FACTORS THAT INFLUENCE MUSCLE TORQUE AFTER SPINAL CORD INJURY

by

CHRISTOPHER SCOTT BICKEL

(Under the direction of Kirk J. Cureton and Gary A. Dudley)

ABSTRACT

Electromyostimulation (EMS) is often used in rehabilitation settings to evoke contractions in paralyzed muscle. Unfortunately, EMS inherently causes greater fatigue than voluntary activation. Muscles that are unloaded are susceptible to contraction-induced muscle injury. Therefore, the purpose of this study is to investigate factors that can influence torque production, such as variable-frequency train stimulation and contraction-induced muscle injury, in patients with spinal cord injury (SCI).

In the first study, able-bodied (AB) and SCI subjects had their Muscle Quadriceps Femoris (m. QF) stimulated with constant-frequency trains (CFT) (six 200-μs square wave pulses separated by 70-ms) or variable-frequency trains (VFT) (identical to CFT except initial interpulse interval separated by 5 ms). After a fatigue protocol that consisted of 180 contractions (50% duty cycle), the isometric peak torque was reduced by 44%, 56%, and 67% in AB, acute SCI, and chronic SCI groups, respectively. The VFTs enhanced the torque-time integral by 18% compared to CFT in AB subjects and VFTs had a much smaller effect in SCI patients.

The second study utilized magnetic resonance (MR) images and EMS to evaluate the susceptibility of paralyzed muscle to contraction-induced injury. MR images were taken of the m. QF prior to, immediately post, and three days post EMS, which consisted of 80 isometric contractions. The relative muscle cross-sectional area (CSA) activated and injured was determined by the number of pixels with an elevated T2 signal. EMS resulted in a decline of peak torque by 66% and 37% for SCI and AB subjects, respectively. EMS activated 66% of AB muscle QF of which 2% of the activated muscle was injured. In contrast, the SCI group had 25% of their activated muscle injured due to EMS.

These results indicate that VFT stimulation does not appear to augment torque in SCI patients to the same extent as AB subjects. They also suggest that electrically-elicited isometric contractions are sufficient to evoke muscle injury in SCI patients.

INDEX WORDS: Spinal cord injury, Electrical stimulation, Fatigue, Muscle injury

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DEDICATION

Dr. Gary A. Dudley

Since July 9, 2002 I have been reminded that "…we rejoice in our sufferings, because we know that suffering produces perseverance, perseverance produces character, character produces hope, and hope does not disappoint us." Romans 5:3

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CHAPTER I

INTRODUCTION

Spinal cord injury (SCI) is a condition that affects approximately 200,000 individuals in the United States, with an additional 10,000 new cases occurring each year [50]. Complete SCI results in the loss of motor and sensory function below the level of injury. Patients suffering from complete SCI are nearly always confined to a wheelchair for the remainder of their life. The lack of both activation and loading of the affected extremities results in smaller muscles with a reduced resistance to fatigue.

Chronic diseases such as cardiovascular disease, obesity, and type II diabetes are tightly linked to physical inactivity [17]. In fact, it has been argued that physical inactivity is now the primary risk factor for the development of cardiovascular disease [18, 48]. It is not surprising then that SCI patients, who are significantly limited in their physical activities, are at a much greater risk (228%) for dying of cardiovascular disease than able-bodied individuals [43]. While there may be several factors that contribute to the greater mortality rate from cardiovascular disease in the SCI population, physical inactivity is clearly one of the most significant.

The muscle atrophy that occurs as a result of SCI is quite remarkable. Studies on the quadriceps femoris (QF) of SCI patients several years after injury indicate the QF is only 1/3 the size of able-bodied (AB) subjects matched for age, height, and weight [38]. The small muscle mass that is observed in patients after SCI is the primary limitation to the patient's ability to consume oxygen during exercise [39]. Electromyostimulation (EMS)

has been used to activate paralyzed muscle for many years and has shown promising results. One of the problems SCI patients encounter during EMS is that the muscle mass activated is too small and is not sufficient to evoke a central cardiovascular response. With that in mind, there is currently great interest in developing programs to increase muscle mass to allow EMS exercise to be more efficacious and potentially improve cardiovascular fitness in the SCI population.

Skeletal muscle that has been unloaded for several weeks is at risk for contraction-induced injury. Studies on both humans and lower mammals have clearly shown that sustained periods of inactivity and unloading put muscle at risk for injury when contractions are initiated [54, 67]. Muscle injury can contribute to declines in torque not associated with muscle fatigue that is due to central or metabolic factors [7]. Magnetic resonance imaging has been used as a tool to measure muscle injury in able-bodied subjects [34, 56]. Other common measures of muscle injury include reports of delayed onset muscle soreness and muscle enzymes circulating in the blood [25]. Complete SCI patients do not have sensation and, therefore, cannot report soreness scores and serum creatine kinase levels do not provide specific information regarding the extent of muscle injury. Therefore, MRI is a logical choice to measure muscle injury in this population. The extent of muscle injury in SCI patients using EMS has yet to be investigated and is addressed in this research.

There are other factors that contribute to the SCI muscle's ability to produce torque during exercise. EMS inherently induces more fatigue that would occur during voluntary activation [2]. This is primarily due to the synchronous stimulation of fast and slow motor units during electrical stimulation which leads to much greater force loss than

orderly (slow to fast), asynchronous recruitment during voluntary effort. There is currently great interest in finding methods to reduce torque loss during EMS. Variable-frequency train (VFT) stimulation has been shown to reduce fatigue in AB individuals [10, 14, 64]. VFTs take advantage of the "catch-like" property of skeletal muscle by varying the frequency within a train of stimulation. Short, then long, inter-pulse intervals (IPI) within a stimulation train enhance the rate of rise in torque, as well as peak torque, and therefore the torque-time integral by as much as 30% in fatigued muscle [14, 60]. This type of stimulation has yet to be tested in SCI patients, a population that relies on EMS for muscle activation and in whom fatigue is highly problematic.

Purpose

Patients with SCI are considered to be prime candidates for EMS therapy to improve their health and wellness and to potentially increase their function. One potential problem that needs to be considered prior to starting an SCI patient on an EMS training program is that they may be more susceptible to muscle injury than their able-bodied counterparts. Simple isometric contractions may cause significant muscle damage in paralyzed muscle. MRI can be used to assess the amount of muscle activated after an exercise bout as well as to assess the amount of muscle damage that may occur for up to seven days following eccentric exercise. The second problem that needs to be addressed is the increased fatigue that occurs after SCI, which is further complicated by the large amount of fatigue that occurs with electrically-induced muscle contractions. Therefore, this study has two primary objectives: 1) To evaluate the efficacy of variable-frequency

train stimulation in paralyzed muscle and 2) To evaluate the extent of exercise-induced injury to paralyzed muscle.

Specific Aims:

1. To determine if VFT stimulation augments force in fatigued paralyzed muscle to the same extent as non-paralyzed muscle.
2. To determine if electrically-induced isometric muscle contractions cause muscle injury in paralyzed and non-paralyzed muscle.

Hypotheses:

1. SCI patients show greater fatigue over repeat bouts of isometric contractions as well as within a single bout.
2. VFT stimulation results in greater torque-time integral, peak torque, and a shorter rise time than CFT stimulation in SCI subjects who have been injured less than one year.
3. Eighty electrically induced, isometric contractions causes muscle damage as determined from MRI.
4. The amount of muscle injured after electrically-elicited isometric contractions is relatively greater for SCI than able-bodied individuals.

Limitations of the Study

One of the limitations to this study is that it only provides information as to whether or not isometric contractions induce muscle injury in spinal cord injured subjects. It does not directly provide information about whether muscle injury limits hypertrophy, but it provides data concerning whether or not contraction-induced muscle injury is an issue in this subject population. Further studies need to be conducted; taking into account the extent of muscle injury that can be caused by contractions, to see if this knowledge improves the training regimens of paralyzed muscle. The VFT stimulation might augment force in a fatigued paralyzed muscle, but future studies will also need to be conducted using them for a training program to see if they are, in fact, beneficial.

CHAPTER II

REVIEW OF THE LITERATURE

Over 200,000 people in the United States have suffered a spinal cord injury (SCI), with an additional 10,000 individuals injured each year [50]. SCI is a condition that primarily affects young adults with ~55% of injuries occurring in individuals between the ages of 16 and 30 years of age [51]. The American Spinal Injury Association has developed a classification system based on impairments for individuals with SCI. The scale ranges from A-D, with ASIA A, complete SCI, considered the most severe where the individual has neither motor nor sensory function below the level of injury. ASIA B, C, and D are considered incomplete and are graded in severity with ASIA D being the least severe because the individual has partial preservation of motor function and at least half of the key muscles below the level of injury have a muscle grade of 3 or more.

Skeletal muscle adaptations to complete SCI

Complete SCI has been shown to have many deleterious effects to skeletal muscle below the level of injury. For example, SCI causes tremendous muscle atrophy, decreases force output, and reduces the resistance to fatigue in affected skeletal muscle [21-24, 36, 38, 49, 57, 59]. Grimby et al[36] was one of the first to describe the adaptations to skeletal muscle fibers in patients years after their injury. They biopsied the vastus lateralis, gastrocnemius, soleus, and deltoid muscles of patients with complete SCI and found that the fibers of the muscles below the level of injury were small in size, and low in succinate

dehydrogenase (SDH) and phosphofructokinase (PFK) activity. Not only did the muscles have low amounts of enzymes involved in ATP resynthesis, the affected muscles also had a high percentage of type II fibers, on the order of 90%. In contrast, the gastrocnemius and vastus lateralis muscles of able-bodied humans are on average, typically only 50% type II fibers, with the soleus being approximately 30% fast fibers [35]. The deltoid muscle from the SCI patients exhibited typical percentages of fiber types and enzyme levels. These results indicate that the affected muscle after SCI has a much greater energy demand, as it has been reported that the energy cost of contraction is greater in type II fibers [27, 37]. Couple the greater energy demand with the reduced ability to supply ATP and it is obvious that the SCI muscle will have a low resistance to fatigue.

