CONTRACTILE PROPERTIES OF THE SOLEUS, TIBIALIS ANTERIOR AND THENAR MUSCLES IN INDIVIDUALS WITH SPINAL CORD INJURY

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Abstract

To examine the effects of purported fiber-type transformation following spinal cord injury (SCI), twitch contractile properties of the soleus, tibialis anterior (TA) and thenar muscles were examined in individuals with chronic (> 4 years) SCI. Furthermore, the force-frequency relationship, fatigue and posttetanic potentiation (PTP) of the paralyzed TA muscle were also evaluated. Nine adults with SCI (22-59 yrs; lesion level C3-T2) and 9 age- and gender-matched able-bodied controls (AB) participated in this study.

On the first visit to the laboratory, the maximum twitch response for all three muscles was determined by delivering a series of single stimuli with gradually increasing intensity. For evaluation of PTP, tetanic stimulation (100Hz) was applied to the TA for 5 seconds followed by single twitches delivered at 5 seconds after tetanus and then at 30-second intervals for 4 minutes posttetanus. On a second visit, the force-frequency relationship (FFR) and 15Hz fatigue of the TA was evaluated. One second bursts ranging from 1-100Hz were delivered randomly with 2 minutes of rest in between each frequency for assessment of FFR. Following a 10-minute rest period, the first fatigue protocol was given, consisting of 1-minute of tetanic stimulation at 15Hz. At the third session, the 30Hz fatigue of the TA was performed, consisting of 1-minute of tetanic stimulation at 30Hz.

In the soleus muscle, the AB had a higher peak twitch torque (PT) and M-wave

amplitude compared to the SCI group (14.2 ± 3.9Nm vs. 8.9 ± 6.1Nm; $p = 0.058$, and 13.5 ± 5.3mV and 5.5 ± 4.0mV; $p < 0.05$, respectively). Contractile speed was not significantly different between groups. Time to peak torque (TPT) was longer in AB (111.5 ± 15.4ms) compared to the SCI (76.7 ± 25.0ms; $p<0.05$) due to the larger twitches; however, the rates of torque development (RTD) were similar between groups. In the TA muscle, AB and SCI had similar PT (2.8 ± 0.5Nm and 3.2 ± 1.2Nm, respectively). TA contractile properties were faster in SCI, as seen by significantly shorter TPT and faster RTD ($p<0.05$). M-wave amplitude of AB was significantly greater than the SCI group, 8.3 ± 2.6mV versus 4.2 ± 1.7mV, respectively ($p<0.05$). Finally, in the thenar muscle, PT appeared to be smaller in AB compared to SCI, 1.7 ± 0.8Nm versus 2.9 ± 1.3Nm, respectively ($p = 0.094$). The RTD was faster in the SCI group compared to AB ($p<0.05$).

Evaluation of FFR revealed that the curve of the SCI was shifted to the left of that of AB. The F50 (frequency required to elicit 50% of maximum peak torque) was significantly lower in the SCI compared to AB, 6.7 ± 3.4Hz and 16.7 ± 4.1Hz, respectively ($p<0.05$). Following the fatigue protocols, SCI group tended to fatigue more rapidly and to a greater extent than AB at both frequencies, however this was only significant at 15Hz. The M-wave declined with fatigue (30Hz) in both groups, but this decline tended to be more rapid in SCI.

For the assessment of PTP, both groups started off with similar baseline twitches in their TA muscle (2.7 ± 0.3Nm and 2.9 ± 0.8Nm, respectively). At 5 seconds following tetanus, PT was significantly greater in both groups, but the amount of potentiation was greater in SCI versus AB ($p = 0.058$). Over the 4-min recovery period, PT declined in

both groups until it was no longer significantly greater than baseline by 3 minutes 30 seconds. The potentiated twitch of both groups was faster than at baseline. RTD increased significantly by an average of 56% in the AB group and 91% in the SCI group and was significantly greater in SCI compared with AB at 30-150 seconds post-tetanus ($p<0.05$). At 5 seconds post-tetanus, RTR was significantly faster in both groups and had increased by 77% and 53% in the AB and SCI groups, respectively. The recovery of RTD and RTR over the 4 minutes occurred more rapidly in AB versus SCI.

In conclusion, changes in contractile properties following SCI differ between muscle groups; faster contractile properties indicative of fiber type transformation are more evident in TA and thenar muscle groups, compared with the soleus. The smaller M-waves seen in the lower extremities support the significant muscle atrophy following SCI. Furthermore, the predicted transformation towards a higher proportion of fast-twitch fibers following paralysis was supported by a trend for decreased fatigue resistance and significantly greater PTP in the SCI group. The FFR data, however, did not support this predicted fiber type transformation, shifting to the left instead of the right. This leftward shift of FFR has been reported in other paralyzed human muscle presenting with faster contractile speeds; the mechanisms behind this warrant further investigation.

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List of Abbreviations

½RT	Half Relaxation Time
AB	Able-bodied
ASIA	American Spinal Injury Association
BWST	Body Weight-supported Treadmill Training
CSA	Cross Sectional Area
DF	Dorsiflexion
FES	Functional Electrical Stimulation
FFR	Force-frequency Relationship
IMF	Intramuscular Fat
LBM	Lean Body Mass
MHC	Myosin Heavy Chain
MVC	Maximal Voluntary Contraction
PAP	Postactivation Potentiation
PF	Plantarflexion
PFP	Post-fatigue Potentiation
PT	Peak Torque
PTP	Posttetanic Potentiation
RTD	Rate of Torque Development
RTR	Rate of Torque Relaxation
SCI	Spinal Cord Injury
ST	Spinal Cord Transection
TA	Tibialis Anterior
TPT	Time to Peak Torque

