence and subjective well being.[170] Giangregorio *et al.*[109, 171] and Stewart *et al.*[39] have also shown that locomotor training involves sufficient mechanical loading to induce muscle plasticity, increasing muscle size and altering the muscle phenotype both after acute and chronic incomplete-SCI. Interestingly, studies involving animal models of incomplete-SCI have also shown that LMT has the potential to augment the force generating capabilities of affected lower hind limb muscles. [111,114] To our knowledge, no study has systematically investigated the effect of locomotor training on lower extremity muscle force

production and instantaneous power in persons with incomplete-SCI. Mostly; studies rely on manual muscle tests and ASIA motor scores to assess voluntary strength in persons with incomplete-SCI. However, ASIA scores have been criticized to lack sensitivity and to have a limited ability as indicators of neuromuscular recovery in chronic SCI.[54,78,143,168,169]

Therefore, the purpose of this study was to determine the effect of nine weeks of locomotor training on lower extremity muscle function in persons with chronic incomplete-SCI using isokinetic dynamometry. Specifically, we measured peak isometric torque, torque developed within the initial 200 ms of contraction ($Torque_{200}$) and the average rate of torque development (ARTD) in the knee extensor and ankle plantar flexor muscle groups. In addition, we quantified voluntary activations deficits using superimposed electrical stimulation. The knee extensor and plantar flexors muscles groups were selected for study because of their purported role during human locomotion.

5.2 Methods

5.2.1 Subjects

Five persons (one woman, four men) with chronic motor incomplete-SCI underwent nine weeks (45 sessions, 5-times /week) of locomotor training. A summary of the subject's demographics is provided in Table 5-1. Criteria for inclusion included: 1) age 18-70; 2) history of SCI as defined by the American Spinal Injury Association (ASIA) Impairment Scale categories C or D; 3) first time traumatic SCI at cervical or thoracic levels (C4-T12) resulting in upper motor neuron lesions in the lower extremity; 4) medically stable and asymptomatic for bladder infection, decubitis, cardiopulmonary disease or other significant medical complications prohibiting testing and/or training; and 5) if using anti-spasticity medication, agreement to maintain current levels throughout the study. Exclusion Criteria were as follows: 1) participation in a rehabilitation or research protocol that could influence the outcome of this study. Prior to

participating in the study, written informed consent was obtained from all subjects, as approved by the Institutional Review Board at the University of Florida, Gainesville.

5.2.2 Locomotor Training Protocol

The locomotor training intervention consisted of 45 training sessions (5x/ week) spread over nine weeks, with each session consisting of 30 minutes of step training on the treadmill with body weight support (BWS) immediately followed by 20 minutes of level overground walking and community ambulation training. Including pre-training stretching, donning/doffing the harness, and additional time spent on the treadmill for stand training and standing rest breaks, the total session duration was approximately 75 to 90 minutes per day. Each subject completed all of the training sessions. With the aid of the body weight support, treadmill speed and manual trainers, the treadmill training environment facilitated delivery of locomotor specific practice.[79,168] Trunk, lower limb, and upper limb kinematics were consistently assisted and/ or monitored by trainers to assure appropriateness in relation to normal walking. Speed of treadmill stepping was kept in a range consistent with normal walking (2.2-2.8 miles/hr). Progression of training was achieved by decreasing BWS, altering speed, increasing trunk control, decreasing manual assistance for limb control and increasing the time spent walking on the treadmill per bout. A more detailed description of the training principles, parameters and progression has been provided by Behrman & Harkema *et al.* 2000.[79] Overground training consisted of an immediate assessment of the participant's ability to stand and/or walk independently overground and an evaluation of the deficits limiting achievement of this goal. These deficits became the focus for goal setting in the next day's training session. Additionally, overground training addressed translation of the skills from the treadmill to the home and community identifying practical ways for the participants to incorporate new skills into everyday activities.

5.2.3 Experimental Protocol

5.2.3.1 Strength assessment

Voluntary contractile measurements were determined in the self-reported more-involved and less-involved limbs for the knee extensor and plantar flexor muscle groups before and after locomotor training, using a Biodex System 3 Dynamometer. Testing was performed with subjects seated with hips flexed to ~85°, as previously described. [53] For knee extensor testing, the knees were flexed to ~90° and the axis of rotation of the dynamometer was aligned with the axis of the knee joint and the lever arm secured against the anterior aspect of the leg, proximal to the lateral malleolus. Plantar flexor testing was performed with the knee flexed at ~30° and the ankle at ~0° plantar flexion. The anatomical axis of the ankle was aligned with the axis of the dynamometer, while the foot was secured to the footplate with straps placed at the forefoot and ankle. Proximal stabilization for all testing was achieved with straps across the chest, hips and thigh.

5.2.3.2 Voluntary contractile measurements

Prior to testing, subjects performed three warm-up contractions to become familiar with the testing procedures. Subjects then performed three maximal voluntary isometric contractions (~5 seconds each with 1 minute rest intervals) while being given verbal encouragement. Peak torque was defined as the highest value obtained during the 3 maximal contractions. In the event that the peak torque values differed by more than 5%, additional contractions were performed. In addition to peak torque we also determined the absolute torque generated during the initial 200ms of contraction ($Torque_{200}$) as well as the average rate of torque development (ARTD) during the contractile effort, as previously described. [53]

5.2.3.3 Voluntary activation deficits

Voluntary activation deficits were determined using the twitch interpolation method. [152] Briefly a single biphasic, supra-maximal electrical pulse was delivered at rest and during maximal voluntary isometric contraction. Voluntary activation deficit was calculated using the ratio between the torques produced by the superimposition of a supra-maximal twitch on a peak isometric contraction (a) and the torque produced by the same stimulus in the potentiated, resting muscle (b).

Voluntary activation deficit (%) = (a/b)*100.

Electrical stimulation was elicited using a Grass S8800 stimulator with a Grass Model SIU8T stimulus isolation unit. Electrically induced contractions were delivered through two 3.0" by 5.0" self-adhesive neuromuscular stimulation electrodes placed over the proximal and distal portions of the muscle group being tested. The stimulator and the dynamometer were interfaced with a personal computer through a commercially available hardware system (Biopac MP150 system) sampling at 400Hz and data were analyzed with commercially available software (AcqKnowledge 3.7.1).

5.2.4 Statistical Analyses

A longitudinal, prospective case series was used in which participants completed nine-weeks of locomotor training. Individual data have been summarized in tables and as plots.

5.3 Results

5.3.1 Voluntary Contractile Measurements

All individuals with chronic incomplete SCI demonstrated a significant improvement in their ability to generate peak isometric torque following locomotor training. The most robust increase in isometric peak torque production was observed in the ankle plantar flexor muscles (average increase 43.9±20.0%) of the self-reported more involved limb, followed by the knee

extensor muscles of both the more involved (21.1±12.3%) and less involved (19.8±6.3%) limb. Individual gains in peak torque ranged from 8% to 45% in knee extensor and 14% to 98% in the plantar flexor muscle groups. Note that four out of five subjects showed an increase in isometric peak torque in at least three of the tested muscle groups. Individual torque data prior to and after nine weeks of locomotor training are summarized in Table 5-2.

Both indices of explosive muscle torque generation, ARTD and $Torque_{200,}$ showed large improvements in the ankle plantar flexor and knee extensor muscles with locomotor training. In particular, large bilateral improvements in plantar flexor $Torque_{200}$ measures were realized, with average relative improvements of 587±247% and 219±126% in the more-involved and less-involved limbs, respectively. Individual increases in ankle plantar flexor $Torque_{200}$ ranged from 8% to 835%. A more variable response was noted in the knee extensors with some subjects showing an enhancement in the more involved limb (subjects 2, 3 and 5) and others in the less involved limb (subject 1 and 4). $Torque_{200}$ data for both the knee extensors and ankle plantar flexors are presented in Figures 5-1A-D. ARTD values showed a similar pattern with relatively larger improvements in the ankle plantar flexor muscles compared to the knee extensors (Table 5-2). The mean ARTD in the ankle plantar flexor muscles improved from 36.3 ± 16.5Nm/s to 46.9 ± 13.3 Nm/s in the more involved limb and from 68.2 ± 23.2 Nm/s to 102.8 ± 32.7 Nm/s in the less involved limb. The mean ARTD in the knee extensor muscles increased from 207.9±112.9 Nm/s to 252.1±115.7Nm/s and from 325.5±132.6Nm/s to 392.7±137.0Nm/s.

5.3.2 Voluntary Activation Deficits

All subjects showed voluntary activation deficits in both the knee extensor and ankle plantar flexors muscles prior to LMT. Interestingly, a significant training effect was noted in the ability to voluntarily activate the bilateral knee extensor muscle groups as well as the more-involved

plantar flexor muscles. Mean activation deficits in the knee extensors improved from 63±15% to 43±10% and from 41±16% to 31±16% in the more involved and less involved sides, respectively. Only one subject (subject 4), the subject with the highest pre-LMT knee extensors strength, did not show any improvement in knee extensor activation deficit after LMT. Similar to the knee extensors, activation deficits in the more involved plantar flexors improved from 61±10% to 41±11% after nine weeks of locomotor training. Individual data are summarized in Figure 5-2A & B.

5.4 Discussion

The results of this case series suggest that nine weeks of locomotor training in persons with chronic motor incomplete SCI results in positive alterations in lower extremity skeletal muscles that include an improved ability to generate both peak and instantaneous torque about the knee and ankle joints. Interestingly, increases in force production were more pronounced in the ankle plantar flexor muscles versus the knee extensor muscles, consistent with previous literature suggesting that the ankle plantar flexors are critical for propulsive force generation during locomotion and experience high loads.[159,172] Superimposed electrical stimulation further showed that improvements in muscle strength with locomotor training are accompanied with a decrease in voluntary activation deficit.

A myriad of physiological changes occur in persons as a results of traumatic spinal cord injury. Many of these changes are due to direct effects of the injury (i.e. neural circuitry disruptions), while others are linked to pharmacological side effects or due to the lack of neuromuscular activity and loading. Among the physiological changes is a dramatic loss in the ability to voluntarily produce muscle force, leading to impaired motor function and disability. We previously demonstrated that isometric peak torque generation in the knee extensor and

plantar flexor muscle groups is reduced by about 70% in person with chronic incomplete SCI (>1 year), compared to age- gender- and body weight- matched control subjects.[53] Individuals in the present study demonstrated similar reduced plantar flexor and knee extensor peak torque values prior to locomotor training. Forty-five sessions of locomotor training resulted in a robust increase in isometric peak torque production in the ankle plantar flexor muscles (average increase 43.9±20.0%) of the self-reported more involved limb and the knee extensor muscles of both the more involved (21.1±12.3%) and less involved (19.8±6.3%) limb. The ability to improve peripheral muscle strength in persons with incomplete-SCI seemingly adds to the positive attributes previously contributed to this experimental therapeutic intervention. In addition to peak torque generation, we suggest that the functionally more relevant characteristics of muscle torque production in person with incomplete SCI are represented by the indices of explosive or instantaneous strength, ARTD and $Torque_{200}$. ARTD represents the average rate of contractile torque development during maximum voluntary contraction, while $Torque_{200}$ measures the absolute torque generated within the initial 200ms of contraction. We previously showed that both ARTD and $Torque_{200}$ are significantly reduced in persons with incomplete SCI, with more pronounced deficits in the ankle plantar flexor muscles compared to the knee extensor muscles.[53] In particular large deficits were noted in the $Torque_{200}$ of the ankle plantar flexor muscles with an 11.7 fold difference between the $Torque_{200}$ measured in the self-reported more involved limb and a 5 fold difference in the less-involved limb compared to control muscles.

With nine weeks of locomotor training large improvements in both measures of instantaneous muscle strength were noted. In particular, large bilateral improvements in plantar flexor $Torque_{200}$ measures were realized, with average relative improvements of ~600% and 200% in the more-involved and less-involved limbs, respectively. Smaller and less consistent

relative gains were realized in the knee extensor muscle group. The large increase in the $Torque_{200}$ of both ankle plantar flexor muscle groups with locomotor training deserves special attention, given these muscles' importance during bipedal walking. At a speed commonly deemed necessary for persons to safely ambulate in the community (1.2 m/s),[173] a time window of only about 200ms is available to generate the necessary concentric torque in the plantar flexor muscle group to produce forward propulsion.[159] Data from our previous and current study combined indicate that the torque produced by the ankle plantar flexors in this time window is significantly reduced in persons with incomplete SCI and can be considerably improved with intense locomotor training.[53] An improved ability to generate instantaneous torque may be critical to facilitating functional recovery and ambulation in patients with incomplete SCI. The suggested importance of plantar flexor muscle torque generation for improving ambulation in persons with central nervous system injuries is not new and has been reported in persons post-stroke.[172,159]

The ability to drive α-motoneurons to elicit maximal muscle recruitment is often referred to as maximal voluntary activation and can be estimated using superimposed electrical stimulation, a method commonly implemented in a variety of populations.[154,155,174] In a previous study, we measured voluntary activation deficits ranging between 42% and 66% in the lower extremity muscles of incomplete-SCI subjects, whereas control subjects showed a ~5% voluntary activation deficit.[53] Similar voluntary activation deficits were found in this study prior to locomotor training. Interestingly, voluntary activation deficits were partially attenuated following 45 sessions of locomotor training (30-40% post-training), even though they did not return to normal values. In particular in the knee extensor muscles bilateral improvements in voluntary muscle activation contributed significantly to gains in muscle force production, while

muscle cross-sectional area was relatively unchanged. Improvements in muscle activation in persons with incomplete SCI with locomotor training have also been reported using iEMG.[165] Of interest to note is that voluntary activation deficits can also be observed following disuse or immobilization. However, in these models the phenomena is transient and normalization in muscle activation is typically observed after 3 to 4 weeks of rehabilitation.[175]

Despite the measured increases in instantaneous and peak force production, and improvements in voluntary activation only one of the five participants in this study showed any change in their lower extremity motor scores (LEMS) after locomotor training. In specific, subject 3 improved his LEMS score from 35 to 38. In all other subjects no change in LEMS score could be detected. These data are consistent with other locomotor training studies, which often fail to demonstrate a change in ASIA scores with training in persons with chronic injuries.[79,168,169] We believe that the lack of change in ASIA motor scores in the present study reflects a limitation in the measurement tool. Compared to isokinetic dynamometry, manual muscle tests have a limited inter-rater reliability and have been criticized to lack sensitivity, especially at scores above 3 (out of 5).[54,143] Others have argued that while ASIA scores are valuable in predicting motor recovery in acute patients, they may be less powerful as measures of neuromuscular recovery in chronic SCI.[110,176-178]

In conclusion, nine weeks of locomotor training resulted in improved lower extremity skeletal muscle function in persons after incomplete spinal cord injury. Specifically, extensor muscles about the ankle and knee joint demonstrated an improved ability to generate both peak and instantaneous torque. Relative gains in muscle function were greatest in the ankle plantar flexor muscles, consistent with their critical role for propulsive force generation and high loading during locomotion. Ankle plantar flexor muscles also showed a significant increase in maximal

CSA, while increases in knee extensor force production were mainly linked to improvements in voluntary muscle activation. Finally, we suggest that skeletal muscle alterations contribute to the functional improvements reported with locomotor training in person with incomplete-SCI.