The work by Grimby et al. in the late 1970's has been further supported by others more recently. Martin et al.[49] biopsied the tibialis anterior (TA) in SCI patients who were 2-11 years post SCI. They reported that the TA, which is typically 70% slow fibers in the average able-bodied person, was 85% type II in the SCI subjects [35, 49]. Thus, there was a significant shift in the fiber type composition of the affected TA. Martin et al. also reported that the fibers were low in SDH activity and had a reduced capillary-to-fiber ratio by 35%. Rochester et al.[57] followed up the work by Martin et al. on the TA and found similar changes to the TA after SCI. The conclusion, based on the work of many different groups of researchers in regards to the skeletal muscle fiber composition in patients with SCI, is that the fibers become predominantly fast, small in size, and have a reduced ability to produce ATP.

The previous studies mentioned have all been cross sectional in design, so the time course for the alterations to human skeletal muscle fibers due to SCI was not known

until recently. Castro et al.[23] biopsied the VL in complete SCI patients starting at 6 weeks post-injury and following patients for up to 6 months. Interestingly, they reported that the fibers were much smaller in size compared to AB subjects, but the only fiber type change found was a shift from type IIa to IIb. This study also reported that SDH and glycerophosphate dehydrogenase (GPDH) levels actually increased during the first 6 months after SCI. Fibers were small in size, there was no shift from type I to type II fibers and the enzymes of energy supply were elevated. Surprisingly, this had no effect on fatigue resistance and the subjects actually showed much greater fatigue than AB controls at all timepoints up to 6 months after injury. This led the researchers to conclude that the mechanisms of fatigue in patients with SCI may not be similar to that of AB individuals.

The atrophic response after SCI can also be assessed grossly with magnetic resonance imaging (MRI). The changes in whole muscle size as a result of SCI can be seen very early after injury. Castro et al.[21] followed acute SCI patients over time and found that 6 months after injury, the quadriceps femoris (QF) was approximately 1/2 the size of able bodied controls as measured from magnetic resonance images (MRI) of the thigh. They reported significant atrophy in most muscles of the lower extremity, including the QF, hamstrings, gastrocnemius, and soleus. The only muscle that did not show significant atrophy was the tibialis anterior. The lack of atrophy in the TA could partly be explained by the constant stretch applied to it from the patient consistently being in a plantar flexed position. This may have slowed down the atrophic response because as mentioned from the biopsy studies, the TA muscle fibers are indeed smaller several years after injury [49, 57]. The alterations in CSA are even more obvious several

years after SCI. Hillegass and Dudley[38] used MRI to study chronic SCI patients who were on average, four years post complete SCI. The SCI patients' average QF CSA was about 1/3 the size of able-bodied controls matched for age, height, and weight. Thus, cross sectional and longitudinal studies indicate that muscle atrophy can be seen in individual muscle fibers and whole muscles with the use of MRI as early as 6 weeks post injury.

The reduced muscle size after SCI appears to be more severe than any other model of skeletal muscle unloading. For example, when comparing studies that have implemented either unilateral lower limb suspension (ULLS) or bed rest, the fiber atrophy in six weeks does not appear to be as extensive as that found in patients with SCI. Six weeks of ULLS resulted in ~10% reduction in fiber CSA across all fiber types [3]. Taking a different approach to unloading, 6 weeks of bedrest has been reported to induce a 17% reduction in fiber CSA [9]. The largest effect of unloading on muscle atrophy has been seen in patients with complete SCI. Castro et al. report a 35% reduction in fiber CSA across all fiber types when comparing fibers 6 weeks after complete SCI to those of height, weight, and age matched controls [23].

Muscle fatigue after SCI

As mentioned previously, the affected muscle fibers after SCI also have a decreased resistance to fatigue. Shields studied the fatigability of the paralyzed human soleus, a muscle that is highly fatigue resistant in AB persons [63]. It was reported that the chronically paralyzed soleus muscle of SCI patients had very low fatigue resistance and showed about an 80% decrease in torque after a modified Burke fatigue protocol [63]. Five

minutes into recovery, torque had only recovered by 60% in Shields' study. Hillegass and Dudley[38] used electromyostimulation (EMS) to determine the force generating ability of the available muscle over repeat bouts of isometric contractions. A consistent finding in their study was that the muscle maintained an ability to generate torque, but the level of force that could be developed was limited by the tremendous muscle atrophy that accompanies SCI. The SCI subjects could produce torque initially, but after the onset of fatigue, subjects had an inability to recover between sets.

The reduced size and increased fatigue in muscle after SCI appears to be quite severe compared with any other model of disuse. One interesting issue in regards to reduced force production after SCI is the inability of these patients to recover between sets to the same extent as able-bodied controls [38]. One suggestion to explain this fatigue is that the chronic SCI muscle has converted to a faster muscle with a low mitochondrial content thus reducing the ratio of energy supply to energy demand. Based on previous studies, this is a valid argument, as it is widely accepted that chronic SCI muscle is predominately fast [24, 36, 49, 58, 59, 61]. Another potential aspect that may contribute to the incomplete recovery between sets and the overall fatigue is the possibility of contraction-induced muscle injury. Based on the results of unloading studies in animals and humans, one would predict that muscle after SCI would be highly susceptible to exercise-induced damage [54, 67]. Studies of lower mammals have shown that 15 days of hindlimb suspension is sufficient to predispose the m. soleus to contraction induced muscle damage [67]. Warren et al[67] showed a 15% reduction in the force generating ability of the soleus after just 15 isometric contractions. Eccentric contractions are most likely to cause contraction-induced muscle damage in the able bodied population [30], but it has been

suggested that eccentric actions are NOT necessary to evoke damage in unloaded muscle [67].

Muscle damage is the most common injury associated with physical activity [41]. The unloading of skeletal muscle causes muscle fiber atrophy, decreases in strength and increases the muscle's vulnerability to exercise induced dysfunction and muscle injury [29, 36, 49, 54, 66, 67]. Studies of lower mammals have shown that mild activity such as reambulation after hindlimb suspension evokes more extensive muscle injury than would normally be expected of such a simple task [44, 45]. In humans, it has been shown that when one of the lower limbs is unloaded for 6 weeks, the unloaded limb is more susceptible to eccentric exercise-induced muscle injury than that of the loaded limb [54]. The fact that muscles are predisposed to injury after prolonged periods of immobilization or unloading may be problematic in a rehabilitation setting, where muscles are routinely subjected to exercise after such conditions. Currently, there is great interest in improving the overall condition of muscles in patients with spinal cord injury, but the issue of contraction-induced muscle injury has yet to be addressed.

MRI measurements of muscle activation and injury

Magnetic resonance imaging (MRI) has been used to measure muscle activation and exercise-induced muscle injury [1, 2, 4-6, 8, 26, 28, 32-34, 53, 54, 56, 62, 65]. Immediately following contraction there is an increase in the signal intensity and longer T2 relaxation times which are associated with increased contractile activity [1, 2, 32]. These same changes also peak 2-6 days after an exercise that causes muscle injury [47, 65]. Delayed changes in the MR signal correlate well with increased muscle soreness and serum creatine kinase

(CK), both of which are used as indicators of muscle fiber injury [25, 47, 65]. It has long been established that the acute T2 increase with exercise is due to changes in the intracellular water chemistry [52]. The second T2 increase in muscle that arises after activities which evoke muscle damage follows a time-course that is consistent with other markers of injury. The delayed increase has been assumed to reflect edema as well. One problem with the assumption that the delayed T2 increase is due to edema is that a few studies have shown a persistent increase in T2 for 2-3 months after injury [34]. Three months is more than enough time for the acute inflammatory response to subside so the adaptation may reflect a chronic adaptation to exercise induced muscle injury. In fact, Foley et al[34], studied this long-term adaptation and concluded the edema may not be the primary factor contributing to the long-term adaptation, but the mechanisms still remain unclear. What is certain is that the T2 increases 2-6 days after a damaging bout of exercise.

Prior et al[56] recently used MRI to measure both the muscle activation and subsequent muscle injury that developed after voluntary single leg eccentric-only contractions. Their goal was to relate the increase in T2 that developed several days after injury to the increase of T2 that occurs due to muscle activation. They actually found that there was not a relationship between the magnitude of T2 increase immediately after exercise and that which occurs due to muscle injury [56]. This was probably due to a disproportionately greater amount of muscle injury to a muscle that is rarely loaded, in this case, the rectus femoris. The interesting part of the study is that they attempted to quantify both muscle activation and injury in the same muscles. Because SCI patients are likely to train with EMS, the question can be asked: how much of the recruited muscle would be damaged during electrically elicited contractions? Complete SCI patients do

not have sensation so therefore they cannot report soreness scores and serum CK levels do not provide specific information regarding the extent of muscle injury, therefore MRI is a logical choice to measure muscle injury in this population.

Electromyostimulation and fatigue

Electromyostimulation (EMS) is a rehabilitation technique that is used after SCI to evoke muscle contractions in paralyzed muscle. When used appropriately, the electrically-induced contractions can cause an increase in heart rate, oxygen uptake, and other typical acute responses to exercise [31, 55]. EMS has also been used to evoke training adaptations within paralyzed muscle that are typical after similar training in able-bodied individuals undergoing an exercise program. One training adaptation that has shown conflicting results in SCI patients is muscle hypertrophy. Although a few studies have used EMS to evoke minimal muscle hypertrophy after chronic SCI, there are others that show no increase at all [24, 49]. What limits the progress of muscle hypertrophy after chronic SCI? One possible confounding factor could be the incidence of contraction-induced muscle injury to the fibers being studied. If the muscle is damaged during training, the force output may be compromised and the training stimulus may not be sufficient to evoke the desired response.