Chapter 1
Introduction

1.1 Spinal Cord Injury

Spinal cord injury (SCI), resulting in permanent paralysis or neurological deficit, occurs in approximately 35 per 1 million population annually in Canada (Pickett et al., 2003) and is approximately four times more likely in men than women (Sipski and Richards, 2006). A higher incidence of SCI among younger individuals is also observed, since injury typically occurs in the early 30's (Sipski and Richards, 2006). The leading causes of acute SCI include motor vehicle crashes, falls, violence and sports (Pickett et al., 2003; Sipski and Richards, 2006). Trauma to the spinal cord affects the body's normal motor, sensory and/or autonomic function below the level of lesion (Somers, 1992). The degree of impairment depends on the severity and location of the injury (Marieb, 2002) which can be classified by the American Spinal Injury Association (ASIA) Impairment Scale (Maynard et al., 1997). This classification system is based on a motor and sensory examination of neurological function (Maynard et al., 1997). The ASIA Impairment Scale classifies patients into 5 categories: A – No function preserved in the sacral segment; B – Sensory function only below the neurological level; C – Some sensory and motor function below the neurological level; D – Useful motor function; E – Normal sensory and motor function (Maynard et al., 1997). The neurological level is the most caudal segment of the spinal cord with normal sensory and motor function on both

sides of the body (Maynard et al., 1997). At times the injury is simply termed complete or incomplete. A complete injury refers to the condition where there is an absence of sensory or motor function in the lowest sacral segment (ASIA A). While an individual with an incomplete injury has partial preservation of sensory and/or motor function below the neurological level (ASIA B-D; Maynard et al., 1997). Individuals that sustain cervical injuries, whether complete or incomplete, are not only limited by paralysis of lower extremity and trunk muscles but upper extremity muscles are also affected resulting in weaker supportive function of the arms and hands (Wirz at al., 2006). In the past few decades, due to new technologies and discoveries in the medical field, the life expectancy for individuals with SCI has increased dramatically (Krause et al., 2004). By gaining further insight into the many characteristics associated with SCI, there is a better probability to remediate (or possibly reverse) some of the effects resulting from injury to improve functional status.

It is well documented that below the level of lesion, there are significant muscular changes that occur in response to SCI (Gerrits et al., 1999). The changes resulting from paralysis can be characterized as morphological, metabolic or contractile in nature (Hartkopp et al., 2003). These adaptations, such as the striking decrease in muscle mass, can be the result of disuse due to the interruption of descending spinal motor pathways (Gerrits et al., 1999). There is an array of muscular responses that must not only be examined individually but also in relation to one another. Some research has focused on the changes in fiber type expression and composition; for example, it has been established that muscle morphology changes occur throughout the several months

following spinal cord lesion (Lotta et al., 1991; Round et al., 1993), affecting fiber type distribution, contractile characteristics and fatigability. It is important to understand the many changes in skeletal muscle properties and characteristics after SCI in order to optimize rehabilitation strategies.

1.2 Muscle Atrophy

One of the most commonly observed adaptations following spinal cord injury is skeletal muscle atrophy. Atrophy is caused by a functional denervation due to the disruption in the descending motor tracts (Gordon and Mao, 1994). The muscle atrophy is accompanied by significant decreases in both upper and lower extremity strength, which can severely limit functional performance in an individual with SCI. For individuals with cervical SCI in particular, decreased muscle strength in the upper limb muscles can make necessary daily activities, such as transfers and reaching for overhead objects, difficult (Thomas et al., 1997). Being wheelchair-dependent contributes to the significant muscle weakness and functional denervation in lower limb muscles, in people with SCI. For example, Jayaraman *et al.* (2005) found that persons with incomplete SCI (ASIA C or D) generated approximately 70% less peak torque in the knee extensor and plantarflexor muscles compared with able-bodied controls.

The loss in muscle mass following SCI occurs relatively quickly. During the first year following SCI there is significant reduction in not only lower body and gluteal lean body mass (LBM) but also total body LBM (Baldi, et al., 1998). Muscle atrophy, generally expressed as a decrease in muscle area, fiber area or number, seems to reach a

steady state by approximately 1 year after SCI (Round, et al., 1993). An important factor when assessing muscular atrophy in individuals with SCI is the accumulation of intramuscular fat (IMF). Recently Gorgey and Dudley (2007) found that between the 6th week and 3rd month post injury, thigh IMF cross sectional area increased by 26% in individuals with incomplete SCI (ASIA B or C). At 6 weeks after injury, thigh cross sectional area (CSA) had decreased by 33% but without correcting for IMF this decrease may be underestimated by approximately 6% (Gorgey and Dudley, 2007). Measuring both muscle and fat CSA, Giangregorio *et al.* (2005) found that before participating in a training program thigh and calf muscle CSA of individuals that had sustained SCI 2-6 months prior were on average 60 and 65% of control values, respectively. Following 48 sessions of body weight supported treadmill training, thigh muscle CSA increased to 72% of control values and calf muscle CSA increased to 79% of control values (Giangregorio et al., 2005). It is apparent that muscle atrophy occurs soon after SCI, in fact, it has even been detected as early as in the first 3 weeks following SCI in the quadriceps muscle (Taylor, et al., 1993). Thus, it has been suggested that rehabilitation efforts to diminish the degree of muscle atrophy following SCI should be initiated very soon after the injury (Taylor, et al., 1993).

Studies measuring single fiber CSA and diameter have presented similar findings to the whole muscle research. Smaller muscle fibers, type I and II, have been reported in the paralyzed vastus lateralis, gastrocnemius and soleus muscles (Grimby, et al., 1976). In addition, studies that distinguish between atrophy of type I and II fibers have provided further insight into the progression of muscle atrophy following SCI. Castro *et al.* (1999)

found that the average fiber CSA in the vastus lateralis decreased during weeks 6 to 24 after injury in individuals with complete SCI. More specifically, the CSA of all fiber types (I, IIa, IIax + IIx) decreased significantly from week 6 to 11 but only type IIa fibers continued to diminish until week 24, demonstrating preferential atrophy of type II fibers (Castro et al., 1999). Although these findings are consistent with other studies examining fiber size in the first 6 months following injury, when changes in fiber size are tracked for a longer period of time differences emerge between fiber types. Scelsi *et al.* (1982) monitored the progressive decline in fiber diameter in the rectus femoris muscle from 1 to 17 months following complete SCI. In the earlier months preferential atrophy of type II fibers was similarly observed but in the later months following SCI there was a predominance of type I atrophy (Scelsi et al., 1982). This two stage process was also observed in the gastrocnemius medialis and soleus muscles, where from months 1 to 6 there was predominantly type IIa atrophy and from months 8 to 10 predominately type I atrophy (Lotta et al., 1991).