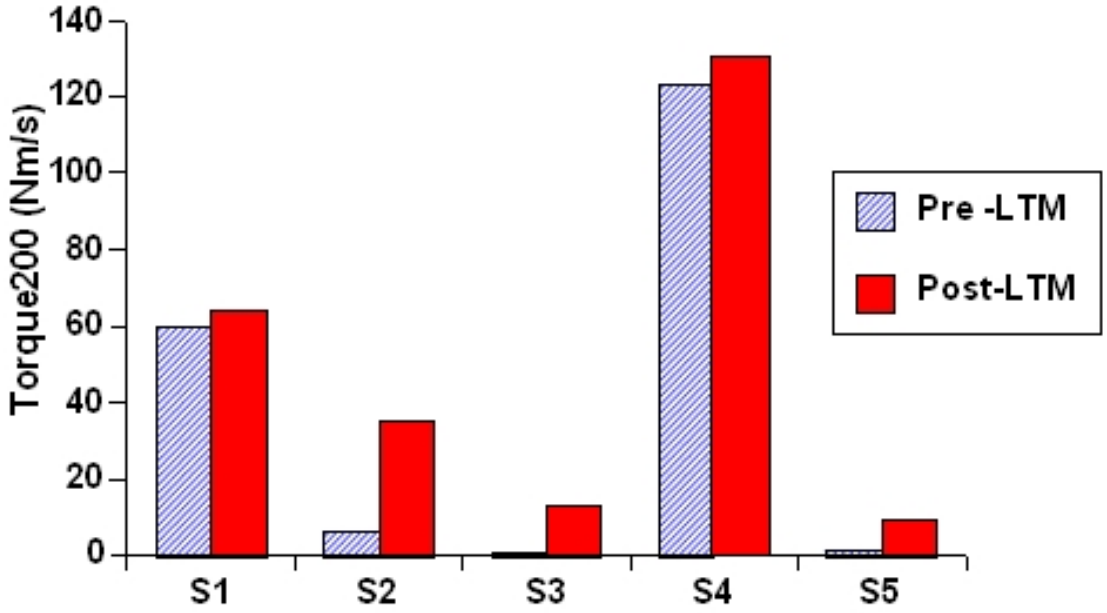
Table 5-1. Characteristics of incomplete SCI subjects

	Age (yrs)	Height (cm)	Body Mass (kg)	ASIA^{R} Impairment Classification	Level of Injury	Duration of injury (months)	Mobility status	LEMS (pre-LMT)	LEMS (post-LMT)
S1	44	154.9	74.8	C	C6	20	Power-wheelchair	33/50	34/50
S2	21	185.4	68.0	D	T4	8	Bilateral-canes	44/50	44/50
S3	48	198.6	77.0	D	C6	39	Bilateral-crutches	35/50	38/50
S4	58	183.0	90.7	D	C4	14	Wheelchair	45/50	45/50
S5	36	176.9	83.9	C	C6	16	Wheelchair	17/50	17/50

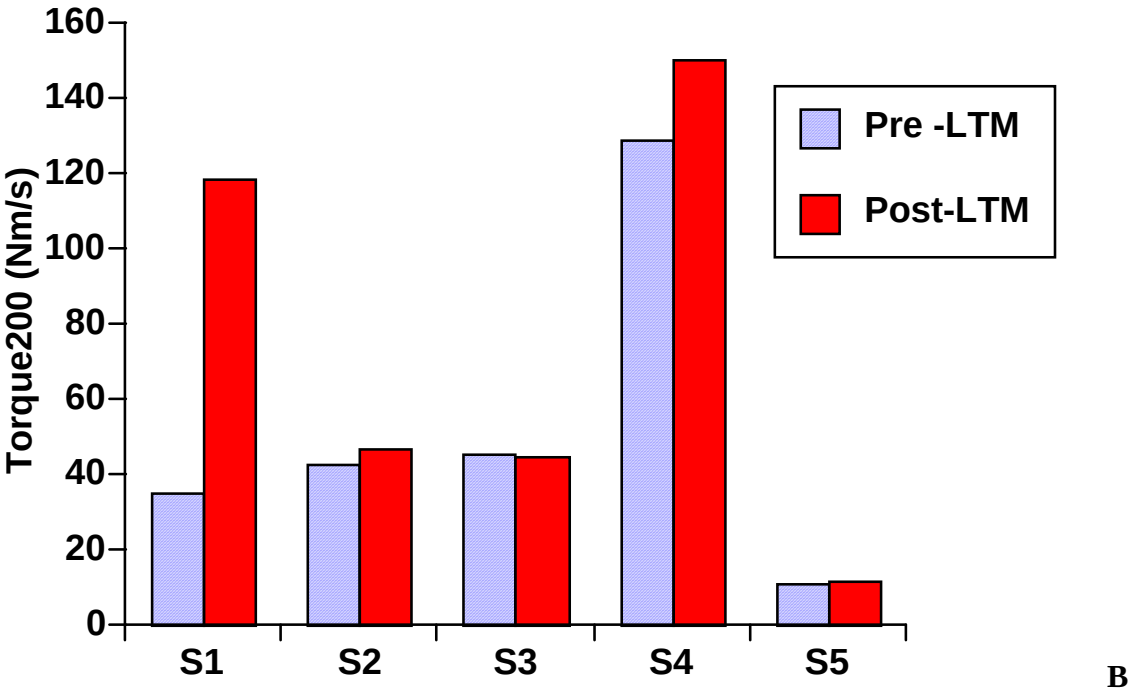
Table 5-2. Values of isometric peak torque and average rate of force development

	Isometric Peak Torque				
	S1	S2	S3	S4	S5
Knee Extensors					
More Involved					
Pre-LMT	35.8	95.8	26.8	176.5	10.8
Post-LMT	42.6	84.6	39.0	181.8	15.7
Less Involved					
Pre-LMT	65.0	136.5	62.6	179.0	15.7
Post-LMT	70.3	190.5	78.0	199.9	18.0
Plantar Flexors					
More Involved					
Pre-LMT	11.7	45.5	19.4	65.2	12.5
Post-LMT	23.3	51.9	29.1	63.3	20.0
Less Involved					
Pre-LMT	24.8	52.3	42.7	90.2	21.9
Post-LMT	35.5	60.7	51.7	82.5	21.0

	Average Rate of Torque Development				
	S1	S2	S3	S4	S5
Knee Extensors					
More Involved					
Pre-LMT	92.6	281.5	62.3	572.1	30.8
Post-LMT	182.5	337.1	81.8	613.3	46.0
Less Involved					
Pre-LMT	270.4	338.0	233.1	727.1	58.1
Post-LMT	325.9	588.1	239.2	746.8	63.3
Plantar Flexors					
More Involved					
Pre-LMT	17.8	27.4	20.0	94.7	21.2
Post-LMT	30.8	44.4	45.9	91.2	22.2
Less Involved					
Pre-LMT	36.5	47.0	103.8	130.3	22.9
Post-LMT	101.0	82.7	105.0	203.5	21.9



A



B

Figure 5-1. Torque200 (Nm) measured in the knee extensor muscle group of the (A) more involved and (B) less involved limb of individuals with incomplete-SCI before (pre-LMT) and after locomotor training (post-LMT).

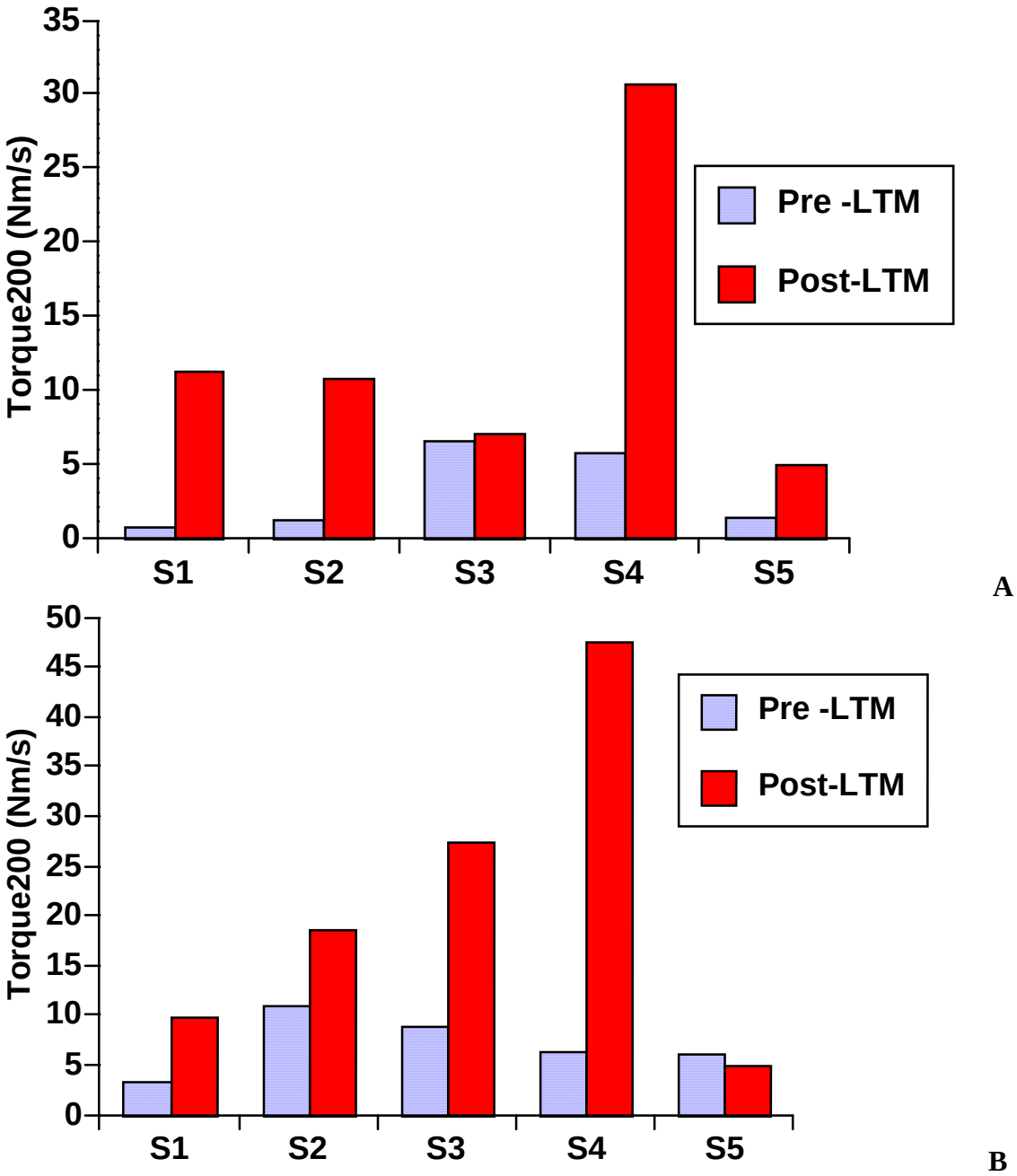


Figure 5-2. Torque200 (Nm) measured in the plantar flexor muscle group of the (A) more involved and (B) less involved limb of individuals with incomplete-SCI before (pre-LMT) and after locomotor training (post-LMT).

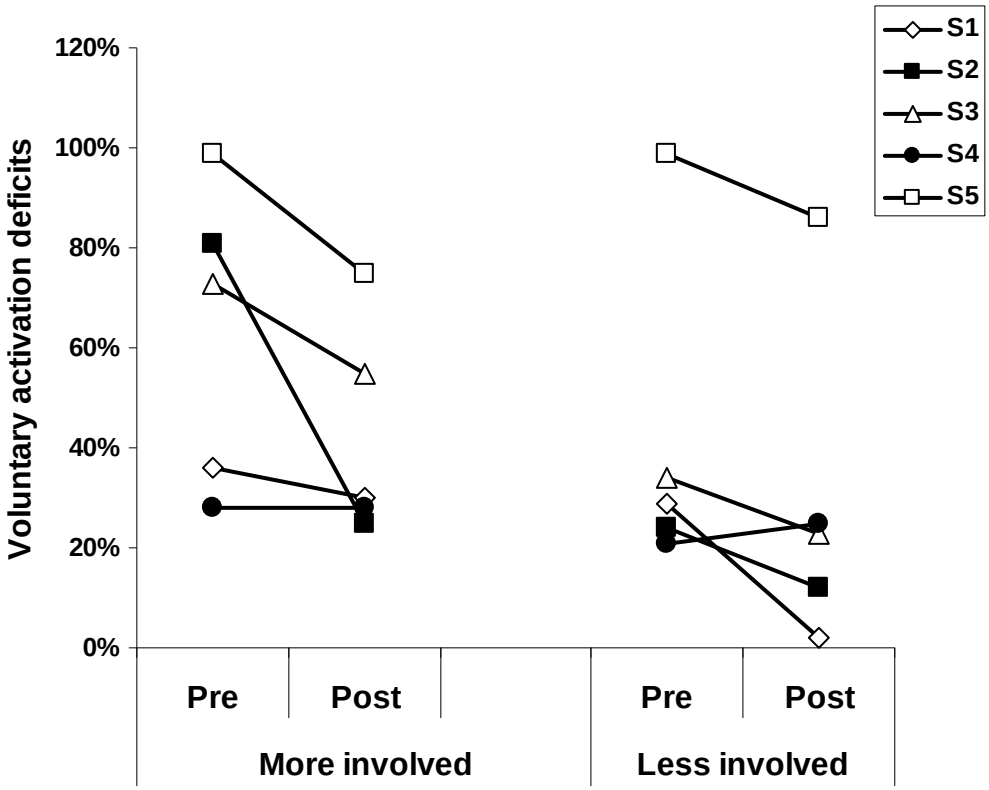


Figure 5-3. Voluntary activation deficits (%) measured in the knee extensor muscle group, of the more involved and less involved limb of individuals with incomplete-SCI before (Pre) and after locomotor training (Post).

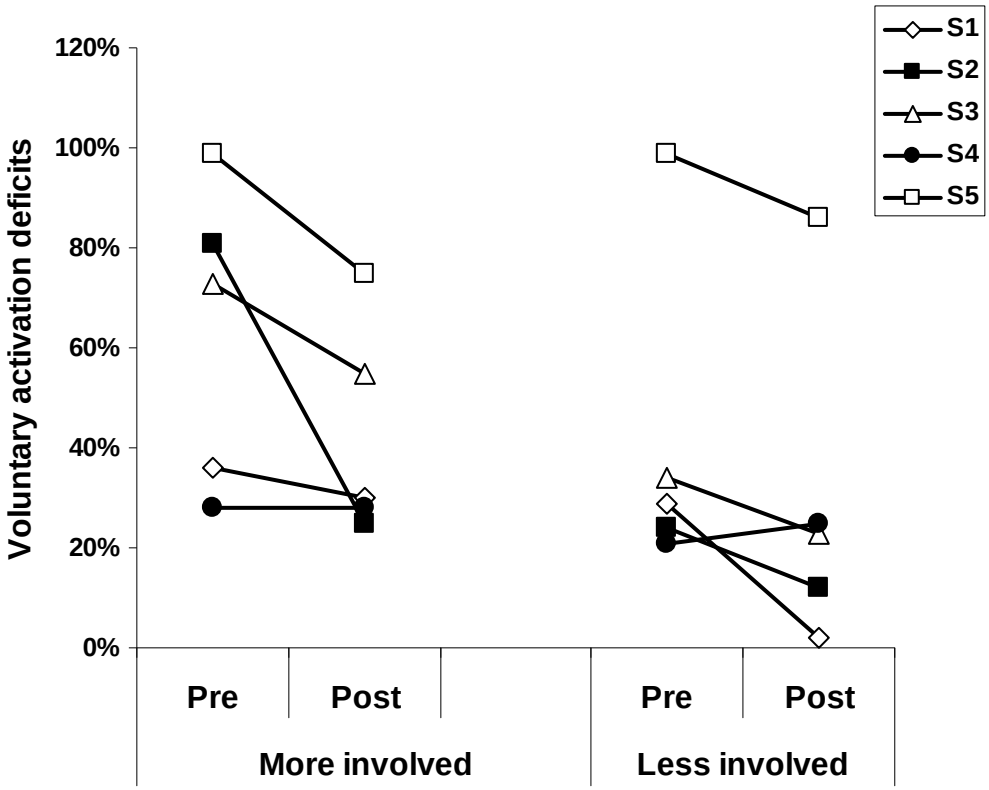


Figure 5-4. Voluntary activation deficits (%) measured in the plantar flexor muscle group of the more involved and less involved limb of individuals with incomplete-SCI before (Pre) and after locomotor training (Post).

CHAPTER 6
RESISTANCE TRAINING AND LOCOMOTOR RECOVERY AFTER INCOMPLETE SPINAL CORD INJURY: A CASE SERIES

6.1 Introduction

The proportion of persons that suffer a spinal cord injury (SCI) resulting in an incomplete lesion has risen dramatically over the past 20 years. As a result ~55% of the new injuries sustained in the United States are now classified as incomplete. In addition, the life expectancy for persons with an incomplete injury is higher than after a complete SCI and is approaching that of non-injured persons, regardless of age at injury.[1] As such, the increased incidence and prevalence of persons with this type of injury necessitates a comprehensive understanding of the adaptations that occur and the potential for rehabilitative interventions to impact persons with incomplete-SCI. Unfortunately, despite the proportion of persons sustaining and subsequently living with incomplete SCI, the preponderance of scientific literature describing the physiological and functional adaptations to SCI involves persons with complete injuries.

Accordingly, limited data are available that describe motor function and its impact on functional ability in this large subject cohort. The ability to independently ambulate is a primary goal of many persons after SCI. However, even though a large number of individuals with incomplete SCI regain some ability to walk, limitations in gait speed may make this method of mobility impractical for activities of daily living. Slow speed combined with other mobility deficits (*e.g.* difficulty climbing stairs, curbs, etc...), could negate the ability to safely ambulate in the community, resulting in a perceived disability. Interestingly, rehabilitation practice focusing on compensatory approaches to locomotion has largely been based on the prevailing assumption that neural as well as functional recovery is limited in persons with chronic SCI. However, recent evidence from both animal and human studies indicates that with the appropriate training stimuli,

neural as well as muscular plasticity can be induced even years after injury [47,140] Improvements in functional ability, however, vary greatly and the incidence of disability remains high.[52,141]

Previous data suggest that persons after incomplete SCI produce less voluntary torque about the knee and ankle than non-injured controls. Perhaps more importantly, impairments in the ability to produce torque in a timely manner as well as a reduced walking velocity is also common to these persons.[53] It is our belief that reduced muscle power generation significantly impacts locomotor function and that functional recovery can be facilitated with rehabilitation interventions that attenuate this impairment. Specifically, the ankle plantar flexor and knee extensor muscle groups are of interest primarily because of their purported roles during bipedal locomotion, with torque demands at these joints during walking representing the two highest in the lower extremity. As such, the potential for impaired torque production about these joints to be a limiting factor in locomotor performance seems high.