Another impairment associated with SCI is a reduced resistance to fatigue. This phenomenon is particularly problematic during electrical stimulation of paralyzed human skeletal muscle. The synchronous stimulation of fast and slow motor units during electrical stimulation leads to much greater force loss than orderly (slow to fast), asynchronous recruitment during voluntary effort [2, 42]. With the order of recruitment

being altered and the muscle already having a decreased resistance to fatigue, the paralyzed muscle is at a disadvantage in performing repeated, high force contractions. Variable frequency trains (VFT) that take advantage of the "catch-like" property of skeletal muscle have been used to counter fatigue in able-bodied individuals [11-16, 46, 60]. Short then long interpulse intervals within a stimulation train enhance the rate of rise in torque as well as peak torque, and therefore the torque-time integral, especially in fatigued muscle [11, 12, 14, 16, 19, 46, 60]. While this type of EMS has been suggested to be ideal for patients with SCI, there are only a few published studies on SCI patients that use stimulation with varying frequencies within a train [20, 40]. The two published studies that used stimulation with varying frequencies did not use VFT stimulation that takes advantage of the "catch-like" property of skeletal muscle. Studies have repeatedly shown that a VFT with a brief interpulse interval of 5 ms followed by longer interpulse intervals of 70 ms is ideal for the able-bodied population to enhance force in a fatigued muscle from 19-36% [14, 60]. Karu et al.[40] has also reported that a 5 ms interpulse interval for a doublet train is ideal to enhance the time to fatigue by 36% in able-bodied subjects. Four SCI patients were also included in the study by Karu et al., and they report that there were no major differences between the able-bodied and SCI subjects.

In summary, there are multiple factors that influence the torque production of SCI muscle. There are changes to the morphological, biochemical, and mechanical properties of SCI muscle, which have negative effects on the muscle's ability to generate torque. Muscle fatigue is problematic due to the inherent nature of EMS as well as the observation that the mechanisms of fatigue in SCI muscle may not be the same as AB muscle. For example, contraction-induced muscle injury may limit force production

during repeated bouts of stimulation. One countermeasure to fatigue that has shown promise in reducing fatigue in AB muscle is the use of variable frequency train stimulation. VFTs have not yet been proven to be efficacious in SCI muscle. These issues are addressed in the following chapters.

CHAPTER III

VARIABLE FREQUENCY TRAIN STIMULATION OF SKELETAL MUSCLE AFTER SPINAL CORD INJURY[1]

[1]Bickel, C. Scott, Jill M. Slade, Leslie R. VanHiel, Gordon L. Warren and Gary A. Dudley. To be submitted to *J. Rehabil. Res. and Dev.*

Abstract

Skeletal muscle after spinal cord injury (SCI) becomes highly susceptible to fatigue. Variable-frequency trains (VFT) enhance force in fatigued human skeletal muscle of able-bodied (AB) individuals. VFTs do this by taking advantage of the "catch-like" property of skeletal muscle. However, mechanisms responsible for fatigue in AB and SCI subjects may not be the same, and the efficacy of VFT stimulation after SCI is unknown. Accordingly, we tested the hypothesis that VFT stimulation would augment torque-time integral in SCI subjects. M. Quadriceps femoris (QF) was stimulated with constant frequency trains (CFT) (six 200-μs square wave pulses separated by 70-ms) or variable frequency trains (a train identical to the CFT except that the first two pulses were separated by 5 ms) in SCI and AB subjects. After 180 contractions (50% duty cycle), isometric peak torque decreased 44%, 56%, and 67%, in AB, acute SCI, and chronic SCI groups, respectively. In fatigued muscle, VFTs enhanced the torque-time integral by 18% in AB subjects, 6% in chronic SCI patients, and had no effect in acute SCI patients when compared to the corresponding CFT. The much faster rise times in SCI subjects (~80 ms vs. 120 ms in AB subjects) probably contributed to the inability of VFTs to enhance torque-time integrals in SCI patients. The results suggest that the use of VFT stimulation in patients with SCI may not be as efficacious as it is in able-bodied persons.

Key words: Electrical stimulation, fatigue, spinal cord injury, catch-like property

Introduction:

Affected skeletal muscle after spinal cord injury (SCI) has a reduced ability to generate and maintain force. Significant muscle atrophy and conversion to a fast fiber composition with low levels of oxidative enzymes has been reported to contribute to the muscle's compromised performance after SCI [13, 18, 21]. However, Castro et al[9] has reported increased fatigability after SCI unrelated to changes in metabolic enzymes associated with ATP synthesis. Electrical stimulation is often used in the rehabilitative setting for training of paralyzed muscle. However, activation with electrical stimulation will inherently cause more fatigue than contractions of voluntary effort [1]. This is probably due to the repetitive, synchronous stimulation of fast and slow motor units during electrical stimulation in apparent disregard for the size principle regarding the orderly recruitment of motor units and the inability to recruit additional motor units to offset fatigue [1, 16]. Thus, the ability of a patient with spinal cord injury to do multiple sets or repetitions of activities is compromised due to 1) the nature of the muscle itself and 2) the means of activation.

In light of the aforementioned, developing a means to counter force loss during electrical stimulation of paralyzed muscle would be beneficial. Variable frequency train (VFT) stimulation has received considerable interest for countering fatigue in skeletal muscle of able-bodied (AB) individuals [4, 6, 22, 23]. VFTs take advantage of the "catch-like" property of skeletal muscle by varying the frequency within a train of stimulation [5, 7]. Short, then long, inter-pulse intervals (IPI) within a stimulation train enhance the rate of rise in torque, as well as peak torque, and therefore the torque-time integral by as much as 30% in fatigued muscle.

The presence of an initial, brief IPI has been reported to occur during voluntary activation of human motor units [3, 12, 19]. Therefore, the constant frequency pattern of stimuli that is used during conventional electrical stimulation may not be similar to what occurs under voluntary conditions. This difference may also be amplified in fatigued muscle, as Griffin et al.[12] have reported that the prevalence of initial, brief IPIs becomes more common as the muscle fatigues. Thus, VFT stimulation may be more similar to voluntary motor unit activation in regards to pulse frequency than is CFT stimulation, which is typically used clinically. It should also be noted that despite VFT stimulation having similar pulse frequencies to voluntary motor control, the method of activation is still quite different because electrically-stimulated motor unit activation does not adhere to the size principle.

The above issues raise the question as to whether VFT stimulation can augment torque in subjects with SCI, a patient population that could benefit from an optimal stimulation pattern. Accordingly, we tested the hypothesis that VFT stimulation would enhance the torque-time integral in patients with complete spinal cord injury. Subjects either months or years after SCI had their m. quadriceps femoris (QF) subjected to electrical stimulation with both VFT and CFT stimulation before and after a fatigue protocol. Because the muscles of SCI subjects produce very low levels of force as compared to (AB) individuals (~10-15% of AB maximal voluntary contraction), we subjected AB subjects to the same protocol starting with similar initial peak torques.

Methods:

Spinal cord injured patients were screened prior to participation to ensure that their m. quadriceps femoris (QF) could elicit modest force from the electrical stimulation without simultaneous muscle spasms that would interfere with the testing trains. Twenty-two SCI subjects completed all phases of the tests without complications. The SCI subjects were further divided into two groups, acute (injured < 1 year) and chronic (injured > 1 year). The acute SCI group (SCI-A) included 10 subjects (28 ± 2 yr, 177 ± 4 cm, 77 ± 6 kg, 1 female, mean ± SE) with their level of injury ranging from C3-T8 and the average weeks post injury was 23 ± 5. The chronic SCI group (SCI-C) included 12 subjects (36 ± 6 yr, 180 ± 2 cm, 78 ± 4 kg, 2 females) on average 8 ± 2 years post injury with the level of injury ranging from C6-T9. An AB control group was also studied (26 ± 1 yr, 175 ± 3 cm, 83 ± 7 kg, 1 female). Subjects had no history of lower-extremity pathology and signed informed consent prior to testing. The methods were approved by the Institutional Review Boards of both the University of Georgia and Shepherd Center.

QF experimental setup: The m. QF was studied during stimulated isometric contractions essentially as described previously [1, 2, 8, 10, 11, 14, 15, 24]. Briefly, subjects were seated in a custom built chair with the left hip and knee secured at approximately 90° of flexion. The leg was firmly secured to a rigid lever arm with an inelastic strap to ensure that m. QF would perform only isometric contractions. The moment arm was established by placing a Rice Lake 2000A load cell (Rice Lake Weighing Systems, West Coleman Street, Rice Lake, WI, USA) parallel to the line of pull and perpendicular to the lever arm. Two 8- x 10-cm surface electrodes (Uni-Patch, P.O. Box 1271, 1313 West Grant Boulevard, Wabasha, MN, USA) were placed on the distal m. vastus medialis and the

proximal m. vastus lateralis to allow sufficient recruitment of m. QF as done previously [1, 4].

Electrical stimulation and force recordings: An electrical stimulator (model DS7AH, Digitimer Limited, 37 Hydeway, Welwyn Garden City, Hertfordshire, AL7 3BE, England) was triggered with a personal computer using an A/D board (model KPCI 3108, Keithley Instruments, 28775 Aurora Road, Cleveland OH, USA) and a customized program written with TestPoint software (v4.0, Capital Equipment Corporation, 900 Middlesex Turnpike, Billerica, MA, USA). The stimulator delivered six 200-μs square wave pulses with either a CFT or a VFT. The CFT consisted of six pulses separated by a 70-ms interpulse interval (IPI) while the VFT had a 5-ms IPI between the first and second pulses followed by four additional pulses separated by 70-ms IPIs. The VFT utilizing only one brief IPI was chosen because it has been reported to augment force in fatigued human skeletal muscle [6]. Torque from the load cell was sampled at 10-kHz by computer using the A/D board.

Experimental procedure: The m. QF of each subject was investigated. The current necessary to elicit ~25% of the SCI subjects' estimated maximum voluntary contraction (MVC) was determined. Estimated MVC for each SCI subject was set as the torque equal to 1.3 times body weight because maximal voluntary torque for knee extension approximates 130% of body weight in able bodied individuals [1, 11, 14]. If 25% of the subject's estimated MVC could not be attained, the maximum amount of torque that could be evoked was used. AB subjects were tested after all SCI subjects completed the study with the goal of matching force levels with the acute SCI group, as their muscles would be expected to be similar in fiber composition [9]. Subsequently, m. QF

was potentiated with 6-pulse CFTs that were delivered one every 5 seconds until force plateaued. When the muscle was highly potentiated, a CFT and a VFT were delivered. The m. QF was then fatigued using 180 six-pulse CFTs delivered at a 50% duty cycle, which results in substantial fatigue in both SCI and AB subjects. Immediately following the 180^{th} train, a CFT and a VFT were delivered in random order.