Further research has shown that the acute changes observed in muscle mass were relatively maintained in individuals with chronic SCI (>1 year post-injury). Round and colleagues (1993) found that the mean fiber area of both type I and II fibers were reduced in the paralyzed quadriceps muscle, ranging from close to or below that of the lower limit of normal values, although type I fibers were not found in all SCI participants. The time since injury ranged from 11 months to 9 years but no significant relationship was found between mean fiber area and time post-injury (Round et al., 1993) indicating a steady-state was achieved by the first year following SCI. Ditor et al. (2004) also found a

significant decrease in mean fiber area of all fiber types (I, IIa and IIx) when comparing fiber areas from the vastus lateralis of 6 individuals with complete SCI (1-10 years post-injury) to literature values for able-bodied controls. In contrast to the studies of lower limb muscles, no difference in fiber type area has been found in the anterior deltoid muscle of individuals with SCI compared with controls (Schatz et al., 1997). However, the comparison was made using a pooled SCI group consisting of untrained and endurance trained tetraplegics and paraplegics (4 subgroups). Comparisons made between the SCI groups revealed that the paraplegic group has significantly larger (10-37%) type I fiber area than the tetraplegic group (Schatz et al., 1997). The preservation of fiber area may be due to the fact that the anterior deltoid muscle is active during wheelchair propulsion and that almost half of the SCI subjects were involved in endurance wheelchair-training (Schatz et al., 1997).

Although a decrease in muscle mass is generally observed in paralyzed lower limb muscles, Malisoux *et al.* (2007) found no difference in fiber area of the vastus lateralis between chronically paralyzed individuals and able-bodied controls. The absence of fiber atrophy is somewhat surprising; however, Malisoux and colleagues (2007) suggested that the wheelchair dependent individuals may have limited fiber atrophy due to their continual seated position which places the vastus lateralis muscle at a lengthened state. It has also been previously suggested that the spinal reflexes developed following SCI resulting in sporadic and at times aggressive muscle contractions of the lower limbs may serve to maintain muscle area (Round et al., 1993). This may have also contributed

to the finding that fiber atrophy was absent in the vastus lateralis of chronic SCI individuals in the study by Malisoux *et al.*(2007).

1.3 Fiber Type

Human skeletal muscle is comprised of two main types of fibers, each producing ATP by different mechanisms. Type I fibers are slow twitch, fatigue resistant fibers that produce ATP more slowly and type II fibers are fast twitch fibers that are more susceptible to fatigue (Whiting and Rugg, 2006). Type II fibers metabolize ATP more quickly and ATPase activity of the myosin isoform in this fiber is high (Whiting and Rugg, 2006). Fast fibers are further subdivided into type IIa and type IIx fibers. Type IIx are the fastest fibers and are recruited during rapid accelerations and very short maximal contractions. Activity level imposed by the motor neuron plays an important role in fiber type expression in muscle fibers (Pette and Vrbova, 1992).

In addition to muscle wasting following SCI, the skeletal muscle also undergoes fiber type transformation. In the first 6 months following injury, the percentage of type IIa fibers in the vastus lateralis muscle of individuals with complete SCI decreased, the percentage of type IIax + IIx fibers increased and type I fiber percentage showed no change (Castro et al., 1999). In a longer-duration study, Lotta *et al.* (1991) examined fiber type transformation in the gastrocnemius medialis and soleus muscle from 1-10 months following complete SCI. From month 1-6 a similar decrease of type IIa fibers was observed but from months 8-10 a reduction of type I fibers and higher percentage of type IIx fibers was found in both muscles (Lotta et al., 1991). Similar findings have been

observed in the vastus lateralis of individuals with chronic SCI (3-25 years post-injury) with an average of 91.3 ± 3.6% type II fibers (Chilibeck at al., 1999). This is significantly greater than the relatively equal mix of type I and II fibers found in the vastus lateralis of the able-bodied control. However, the transition to the faster fiber types may not be complete in the first year after injury. For example, Round *et al.* (1993) found that the proportion of type I fibers in the quadriceps of paraplegics was relatively maintained at one year post-injury, but that participants with longer duration injury (3-9 years) displayed significant reductions or complete loss of type I fibers with a predominance of type IIx fibers.

Fiber typing classification can also be performed by determining the distribution of fast and slow myosin heavy chain (MHC) isoforms. Type I fibers have slow MHC isoforms, type II fibers have fast MHC isoforms and type IIx fibers express both (Staron and Pette, 1987). By assessing MHC isoform expression in the vastus lateralis muscle in individuals with acute SCI, Burnham *et al.*(1997) not only demonstrated the change from type I to type II fibers but also observed a transitional phase. In the first month following SCI, MHC isoform expression remained relatively stable with a higher or equal percentage of slow MHC compared to fast MHC. A transitional period took place between 1-20 months, where single fibers expressed both MHC isoforms. The vastus lateralis muscle in SCI participants injured for longer than 73 months were characterized by a predominance of fibers expressing only fast MHC isoforms indicating that a steady-state was achieved some time between month 20 and 73 post-injury. Similarly Malisoux and colleagues (2007) found that individuals with chronic SCI (3-28 years post-injury)

expressed significantly less MHC isoforms for type I and IIa fibers with almost a full transition to type IIx fibers in the paralyzed vastus lateralis muscle. Additionally a higher percentage of MHC isoforms for Type IIa/IIx was expressed in the SCI individuals compared to the controls, 34 ± 5% and 23 ± 3% respectively, though not statistically significant ($p = 0.06$). It was suggested that the transition of MHC isoform composition is as follows: MHC I to MHC IIa to MHC IIx (Malisoux et al., 2007). However it has been shown that MHC expression can be quite variable in individuals with chronic SCI. Gerrits *et al.* (2003) found that the vastus lateralis of some subjects with complete SCI (>2 years post injury) expressed a predominance of MHC IIx while others expressed relatively high levels of MHC I (between 36-63%). Although this variability could not be accounted for, many possible explanations were suggested including spasticity, hormone influences and muscle enzyme activity (Gerrits et al., 2003).