The common goal of resistance training programs is to increase maximal strength in the trained musculature. In addition, the focus of plyometric training, which incorporates high-velocity stretch-shortening type contractions, has been to improve performance in activities requiring fast contractions (e.g. jumping or sprinting).[179,180] The combination of these two types of training has been shown to be effective in improving both maximal strength as well as muscle power production and [179,181] result in improved jump height and sprint speed in neurologically healthy individuals . Interestingly, the potential for rehabilitative-training induced changes in muscle strength and power to affect functional ability after incomplete SCI is largely unstudied. In addition, whether potential increases in muscle function in these persons identified during strength testing are reflective of improved muscle power output during functional tasks is unknown and of obvious value. Accordingly, the challenge is to now develop, evaluate and

implement strategies that maximize neuromuscular plasticity in individuals after incomplete SCI with the hopes of resultant improvements in functional capacity and a subsequent decreased disability. As such, the purpose of this study was to determine if improvements in muscle function accompanied by improvements in locomotor ability can be realized following a combined resistance and plyometric jump training program in persons with chronic incomplete SCI.

6.2 Methods

6.2.1 Subjects

Three independently ambulatory males with chronic motor-incomplete SCI participated in this study. Criteria for inclusion included 1) age 18-70; 2) first time SCI (C5-T10); 3) medically stable and asymptomatic for bladder infection, decubitis, cardiopulmonary disease or other significant medical complications prohibiting testing and/or training; 4) if using antispasticity medication, agreement to maintain current levels throughout study; Exclusion criteria were 1) participation in a rehabilitation or research protocol that could influence outcomes of this study. 2) history of congenital SCI or other disorders that may confound treatment, study, and/or evaluation procedures; Prior to participation, written informed consent was obtained from all subjects, as approved by the Institutional Review Board at the University of Florida.

Subject 1, a 22 year-old male (69 kg, 185 cm), suffered a traumatic SCI (T4, 17 months post-injury) and was classified as American Spinal Injury Association (ASIA) impairment level D, with a lower extremity motor score (LEMS) of 44/50. Prior to RPT this subject had a self-selected gait speed of 0.71 m/s and a maximal gait speed of 1.01 m/s. This subject completed 29 sessions of RPT over the 12-week study period.

Subject 2, a 61 year-old male (93 kg, 189 cm), suffered a traumatic SCI (C5, 27 months post-injury) and was classified as ASIA D with a LEMS of 48/50 prior to RPT. Subject 2 had a

self-selected gait speed of 0.82 m/s and a maximal gait speed of 1.18 m/s. Subject 2 completed 30 sessions of RPT over the 12-week study period.

Subject 3, a 58 year-old male (88 kg, 178 cm), suffered a traumatic SCI (C5, 24 months post-injury) and was classified as ASIA D with a LEMS of 35/50. Prior to RPT this subject had a self-selected gait speed of 0.78 m/s and a maximal gait speed of 1.06 m/s. This subject completed 30 sessions of RPT over the 12-week study period.

6.2.2 Resistance Training Program

Lower extremity progressive resistance training was 12 weeks in duration and subjects completed 2-3 sessions/week for a total of 30 sessions. Resistance exercises included unilateral leg press, knee extension/flexion, hip extension/flexion and ankle plantar-flexion exercises performed on adjustable load weight machines. During the initial training session a predicted 1-repetition maximum (1-RM) was calculated for each subject and for each exercise. 1-RM was determined using a prediction table based on a single set to volitional failure with load that allowed between 6 and 12 repetitions. During subsequent training sessions, subjects performed 2-3 sets of 6-12 repetitions at a relative intensity of ~70-85% of predicted 1-RM. Maximal strength was evaluated weekly to assess for training-related improvements and exercise loads were adjusted accordingly. Specifically, if the subject achieved the target number of repetitions for all prescribed sets of a given exercise, a new predicted 1-RM was prescribed and resistance was increased for subsequent training sessions.

6.2.3 Plyometric Training

Unilateral plyometric jump-training exercises were performed in both limbs in a supine position on a ballistic jump-training device (ShuttlePro MVP ®, Contemporary Design Group, Figure 1). Session intensity for this exercise was modified by changing either the resistance or the number of ground contacts and progressed over the training period, accordingly. Briefly,

after familiarization with the training device, subjects completed a total of 20 unilateral =ground contacts (e.g. jumps) with each limb at a resistance of ~25% of body mass. Thereafter, upon successful completion of at least 20 ground contacts per limb (e.g. complete clearance from foot plate), resistance was increased in increments of 10 lbs. When a new resistance was set, repetition goal was set at 10 ground contacts per limb for the initial session. Subsequent sessions allowed for up to 20 contacts per limb. Thus, a minimum of two sessions at a given resistance was required before load was increased. Resistance was held consistent between limbs throughout the training program.[182,183]

6.2.4 Dynamometry

Strength measurements were performed in the PF and KE muscle groups using a Biodex isokinetic dynamometer (Biodex Corp., Shirley, NY). PF strength was assessed with subjects seated in a semi-reclined (~70° hip flexion) position, with the knee flexed ~15° and the ankle in an anatomical neutral position (0° of plantar flexion). The axis of the dynamometer was aligned with the lateral malleolus, and the foot was secured with straps placed at the forefoot and ankle. Proximal stabilization was achieved with straps across the chest, hips, and knee. KE strength assessments were performed with subjects seated in the same position used for PF testing, with the exception that the knee was flexed to 90°. The axis of the dynamometer was aligned with the knee joint line, and the leg was secured to the lever arm.

Peak torque (Nm) was defined as the highest isometric torque achieved during 3 maximal contractions (~3 sec contractions separated by a minimum of 60 seconds rest). In the event that the peak torque values during the three trials differed by more than 5%, additional contractions were performed. In addition to peak torque, values for $T_{20\text{-}80}$, $torque_{200}$ and ARTD were also determined both pre- and post-RPT. These measures were used as indices of a subjects' ability to produce torque in an explosive manner and account for potential differences in both the timing

and magnitude of torque production. $T_{20\text{-}80}$, used to represent the time to peak tension, was defined as the amount of time to generate from 20% to 80% of peak isometric torque. This time interval was chosen to minimize potential errors in the determination of the precise onset and nadir of torque development while still representing a majority of the time interval for achieving maximal torque production. Average rate of torque development (ARTD) was defined as the average increase in torque generated in unit time (Nm/s), and was calculated over the same interval as $T_{20\text{-}80}$. Hence ARTD was calculated through numerical differentiation as

$$ARTD = \frac{1}{N}\sum_{i=1}^{N}\frac{\delta f_i}{\delta t}$$

where N is the total number of time slots for numerical differentiation, δf_i is the change in torque in the time slot $\boldsymbol{i}$ and δt is the unit time duration for a slot. $Torque_{200}$ was defined as the absolute torque reached at 200ms during a maximal voluntary contraction (Nm).

$Torque_{220}$ was defined as the absolute amount of torque generated during the initial 220ms during a maximal voluntary contraction and is based on the calculated time that is available for concentric torque generation during a typical gait cycle at a speed designated necessary for community ambulation[159]. For example, the speed commonly deemed necessary for persons to safely ambulate in the community is 1.2 m/s [159]. At this speed, the time it takes to complete one gait cycle (i.e. right heel strike to right heel strike) is ~1.1 seconds. Given that the plantar flexor muscles are reported to be active for ~40% of the gait cycle and approximately 1/2 of this active time is spent generating concentric torque, roughly 200 milliseconds is available for force generation (e.g. propulsion) by this muscle group.

6.2.5 Voluntary Activation Deficits

Voluntary activation deficits were determined using the twitch interpolation method.[152,184] Briefly, a single biphasic, supra-maximal pulse (600μsec pulse duration) was delivered at rest

and during maximal voluntary isometric contraction. Voluntary activation deficit was calculated using the ratio between the torques produced by the superimposition of a supra-maximal twitch on a peak isometric contraction (a) and the torque produced by the same stimulus in the potentiated resting muscle (b). Voluntary activation deficits were expressed as: voluntary activation deficit (%) = (a/b)*100.

6.2.6 Locomotor Data Collection

Subjects performed repeated 10 meter walks over a 14 ft. long mat (Gait Rite) that measures the geometry and the applied pressure of each footfall as a function of time in order to determine both self-selected and maximal overground walking speed (3 trials each). Gait analyses were performed 3 months prior to training as well as at both pre- and post-RPT time points. Multiple baseline tests were conducted to control for improvements resulting from natural recovery.

6.3 Results

6.3.1 Dynamometry

All subjects demonstrated improvements in peak torque production, $T_{20\text{-}80}$, $torque_{200}$ and ARTD during post- versus pre-RPT dynamometric testing. On average, RPT resulted in a 35.0 ± 9.1% and 28.9 ± 4.4% improvements in peak isometric torque production in the PF and KE muscle groups, respectively. Individual gains ranged from 17% to 76% in the plantar flexors and from 22% to 45% in the knee extensors. Time to peak tension, represented by $T_{20\text{-}80}$, decreased from 470.8 ± 82.2 ms to 312.0 ± 65.7 ms in the PF and from 324.5 ± 35.4 ms to 254.2 ± 34.5 ms in the KE muscle groups following training. In addition, both indices of muscle power generation, ARTD and $torque_{220}$, were noticeably improved following training. Of interest to note is that both $torque_{220}$ and ARTD showed more pronounced improvements in the PF

compared to the KE muscles with training. Specifically, a 62.1% and 122.2 % improvement in $torque_{220}$ and ARTD were seen in the PF muscles, with only a 33.4% improvement in $torque_{220}$ and a 66.4 % improvement in ARTD in the KE muscle group. In addition, the largest relative gains in indices of explosive muscle strength ($T_{20\text{-}80}$, $torque_{200}$ and ARTD) occurred in the PF muscle group of the more-involved limb. Peak torque, $torque_{200}$, $T_{20\text{-}80}$ and ARTD data are summarized in Table 6-1.

6.3.2 Voluntary Activation Deficits

Significant voluntary activation deficits were noted in both the PF and KE muscle groups prior to training. RPT resulted in reductions in activation deficits in both the PF and KE muscle groups in each subject. Individual data for activation deficits are presented in Table6-1. Although significant bilateral asymmetries existed prior to and following the intervention, these differences were seemingly attenuated in both muscle groups following RPT.

6.3.3 Locomotor Analyses

Values for maximum and self-selected gait speeds did not differ by more than 0.04 m/s and 0.02 m/s, respectively, for any of the subjects in this study when comparing tests done 3 months prior to the onset of training and immediately prior to training. Following RPT, a 36.1 % average increase in maximum gait speed and a 34.7% average improvement in self-selected gait speed were realized.

6.4 Discussion

The results of this study suggest that a combination of resistance and plyometric training in persons with motor incomplete SCI results in bilateral improvements in 1) peak torque production, 2) time to peak torque and 3) rate of torque production in the plantar flexor and knee extensor muscle groups. These improvements in muscle function can be attributed to both an increase in muscle cross-sectional area as well as an increased ability to voluntarily activate

affected skeletal muscles. Interestingly, the magnitude of improvement in these outcomes was most pronounced in the more- versus the less-involved limb and in the PF versus the KE muscle group. In addition, improvements in both self-selected and maximum gait speeds were realized and were explained by increased propulsion in the more-involved limb as well as increased lower extremity joint powers, suggestive of improved task specific muscle function (i.e. during walking).

Injury to descending spinal pathways as well as decreased activation history both has the physiological consequence of reducing the ability to voluntarily activate affected skeletal muscles. Although restoration or repair of the injured spinal cord is not a reasonable expectation with training, the potential to improve deficits resulting from disuse seems likely and has been demonstrated after periods of inactivity in other populations.[160,185,186] In this study, significant activation deficits existed prior to RPT that are comparable to other models of disuse (i.e. cast immobilization, limb-suspension).[187] Interestingly, these deficits were partially attenuated with training and this enhancement of neural function could serve to explain a portion of the strength gains realized post-RPT. In addition to enhanced neural transmission, muscle hypertrophy post-RPT cannot be ignored as a mechanism for improved muscle torque production during both dynamometric testing as well as during walking. However, though significant skeletal muscle hypertrophy (*e.g.* larger effector) might suggest improved torque generation independent of the activation pattern, the magnitude of strength gains would suggest that the majority of these gains were accounted for by means other than muscle hypertrophy.

In this study we chose to examine the morphological and contractile characteristics of the ankle plantar flexor and knee extensor muscle groups primarily because of their purported roles during bipedal locomotion. Torque demands at these joints during walking are the two highest in

the lower extremity. In addition, we have previously shown that torque generation about these joints is limited in persons after incomplete SCI.[53] Similarly, subjects in the present study presented with reduced PF and KE peak torque values prior to RPT, as well as a reduced gait speeds. Interestingly, marked improvements in PF and KE isometric torque generation and gait speed were realized following RPT. However, post-RPT measures of peak torque about these joints as well as maximum gait speeds are still reduced relative to control values [53], thereby suggesting the potential for further functional improvements if additional increases in torque production by these muscle groups can be realized.

In addition to absolute torque production, a likely mechanism explaining impaired muscle function during locomotor tasks may be an inability to produce properly graded and timed muscle output. This impairment has been identified in this and other populations with central nervous system dysfunction [53,188-190] and shown to relate to reduced gait speed.[190] The combination of a prolonged time to peak torque and a decreased ability to generate maximal torque in these persons suggests that at least some of limitations in gait speed in persons with incomplete SCI might result from impaired muscle function. However, the dramatic improvements in muscle function demonstrated in the present study highlight the potential for this type of training to attenuate existing deficits in neuromuscular function and facilitate functional improvements.

Recent therapeutic interventions examining gait in persons after CNS injury have largely focused on the task specificity of training with little focus on impairment level deficits [151,168,191] Although the rationale for task-specific training interventions to result in improvements in motor function is quite strong and shown to be effective in producing cortical reorganization [192,193] we feel that in-vivo muscle function is also limiting in these persons and appropriate training can

also induce neuroplastic changes in these tissues that facilitate locomotor improvements by improving the element of muscle function dictated by locomotor task performance. Accordingly, given that few studies have attempted to examine the relationship between lower extremity strength and gait in persons after incomplete SCI, comparisons to other populations with CNS involvement yield valuable information. For example, data examining the relative importance of lower extremity strength in persons after stroke demonstrate significant correlations between the strength of the paretic hip flexors (r = .57), knee extensors (r = .41) and primarily the ankle plantar flexors (r = .85), with maximal gait speed.[172,194] In addition, previous simulation work suggests that force production by the soleus and gastrocnemius is critical to trunk forward progression, swing initiation and power generation during gait.[159,195] Thus, one might predict slower gait speeds if force production by these muscles is abnormal during locomotion. Indeed, the negative impact of reduced plantar flexor function is supported by experimental data. For example, Lamontagne *et al.* suggested that more than 50% of the variance in gait speed in persons post-stroke was explained by the peak activation of the medial gastrocnemius. In addition, Mulroy *et al.* demonstrated that ankle moments were substantially reduced in two groups of hemiparetic persons compared to slow walking controls, with household walkers having reduced moments relative to limited community walkers.[172] These same investigators also found that at two different time points, walking speed was strongly associated with plantar flexor voluntary strength. Specifically, deficits in plantar flexor strength were pronounced, with the slow subject group (~10% of normal age-matched speed) demonstrating strength equal to ~18% of normal age-matched strength upon admission to rehabilitation. Interestingly, at six months post stroke, plantar flexor strength increased to 22% of control value, an increase of ~20%, and was associated with increased walking speed (~ 20%). Thus, these data provide support to

suggest a relationship may exist between changes in plantar flexor strength and gait speed, at least at slow velocities. Interestingly, the relative gains in plantar flexor strength in the present study (35.0%) are almost identical to the increases in fastest (36.1%) and self-selected (34.7%) gait speeds post- RPT.