Torque-time integral, peak torque and the time from 20% to 80% of peak torque (T20-80) were determined from the torque recordings. A 2 x 2 (time x train type) repeated measures analysis of variance was run on each variable using SPSS (v. 10.0). Gain scores were calculated for the percent augmentation between the post fatigue trains (VFT vs. CFT) to determine if group differences existed.

Results:

Muscle fatigue: Stimulation prior to fatigue resulted in similar initial torques for all three groups (Table 3.1, $p > 0.05$). The relative torque decline during the 180 CFT contraction protocol showed group differences with AB showing less fatigue than the chronic SCI group ($p < 0.05$). The trend for the groups was that AB fatigue was less than the SCI-A followed by the SCI-C; 44%, 56%, and 67%, respectively.

Torque-time integral: All groups showed significant time-by-train interactions for the torque-time integral ($p < 0.05$). In the AB group prior to fatigue, the CFT torque-time integral was 3% greater than that for the VFT, but after the fatigue protocol, the VFT torque-time integral was 17% greater despite the fact the VFT is 65 ms shorter in duration (Figure 3.1). In contrast, the CFT had higher torque-time integrals, 10% and 6% for both the pre and post fatigue conditions, respectively for the SCI-A group. The SCI-C group demonstrated a 12% greater torque-time integral for the CFT when compared to

the VFT prior to fatigue, but after fatigue the VFT enhanced the torque-time integral by 6%. (Figure 3.1).

Peak Torque: Greater peak torque can contribute to the augmented torque-time integral that is evoked by VFT stimulation in fatigued muscles (see Figure 3.2). Peak torque responses to CFT and VFT stimulation were different among groups (Table 3.1). The AB group had a significant time-by-train interaction for peak torque ($p < 0.05$), with the VFT eliciting a peak torque 18% greater than that for the CFT in the fatigued QF. The SCI-A group showed neither a time-by-train interaction ($p > 0.05$) nor a main effect for train ($p > 0.05$), indicating that the type of train did not affect peak torque. The SCI-C group did not show a time-by-train interaction ($p > 0.05$) but did have main effects for type of train and time indicating that the train effect (higher peak torque for VFT) was the same both pre and post fatigue.

Time from 20% 80% of peak torque: Another factor that can contribute to an enhanced torque-time integral by VFT stimulation in fatigued muscle is a more rapid rise time (see Figure 3.2). The T20-80 for the AB group had a significant time-by-train interaction. The CFT T20-80 was 44% slower after the fatigue protocol when compared to the pre CFT T20-80, while the VFT T20-80 slowed by only 11% with fatigue (Table 3.1). The fact that the CFT showed such increased slowing of contraction compared to VFT probably contributed to the enhanced torque-time integral seen most remarkably in the fatigued muscles of the AB group. When comparing the T20-80 in the fatigued state, the VFT is 60% shorter than the CFT. The SCI groups did not show the same advantage of the VFT over the CFT in the T20-80 as for the AB group. The T20-80 post fatigue

when comparing VFT to CFT was 26% and 37% shorter in the SCI-A and SCI-C groups, respectively (Table 3.1).

Torque-time integral over the first 200ms (TT200): To verify that the greater torque-time integral observed for the VFT is primarily due to a more rapid rise time, we calculated the torque-time integral over the first 200 ms of each contraction (Table 3.1). This revealed in the AB subjects an 86% greater TT200 for the VFT compared to CFT in the fatigued muscle. The SCI-A and SCI-C groups showed much less enhancement of the VFT over the CFT on TT200, 27% and 41%, respectively.

Discussion:

The major finding of this study was that VFTs failed to enhance the torque-time integral in fatigued, paralyzed skeletal muscle. This could be due to the finding that the rise times were already so fast in the patients with SCI during CFT stimulation that providing an initial, brief IPI did not reduce the T20-80 or increase peak torque enough to augment the torque-time integral. The TT200 data supports this argument because the advantage of the VFT over the CFT was much smaller in the SCI groups. For the VFT to have an advantage over the CFT, the T20-80 must be shorter for the VFT and/or peak torque higher to counter the fact that the VFT train is 65 ms shorter than the CFT since the two trains have the same number of pulses. An analogy can be made to VFT stimulation in fresh muscle of able-bodied subjects. The VFT does not augment the torque-time integral in fresh muscle because a 60 ms increase in rise time with VFTs is not sufficient to counter the 65 ms longer train duration of the CFT. After fatigue, there is a slowing of contraction, which is made evident by much longer rise times, and the VFT exposes this by enhancing the rise time (by ~80 ms). This leads to the novel aspect

of VFT stimulation, which is an increased torque time integral despite the difference in train duration (~20%, see Figure 3.2).

The contraction rise times of the SCI subjects studied were already so fast that a shorter IPI at the beginning of the train was not sufficient to increase the torque-time integral. Even after fatigue, although there was ~60% increase in the T20-80 (compared to pre fatigue) of SCI subjects, the rise times were still about the same as for fresh muscle of AB subjects and, as previously mentioned, VFTs do not augment torque-time integrals of fresh AB muscle. The post fatigue VFT T20-80 were about 40 ms and 100 ms shorter in SCI and AB groups, respectively, when compared to their corresponding post fatigue CFT. It is evident that a 40 ms reduction is not sufficient to counter the 65 ms longer train duration of the CFT. Therefore, the brief IPI of the VFT was not adequate to counter fatigue in SCI muscle when the VFT has the same number of pulses as the CFT.

Might the VFT stimulation be advantageous if the train duration was increased by adding an additional pulse? This could potentially show benefit by increasing the torque-time integral as compared to CFTs, but adding an additional pulse could also increase fatigue. It has been suggested that fatigue might be related to the total number of pulses given, therefore, adding additional pulses to a train may cause more fatigue over time [17]. As Russ and Binder-Macleod[22] point out, adding an additional pulse may also cause more fatigue over time by decreasing the amount of rest between trains. Adding additional pulses to the VFT seems contrary to their potential benefit of reducing fatigue. Another potential manner in which the VFT could be shown to augment the torque-time integral in SCI patients is to shorten the train duration of both the CFT and VFT. For example, if a four-pulse train was used, the CFT would be 210 ms compared to a 145 ms VFT. The

initial pulse would be relatively more meaningful and a 40 ms reduction in rise time might significantly enhance the torque time integral for the shortened trains. The problem with having such a short train is that in terms of clinical electrical stimulation, the contraction may not be long enough to perform any functional activities.

It should be appreciated that spinal cord injury has a dramatic effect on skeletal muscle not equaled by many other conditions. The chronically injured muscle has a higher proportion of fast fibers that are small in size and that are low in enzymes involved in ATP resynthesis, thereby making them highly susceptible to fatigue. While VFT stimulation with the parameters used in the present study failed to show significant benefit in the SCI sample, they may still be useful in conditions not quite as extreme and, indeed, the VFTs have been shown to reduce fatigue in AB subjects. It is also possible that the mechanism(s) of fatigue may not be the same in SCI and AB subjects. Castro et al.[9] reported that muscle fatigue was unrelated to differences in the content of enzymes involved in ATP resynthesis. These authors suggested that the fatigue found in SCI patients may be partly due to contraction-induced muscle injury. In fact, short-term unweighting has been shown to increase the vulnerability to exercise-induced muscle injury in humans and lower mammals [20, 26]. It has also been reported that fibers 6 months post SCI have mismatched sarco(endo)plasmic reticulum calcium-ATPase and myosin heavy chain isoforms which could influence the fatigability of fibers [25]. Thus, SCI muscle may need to be conditioned first, by gradually increasing contractile activity over time, prior to the use of VFTs.

In conclusion, numerous studies, including the present one, suggest that VFTs augment the torque-time integral in the fatigued m. QF; this was certainly the case in the

AB group and to a lesser extent the SCI-C group, but it was not the case in the SCI-A group. Because SCI represents one of the most extreme conditions that human skeletal muscle is subjected, other conditions in which the muscle fibers have not been altered to the same extent may still benefit from these stimulation parameters.

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Table 3.1. Mechanical responses of m. quadriceps femoris for able-bodied (AB), acute SCI (SCI-A), and chronic SCI (SCI-C) to variable frequency train (VFT) and constant frequency train (CFT) surface electrical stimulation immediately pre and post 180 CFTs.

Table 3.1, legend
Values are mean ± SE, n = 10 (AB), 10 (SCI-A), and 12 (SCI-C). CFT, six 200-μs square wave pulses separated by 70 ms. VFT, first IPI only 5 ms.