A few studies have indicated that specialized muscle training might result in a reversion of fiber-type distribution to a more 'normal' state. One method used to restore functional movements in paralyzed muscle is functional electrical stimulation (FES). In order to counter the effects of chronic SCI, FES activates paralyzed muscle to induce improvements in muscle strength and endurance (Scott et al., 2006). In addition, the effect of FES on histochemical properties of paralyzed muscles has also been evaluated. The percentage of type IIx fiber and MHC IIx in the paralyzed vastus lateralis muscle has been shown to significantly decrease (by 63% and 24%, respectively) following a 10-week FES training program (Crameri et al., 2002). A FES exercise training program of longer duration (1 year) also produced changes in fiber type distribution, with an

increased percentage of MHC IIa and a decrease in the percentage of MHC IIb (Mohr, et al., 1997). In contrast, Chilibeck *et al.* (1999) found that following 8 weeks of FES exercise training, a predominance of type II fibers was still evident in the paralyzed vastus lateralis muscle, although decreasing slightly from 91% to 88%. Similarly, the mean proportion of MHC I, II and IIx (with a predominance of MHC IIx) in the paralyzed quadriceps muscles were not significantly different following 12 weeks of FES (Gerrits et al., 2003). As the transition from type I to type II fibers appears to take months or even years, it is possible that intramuscular adaptations are more likely to occur following a FES program of longer duration. A change in muscle phenotype has also been produced following body weight-supported treadmill training (BWST). Stewart *et al.* (2004) found that following 68 sessions (average of 3 sessions/week) of BWST, the percentage of type IIa fibers had increased and type IIax/IIx had decreased while the percentage of type I fibers remained the same. In a study by Schantz *et al.* (1997) fiber type distribution of the deltoid muscle was examined in both endurance trained paraplegics and tetraplegics, who had trained for an average of 6 ± 3 and 12 ± 7 years prior to the study, respectively, and untrained paraplegics and tetraplegics. Surprisingly, the percentage of type I fibers was higher in all SCI groups compared to untrained able-bodied controls. This difference in fiber type distribution was significant for all SCI groups, except for the trained paraplegics. No significant difference in the percentage of type I fibers was observed when comparing trained versus untrained SCI individuals. Though the predominance of type I fibers is unusual, it was suggested that it may be a

response to the added strain placed on the deltoid muscles following a higher level injury (Schantz et al., 1997).

1.4 Twitch Contractile Properties

1.4.1 Animal Studies

Contractile properties in paralyzed muscle have been studied extensively in different animal models, with rat as the most prevalent. Animals that have undergone surgical spinal cord transection (ST) are an appropriate model to assess changes in muscle characteristics that occur following a reduction in neuromuscular activity. Although general trends towards muscle wasting and faster contractile speeds are observed, many inconsistencies exist across species and even muscle groups.

The most consistent observations following ST is muscle atrophy of the paralyzed muscles. The tibialis anterior of rats, following ST at the level of T9, showed a significant decrease in muscle CSA at 2 weeks post injury (Frontera et al., 2006). Similarly the paralyzed rat soleus muscle also exhibits significant muscle atrophy compared to controls (Chatzisotiriou et al., 2005; Lieber et al., 1986b; Midrio et al., 1988). At 58 weeks following ST, rat soleus muscles (typically comprised of >80% type I fibers) exhibited properties of fast, fatigable muscles, suggesting selective type I atrophy (Lieber et al., 1986a; 1986b).

Faster contractile speeds have also been observed in the rat soleus muscle from 1 to 12 months after ST (Chatziotiriou et al., 2005; Davey et al., 1981; Lieber et al., 1986b;

Talmadge et al., 2002). Decreases in time to peak torque ranged from 19-65% in paralyzed soleus of rats (Danieli Betto and Midrio, 1978; Lieber et al., 1986b; Midrio et al., 1988; Talmadge et al., 2002) and mice (Landry, Frenette and Guertin, 2004) whereas half relaxation time has been shown to either decrease in rat and mouse soleus after ST (Landry et al., 2004; Talmadge et al., 2002) or remain the same (Danieli Betto and Midrio, 1978; Midrio et al., 1988). Faster contractile properties are also exhibited after spinal cord disruption in cat soleus (Cope et al., 1986) and cat medial gastrocnemius (Mayer et al., 1984). Approximately 7-10 months following ST, it has been reported that the time to peak torque is 38-41% shorter and half relaxation time is 33-50% shorter in cat soleus muscle (Roy et al., 1984; 1998). The contractile properties of the cat soleus muscle, a slow muscle, undergoes greater adaptations following ST than does the cat medial gastrocnemius muscle, a fast muscle, while the cat tibialis anterior is only minimally affected several months following ST (Roy et al., 1984; 1999). In contrast, conflicting results were found during analysis of contractile properties of the tail muscle of rats that had undergone S2-level ST (Harris et al., 2006). Eight months following injury, peak muscle twitch was characterized by a 48.9% increase in time to peak torque and 150% increase in half rise time, compared to normal rats. It was suggested that the apparent slowing of contractile properties may be due to the chronic spasticity characteristic of the observed tail muscle (Harris et al., 2006). The decrease in contractile speed was surprisingly accompanied by a significant increase in fatigability. Harris *et al.* (2006) suggested that spasticity may serve to preserve or even enhance slow muscle properties but may not affect fatigability.

Evaluation of fatigability in the paralyzed rat soleus muscle has shown that the muscle becomes more fatigable at 3 and 6 months post ST (Talmadge et al., 2002). However, assessment of fatigue in the cat model has produced some conflicting results. In the paralyzed soleus muscle, a small but significant increase in fatigability was observed approximately 7 months following ST in adult cats (Roy et al., 1998). However Cope *et al.* (1986) found that cat soleus muscle, showing limited conversion of type I to type II fibers following ST, were still fatigue resistant even though they also exhibited faster contractile speeds. Similarly, minimal or no change in muscle fatigue was observed 1 year after ST in cat soleus muscle (Baldwin et al., 1984).