In conclusion, the importance of the proposed work revolves around the fact that little is known about the extent to which skeletal muscle plasticity may impact functional outcomes after incomplete SCI. The desire "to be more normal" with respect to locomotor ability is one that many persons after this type of injury possess. Accordingly, the development of appropriate rehabilitation strategies that target improvements in locomotor ability with the goal of increasing functional independence could have a tremendous impact on this population. The data in the present study provide support for the use of physical rehabilitation interventions aimed at attenuating neuromuscular impairments as a means for improving not only gait speed but also the strategies utilized by these persons to ambulate. As such, we suggest that the benefits reported following a combination of resistance and plyometric training represent a first step in the use of these modalities to facilitate the recovery of motor function and functional ability in this population. Although, we report significant gains in strength and gait speed following 12 weeks of RPT, at this point we do not know if the subjects in thus study reached a plateau in any of the outcomes measured. Therefore, future studies examining the impact of physical rehabilitation training programs after incomplete SCI should focus on the optimal volume (e.g. duration and frequency) and intensity of training, as well as the potential of this type of training to serve as an adjunctive therapy in the overall treatment of these persons. In addition, these studies need not only focus only on gait, but other functional outcomes (e.g. stair climbing, sit to stand) as well as

the potential psychosocial benefits (i.e. community integration) that likely parallel increased functional capacity.

Table 6-1. Pre- and post-RPT isometric torque data for the plantar flexor and knee extensor muscle groups.

	Pre-RPT					*Post-RPT*				
KNEE EXTENSORS	**Peak Torque**	**ARTD**	**Torque$_{200}$**	**T_{20-80}**	**Activation Deficit (%)**	**Peak Torque**	**ARTD**	**Torque$_{220}$**	**T_{20-80}**	**Activation Deficit (%)**
More-involved										
S1	99.8	282.4	67.5	283.0	39.0	125.7	478.6	88.9	241.6	31.0
S2	100.3	204.6	44.1	440.8	34.0	123.8	497.4	71.0	210.6	25.0
S3	65.1	196.1	28.1	370.4	50.0	81.6	244.5	30.5	280.4	35.0
Less-involved										
S1	136.4	482.1	78.2	254.1	32.0	177.6	706.2	108.5	250.3	29.0
S2	143.9	501.8	69.7	240.7	20.0	176.1	827.3	102.4	300.9	14.0
S3	112.5	330.9	53.8	360.2	19.0	162.7	570.5	54.2	215.5	18.0
	Pre-RPT					***Post-RPT***				
PLANTAR FLEXORS	**Peak Torque**	**ARTD**	**Torque$_{220}$**	**T_{20-80}**	**Activation Deficit (%)**	**Peak Torque**	**ARTD**	**Torque$_{220}$**	**T_{20-80}**	**Activation Deficit (%)**
More-involved										
S1	45.4	59.1	13.7	807.3	36.0	56.1	95.7	22.8	587.7	28.0
S2	27.3	50.4	12.1	430.1	42.0	36.1	119.5	27.5	240.1	31.0
S3	17.0	28.6	5.6	490.9	41.0	26.8	102.9	9.5	280.6	28.0
Less-involved										
S1	56.7	105.2	14.4	403.2	18.0	66.4	259.8	26.4	252.2	16.0
S2	32.7	95.0	26.7	380.6	34.0	42.8	164.9	34.4	300.4	15.0
S3	33.2	84.5	12.5	315.9	47.0	58.6	256.1	16.9	215.4	41.0

Figure 6-1. Example of plyometric training device.

CHAPTER 7
LOWER EXTREMITY SKELETAL MUSCLE MORPHOLOGY AND FIBER TYPE COMPOSITION FOLLOWING MODERATE CONTUSION SPINAL CORD INJURY AND LOCOMOTOR TRAINING

7.1 Introduction

Spinal cord injury (SCI) is a devastating condition which causes severe long lasting neurological dysfunction and morbidity in humans.[12,52,196] In addition to effects directly related to CNS dysfunction, common problems experienced with SCI are skeletal muscle atrophy and impaired muscle function leading to walking disabilities.[45,52,69,197,198] Animal models of SCI are commonly used to evaluate the pathology of SCI and to ensure the feasibility and efficacy of new therapeutic interventions. Commonly used animal models of SCI include transection, isolation, and contusion injuries.[199,200] While the transection and isolation models successfully reproduce complete SCI, the contusion model is a clinically more relevant model as it is known to closely mimic the mechanism and histopathologic sequela of the majority of current human SCI (>55%, incomplete-SCI), thus making it a relevant model to study.[20,200] In contrast to the complete SCIs in which animals experience significant atrophy and a complete loss of locomotor capabilities, animals with contusion injury[199,201] show some spontaneous recovery of muscle size and regain some locomotor function without any specific therapeutic intervention.[199,202,203]

Locomotor treadmill training has recently gained momentum as a therapeutic intervention to improve lower extremity function and walking after SCI. Locomotor training is based on the principle that stepping can be generated by virtue of the neuromuscular system's responsiveness to phasic, peripheral sensory information associated with locomotion.[72,79,204,205] Although locomotor treadmill training programs promote changes in spinal cord properties, motor unit morphology, and functional recovery[40,198,206,207], the impact of this training intervention towards ameliorating atrophy and improving muscle function after SCI are not clear. Currently, a few

studies have looked at skeletal muscle adaptations after contusion SCI and locomotor training. Min *et al*.2008[208] characterized the longitudinal changes in rat lower hindlimb muscle morphology following contusion SCI and locomotor training by using magnetic resonance imaging over a three month period. The greatest amount of atrophy was observed at 2-week post-injury and locomotor training as early one-week post injury significantly reduced atrophy and improved function. In a follow up study, Stevens *et al.* 2006[65] evaluated therapeutic potential of early locomotor training in the soleus muscle. Locomotor training appeared to ameliorate soleus muscle atrophy and attenuate the shift in myosin heavy composition (MHC) towards faster isoforms. However, this study was limited to the slow postural muscle soleus only and information on other lower extremity muscles is still warranted. Since it is known that different lower extremity muscles adapt differently to unloading conditions based on their specific function role and phenotype, it is important to investigate the influence of locomotor training on muscle size and fiber type distribution in muscles with different functional roles and fiber type composition.

The objectives of this study were 1) to quantify changes in fiber size and fiber type composition following incomplete SCI in lower extremity muscles with different functional roles and fiber type composition in the rat 2) to study the therapeutic influence of one-week of locomotor training on lower extremity muscle with different functional roles and fiber type composition in spinal cord-injured animals.

7.2 Methods

7.2.1 Animals

Twenty-four Sprague–Dawley rats (female, 228–260 g, weighing 250-290gms; Charles River, NJ, USA) were used in this study. Six rats per group were assigned to either a SCI-training group, a SCI-no training group, a control group, or a control training group. Six of the

injured rats received treadmill locomotor training (TM) starting 1 week after SCI, when the surgical staples were removed and soft tissue had healed sufficiently to tolerate training without increasing the risk of trauma at the incision site. Training in the TM group was implemented for 5 consecutive days, 20 min/trial, 2 trials/day. The additional 8 injured rats received no exercise intervention (no TM). The rats were housed in a temperature-controlled room at 21 °C and were provided unrestricted access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

7.2.2 Contusion Spinal Cord Injury

Spinal cord contusion injuries were produced using a protocol described previously. A NYU (New York University) impactor was used to produce the injuries. Briefly, a 10g weight was dropped from a 2.5-cm height onto the T8 segment of the spinal cord which was exposed by laminectomy. The entire procedure was carried out under sterile conditions. All injuries were performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia. Animals received two doses of Ampicillin (100mg/kg) per day for 5 days starting on the day of surgery. To prevent dehydration, subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given Buprenophine (0.05 mg/kg) and Ketoprofen (5.0 mg/kg s.c.) for pain and inflammation over the first 36 hours after SCI. The animals were kept under vigilant postoperative care, including daily examination for signs of distress, weight loss, dehydration, and bladder dysfunction. Manual expression of bladders was performed 2-3 times daily until spontaneous voiding returned (~2 weeks), 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.

7.2.3 Locomotor Treadmill Training

Animals with spinal cord injury were exposed to treadmill locomotor training. Training was started on post-operative day 7. There were two reasons for this. First, on day 8 the surgical staples were removed and soft tissue had healed sufficiently so that trauma could be avoided at the incision site. Second, red porphyrin expression around the eyes, a symptom associated with stress, disappeared within a week post SCI. Therefore, animals could be trained without apparent discomfort and stress at this time.

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 meter/minute) 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 training sessions of 2 hours. Training consisted of quadrapedal treadmill stepping. Body weight support was provided manually by the trainer as necessary. The level of body weight support was adjusted to make sure that the animals' hind limbs did not collapse and was gradually removed as locomotor capability improved. Typically, when all rats had profound paraplegia, assistance was provided to place the rat hind paws in plantar stepping position during training.

7.2.4 Tissue Harvest

Muscle samples will be harvested from the normal control rats, two weeks post injury on the SCI-no training rats, and one week post- training on the trained SCI and trained control rats. The muscles will then be dissected and snap-frozen at resting length in isopentane, pre-cooled in liquid nitrogen and stored at –80 ^{0}C.

7.2.5 Immunohistochemical Measures

Cryostat sections (10μm) in a transverse plane were prepared from the central portion of the soleus, TA, EDL and Gastroc muscles taken from both legs 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 abs (BA-D5, SC-71, BF-F3, and BF-35) were selected on the basis of their reactivity toward adult MHC. Sections were incubated with rabbit anti-laminin and one of the anti-MHC antibodies (4°C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and Fitc-conjugated anti-mouse IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands). Stained sections were mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4°C to diminish fading. Stained cross-sections were photographed (10x magnification) by using a Leica fluorescence microscope (Leica Microsystems, Bannockburn, IL) with a digital camera. A region of the stained serial sections from each muscle was randomly selected for MHC composition analysis. The proportions of each fiber type were determined from a sample of 150–250 fibers across the entire section of each muscle. The pixels setting used for conversion of pixels to micrometers were 1.5 pixels to 1 μm^2 for a 10x objective. The average fiber CSA of all the circle fibers was determined. However, in order to minimize the risk of including nonmuscle tissue, areas consisting of less than 100 pixels were excluded from the analysis.

7.2.6 Data Analysis

All statistical analyses were performed with SPSS, Version 13.0.1. Tests for normality will be performed on all of the measured variables before proceeding with tests of statistical inference. Results are expressed as mean ± standard error of mean. One-way ANOVA was used

to test for differences among the four experimental groups. In an effort to control for multiple comparisons, post-hoc analysis was implemented. For all analyses, significance was established when $p< 0.05$.

7.3 Results

7.3.1 Effects of Incomplete- SCI and Locomotor Training on Fiber Crossectional Area (CSA)

The effect on SCI on fiber size was determined in four different hindlimb muscles-Soleus, Tibialis Anterior (TA), Extensor Digitorum Longus (EDL), and Gastrocnemius (Gastroc). Immunohistochemistry was used to quantify the changes in these lower extremity muscles's size after contusion SCI. Measurements were made in animals two weeks after SCI (SCI group) and in animals with one week of locomotor training one week after SCI (SCI +locomotor training group). The degree of atrophy in this study seemed not to be muscle phenotype or functional role specific. The slow extensor soleus showed the maximum atrophy followed by the predominantly fast flexor EDL, then the mixed extensor gastrocnemius, and finally the fast flexor TA. Two weeks following SCI, the soleus showed significant reduction in fiber CSA (~29%) in comparison to un-trained controls ($p <0.05$, Fig. 7-1). Locomotor training lead to a significant increase in soleus CSA in comparison to untrained SCI group. This change in CSA observed after locomotor training was not significantly different from the control group. Interestingly, one week of locomotor training in the control rats did not result in any change in fiber CSA.

As shown in Figure 7-2, SCI also produced a significant loss in muscle fiber CSA in the EDL in comparison to the control group (~28%, $p<0.05$). Locomotor training resulted in significant increase in fiber CSA compared to the untrained SCI group. Even though the training intervention was only partially effective in restoring fiber CSA towards control levels, the

difference between the control and SCI + locomotor training groups were not significantly different ($p<0.05$).

Gastrocnemius being an extremely large muscle, the fiber CSA was determined from a sample of 150–250 fibers located at areas which mostly stained positive for MHC type I. This method was chosen also to help us study fiber type transformation in the Gastroc muscle following moderate contusion SCI. Two weeks of SCI resulted in significant reduction in average fiber CSA in the predominantly slow Gastroc (~22%, $p<0.05$). Interestingly, locomotor training resulted in no change in average fiber CSA in the Gastroc in comparison to the untrained-SCI group ($p<0.05$). However, we feel our results did not substantially justify the influence of locomotor training in restoring fiber CSA in the Gastroc because our study was specific to areas containing only type I MHC fibers and we strongly feel that the training might have significantly influenced the other MHC fiber types (results not reported, Fig.7-3).

Finally in the TA muscle, two weeks of contusion SCI resulted in a non-significant ~12.6% decrease in average CSA in comparison to controls,($p<0.05$, Fig.7-4). Locomotor training once again resulted in restoring muscle CSA in the TA towards pre-injury levels. No changes in CSA were observed in the control trained group compared to the control group. Overall, these results indicate atrophy following incomplete-SCI is fiber type and functional role specific with slow-extensor showing maximum atrophy while the fast-flexor showing the least amount of atrophy. In addition, locomotor training significantly contributed in reducing the extent of atrophy in all lower extremity muscles except the gastrocnemius in spinally contused-animals.

7.3.2 Effects of Incomplete- SCI and Locomotor Training on Fiber Type Composition

The myosin heavy chain (MHC) molecule is an actin-based protein which plays an important role in specifying skeletal muscle contractile properties. Therefore, we used MHC staining to identify fiber type composition in animals following SCI and locomotor training.

The soleus muscle from the control untrained animals primarily contained fibers reacting exclusively with type I monoclonal antibody (mAB) (~85%), indicating slow MHC isoforms, and a small percentage of fibers reacting with type IIa mAb, exclusively. Two weeks following moderate T8 contusion SCI, the proportion of type I fibers was reduced by ~10% compared to controls and subsequently the reduction in type I fibers was replaced by fibers that co-expressed both MHC-I and MHC-IIa and IIa and IIx (mixed fibers). Locomotor training prevented the appearance of fibers that co expressed both type IIa and IIx in the soleus. In addition the proportion of fibers that were stained positively with both types I and IIa were lower in the SCI-trained animals than the SCI-untrained animals (Fig.7-5).

The TA muscle from untrained controls primarily consisted of fast MHC isoforms (i.e. fibers reacting exclusively with type IIb mAb [~50%], followed by the type IIx [~25%] and IIa [~20%]), and only a small percentage of pure type I fibers (Fig.7-6). In the SCI no –training group, the proportion of type IIb fibers were higher by ~15% compared to the controls, while the proportion of IIx and IIa fibers were lower. In addition, the TA from SCI animals also showed mixed fibers that co-expressed both MHC-I and MHC-IIa and IIx and IIb, which were not present in the controls. Locomotor training one week post-SCI resulted in the MHC fiber type distribution recovering towards phenotypes represented by control TA muscles. In addition, there were also a higher proportion of mixed fibers which co-expressed type IIx and IIa instead of the faster IIb and IIx as seen in the SCI-untrained group. There was no difference in fibers that expressed only type I among the SCI trained and untrained and control groups.

The EDL muscle is a mixed fast muscle containing primarily of fast MHC isoforms. The percentage composition of types I, IIa, IIx and IIb MHC isoforms in the EDL of control rats was ~4±1, 16±3, 34±2, and 45±3% respectively (Fig.7-7). Two-weeks of contusion

SCI shifted the MHC profile toward faster isoforms i.e. the type IIb from 45 to 48% and the type IIx from 34-28%. The other changes include the appearance of IIa + IIx and IIx + IIb fibers. One-week of locomotor training resulted in a significant decrease of the type IIb fibers from 45-32% and increase in the type IIa from 16-21% in comparison to SCI untrained group. Furthermore, in comparison to the SCI untrained group, locomotor training resulted in the reduction of IIx + IIb fibers which seemed to be replaced by an increase in the I + IIa fibers. Even though, there seems to be a slight shift in MHC isoforms after training towards the slower isoforms, these data suggest that type IIb is the default MHC isoform in the EDL both after SCI and training, while training seems to have a positive influence in causing some shift in the MHC isoform from fast to slow just one-week following contusion SCI.

The gastrocnemius is a muscle which is significantly compartmentalized relative to fiber type composition. In order to study changes in fiber type composition with contusion SCI, we choose to study only areas with the Gastroc which stained mainly for type I fibers. In the control rats, our regions of choice compromised 54±6, 28±3, 15±4, and 3±1% pure type I, IIa, IIx and IIb fibers respectively. Following of two weeks of moderate contusion SCI, the MHC isoform distribution was nearly even across groups. There was a ~28% type I, ~20% IIa, ~31%IIx, and ~22% IIb fibers. Interestingly, there was no appearance of fibers co-expressing two MHC isoforms. However, the trend was different following one-week of locomotor training one week post-SCI. The type I, IIa, IIx and IIB fibers were approximately 51%, 23%, 17%, and 9%. In summary, the early training intervention just one-week post-SCI seemed to change the expression of MHC in the Gastroc comparable to control levels (Fig.7-8).