Variable	Group	Train	Pre	Post
Peak Torque (N·m)	AB	CFT	26.7 ± 1.1	15.0 ± 0.7
		VFT	28.3 ± 1.1	17.7 ± 0.7
	SCI-A	CFT	26.2 ± 2.7	11.1 ± 1.0
		VFT	26.6 ± 2.8	11.5 ± 1.0
	SCI-C	CFT	29.6 ± 4.5	9.3 ± 1.5
		VFT	30.8 ± 4.6	10.9 ± 2.1
T20-80 (ms)	AB	CFT	121 ± 7	174 ± 8
		VFT	63 ± 5	70 ± 9
	SCI-A	CFT	85 ± 8	129 ± 12
		VFT	56 ± 5	95 ± 13
	SCI-C	CFT	72 ± 5	121 ± 9
		VFT	49 ± 4	76 ± 9
Torque-time integral – initial 200ms TT200 (N·m·s)	AB	CFT	2.3 ± 0.1	1.1 ± 0.1
		VFT	3.3 ± 0.2	2.0 ± 0.1
	SCI-A	CFT	2.7 ± 0.3	0.9 ± 0.1
		VFT	3.1 ± 0.4	1.1 ± 0.1
	SCI-C	CFT	3.2 ± 0.5	0.8 ± 0.1
		VFT	3.8 ± 0.6	1.2 ± 0.3

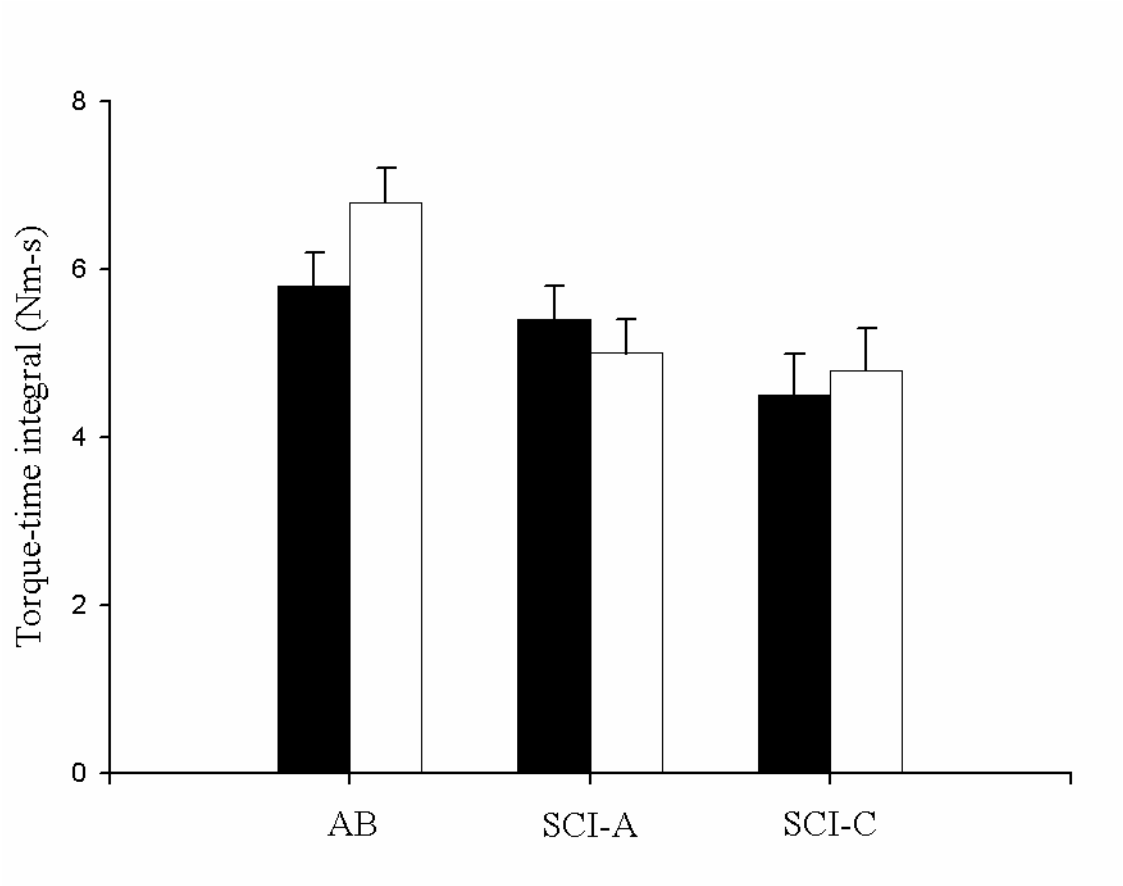

Figure 3.1: Torque-time integral for the post fatigue trains in all three groups. Filled bars represent constant frequency trains (CFT) and open bars are variable frequency trains (VFT). Augmentation by VFT was significantly greater in AB than SCI-A and SCI-C groups ($p < 0.05$)

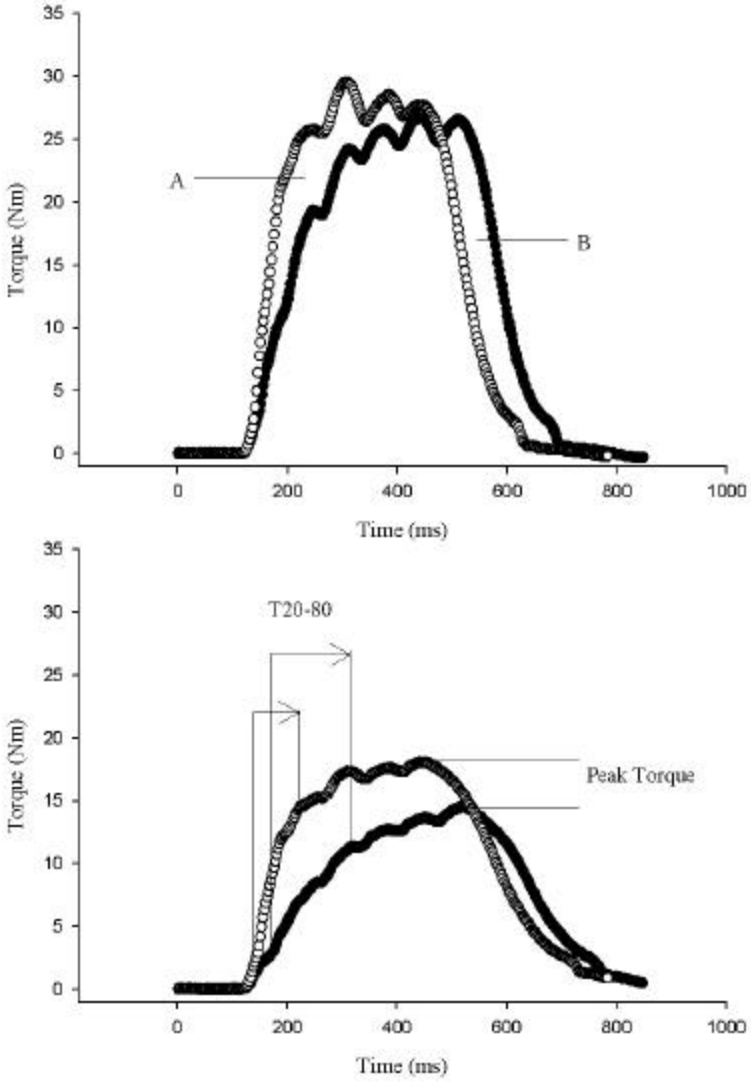

Figure 3.2: Representative torque tracings from an able-bodied subject for VFT (open circles) and CFT (closed circles) stimulation pre (upper panel) and post (lower panel) fatigue. Note that area A and B are about the same size in the upper panel, whereas in the lower panel, the corresponding A and B regions are markedly different. The torque-time integral (area under the curve) is increased by either reducing the T20-80 and/or increasing peak torque.

CHAPTER IV

LONG-TERM SPINAL CORD INJURY INCREASES SUSCEPTIBILITY TO ISOMETRIC CONTRACTION-INDUCED MUSCLE INJURY[1]

[1]Bickel, C. Scott, Jill M. Slade, and Gary A. Dudley. To be submitted to *Eur. J. Appl. Physiol.*

Abstract

Complete spinal cord injury (SCI) results in inactivation and unloading of affected skeletal muscles. Unloading causes an increased susceptibility of muscle to contraction-induced injury. This study used magnetic resonance imaging (MRI) to test the hypothesis that isometric contractions would evoke greater muscle damage to the m. quadriceps femoris (QF) of SCI subjects than that of able-bodied (AB) controls. MR images were taken of the m. QF prior to, immediately post, and three days post electromyostimulation (EMS). EMS consisted of 5 sets of 10 isometric contractions (2 s on/6 s off, 1 min between sets) followed by another 3 sets of 10 isometric contractions (1 s on/1 s off, 30 s between sets). Average muscle cross-sectional area (CSA) and the relative areas of stimulated and injured muscle were obtained from MR images by quantifying the number of pixels with an elevated T2 signal. SCI subjects had significantly greater relative area (90 ± 2 vs. 66 ± 4 %, $p < 0.05$) but a lesser absolute area (16 ± 3 cm^2 vs. 44 ± 6 cm^2, $p < 0.05$) of m. QF stimulated than AB controls. During EMS, peak torque was reduced by 66% and 37% for SCI and control subjects, respectively. Three days post EMS, there was a greater relative area of stimulated m. QF injured for the SCI subjects (25 ± 6 vs. 2 ± 1 %, $p < 0.05$). Peak torque remained decreased by 22% on day 3 in the SCI group only. These results indicate that affected muscle years after SCI is more susceptible to contraction-induced muscle damage compared to AB controls. They also support the contention that electrically-elicited isometric contractions are sufficient to cause muscle damage after a prolonged period of inactivity.

Key words: spinal cord injury, magnetic resonance imaging, electrical stimulation

Introduction:

Complete spinal cord injury (SCI) results in inactivation and subsequent unloading of affected skeletal muscle. Fibers of affected muscles have been reported to be small in size [9, 25], predominantly fast twitch [17, 34], and have a low resistance to fatigue [8, 33]. These factors contribute to the limited ability of muscle in SCI patients to produce and maintain torque, which could make training affected muscles to elicit muscle hypertrophy difficult to obtain. Another confounding factor that could potentially impact a training program for SCI patients, is an increased vulnerability to contraction-induced muscle injury after SCI, due to the extreme unloading and inactivation.

It is generally known that unloading causes affected muscles to be predisposed to contraction induced injury [29, 40]. Studies of lower mammals have shown that hindlimb suspension [21, 22, 40], immobilization [5], and spaceflight [31] put muscles at a higher risk of injury. Tasks that appear rather mild can evoke muscle injury that is uncharacteristic of the activity when performed under normal conditions. For example, reambulation after hindlimb suspension caused sarcomere lesions that were not observed in muscles studied after hindlimb suspension alone [22]. Human studies have also illustrated that unloading predisposes the muscle to greater muscle injury. Ploutz-Snyder et al.[29] found that eccentric contractions with relatively light loads caused muscle injury in the suspended limb of able-bodied individuals after unilateral lower limb suspension (ULLS).