The change in fiber-type distribution following ST should not only have an effect on fatigability but it would be predicted that this should also affect the force-frequency relationship (FFR). As predicted, a right-ward shift of the FFR, characteristic of faster muscles, has been observed in rat soleus muscle from 3-13 months following ST (Danieli Betto and Midrio, 1978; Lieber et al., 1986; Talmadge et al., 2002). Similarly a rightward shift in FFR has also been reported in paralyzed cat soleus muscle (Roy et al., 1985).

Although the research done on animal models of SCI is informative, the information cannot necessarily be extrapolated to humans. The nature of the injury to the spinal cord in humans is usually unique to each individual, and does not necessarily match the surgical transection of the cord done in animal models. Thus, while one can use the observations made in animal models as a template or reference, it is essential to examine changes in contractile properties in paralyzed human muscles before forming conclusions about adaptations made following SCI.

1.4.2 Human Studies

Generally speaking, similar changes in muscle contractile properties as seen in the animal model occur after SCI in humans. Paralyzed muscle usually displays a decrease in torque production and generally faster contractile properties prior to fatigue, including shorter time to peak twitch torque and a decrease in half relaxation time (Gerrits et al., 1999; 2001; Shields, 1995; 2002).

1.4.2.1 Time-course of Changes in Contractile Properties after SCI

As would be expected, changes in contractile properties do not occur immediately following SCI but rather after changes in muscle mass and fiber type have occurred, possibly after 1-2 years post-injury. On average, a 20 ms shorter time to peak twitch torque and 25% shorter twitch half relaxation time has been reported in the soleus of individuals with chronic SCI when comparing to acutely injured (6 weeks post-injury) individuals (Shields, 2002). In 1997, Shields and colleagues compared the contractile properties, elicited by both single twitches and tetanic stimulation, of 9 individuals with chronic SCI (> 3 years) and 4 acutely injured individuals (< 5 weeks). Although both the chronic and acute groups displayed similar peak torques, the individuals with chronic SCI had faster contraction and relaxation times (Shields et al., 1997).

1.4.2.2 Adaptations following chronic SCI

Several studies have examined twitch contractile properties in the paralyzed lower limb muscles, especially the quadriceps femoris muscle. Recently it has been

demonstrated that paralyzed quadriceps femoris muscles of young individuals with chronic SCI (aged 11-24) generate a peak twitch force equal to only 62% of that produced by able-bodied controls (Scott et al., 2006). In addition, time to peak force and half relaxation time (time taken for force to decline to 50% of max) were 14% and 38% faster, respectively, in subjects with complete SCI (ASIA A-B; Scott et al., 2006) However, the rates of torque development and relaxation were not measured. Similar findings were reported by Gerrits *et al.* (1999), even though contractile speed was determined from isometric tetanic contractions rather then single twitches. The normalized maximal rate of force rise was approximately 50% faster and the half relaxation time (time taken for force to go from 50% to 25% of peak force) was approximately 20% shorter in the SCI subjects compared to the able-bodied controls (Gerrits et al., 1999). In a follow-up study, Gerrits and colleagues (2001) found that the quadriceps muscle of individuals with chronic SCI displayed increased contractile speed with a significantly higher rate of force rise (from 10Hz stimulation), although no difference in half relaxation was observed. It was also observed that day to day variability was lower in the participants with SCI compared to able-bodied controls. The tibialis anterior muscle has also been studied, although the results are not quite as consistent as those seen with the quadriceps muscle group. Faster contraction and relaxation along with a decrease in force has been reported in the paralyzed tibialis anterior muscle compared to controls (Rochester et al, 1995a), and a similar trend was observed by Stein and co-workers (1992). The maximum M-wave of the paralyzed tibialis anterior muscle

has also been shown to be significantly smaller than in able-bodied controls (Stein et al, 1992).

In a study by Jayaraman and colleges (2005), muscle function was examined in both knee extensor and plantarflexor muscle groups in individuals with incomplete SCI. Although an obvious deficit in voluntary torque production was observed, no differences were found between participants with SCI and able-bodied controls in electrically elicited contractile properties, including rates of rise and relaxation (Jayaraman, 2005). These findings conflict with previous studies demonstrating faster contractile speeds in both of these muscle groups following SCI. However the participants in that study were classified as mostly ASIA D and some ASIA C, and some were capable of ambulation over ground. In addition participants were between 7-39 months post injury and thus it may have been too soon post-injury for all of the SCI participants to have undergone fiber type transformation and adaptation of contractile properties.

Although most studies exploring changes in contractile properties after SCI in humans focus on lower limb muscles, there are a few studies that have concentrated on thenar muscles. One study assessing contractile properties of chronically paralyzed thenar muscles (Thomas, 1997) found it was necessary to split SCI subjects into 2 groups based on M-wave amplitude, post hoc. Some of the SCI participants had M-wave amplitudes that were equal to controls and some had significantly lower M-wave amplitudes. It was assumed that the natural division of M-wave amplitude was an indicator of muscle loss since M-wave amplitude is a property often used to assess muscle loss and weakness in individuals with SCI (Thomas, 1997). The SCI group with low M-wave amplitude also

had significantly lower tetanic forces compared to able-bodied controls. Twitch force was only slightly lower while time to peak torque and twitch half relaxation times were longer, though not significantly. This was in contrast to the SCI participants with relatively higher M-wave amplitudes, presumably related to minimal muscle loss. These participants had similar values to controls in tetanic force, time to peak torque and twitch half relaxation time (Thomas, 1997). In addition, significantly stronger twitch forces were also observed in the SCI subgroup with higher M-wave amplitudes compared to controls. Thus the adaptations observed in twitch contractile properties of thenar muscles may differ from those of human lower limb muscles. In a more recent study assessing twitch properties in thenar motor units of individuals with chronic SCI, a slowing of conduction velocity and twitch contraction time were also observed (Häger-Ross, Klein and Thomas, 2006). Motor units of paralyzed thenar muscles also displayed small EMGs and an elevated twitch-tetanus ratio resulting from stronger twitch forces and weaker tetanic forces (Häger-Ross et al., 2006). It was suggested that the slow conduction velocities could be a result of deficits in the axon or muscle while the prolonged contraction times could be due to changes in calcium release or sensitivity of the actin-myosin to Ca^{2+} following chronic SCI (Häger-Ross et al., 2006).