7.4 Discussion

One of the major problems associated with spinal cord injury (SCI) irrespective of the type of injury is loss of muscle mass as manifested by a reduction in cross-sectional area (CSA). This

reduction in CSA has been historically accompanied by fastening of the muscle contractile properties manifested by an increased expression of faster myosin heavy chain (MHC) isoforms.[20,58,209-212] However, these adaptations seen vary based on the functional role or fiber type composition of the observed skeletal muscle.[20,65,213] To better understand the impact of contusion-SCI on skeletal muscle mass and phenotype, we studied changes in fiber CSA and MHC composition in four lower extremity muscles with different functional roles and fiber type compositions. In addition, the therapeutic influence of locomotor training was also examined in restoring muscle mass and attenuating the change in MHC composition. The findings of the current study demonstrate that contusion SCI results in significant atrophy in all lower extremity muscles (soleus, extensor digitorum longus, tibialis anterior and gastrocnemius), and this was accompanied by a shift in MHC composition in all the muscles towards faster isoforms. Interestingly, locomotor training was effective in restoring muscle mass and MHC composition to pre-injury levels.

Numerous studies have been conducted in looking at the loss of muscle mass following SCI.[63,208,213,214] The majority of these studies have been performed following spinal transection or spinal isolation were minimal loading or minimal muscle activity was recorded following the injury. In a few studies similar to our study, were changes in muscle mass were quantified two-weeks following spinal transection and isolation they observed an atrophy of ~41-50% in the soleus, ~36-49% in the medial Gastroc, ~45% in the TA, and ~40% in the EDL.[23,63,215,216] These studies have indicated that muscle adaptive responses following transection and isolation SCI are similar in the early stages of atrophy (14-15 days), however following chronic inactivity muscle-specific atrophic response is more in the slow-twitch muscles compared to the fast-twitch, and more in the extensors compared to the flexors. [23,63,215,216] In comparison, only a few studies have

looked at skeletal muscle morphology following contusion SCI and results from these studies are conflicting. In the first study by Hutchinson *et al*. 2001[20] reported a decrease of 20-25% and 16-21% in all lower extremity muscles, at 1 and 3 weeks following moderate contusion SCI. In this study they reported that muscle atrophy occurred in flexor as well as the extensor muscle and that the extent of atrophy was similar in the fast and the slow muscles. In contrast Min *et al.* 2008[208], using MRI observed at 2 weeks post contusion SCI showed a hierarchal pattern of atrophy, with the extensor triceps surae having more atrophy than flexors muscles. In the current study, at 2-weeks post contusion injury, atrophy quantified through fiber CSA showed the following hierarchy of atrophy: soleus>EDL>Gastroc>TA. The overall atrophy of ~12-29% was observed in all the lower extremity muscles. Significant difference in fiber CSA after contusion SCI was observed only between the slow-extensor soleus and fast-flexor TA. In summary, in this study we demonstrated that there was significant atrophy in all lower extremity muscles 2 weeks following contusion SCI and the extent of atrophy measured through fiber CSA was maximal in the soleus but similar between a slow-twitch and fast-twitch muscle and also similar between a extensor and flexor. We feel this muscle response may be attributed to the spontaneous recovery and muscle activity observed following the contusion injury.

The appearance of the different MHC isoforms in a muscle plays a defining role in regulating the contractile and histochemical characteristics of the muscle.[5,24,209] The maximal velocity of shortening of muscle at least in part is dependant on the MHC composition of the muscle. Research over the years has identified atleast four different MHC isoforms being highly expressed in rat muscles. [5,24,209] They have been identified as MHC-I, IIa, IIx and IIb isoforms. Hybrid fibers which coexpress multiple MHC isoforms also exist. Reduction in loading and neuromuscular activity following SCI leads to fastening of the muscle contractile properties

resulting from an increased expression of faster MHC isoforms.[62,213,217,218] There is also an increased expression of hybrid fibers which co-express different MHC isoforms. Findings of our current study are consistent with other studies performed following contusion injury, like studies by Hutchinson *et al*. 2001[20] and Stevens *et al*. 2004[65] who reported increases in faster MHC isoforms and appearance of hybrid fibers co-expressing different MHC isoforms two weeks following contusion SCI. Specifically in our current study, the soleus had an increased expression of IIx fibers and also the appearance of hybrid fibers expressing faster isoforms, while both the TA and EDL had increases in IIb MHC expression. In the Gastroc the fibers which were predominantly type I shifted to expressing equal levels of all MHC isoforms. To summarize, the findings of our study are consistent with those of other studies indicating that 2 weeks post-SCI there is a significant shift in MHC composition in all lower extremity muscles irrespective of functional role or fiber type to switch to faster isoforms. We feel the influence of injury in modulating MHC composition is similar across all muscle groups at earlier time points (2-weeks) and this might turn muscle specific at more chronic time points.

Motor recovery following spinal cord injury can be enhanced or accelerated by locomotor treadmill training.[72,79,204,205] Locomotor training uses the principles showing that rhythmic loading of the limbs and force feedback from the hindlimb muscles induces task appropriate activity-dependent plasticity. Following moderate contusion in rats, locomotor training has been shown to induce substantial hindlimb muscle and motor recovery.[65,114,208] Locomotor training using treadmill has also produced significant improvement in locomotor recovery (limb axis, base of support, BBB locomotor scale) compared with those of untrained injured controls.[65,114,208] In the current study, we monitored the impact of one-week of locomotor treadmill training on the lower extremity muscles one week-post mid-thoracic spinal cord contusion injury

by studying changes in fiber CSA and MHC composition. In this context our findings are unique and suggest that early locomotor training can be effective in halting the atrophic process and improving the rate of recovery by restoring fiber CSA and phenotype of lower extremity muscles following contusion SCI. At the end of one week of locomotor training, no significant differences in fiber CSA were noted between the locomotor trained group and the control group in all the lower extremity muscles, except for the gastrocnemius which showed slightly lower CSA values. One possible explanation for the apparent smaller changes in the Gastroc is that measures for CSA were restricted to only the type I fibers and we feel that the training could have impacted fibers of other phenotypes which are predominant in the Gastroc and if we had averaged CSA across fibers of all the phenotypes we would have had a significant training impact. An interesting finding in this study is early training intervention resulted in similar rates of recovery in fiber CSA in all lower extremity muscles irrespective of their functional role or fiber type composition. In addition, locomotor training was effective in attenuating the shift MHC composition towards faster isoforms. There was a significant recovery in the proportion of fibers expressing slower isoforms in all the four lower extremity muscles. The restoration of the slow MHC phenotypes following locomotor training may also reflect a potential modulatory decrease in the velocity of shortening in the muscle.

In summary, the findings of this study are encouraging because they demonstrate that skeletal muscle atrophy and changes in muscle phenotype following two-weeks contusion SCI are similar across muscles with different functional roles and fiber types and early locomotor training starting one week post-SCI is effective in restoring fiber CSA and MHC isoform phenotype irrespective of the muscle functional role or fiber type. Although there are limitations in using animal models to understand human SCI recovery with locomotor training, the present

study demonstrates that early training interventions will be effective in ameliorate the debilitating effects of SCI in all the lower extremity muscles.

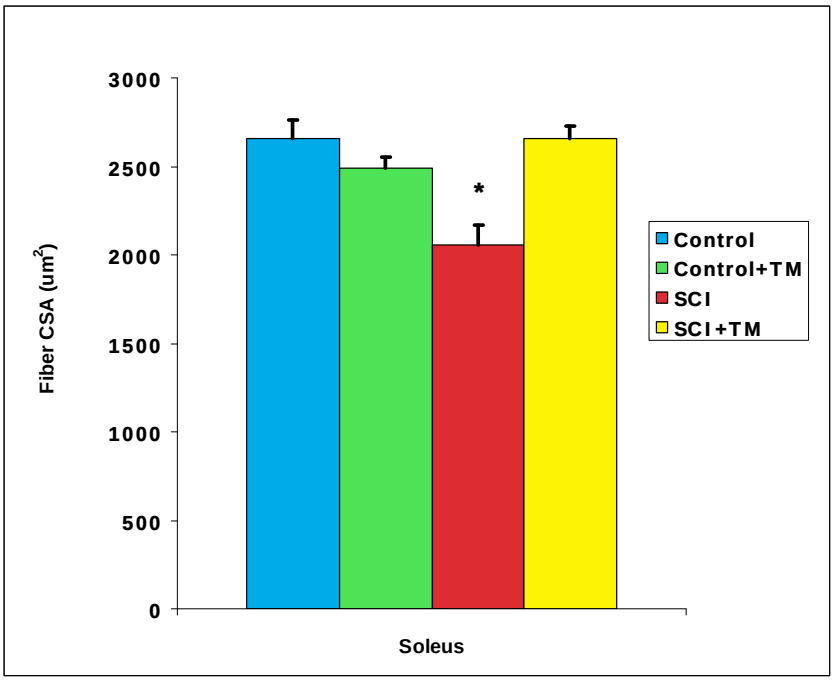


Figure 7-1. Average soleus muscle fiber CSA for control, control+TM, SCI no TM, and SCI + TM groups at 2 weeks post SCI. *Significantly smaller average muscle fiber CSA in SCI no TM compared to control, control+TM, and SCI + TM groups, $p<0.05$.

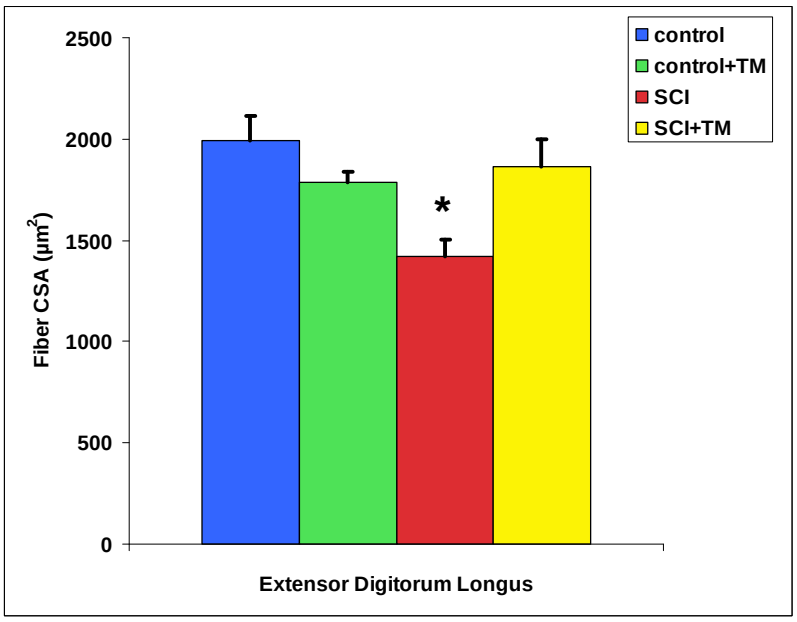


Figure 7-2. Average EDL muscle fiber CSA for control, control+TM, SCI no TM, and SCI + TM groups at 2 weeks post SCI. *Significantly smaller average muscle fiber CSA in SCI no TM compared to control, control+TM, and SCI + TM groups, $p<0.05$.

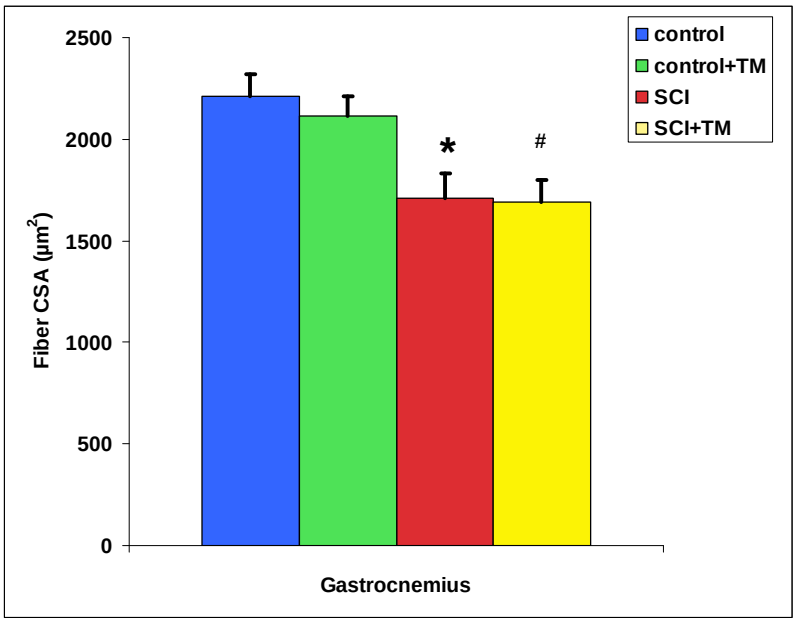


Figure 7-3. Average gastrocnemius muscle fiber CSA for control, control+TM, SCI no TM, and SCI + TM groups at 2 weeks post SCI. *Significantly smaller average muscle fiber CSA in SCI no TM compared to control and control+TM groups, $p<0.05$. #Significantly smaller average muscle fiber CSA in SCI + TM compared to control and control+TM groups, $p<0.05$.

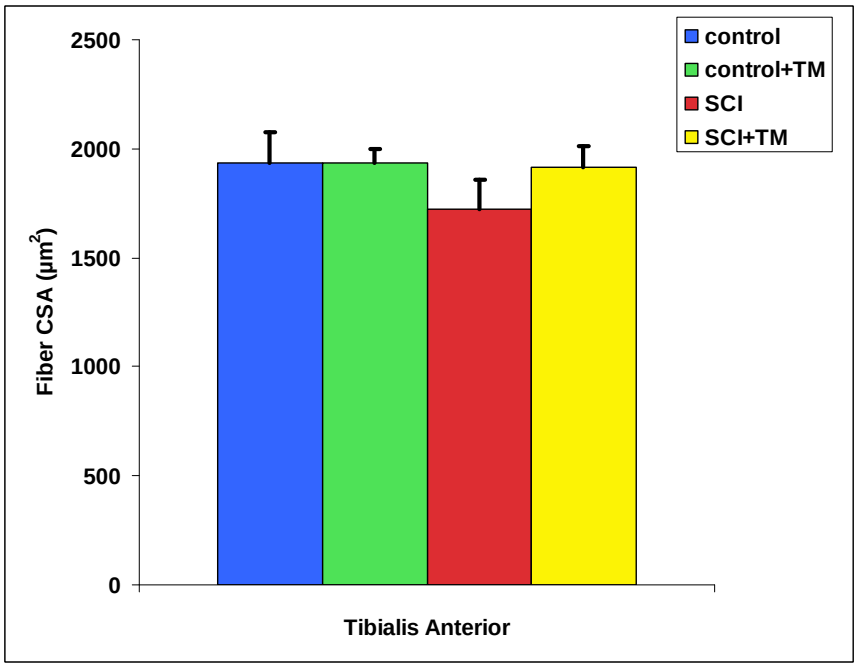


Figure 7-4. Average TA muscle fiber CSA for control, control+TM, SCI no TM, and SCI + TM groups at 2 weeks post SCI.

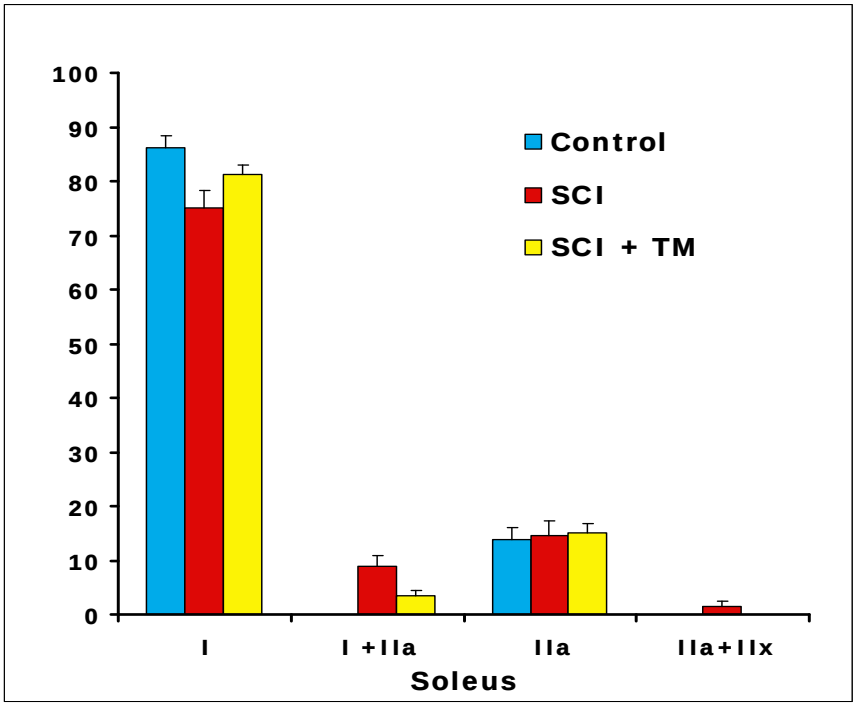


Figure 7-5. MHC based fiber type composition of rat soleus from control, control+TM, SCI, and SCI+TM groups.