Magnetic resonance imaging (MRI) has been used to measure muscle activation[1-4, 12, 15, 36] and exercise-induced muscle injury [6, 14, 16, 28-30, 38]. Immediately following a bout of contractions there is an increase in signal intensity and T2 relaxation times which are associated with the increased contractile activity [1, 2, 15]. Adams et al.[1, 2] demonstrated the

unique ability of MRI to map and quantify the pattern of muscle activation after electromyostimulation (EMS). The same changes in signal and T2 also peak 2-6 days after an exercise bout that induces muscle injury [24, 38]. Delayed changes in the MR signal correlate with other indicators of muscle fiber injury, such as delayed onset muscle soreness [38].

The use of EMS for the training of SCI patients has received considerable attention for many years. The potential for contraction-induced muscle injury in this population should be considered due to the long period of inactivity and unloading that the affected muscles of these patients endure. In the present study, MRI was used to assess the amount of muscle activated and injured after one bout of EMS consisting of 80 isometric contractions. We hypothesized that SCI patients would experience greater muscle injury after a bout of EMS not expected to cause injury to the muscles of able-bodied (AB) controls.

Methods:

Subjects: Eight AB (2 female) and eight SCI (1 female) subjects participated in this study. SCI level of injury ranged from C5-T9 and the average time post injury was 10 ± 3 years. Descriptive data on all subjects is listed in Table 1. SCI and AB subjects had no history of lower extremity pathology and signed informed consent prior to testing. Both groups were asked to refrain from ingesting non-steroidal anti-inflammatory medications and the AB group was not currently involved in lower extremity resistance exercise. All methods were approved by the Institutional Review Boards of the University of Georgia and Shepherd Center. Briefly, subjects had MR images of the left

thigh taken prior to, immediately after, and 3 days post electrical stimulation that evoked 80 isometric contractions.

QF experimental setup: The m. QF was stimulated essentially as described previously [2, 8, 9, 19]. Subjects were seated in a custom built chair with the hip and knee secured at approximately 90° of flexion. The leg was firmly secured to a rigid lever arm with an inelastic strap to ensure that the knee extensors could only perform isometric contractions. The moment arm was established by placing a load cell (model 2000A, Rice Lake Weighing Systems, West Coleman Street, Rice Lake, WI, USA) parallel to the line of pull and perpendicular to the lever arm. Torque was recorded from the load cell using a MacLab A-D converter (model ML 400, ADInstruments, Milford, MA, USA) sampling at 100 Hz and interfaced with a portable Macintosh computer (Apple Computer, Cupertino, CA, USA).

Electrical stimulation protocol: Two 8 x 10-cm surface electrodes (Uni-Patch, P.O. Box 1271, 1313 West Grant Boulevard, Wabasha, MN, USA) were placed on the distal m. vastus medialis and the proximal m. VL. This electrode placement has been previously shown to allow sufficient recruitment of m. QF in both able-bodied and SCI subjects [2, 19]. A commercial stimulator (TheraTouch model 4.7, Rich-Mar Corporation, Inola, OK, USA) was used for EMS. The initial torque was determined in the following manner. For SCI patients, the maximum torque was determined by increasing current incrementally until torque no longer increased. The AB controls performed a maximum voluntary contraction (MVC) for isometric knee extension prior to EMS. The subjects were highly motivated and all had prior experience with knee extension MVC. Then the current that elicited approximately 60% of isometric knee extension MVC was

determined. The EMS protocol consisted initially of 5 sets of 10 two-second isometric contractions with 6 s and 1 min rest between contractions and sets, respectively, at the current that was initially determined. The rest between contractions and sets was established in an effort to minimize fatigue. At the completion of the 5 sets of 10, another 3 sets of 10 one-second contractions with 1 s and 30 s of rest between contractions and sets were used. The one-second contractions were utilized to ensure significant contrast shift on MRI as it has been reported that the T2 increase is directly related to exercise intensity.[15] For both groups, contractions were evoked with 50 Hz trains of 450 μs biphasic pulses.

The maximum torque that could be evoked via EMS was measured on day 3 in five of the eight SCI subjects. The stimulation set-up and electrode placement was similar to day 1, current was again increased incrementally until torque reached a plateau. Five of eight AB subjects (n = 5) were also re-tested for MVC on day 3, to determine if performance was compromised.

Magnetic resonance imaging (MRI): Standard spin-echo images of the thigh were collected using a 1.5 Tesla super-conducting magnet (Signa, General Electric, Milwaukee, WI) essentially as described previously [1, 2, 6, 13, 18, 27, 29, 37]. Twelve 1-cm thick transaxial images (TR/TE = 2000/30,60) spaced 1-cm apart were collected using a 25-cm-diameter extremity coil. A 256 x 128 matrix was acquired with one excitation and a 20-cm field of view. The proximal aspect of the patella was aligned with the distal portion of the extremity coil for each image to ensure consistent subject position in the magnet over repeat MR images. A test-retest reliability of $r > 0.95$ ($p < 0.05$) for measuring QF CSA has previously been reported for these parameters [2].

MRI analysis: MR images were transferred to computer for calculation of T2 using a modified version of the public domain National Institutes of Health (NIH) Image program (v. 1.52), written by Wayne Rasband at NIH and available from the Internet by anonymous ftp. After spatial calibration (20 cm/256 pixels = 0.078 cm/pixel), a region of interest (ROI) was defined by tracing the outline of the m. QF. The T2 for each pixel within the ROI was determined from the native images. Pixels with a T2 between 20 ms and 35 ms were assumed to represent muscle at rest in the pre-EMS images. The area that was stimulated was assessed in the post-exercise images and reflected by the pixels with an elevated T2 minus pixels in the pre-EMS images with elevated T2. This was done in order to correct for pixels containing material such as fat, which have longer T2 values than muscle, and would be present in both images. Initially, the mean and SD of the T2 of pixels in each pre-EMS image were calculated. Pixels in matching post-EMS images with a T2 greater than the mean plus 1 SD of the T2 of muscle pre-EMS were considered elevated. The CSA of such pixels was determined. CSA values were averaged over eight slices starting with the first slice not containing gluteal muscle and continuing distally for the next seven slices to determine the average absolute and relative CSA of muscle activated. This region of slices has been reported to represent the maximum CSA of m. QF [7, 26]. The CSA of pixels with an elevated T2 was determined from the images taken on day 3 in the same manner to represent damaged muscle.

Statistics: Statistical analyses were run using SPSS (v. 10.0). Variables were analyzed with a one-way ANOVA. Relative CSA of stimulated muscle and resultant torque values were analyzed with simple linear regression. The level of significance was set at $p \leq 0.05$. The data are presented as mean ± SE.

Results

The SCI subjects were slightly older (7 years, $p < 0.05$) than the AB controls but otherwise were similar in height and weight (Table 4.1). Fatigue was greater for the SCI subjects over the entire EMS protocol (Figure 4.1). On average, torque decreased by ~66% for the SCI group, while the AB controls showed a ~36% reduction in torque after EMS. The AB controls showed nearly complete recovery of torque between sets for the first 5 sets, while there was clearly incomplete recovery for the same protocol in the SCI subjects (Figure 4.1). While there was a substantial decrease in torque for both SCI and AB subjects during the 3 sets of one-second contractions, this was expected and desired, to allow a greater contrast shift to be seen on MRI. It has been suggested that the T2 contrast shift associated with muscle activation is directly related to the metabolic activity of the muscle [2].

The absolute average CSA of muscle stimulated in the AB controls was significantly greater than in the SCI patients (44 ± 6 cm^2 vs. 16 ± 3 cm^2, $p < 0.05$), due to the atrophied QF of SCI subjects. However, in a relative sense, there was a higher proportion of the QF stimulated in the SCI group ($p < 0.05$, Figure 4.2 and 4.3). Despite the small m. QF of SCI patients and the long duration since injury, the available motor units responded quite well to stimulation. Overall, EMS torque was predicted by the following equation: torque (Nm) = 3.74 x stimulated CSA (cm^2) – 14.37 ($R^2 = 0.93$, $p < 0.05$, Figure 4.4). The relative QF CSA with an elevated T2 on day 3 was significantly higher for SCI than AB subjects (25% vs. 2% of activated muscle, $p< 0.05$, Figure 4.5). The damaged area was normalized to the amount of muscle activated on day 1. AB

subjects showed no decline in MVC 3-4 days post stimulation (± 5%), yet SCI subjects' electrically stimulated torque was reduced by 22%.

Discussion:

The primary finding of this study was that long-term SCI patients experienced increased muscle damage compared to that of able-bodied controls following a single session of electrically-evoked isometric contractions. The unloading and long-term inactivity of the m. QF in SCI patients resulted in an increased susceptibility to contraction induced muscle injury compared to able-bodied controls. There was a greater relative area of muscle with increased T2 relaxation times in the SCI patients than AB controls (25% vs. 2% of activated muscle) three days after 80 isometric contractions. The increased muscle damage also resulted in compromised torque output on day 3 in the SCI subjects. These findings support the hypothesis that the QF of SCI patients has an increased potential for contraction-induced muscle damage.

These results may help to explain the incomplete torque recovery during EMS that we have observed in our laboratory. In our previous study of chronic SCI patients, incomplete recovery was noted between sets and for at least 60 minutes after surface EMS, which was probably due to muscle fiber injury [19]. We found similar torque declines in a study of SCI patients who had been injured for 6 months and it was concluded that muscle fiber injury might have contributed to their torque declines during isometric actions [9]. This present study further supports the notion that SCI muscle can be injured after isometric contractions and incomplete recovery between sets may be indicative of muscle damage (Figure 4.1).