Interestingly, almost all of the studies examining muscle properties following SCI rely solely on measurements of contractile properties and force generation to support (or not support) the theory of muscle atrophy and fiber type transformation following SCI; relatively fewer studies have used histochemical analyses of muscle to confirm the findings. The study by Rochester et al. (1995a) evaluating changes in contractile

properties following an electrical stimulation program completed a histochemical analysis of the tibialis anterior muscle before and after the intervention. Reported in another paper, biopsies of the paralyzed tibialis anterior muscle revealed an increased proportion in type II fibers together with a decreased oxidative capacity prior to the program (Rochester et al., 1995b).

1.4.2.3 Factors affecting contractile properties in paralyzed muscle

There are several factors that are suggested to influence contractile properties in paralyzed muscle, such as muscle temperature and spasticity. To determine whether muscle temperature influenced contractile properties of the vastus lateralis muscles following SCI, Gerrits *et al.* (2000) evaluated evoked torque production and contractile speed before and after muscle heating (using short-wave diathermy and monitored by a thermocouple inserted into the muscle belly) in individuals with chronic SCI. The measured temperature of the vastus lateralis in individuals with SCI was lower than 'normal' values by approximately 5° (31° vs. 36°, respectively). Heating had no effect on absolute torque in the SCI group, in fact, the evoked torques were similar to the values measured in able-bodied controls (Gerrits et al., 2000). Similarly heating had no effect on the maximal rate of increase in force (Gerrits et al., 2000). Temperature did affect half relaxation time, however, as half relaxation time decreased in the SCI group following muscle heating. In addition, 10Hz and 20Hz responses were significantly less fused following heating. Thus when the paralyzed muscle was heated to approximately 36°, it exhibited shorter relaxation and contraction times (Gerrits et al., 2000). If paralyzed

muscle is compared to control muscle of the same temperature, greater differences in contractile speed may be observed. It was suggested that the colder muscle temperatures of individuals with SCI may have lead to an underestimation of contractile speed in previous studies (Gerrits et al., 2000).

There are also studies that suggest the degree of spasticity can influence the muscle dynamics in individuals with SCI. The changes in contractile properties exhibited in paralyzed soleus muscles of individuals with low degrees of spasticity were found to be greater than those of individuals with high degrees of spasticity (Hidler, Harvey and Rymer, 2002). Following a stochastic stimulation protocol, the low spasticity group displayed a significant increase in contractile speed and generated comparable torque compared to controls. This is in contrast to the high spasticity group that exhibited slower contractile speeds and lower torque generating capacity compared to controls (Hidler et al., 2002). Although high levels of spasticity do not sufficiently build muscle, it may allow for adequate maintenance of contractile dynamics (Hidler et al., 2002). Furthermore it was suggested that it is not possible to draw general conclusions about contractile properties for the whole SCI population but that it is necessary for individuals with SCI to be grouped according to spasticity level prior to examination of muscle characteristics (Hidler et al., 2002).

1.5 Fatigue

1.5.1 Adaptations following SCI

Several studies have revealed that individuals with SCI exhibit reduced resistance to fatigue characterized by a greater force decline and an increase in half relaxation time (Gerrits et al., 1999; 2001; Shields, 2002). In 1995, Shields demonstrated that chronically paralyzed soleus muscle is much more fatigable in comparison to acutely paralyzed soleus muscle, and is characterized by a significant reduction in M-wave amplitude and torque. During a 4-minute fatigue protocol (20Hz burst for 330ms every second for 4 minutes), the torque dropped rapidly in the first 2 minutes with minimal changes in the last 2 minutes. The M-wave amplitude declined significantly, primarily during the last 3 minutes. This fatigue protocol was a modification of the Burke fatigue protocol (Burke et al., 1973), in which a frequency of 20Hz was used instead of 40Hz. Several factors prompted the decision to use 20Hz, including an augmentation in relaxation time preventing the torque from returning to baseline at 40Hz and a decreased incidence of spasms at 20Hz (Shields, 1995). Using the same protocol, similar observations were made in a later study of chronically paralyzed soleus muscle which generated 80% less torque, had 20% reduced M-wave amplitudes and 40% increased M-wave duration following repeated stimulation compared to acutely injured individuals (Shields et al., 1998). A near doubling of half relaxation time has also been observed in the chronically paralyzed soleus muscle during fatigue (Shields et al., 1997). Variations of the fatigue protocol have been used to assess fatigability in other muscles in individuals with SCI as

well. Using a stimulation protocol consisting of 30 Hz trains, Gerrits *et al.* (1999) demonstrated that paralyzed quadriceps muscles fatigued more rapidly than those of able-bodied controls. A significant decrease in force after only 2 minutes and increased prolongation of half relaxation time were also observed (Gerrits et al., 1999). All of these findings are consistent with the impaired endurance capacity observed in muscles composed of predominantly type II fibers.