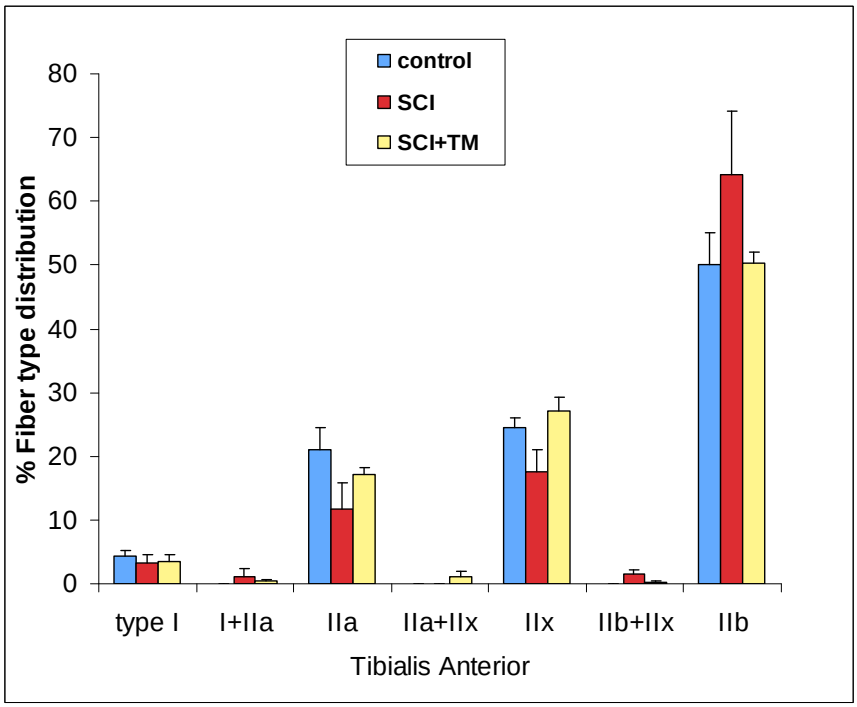


Figure 7-6. MHC based fiber type composition of rat TA from control, control+TM, SCI, and SCI+TM groups.

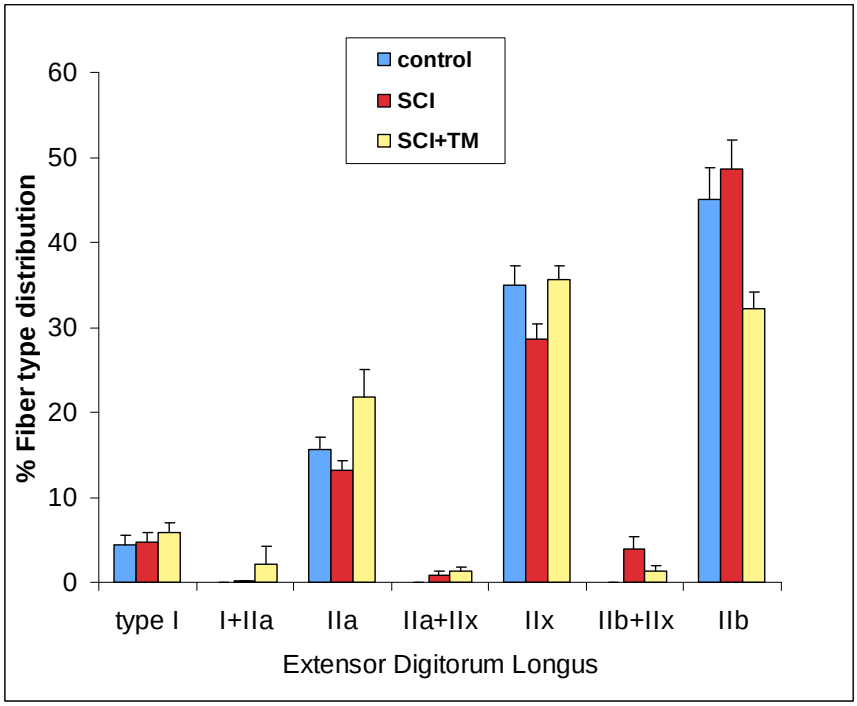


Figure 7-7. MHC based fiber type composition of rat EDL from control, control+TM, SCI, and SCI+TM groups.

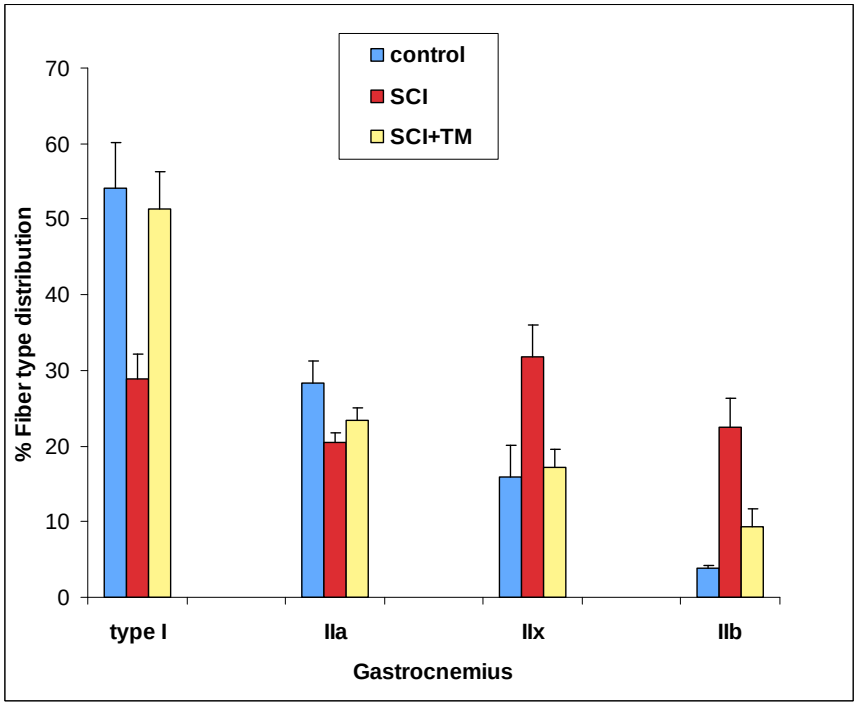


Figure 7-8. MHC based fiber type composition of rat gastrocnemius from control, control+TM, SCI, and SCI+TM groups.

CHAPTER 8
SKELETAL MUSCLE RECOVERY AND REGENERATION FOLLOWING MODERATE CONTUSION SPINAL CORD INJURY AND LOCOMOTOR TRAINING

8.1 Introduction

Incomplete spinal cord injury (SCI) is a debilitating human condition resulting in severe motor and sensory impairments below the level of injury.[52,196,219] In addition to effects directly related to CNS dysfunction, atrophy of skeletal muscle is a common problem associated with incomplete SCI.[45,52,163,206] Animal models of SCI have been used to characterize lesions, study mechanisms of recovery, and to develop and test therapeutic interventions.[14,73,220] Although the majority of current SCI's are incomplete (>55%), most animal studies of skeletal muscle adaptations after SCI have been done following complete spinal cord injuries.[45,93,221] Therefore, it may be relevant to use an animal model of incomplete SCI to study skeletal muscle adaptations and the effects of therapeutic interventions. One such model is the contusion injury model which mimics the mechanism and histopathologic sequela associated with human incomplete SCI.

Skeletal muscle possesses a remarkable ability to recover after damage or disuse atrophy. One way skeletal muscle can recover involves the activation, proliferation, and differentiation of a resident population of myogenic cells called satellite cells.[122,133] These satellite cells induce muscle plasticity by differentiating and fusing to form multinucleated myotubes which repair or replace damaged or lost muscle fibers. Activation of satellite cells seems to require growth factors, such as insulin-like growth factor 1 (IGF-1), which have also been shown to increase muscle protein and DNA content.[122,222,223] Once activated, the upregulation of these cells can be identified by using various molecular markers.[123,128] Activated satellite cells committed to myogenic lineage express the transcription factor Pax-7. The myogenic regulatory factors, MyoD and Myf5 are involved in satellite cell proliferation, and the expression of transcription factor myogenin signals satellite cell terminal differentiation into myotubes. Finally, the appearance of

embryonic myosin signals new fiber formation. Despite the fact that SCI results in significant muscle atrophy, only a few studies have been done to assess satellite cell activity after SCI, and they have been done only after complete SCI. Furthermore, the results of these studies have suggested greater satellite cell activity in slow-twitch extensor muscles than in fast-twitch flexor muscles. Therefore, it may be relevant to study satellite cell activity after incomplete SCI and to do this study in both slow-twitch extensor and fast-twitch flexor muscles.

Locomotor treadmill training has been used as a therapeutic intervention to improve lower extremity function and/or walking after SCI. Although locomotor treadmill training programs promote changes in spinal cord properties, motor unit morphology, and functional recovery,[40,198,206,207] the particular contributions of this therapy towards skeletal muscle plasticity and function are still unclear. Numerous studies have shown that therapeutic interventions like treadmill training, resistance exercise, and cycling training result in the activation of satellite cells.[224-226] However, to our knowledge, no studies have been done to assess the effects of short term locomotor training after incomplete SCI on satellite cell activation and regulation.
The objectives of this study were 1) to investigate the effects of incomplete SCI (moderate contusion model) on satellite cell activity in a slow-twitch extensor and a fast-twitch flexor muscle in the rat and 2) to examine the influence of one week of locomotor training on satellite cell activity in these muscles in spinal cord-injured animals. Satellite cell activity was monitored by measuring IGF-1 and by using various molecular makers.

8.2 Methods

8.2.1 Animals

Twenty-four Sprague–Dawley rats (female, 228–260 g, weighing 250-290gms; Charles River, NJ, USA) were used in this study. Six rats per group were assigned to either a SCI-

training group, a SCI-no training group, a control group, a control training group. Six of the injured rats received treadmill locomotor training (TM) starting 1 week after SCI, when the surgical staples were removed and soft tissue had healed sufficiently to tolerate training without increasing the risk of trauma at the incision site. Training in the TM group was implemented for 5 consecutive days, 20 min/trial, 2 trials/day. The additional 8 injured rats received no exercise intervention (no TM). The rats were housed in a temperature-controlled room at 21 °C and were provided unrestricted access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida

8.2.2 Contusion Spinal Cord Injury

Spinal cord contusion injuries were produced using a protocol described previously. A NYU (New York University) impactor was used to produce the injuries. Briefly, a 10g weight was dropped from a 2.5-cm height onto the T8 segment of the spinal cord which was exposed by laminectomy. The entire procedure was carried out under sterile conditions. All injuries were performed under ketamine (100mg/kg)-xylazine (6.7mg/kg) anesthesia. Animals received two doses of Ampicillin (100mg/kg) per day for 5 days starting on the day of surgery. To prevent dehydration, subcutaneous lactated Ringer's solution (5 ml) was administered after completion of the surgery. Animals were given Buprenophine (0.05 mg/kg) and Ketoprofen (5.0 mg/kg s.c.) for pain and inflammation over the first 36 hours after SCI. The animals were kept under vigilant postoperative care, including daily examination for signs of distress, weight loss, dehydration, and bladder dysfunction. Manual expression of bladders was performed 2-3 times daily until spontaneous voiding returned (~2 weeks), 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.

8.2.3 Locomotor Treadmill Training

Animals with spinal cord injury were exposed to treadmill locomotor training. Training was started on post-operative day 7. There were two reasons for this. First, on day 8 the surgical staples were removed and soft tissue had healed sufficiently so that trauma could be avoided at the incision site. Second, red porphyrin expression around the eyes, a symptom associated with stress, disappeared within a week post SCI. Therefore, animals could be trained without apparent discomfort and stress at this time.

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 meter/minute) 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 training sessions of 2 hours. Training consisted of quadrapedal treadmill stepping. Body weight support was provided manually by the trainer as necessary. The level of body weight support was adjusted to make sure that the animals' hind limbs did not collapse and was gradually removed as locomotor capability improved. Typically, when all rats had profound paraplegia, assistance was provided to place the rat hind paws in plantar stepping position during training.

8.2.4 Tissue Harvest

At the time points indicated above, the soleus and TA muscles of both legs were dissected and snap-frozen at resting length in isopentane, pre-cooled in liquid nitrogen and stored at –80 ^{0}C.

8.2.5 Determination of IGF-I Protein Concentration

Frozen soleus and TA muscles were rinsed with PBS to remove excess blood, homogenized in 20 mL of PBS and stored overnight at -20^{0}C. The homogenates were then centrifuged for 5 minutes at 5000 x g. The supernatants were utilized for measurements of total IGF-1 in a commercially available ELISA kit specific for rodent IGF-I (R&D Systems, Minneapolis, MN). IGF-I concentration was calculated based on a standard curve generated from recombinant rat IGF-I. This kit detects total rodent IGF-I, and the measurements are not affected by the presence of IGF-I binding proteins or IGF-II.[227] This kit has been validated for the determination of rat IGF-I at 30-3000 pg/ml with an intra-assay precision of ~4.3% and an inter-assay precision o f ~6.0%.[227] All samples were measured on a micro-plate reader at 450nm in duplicate.

8.2.6 Immunohistochemistry Measurements

Cryostat sections (10 μm) in a transverse plane were prepared from the central portion of each muscle taken from both legs and mounted serially on gelatin-coated glass slides. Immunocytochemical reactions were performed on cryostat sections with anti-laminin and anti-Pax-7 or anti-embryonic myosin antibody at various dilutions. Rabbit anti-laminin (Neomarker,Labvision, Fremont, CA) was used to outline the muscle fibers. Sections were incubated with rabbit anti-laminin and the anti-Pax-7 (1:300) and anti-embryonic myosin (1:10) antibodies (4°C over night), followed by incubation with rhodamine-conjugated anti-rabbit IgG and Fitc-conjugated anti-mouse IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands). Stained sections were mounted in mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) and kept at 4°C to diminish fading. Stained cross-sections were photographed (10 x magnification) by using a Leica fluorescence microscope (Leica Microsystems, Bannockburn, IL) with a digital camera. Regions of the stained sections from

each muscle were randomly selected for positive pax-7 and embryonic myosin. The proportions of each fiber type were determined from a sample of 150–250 fibers across the entire section of each muscle. The pixels setting used for conversion of pixels to micrometers was 1.5 pixels to 1 μm2 for a 10 x objective.

8.2.7 Western Blot Analysis

Quantification and expression of MyoD, Myf5 and Myogenin will be measured using Western blot analysis. Muscles will be homogenized in a lysis buffer with Fast-Prep homogenizer machine at 13,000 RPM at 40C for five minutes. The supernatant will be preserved for protein assay. Protein will be denatured by heating samples to 95-100 0C for 5 minutes. Protein will be measured using BCA protein assay kit from Pierce. Electrophoresis will be performed by mixing 40-50 µg protein with 5X loading buffer and loading it to 4-15% SDS page gel from Bio-Rad. Protein will then be transferred from gel to nitrocellulose membrane. Blocking will be conducting using 5% non fat dry milk in TBS/T (Tris Buffer Saline, Tween-20). Blot with be incubated with primary antibody overnight at 40C according to manufacturer's instruction. Blot will then be incubated with HRP-conjugate secondary antibody for 40 minutes to one hour at room temperature. Finally protein will be detected using Western Blotting Luminal Reagent from Santa Cruz.

8.2.8 Data Analysis

All statistical analyses were performed with SPSS, Version 13.0.1. Tests for normality will be performed on all of the measured variables before proceeding with tests of statistical inference. Results are expressed as mean ± standard error of mean. One-way ANOVA was used to test for differences among the four experimental groups. In an effort to control for multiple comparisons, post-hoc analysis was implemented. For all analyses, significance was established when $p < 0.05$.

8.3 Results

8.3.1 Effects of Incomplete Spinal cord Injury and Locomotor Training on Insulin-Like Growth Factor-1 (IGF-1) Expression

Activation and regulation of satellite cells seems to require IGF-1. Therefore, an enzyme-linked immuno sorbent assay (ELISA) was used to quantify IGF-1 levels in the slow-twitch soleus and fast-twitch tibialis anterior (TA) muscles. Measurements were made in animals two weeks after SCI (SCI group) and in animals with one week of locomotor training one week after SCI (SCI +locomotor training group). The IGF-1 levels in the soleus muscle were approximately four-fold higher in the SCI group in comparison to the control group ($p<0.01$) (Fig.8-6A). Locomotor training lead to an approximately 2-3-fold increase in soleus IGF-1 levels in comparison to the untrained SCI group. SCI or locomotor training did not affect IGF-1 levels in the TA muscle (Fig.8-6B). These results indicate that there are significant increases in IGF-1 protein levels following SCI in the slow-twitch extensor soleus muscle. In addition, locomotor training results in additional increases in soleus IGF-1 protein levels in spinal cord-injured animals.