Was the increased muscle damage found in this study due to the greater relative amount of muscle activated in the SCI patients? The answer is no. Adams et al.[2] report that the increased force produced by increasing the amplitude of stimulation is due to a greater amount of muscle mass stimulated. Electrical stimulation activates muscle in a synchronous pattern and the stimulus on each motor unit is dependent only on the stimulation parameters. Thus it should be noted that although the SCI patients had a relatively greater amount of the available muscle stimulated, this was still much less absolute muscle than the AB subjects. Neither of which complicate our findings, as each group received the same stimulus (for example 50 Hz, 2 s on/5 s off). Provided there was more muscle activated in the AB group, we would expect there to be no more or less relative damage.

High force eccentric actions are not necessary to evoke fiber injury in muscles that have been subjected to unloading [29, 40]. Warren et al.[40] showed that just 15 tetanic isometric contractions after, but not before, hindlimb suspension in the m. soleus of a mouse was sufficient to evoke force loss. Others have shown elevated plasma creatine kinase (CK) in human SCI patients after a single bout of functional electrical stimulation leg cycling [32]. While plasma CK is not a direct marker of muscle injury, it has been repeatedly shown that elevated CK levels provide support that muscle fibers have been injured [10, 11]. It is well documented that eccentric actions can cause extensive contraction induced muscle injury and muscle atrophy (10% reported by Foley et al [16]) in the able-bodied population. Isometric actions were used in this study due to uncertainty of the extent of damage that might have been caused with eccentric actions. In this study, paralyzed muscle was indeed more vulnerable to injury. Consistent with the current

literature on muscle injury following eccentric exercise, we would expect even more damage with isotonic exercise. Preliminary observations from this laboratory suggested that isometric actions would cause damage yet be minor enough that it would be repaired within a few weeks (unpublished observations). In fact, isometric exercise has been shown to cause small increases in plasma CK levels and muscle soreness in able-bodied subjects [39].

Some researchers have suggested that the unloading alone could cause muscle damage, while others argue it is the activity after the unloading that causes the injury to muscle. In this study the muscle contractions alone caused the muscle injury. These patients were 10 years post SCI, time enough for the atrophic response to reach nadir. Cross sectional studies of SCI patients have shown that the affected muscles may atrophy for up to 17 months after injury [35]. Our study of acute patients showed muscle CSA of the QF to decline the most (~16%) from 6 to 24 weeks of injury and only a 3% decline from 24 to 46 weeks, thereafter [7, 13]. Although it should be noted that at 6 weeks post injury the SCI m. QF is already ~30% smaller than age, height, and weight matched controls [7]. Furthermore, short-term bedrest and ULLS do not alter the MR image contrast of muscle, while activities that cause muscle injury do [23].

MR imaging has proven to be an excellent tool for assessing the extent of a damaging bout of exercise on skeletal muscle. This laboratory, and others have shown that increases in T2 signal 2-3 days after exercise are indicative of muscle injury in both young and old subjects [6, 29]. To our knowledge this is the first study to document contraction-induced muscle injury with MRI in SCI patients who are years after SCI. To further support our MRI data, torque was also reduced by 22% three days after the

contractions in the SCI subjects with essentially no change in MVC of AB subjects at the same time point. As expected the muscle damage resulted in compromised performance several days post activity. Taking together the higher % CSA with elevated T2 and torque reductions on day 3 after EMS in the SCI compared to AB controls, it is reasonable to conclude there was indeed muscle injury.

The clinical and practical significance of these findings are important. Skeletal muscle atrophy is a significant problem associated with SCI and is primarily responsible for limiting oxygen consumption during FES exercise [20]. This might suggest that in order to establish an FES exercise program to evoke significant exercise responses that stress the cardiovascular system, the muscle needs to be made larger. Muscle hypertrophy is generally evoked through relatively few (< 100/week) high force contractions. As illustrated, these types of contractions may initially cause muscle injury. Thus time for recovery and attention to proper progression is needed when training these patients. These data may also help to explain the lack of significant hypertrophy in some training studies of SCI patients.

In summary, isometric exercise evoked significantly greater muscle injury in SCI subjects compared to AB controls as reflected from MRI and torque measurements. This was probably related to the chronic unloading and inactivity that these patients experience. These results also support previous conclusions that contraction-induced muscle injury may have contributed to decrements in force production of both chronic and acute SCI patients. Clinicians should proceed with caution when developing and implementing EMS training programs whose goal is to evoke muscle hypertrophy.

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Table 4.1 Subject characteristics and relative QF CSA data

Values are means ± SE, except for SCI level which is the range; n = 8 for each group. SCI is spinal cord injury, MVC is torque from maximum voluntary contraction.

Variable	Able-bodied	SCI
Age (yrs)	27 ± 1	34 ± 2
Height (cm)	174 ± 4	178 ± 4
Weight (kg)	76 ± 7	76 ± 8
SCI level		C5-T9
Years post injury		10 ± 3
MVC (Nm)	236 ± 27	

Figure legends:

Figure 4.1: Fatigue during EMS. SCI subjects (open circles) had incomplete recovery between sets resulting in about a 66% decline in torque compared to 36% in able-bodied controls (closed circles). Stim 1 protocol was 2 second contractions with 6 s and 1 min rest between reps and sets, respectively. Stim 2 protocol was 1 second contractions with 1 s and 30 s rest between reps and sets, respectively. n = 8 per group.

Figure 4.2: Percent muscle cross-sectional area (CSA) activated with electrical stimulation. Significantly different between groups ($p < 0.05$).

Figure 4.3: Representative single slice, binary T2 map of m. QF for one able-bodied (upper) and spinal cord injured (lower) subject pre (left), immediately post (middle), and 3 days post (right) EMS, respectively. Black represents muscle at rest, borders drawn for clarity. In general, SCI subjects had smaller m. QF CSA but larger relative amount of muscle stimulated and injured.

Figure 4.4: Torque vs stimulated CSA. The average CSA of stimulated muscle was plotted versus the initial torque prior to the EMS protocol. Simple linear regression shows that torque (Nm) can be predicted from the following equation: torque (Nm) = 3.74 x stimulated CSA (cm^2) – 14.37 ($r^2 = 0.93$, $p < 0.05$). All subjects included, n = 16.

Figure 4.5: Damaged muscle as percent of activated muscle with elevated T2 on day 3. Significantly different between groups ($p < 0.05$).

Figure 4.1

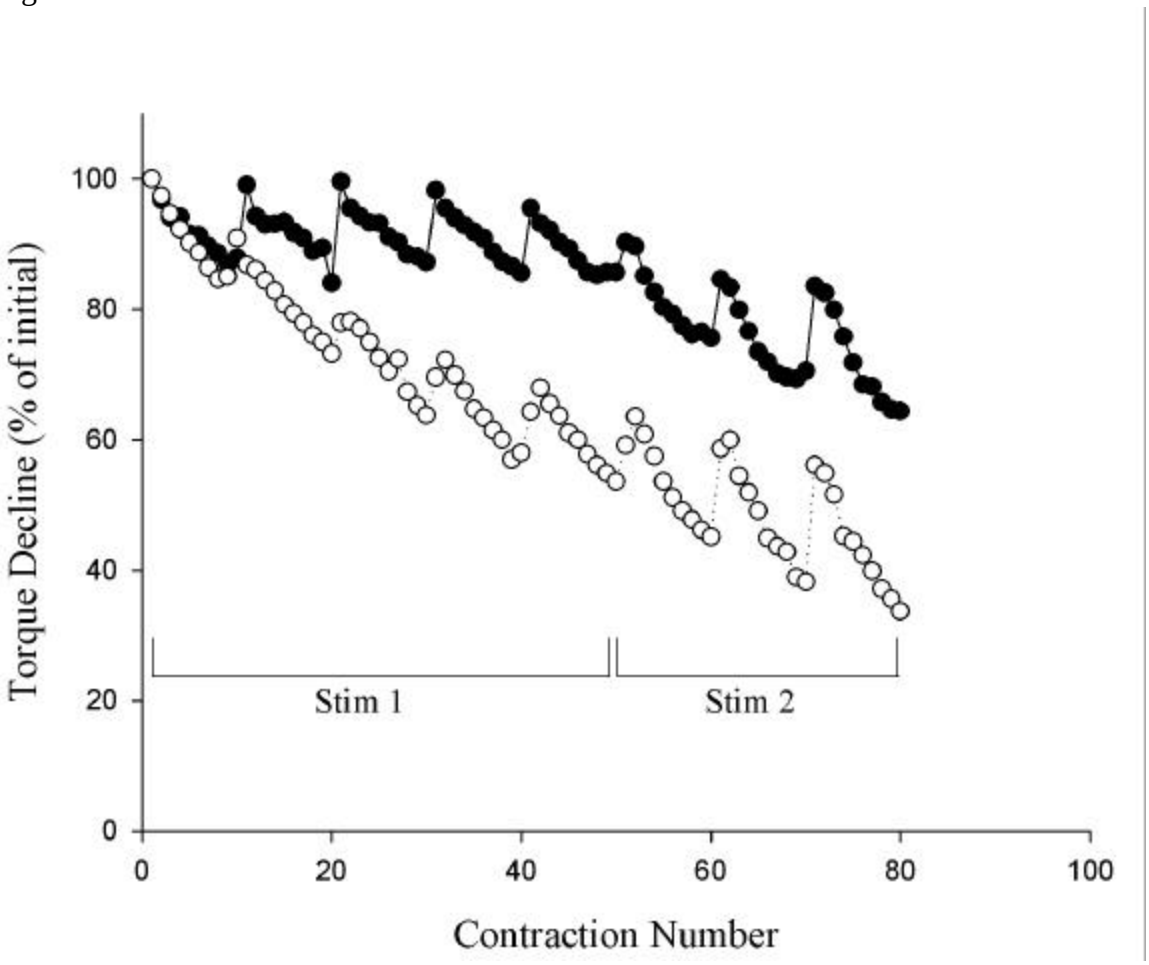

Torque Decline (% of initial)
100
80
60
40
20
0
Stim 1
Stim 2
0
20
40
60
80
100
Contraction Number