Given that the changes in fatigue resistance after SCI likely reflect the changes in fiber type distribution, significant changes in fatigue resistance would not be expected to be as great in acutely injured individuals, as in the chronic population, especially in the first few weeks when there is a predominance of type II atrophy. In support of this, Shields (1995) demonstrated only minimal changes in torque, M-wave amplitude and half relaxation time in the soleus muscles of individuals with acute SCI (within 6 weeks of injury) following a 4-minute fatigue protocol. This is consistent with later findings by Shields et al (1998), also examining the acutely paralyzed soleus muscle. Acutely injured subjects (< 6 weeks) were not significantly fatigued following a 4-minute fatigue protocol (20Hz), only displaying a 12% drop in torque. Additionally there were no changes in any M-wave properties following repeated stimulation (Shields et al., 1998). Castro and colleagues (2000) examined the quadriceps femoris of acutely injured individuals from 6 weeks to 24 weeks post injury and compared fatigability to able-bodied controls. The drop in force following a fatigue protocol was found to be significantly greater in the SCI group than controls, 27% and 9% decrease, respectively. This difference in force loss did not change, however, from 6 to 24 weeks after SCI. Despite the increased fatigue in the

SCI group, there were only minor changes in contractile speed. The time from 20% to 80% of peak torque was not significantly different between groups nor did it change over the time (Castro et al., 2000). The SCI group did have a significantly slower half relaxation time than the controls but this variable did not change over the 18-week period either. In 2006, Shields *et al.* attempted to establish the time course of changes in fatigue resistance for paralyzed human soleus muscles. By determining the Fatigue Index (FI; quotient of torque generated at the beginning and end of a series of repetitive contractions) of individuals with SCI varying from 3 weeks to 12 years post injury, a rapid decline in FI was observed immediately following SCI with a break point occurring at 1.7 years (Shields et al., 2006). At 1.7 years following injury the FI slope changed and only minimal and slow decreases were observed after this time. It was suggested that the increased fatigability that occurs in the first 1.7 years may be due to a predominance of type I fiber atrophy, conversion of type I to II fibers or chronic disuse (Shields et al., 2006).

1.5.2 Effect of Fatigue protocol

The changes in fatigue resistance after SCI are likely very much dependent on the stimulation pattern of the fatigue produced. Using 4 different fatigue protocols at supramaximal intensity (constant and variable pulse pattern at both 40 and 20Hz), Thomas *et al.* (2003) examined the fatigability of the thenar muscle in individuals with chronic SCI compared to able-bodied controls. Significant force reductions in paralyzed muscles were observed in all 4 fatigue protocols, accompanied by significant increases in

half-relaxation time (Thomas et al., 2003). Although control subjects exhibited significant fatigue in all cases as well, the relative reduction in force was significantly greater in the SCI group for 3 out of 4 protocols, and there were no changes in half relaxation time in the control group. Although the control thenar muscles fatigued less following variable stimulation, as was expected, variations in stimulation frequency, pulse pattern and pulse number had little influence on reducing fatigability commonly observed in paralyzed thenar muscle (Thomas et al., 2003).

1.6 Force-Frequency Relationship

1.6.1 Adaptations following chronic SCI

It is expected that paralyzed muscle, associated with a greater percentage of type II fibers, would exhibit a right-ward shift in the force-frequency relationship (FFR). However many studies have reported an unexpected leftward shift in FFR following SCI. A leftward shift would be typical of slow muscles that require lower stimulation frequencies to generate the same relative force as faster muscles (Gerrits et al., 1999). Yet, evaluation of FFR following SCI has revealed a consistent leftward shift in FFR in the paralyzed quadriceps femoris muscle (Gerrits et al., 1999; 2001) and tibialis anterior muscle (Rochester et al., 1995a). A more recent study examining contractile properties in quadriceps femoris found no difference in the FFR between the individuals with chronic SCI and the able-bodied controls in the non-fatigued state (Scott et al., 2006). It was suggested that the most likely explanation of the discrepancy between the studies was the

small samples sizes and variability in paralyzed muscles (Scott et al., 2006). Interestingly, both the studies by Gerrits *et al.* (1999) and Scott *et al.* (2006) observed a significantly elevated twitch-to-tetanus ratio due to elevated twitch force. Although this increase in twitch-to-tetanus ratio currently remains unexplained, these observations mimic those previously found by Thomas (1997) in paralyzed thenar muscles. In addition to exhibiting an elevated twitch-to-tetanis ratio, thenar muscles in individuals with chronic SCI also required lower frequencies to generate the same relative torques as able-bodied controls (Thomas, 1997).

1.6.2 Factors affecting FFR in paralyzed muscle

Some research has focused on certain factors that may play a role in the FFR in muscles of individuals with SCI, such as muscle temperature and joint angle. As mentioned above, the vastus lateralis muscles of individuals with SCI have lower muscle temperatures compared to able-bodied individuals, 31° versus 36°, respectively (Gerrits et al., 2000). However, when they are heated to normal temperatures, they exhibit a rightward shift in force-frequency response (Gerrits et al., 2000). It has been suggested, therefore, that muscle temperature may have been a confounding factor in the previous studies in which paralyzed muscles had force-frequency curves shifted to the left, a property of slow muscles (Gerrits et al., 2000). In a more recent study, Gerrits and colleagues (2005) assessed the effect of knee joint angle on the FFR of the quadriceps femoris. It was hypothesized that if the knee joint was positioned at the optimal angle, a leftward shift in FFR would no longer be seen in the individuals with SCI (Gerrits et al.,

2005). However, contrary to their predictions, a leftward shift in FFR was still observed in the individuals with chronic SCI compared to controls at all muscle lengths (Gerrits et al., 2005). At longer muscle lengths, the force-frequency curve shifted to the left in both SCI and control groups, however the shift was more pronounced in the SCI participants. Thus, Gerrits *et al.* (2005) concluded that the leftward shift observed in paralyzed quadriceps femoris muscles must be an actual feature of the contractile properties of the muscle.

1.7 Posttetanic Potentiation

Following a brief tetanic stimulation the force of a single twitch is greater than it was before the tetanus (Brown and von Euler, 1938). This enhancement of twitch force is known as posttetanic potentiation (PTP). The increase in force that follows repetitive stimulation is greatest immediately after tetanus. Although there is a rapid decline of PTP over time, it is still evident up to 10 minutes after the conditioning tetanus has ended (Brown and von Euler, 1938; Hughes and Morrell, 1957). A similar phenomenon is observed following a brief maximal voluntary contraction (MVC). The gain in torque of a twitch when preceded by an MVC is known as postactivation potentiation (PAP; Vandervoort, Quinlan and McComas, 1983). Twitch potentiation has been observed in both animals (Brown and von Euler, 1938; Tubman and Rassier, 1997; Wilson and Skirboll, 1974) and humans (O'Leary, Hope and Sale, 1997; Rassier, 2000) in a variety of skeletal muscles. The most accepted mechanism for PTP is phosphorylation of the regulatory light chains during the conditioning tetanus (Tubman, MacIntosh and Maki,

1996), most likely causing an increase in sensitivity of the actin-myosin to Ca^{2+} in the subsequent twitch.