8.3.2 Effects of Incomplete Spinal Cord Injury and Locomotor Training on Pax-7

Pax-7 is a transcription factor, and its expression is upregulated in activated and proliferating satellite cells. Therefore, immunohistochemistry was used to study the frequency of Pax-7-positive myonuclei in transverse sections of the soleus and TA muscles (Figs 8-1 A& B). Although the number of Pax-7-positive myonuclei seemed to be increased in the soleus and TA muscles two weeks after SCI, these increases were not significant. However, locomotor training lead to an approximately 2-fold increase in Pax-7 positive-myonuclei in the soleus muscle and an approximately 50% increase in the TA muscle in comparison to the untrained SCI group. These results indicate that locomotor training leads to significant increases in Pax-7-positive myonuclei

in both the slow- twitch extensor soleus and fast-twitch flexor TA muscles in spinal cord-injured animals. Furthermore, it is interesting to note that the number of Pax-7-positive fibers in the soleus muscle was almost 2-fold higher compared to the TA muscle in the SCI and locomotor training groups ($p<0.05$).

8.3.3 Effects of Incomplete Spinal Cord Injury and Locomotor Training on Myogenic Regulatory Factors – (MyoD, Myf5 and Myogenin)

Myogenic regulatory factors (MRFs) are known to regulate satellite cell activity as the cells pass through the different stages of muscle regeneration. Upon satellite cell activation, MyoD and Myf5 are thought to be involved in promoting satellite cell proliferation and progression toward terminal differentiation. Therefore, western blot analysis was used to quantify MyoD and Myf5 protein levels in the slow-twitch soleus and fast-twitch TA muscles. Two weeks after SCI, there was no significant difference in the MyoD or Myf5 protein levels in both the soleus and TA muscles in comparison to the control group. Although we saw increases in MyoD and Myf5 protein levels in both muscles following locomotor training in spinal cord-injured animals, the results were not significant. (Fig.8-2 & 3).

Myogenin levels are known to be upregulated when satellite cells begin their terminal differentiation program. Therefore, we measured myogenin protein levels using western blot analysis. Two weeks after SCI, the myogenin levels in the soleus muscle were approximately 2-3-fold higher in the SCI group in comparison to the control group ($p<0.05$) (Fig.8-4A). However, locomotor training did not result in any change in myogenin levels in the soleus muscle in comparison to the SCI untrained group. SCI or locomotor training did not affect myogenin protein levels in the TA muscle (Fig.8-4B). Overall, these results show that following SCI soleus myogenin levels were increased, but MyoD and Myf5 levels were not significantly altered. In

addition, locomotor training did not significantly impact any of the MRF protein levels in either of the muscles.

8.3.4 Effects of Incomplete Spinal Cord Injury and Locomotor Training on Embryonic Myosin

Expression of embryonic myosin indicates new fiber formation. Therefore we used immunohistochemistry to study the frequency of embryonic myosin-positive muscle fibers in transverse sections of the soleus and TA muscles (Figs 8-5 A& B). Although the number of embryonic myosin-positive fibers seemed to be increased in the soleus muscle two weeks after SCI, the increase was not significant. However, one week of locomotor training lead to an approximately 3-fold increase in embryonic myosin-positive in the soleus in comparison to the untrained SCI group ($p<0.001$). In contrast, SCI resulted in a significant increase in the embryonic myosin-positive-fibers in the TA muscle, while locomotor training in spinal cord-injured animals had no effect ($p<0.01$). It is also interesting to note that the numbers of embryonic myosin positive fibers in the soleus muscle was almost 6-fold higher compared to the TA in the SCI and locomotor training group ($p<0.05$). These results indicate that SCI alone without training results in significant increases in embryonic myosin in the TA, while locomotor training in combination with SCI results in significant embryonic myosin levels in the soleus.

8.4 Discussion

Atrophy in skeletal muscle has been shown to be associated with a loss of myonuclei independent of the manner the atrophy was induced. Satellite cells once activated proliferate and migrate to the site of muscle fiber atrophy and then differentiate to either form a new fiber or help repair the damaged fiber[122,128,228-230] To better understand recovery from contusion-SCI induced atrophy and loss of myonuclei, we studied satellite cell activity in two different muscles, the soleus and the TA. In our current study, we found that there was an increase in satellite cell

activity following contusion SCI. In addition, locomotor training initiated one-week following contusion-SCI further substantially increased satellite cell activity. Furthermore, we found the increase in satellite cell activity to be different in the slow-extensor soleus compared to the fast-flexor tibialis anterior (TA).

Satellite cell activation requires the influence of growth factors.[122,231,232] In our study, we saw significant increases in IGF-1 levels in the soleus following contusion SCI, which was further significantly increased with locomotor training. However, IGF-1 levels remained stable in the TA muscle. We studied the growth factor IGF-1 as it has been shown to stimulate satellite cell activation, proliferation and differentiation in the rat muscle and also to increase myonuclei number and myofiber size.[233,234] In addition, exercise results in elevated IGF-I levels, which could result in an increase in satellite cell activation and a compensatory hypertrophy of skeletal muscle, thereby making it relevant to study IGF-1 levels following SCI and locomotor training.[223,235,236] There are a few studies which involved SCI and IGF-1. In the first study by Resnick *et al.*[237] 2004, IGF-1 levels were up-regulated within the spinal cord following contusion injury. Even though the regions of IGF-1 measurement were different in our studies, we felt since IGF-1 presence is systemic in nature our studies related and both studies reported increased IGF-1 levels following contusion SCI. However, Versteegden *et al.* 2000[238] found no changes in IGF-1 mRNA levels 30 days following transection SCI and cycling training which is different to our results. In retrospect, the authors of the study felt that they waited too long to measure IGF-1 levels and transient increase in IGF could have occurred at an earlier time point. Interestingly, based on our results we feel the increases in IGF-1 following SCI and locomotor training could have triggered satellite cell activity in the soleus, while no significant changes in IGF-1 levels in the TA mirrors the significant lack of satellite cell activity in this muscle.

In the present study, SCI resulted in stimulating similar increases in pax-7-positive myonuclei in both soleus and TA muscles. In addition, locomotor training lead to a further substantial increase in pax-7-positive myonuclei in both soleus and TA, with the soleus almost having the twice the number of Pax-7-positive myonuclei compared to the TA. Pax-7 was quantified in the current study because activated satellite cells express Pax7. [125,229]Most activated satellite cells then proliferate, thereby down regulating Pax7 and then differentiate.[125,239,240] Furthermore, treadmill training is known to stimulate satellite cell activity in skeletal muscle, thereby making it relevant to study Pax-7 activity following SCI and locomotor training. In our results, the soleus and TA had similar levels of Pax7 following contusion SCI. These results are different from that seen in other types of SCI like isolation were they found the slow twitch muscle to have higher satellite cell expression compared to the fast twitch.[216] We however feel the contusion SCI presenting itself with spared spinal tracts and varied activation levels could be the reason for the similar expression of Pax-7 in both slow and fast muscle. However, locomotor training following SCI resulted in twice the number of Pax-7 positives compared to the TA. The reason for this may be due to differences in muscle activity, with the slow soleus being 20-times more active and frequently recruited than the fast flexor TA muscle during locomotor tasks.[241] To summarize, both SCI and locomotor training resulted in activating satellite cells in both the soleus and TA, with the soleus having higher levels of satellite cell activation compared to the TA due to its higher recruitment in loading conditions.

Surprisingly in this study we did not find any significant changes in myogenic regulatory factor (MRF) protein levels in both muscles following SCI or SCI + locomotor training except for myogenin in the soleus. Myogenin protein levels in the soleus were significantly elevated following SCI and SCI + locomotor training in comparison to controls. We studied MRF

proteins as they are transcription factors that influence and modulate the proliferation and differentiation of the satellite cells.[23,113,122,216,238] Specifically, Myogenin is a MRF protein that regulates the terminal differentiation of satellite cells to myoblasts. Based on our myogenin results we suggest that following SCI and locomotor training one-week-post –SCI leads to significant terminal differentiation of satellite cells into myoblasts.[128,242] However there was no difference in myogenin levels between the SCI trained and un-trained group. These results were similar to a study by Versteegden *et al*. 1999[113], who observed similar significant increases in soleus myogenin expression 10 days after transection SCI and cycling training 5 days post-SCI with no differences in myogenin levels between the trained and un-trained SCI groups. We suggest it could be because myogenin proteins levels may have reached their maximum levels following SCI and the training does not cause any further increase. In summary, we feel that even though there were no increases in MyoD and Myf5 protein levels, significant increases in myogenin following SCI and SCI + locomotor training indicate terminal differentiation of satellite cells only in the soleus.

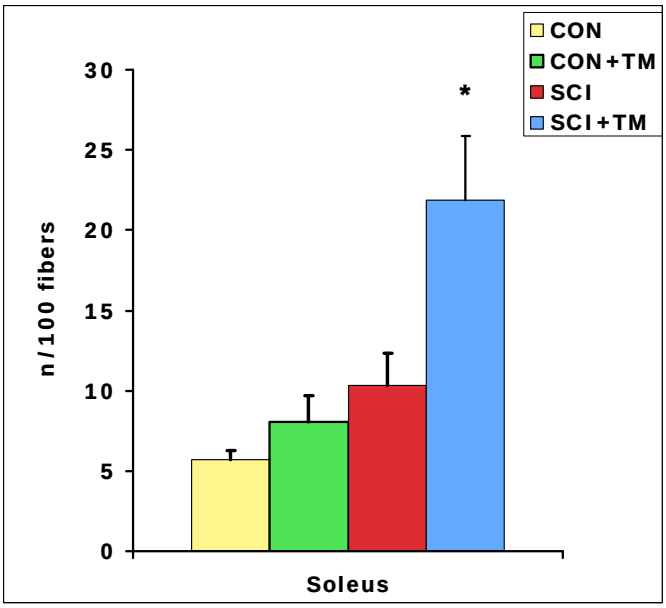
Although after SCI there were a few embryonic myosin positive muscle fibers in the both the soleus and TA compared to no positives in the controls, their numbers were very minimal. However following locomotor training we noticed significant increases in embryonic myosin numbers in the soleus muscle after locomotor training, while in the TA the numbers remained insignificant. In the present study we quantified the developmental isoform of the myosin heavy chains termed as embryonic myosin because it sequentially precedes the appearance of definitive adult myosin heavy chains in rats and is an indicator of new fiber formation.[63,91,213] Based on our results we can suggest that locomotor training following SCI resulted in new fiber formation in the soleus muscle. A point of interest in this study which is similar to a study by Yablonka-

Reuveni *et al.* 1994 [239]is that the numbers of embryonic myosin positive cells in our study were less than half the number of positive satellite cells, indicating that not all the satellite cell descendants entered the phase of terminal differentiation, suggesting muscle plasticity through regeneration does directly related to activated satellite cell numbers especially following contusion SCI.

So in conclusion were satellite cells involved in the exercise induced maintenance of muscle fiber size following contusion SCI? Even though there are limitations in study and alternate theories, we suggest that satellite cell activation to form new fibers could be one of the pathways in which the soleus recovers after contusion SCI and locomotor training. One of the limitations of the study was that MRF levels of MyoD and Myf5 which indicate satellite cell proliferation did not significantly change in both the SCI and locomotor training group in both muscles. A plausible explanation we feel is that these proteins are transiently expressed, and the time points we used only provide snapshots of satellite cells or MRF activity through their entire cycle and hence we might have missed the expression of these proteins. We also have a few suggestions regarding the lack of satellite cell activity in the fast TA. First, there was very less atrophy in the TA to start of with and hence there was less muscle plasticity required to recover from the atrophy. Also, in a fast muscle like the TA, myonuclear number is significantly high and therefore satellite cells may not have been required to restore myonuclear levels.

In summary, atrophy following contusion SCI may be associated with myonuclear loss. Increase in satellite cell activity and new fiber formation might be potential mechanisms to compensate for atrophy and myonuclear loss in the soleus and locomotor training might accelerate the recovery of the soleus muscle through muscle regeneration as a response to increased activity, while in the TA; it might be an order of events which need further

investigation. Overall this study provides more information on the exercise induced contribution of satellite cells towards muscle plasticity through regeneration after moderate contusion SCI.



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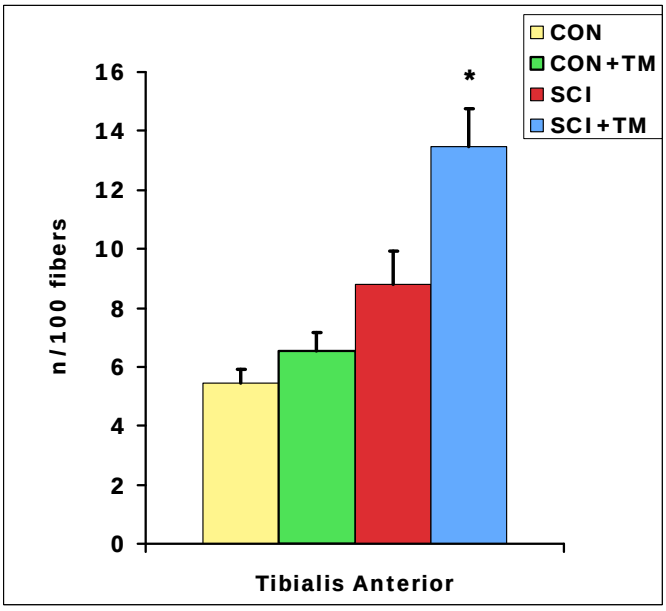
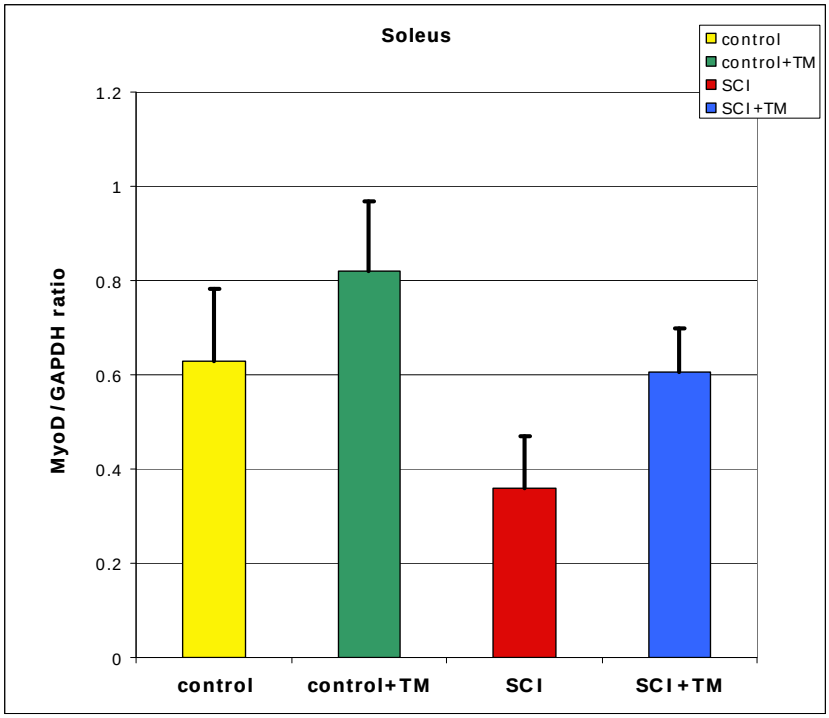


B

Figure 8-1 Pax-7 staining for the (A) soleus and (B) tibialis anterior muscle. * Significant difference between SCI+TM group from the other groups ($p<0.05$)