Figure 4.2

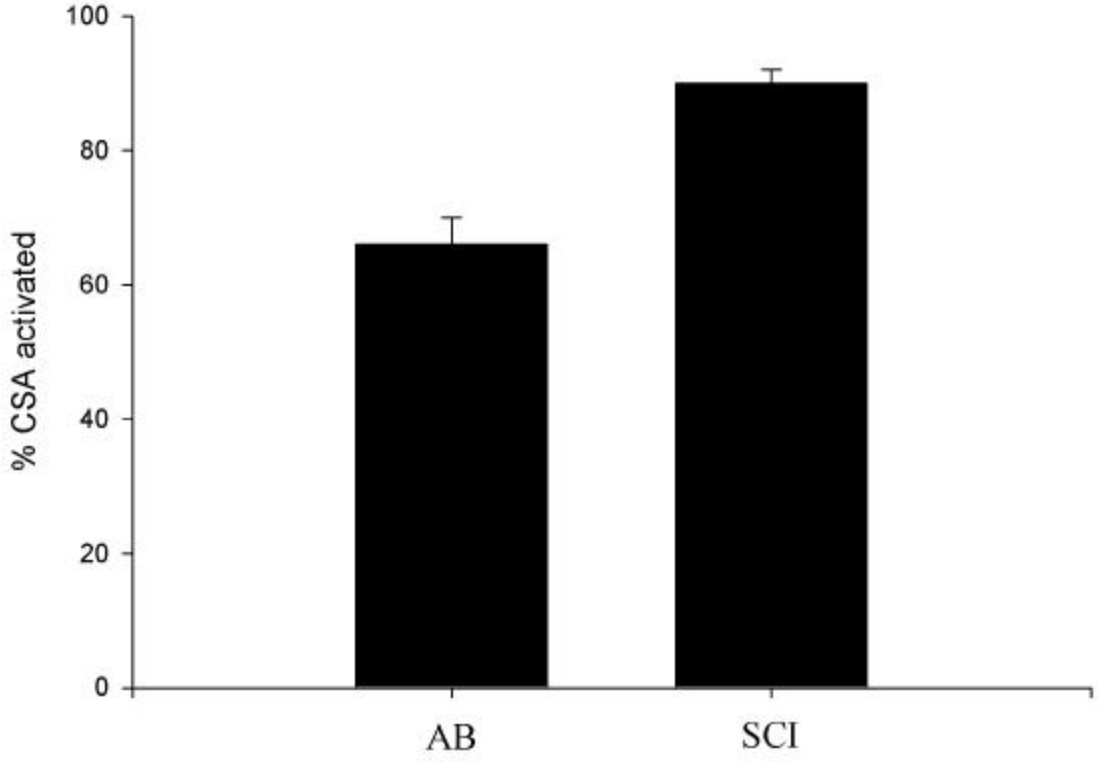


Figure 4.3

Figure 4.4

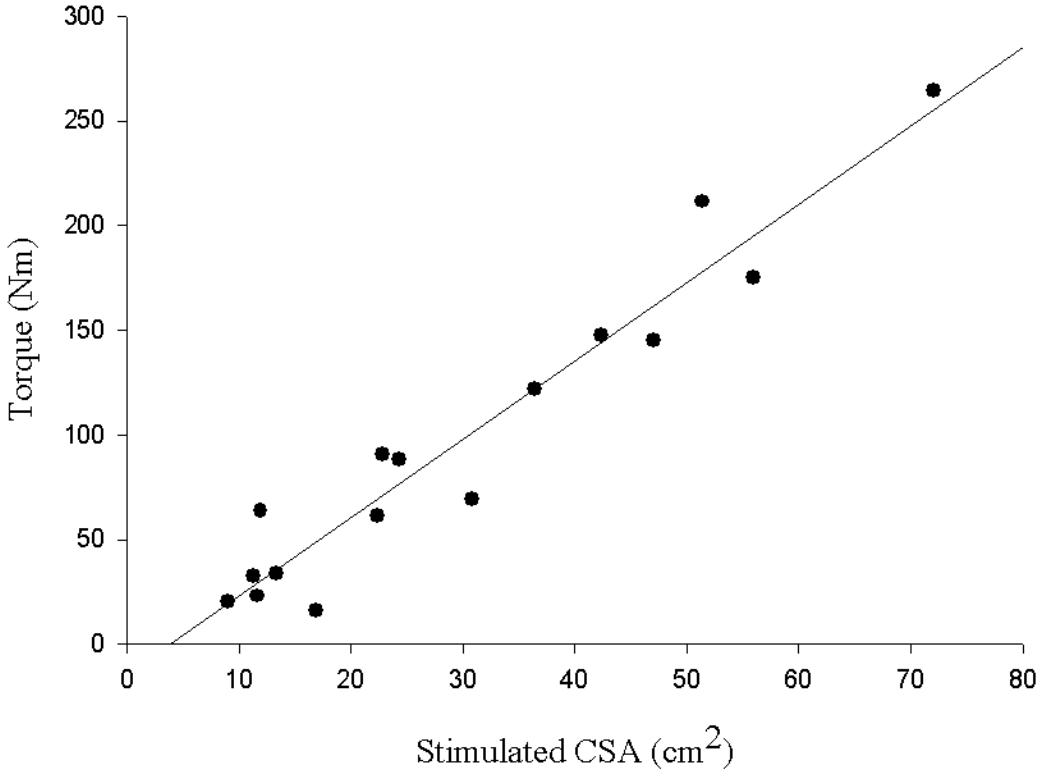
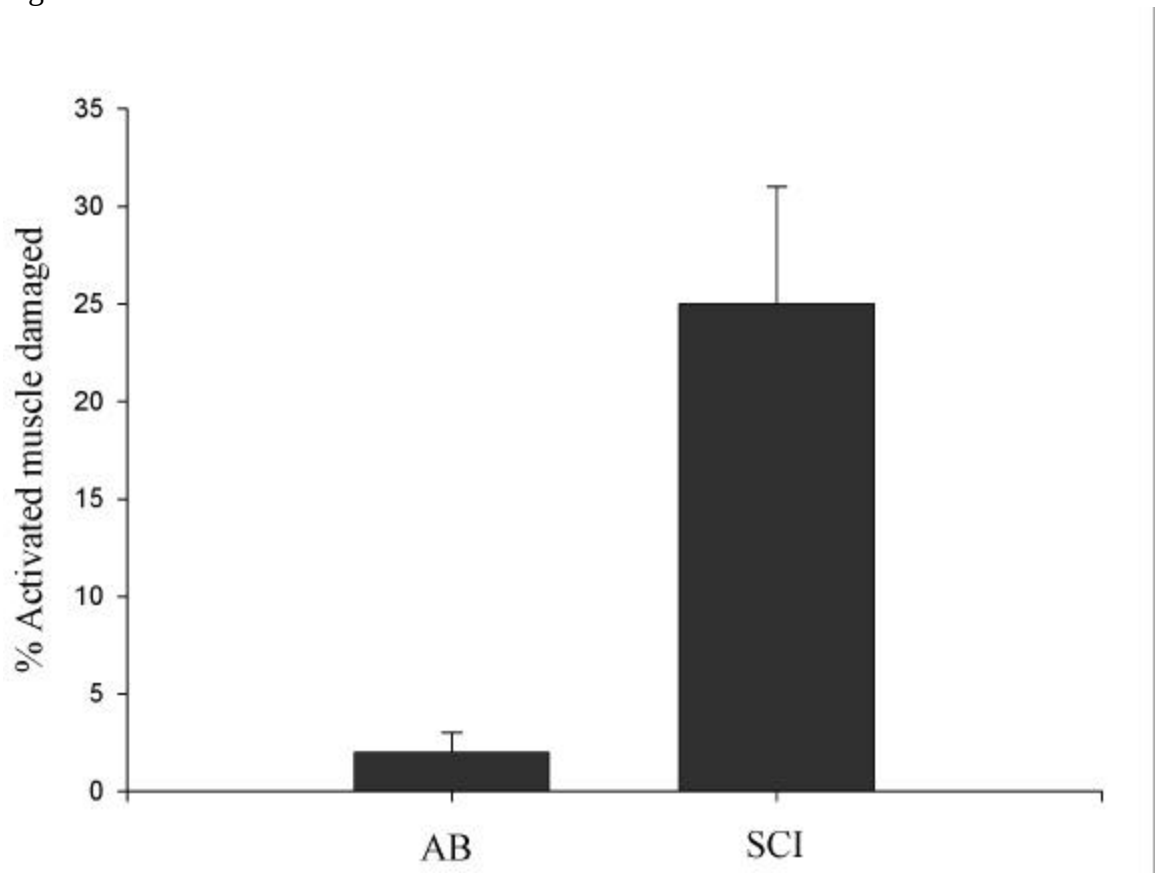

300
250
200
150
100
50
0
Torque (Nm)
0
10
20
30
40
50
60
70
80
Stimulated CSA (cm2)

Figure 4.5



CHAPTER V

SUMMARY

Spinal cord injury (SCI) is a condition that has many negative effects on skeletal muscles below the level of injury. These studies focused on two areas that could potentially influence torque production in SCI patients: contraction-induced muscle injury and variable-frequency train (VFT) stimulation. Because muscle injury has been shown to occur in unloaded muscles of humans and lower mammals, it was necessary to evaluate the extent of muscle injury in SCI patients after a bout of electromyostimulation (EMS). EMS is often used to activate muscles of SCI patients, and there is currently great interest in using EMS for applications such as ambulation and/or exercise training. One problem with EMS is the inherent fatigue that is associated with its use. VFT stimulation is a form of EMS that has shown promise in reducing fatigue in able-bodied (AB) individuals.

SCI patients were subjected to a single bout of EMS and the extent of muscle injury relative to the amount of muscle activated was assessed. This study demonstrated that SCI patients were indeed more susceptible to contraction-induced muscle injury as compared to AB subjects. One novel aspect of this finding was that the injury was evoked with isometric muscle actions, not high-force eccentric muscle actions, which are thought to be the primary cause of contraction-induced damage. VFT stimulation failed to reduce fatigue or enhance the torque-time integral in patients with SCI. This leads to the conclusion that their use in SCI rehabilitation is questionable.

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