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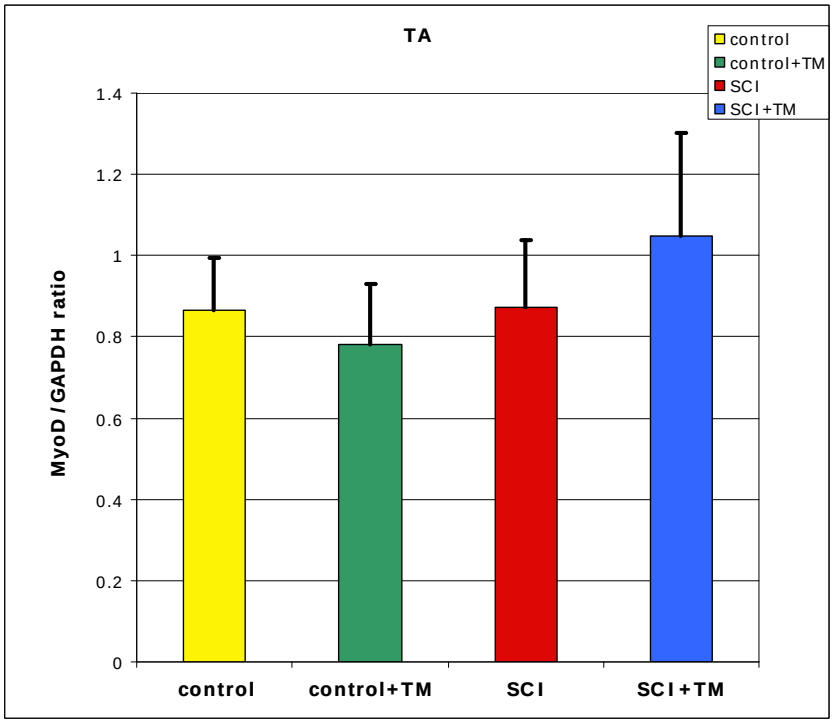
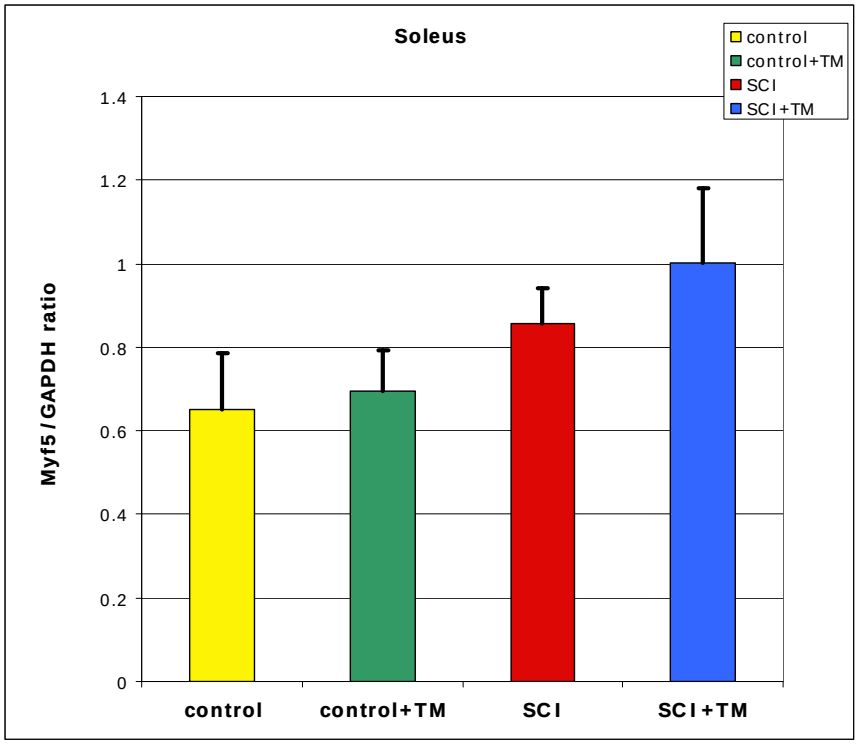


B

Figure 8-2 MyoD protein levels in the (A) soleus and (B) TA muscle.



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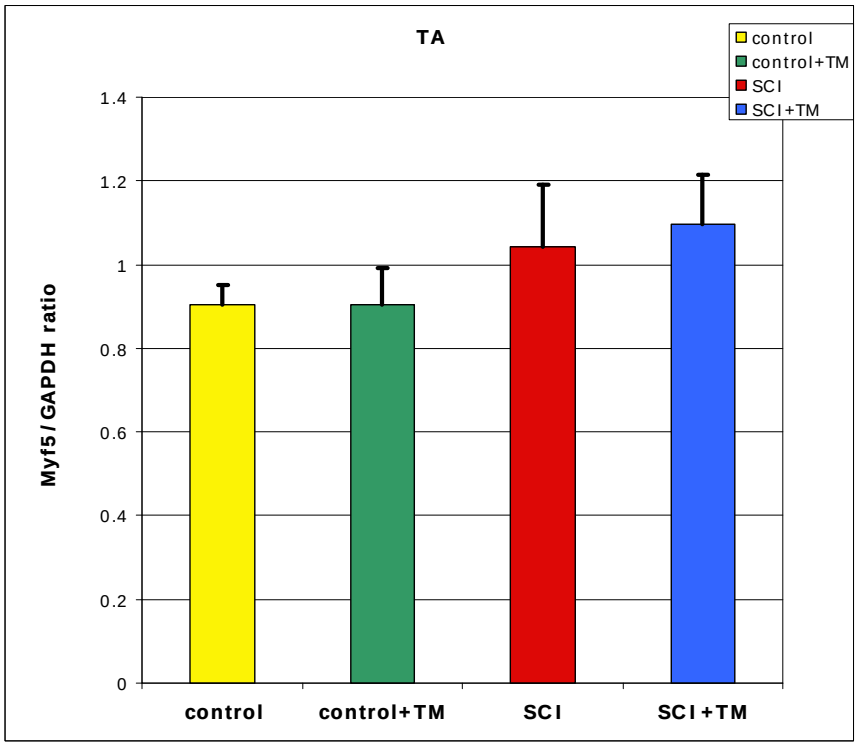
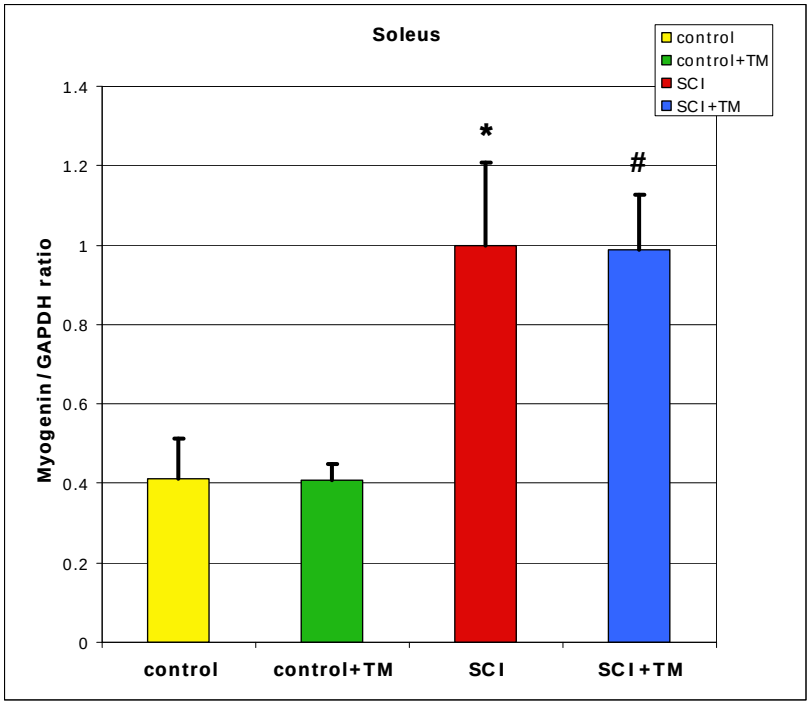


B

Figure 8-3 Myf5 protein levels in the (A) soleus and (B) TA muscle.



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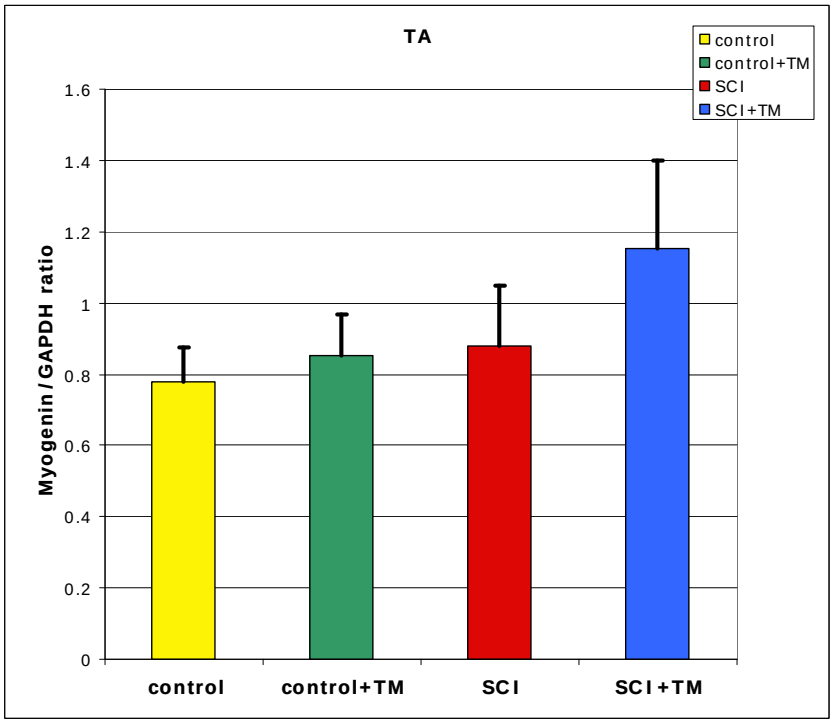
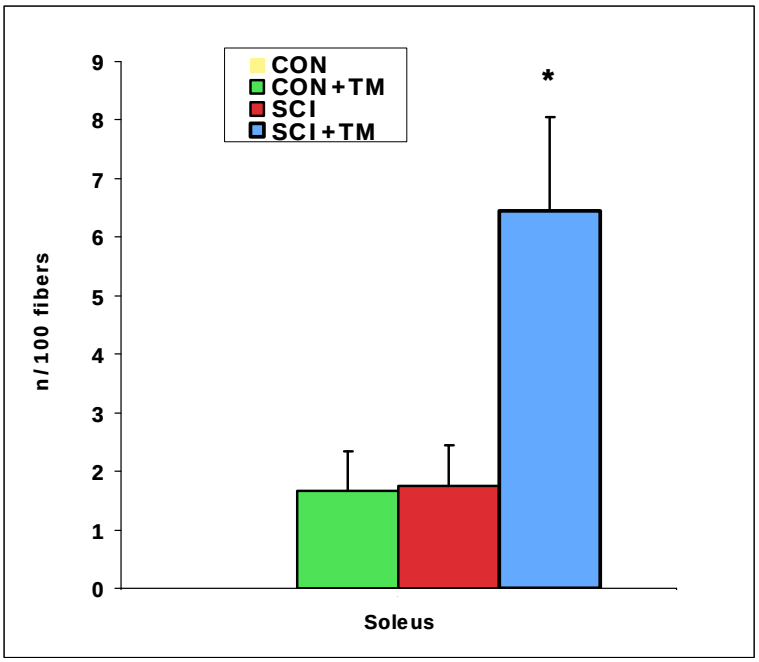


B

Figure 8-4 Myogenin protein in the (A) soleus and (B) TA muscle.* Significant difference between the SCI no training group and the control groups ($p<0.05$). # Significant difference between SCI+TM group from the other groups ($p<0.05$).



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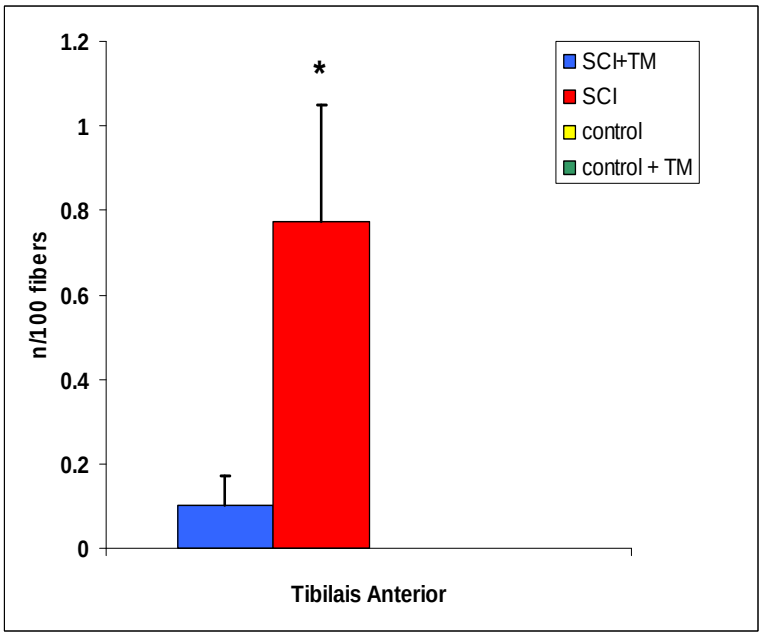
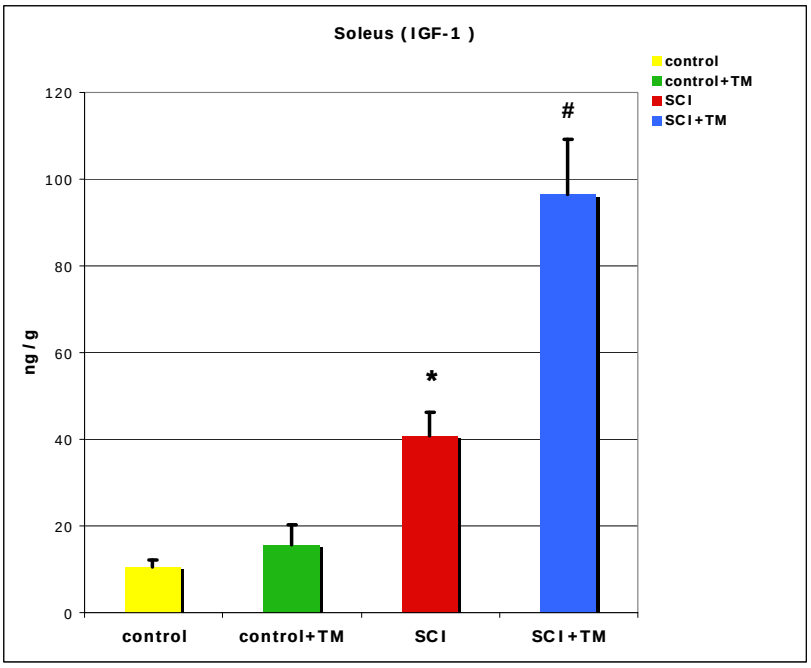


B

Figure 8-5 Embryonic myosin positives. (A) soleus and (B) TA muscle.* Significant difference between the SCI group and the other groups ($p<0.05$).



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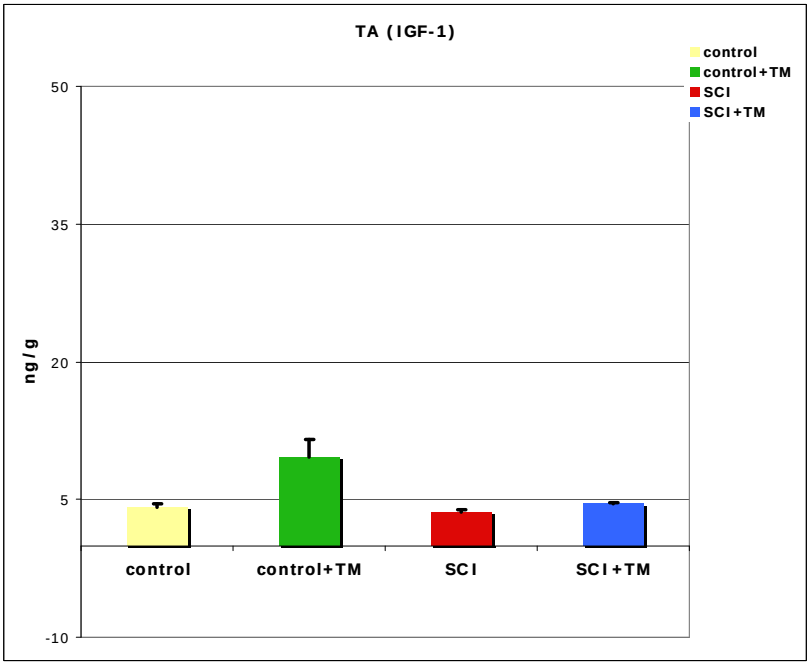

B

Figure 8-6 IGF-1 levels. (A) soleus and (B) TA muscle. * Significant difference between the SCI no training group and the control groups. # Significant difference between SCI+TM group from the other groups ($p<0.05$).

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BIOGRAPHICAL SKETCH

Arun Jayaraman was born in Chennai, India. He received his bachelor's in physical therapy from Dr. MGR Medical University in 2000 and his master's in hospital management from Loyola Institute of Business Administration in 2001. He also worked as an in-patient physical therapist in cardiac rehab in the Institute of Cardio-Pulmonary Diseases in Chennai till the year 2001. He received his advanced master's of science in physical therapy from Georgia State University, Atlanta, GA, in the year 2003. He joined the doctoral program in rehabilitation science at the University of Florida in the fall of 2003.