1.7.1 Factors Affecting PTP

Several factors are known to influence the degree of PTP, involving both the characteristics of the tetanic stimulation and the particular muscle being examined. The intensity, frequency and duration of the conditioning tetanus can affect the magnitude of the potentiation. An increase of any of these three variables results in a greater immediate PTP (Hughes and Morrell, 1957). Muscle fiber type can also influence the extent to which a twitch is potentiated. In animal studies, PTP in muscles with predominately type I slow twitch fibers is smaller or almost non-existent when compared with fast muscles (Buller et al., 1981; Standaert, 1964). In a study by Vandervoort and McComas (1983), it was demonstrated that PTP was greater in the lateral gastrocnemius, with a higher percent of type II fibers, than in the soleus following a 3-second tetanus at 50Hz. In a more recent study examining PAP in human knee extensor muscles, Hamada and colleagues (2000) found that those muscles with the highest PAP following a 10-second MVC also had the greatest percentage of type II fibers. Another factor affecting the level of potentiation is muscle length. Vandervoort *et al.* (1983) observed that the degree of potentiation was greatest when the tibialis anterior muscle was in the shortened position (full dorsiflexion) compared to mid-position and lengthened position (full plantarflexion). In 2000, while examining fatigue and PTP in knee extensor muscles at 3 different lengths, Rassier also demonstrated that PTP was length-dependent. Although PTP was observed at all knee

angles, 39% ± 4, 47% ± 2 and 68% ± 5 at 90°, 60° and 30° respectively (full extension of the knee extensor = 0°), it was greatest at the shortest muscle length. Finally, the magnitude of PTP also increases as the temperature of the muscle increase (Gossen, Allingham and Sale, 2001).

1.7.2 PTP of the Human Tibialis Anterior Muscle

In the last several years PTP in lower limb muscles, more notably the human dorsiflexor muscles, has been investigated more thoroughly than other muscle groups. In 1997, O'Leary and colleagues found that 5 seconds following a 7-second tetanic stimulation at 100Hz, twitch peak torque of dorsiflexor muscles had increased by 45% of the pretetanic value. After this immediate enhancement a drop in PTP was observed at 1 min (28%). This was followed by a slight increase at 2 min to 33% and subsequent decay of PTP over time. It was suggested that the initial decay in PTP may be due partly to fatigue, which quickly fades allowing the level of potentiation to increase once again (O'Leary et al., 1997) Other contractile properties of the pre- and post-tetanic twitches were also compared, including muscle compound action potential (M-wave), twitch rise time and half relaxation time. Initially the M-wave of the potentiated twitches did not differ from that of the pretetanic twitch; however at 2 min the M-wave was significantly elevated (26%) then began to decrease (O'Leary et al., 1997). Furthermore, the posttetanic twitches had a decrease in rise time and half relaxation time compared to the pretetanic twitch (O'Leary et al., 1997). Similarly, a shortening of twitch rise time and

half relaxation time immediately following tetanus was also observed in the study by Gossen and colleagues (2001).

1.7.3 PTP of Paralyzed Human Muscle

Although the enhancement of twitch torque following a tetanic stimulation has been well documented in normal human muscles, the magnitude of PTP in diseased or paralyzed human muscles has been less studied. Following SCI there are significant muscular changes that occur due to injury below the level of lesion (Gerrits et al., 1999). There is well documented evidence in both animal and human studies which shows that type I fatigue resistant fibers transform into type II fibers following SCI (Shields, 1995). Furthermore, paralyzed muscle usually displays a decrease in torque production and generally faster contractile properties prior to fatigue, including shorter time to peak twitch torque and a decrease in half relaxation time characteristic of faster muscles (Gerrits et al., 1999; 2001; Shields, 1995; 2002). Thus, with a predominance of type II muscle fibers and faster contractile properties it would be predicted that after SCI paralyzed muscle should exhibit different PTP characteristics compared to controls.

Reduction of PTP has been documented in patients with myopathy and myasthenia gravis when compared to controls (Krarup, 1983). In 2006, Shields and colleagues examined the degree of post-fatigue potentiation (PFP) in both acutely and chronically (> 2 years) paralyzed soleus muscle, as well as determined the effects of long term electrical stimulation training on PFP of acutely paralyzed muscle. PFP refers to the potentiation of peak forces exhibited by fatigued muscles following repetitive single-

pulse activation or tetanic stimulation (Shields et al., 2006). Thus Shields *et al.* (2006) sought to investigate PTP once the paralyzed muscle had entered low-frequency fatigue (during the fourth bout of electrical stimulation). This is the only study to date that documents potentiation of whole muscle of individuals with SCI. Chronically paralyzed soleus muscle demonstrated PFP (90%) during repetitive activation, while no potentiation was observed in the acutely paralyzed group. One and a half to 2 years of electrical stimulation training was performed on one limb of the acutely paralyzed individuals. When comparing the trained versus the untrained limb after 2 years, the untrained limb exhibited significant potentiation (40%) while the trained limbs did not potentiate (Shields et al., 2006). When taking into account the increase in potentiation observed in muscles consisting of primarily of fast twitch fibers, it is apparent that in this study chronically paralyzed soleus muscle were behaving like fast muscles. This is consistent with the known transformation from type I to II muscle fibers following SCI (Chilibeck at al., 1999; Lotta et al., 1991; Round et al., 1993).

The examination of twitch and tetanic forces in SCI individuals was broadened in a recent study by Häger-Ross and colleagues (2006). In the study by Häger-Ross *et al.* (2006), posttetanic twitches were delivered to thenar motor units of individuals with SCI following 0.5 - 2s stimulation trains at various frequencies. This is the first study to examine post-tetanic potentiation of motor units in the SCI population. Findings indicated that thenar motor units from paralyzed muscle potentiate less than units from able-bodied controls (Häger-Ross et al., 2006). Although this was unexpected, the contractile properties of the thenar motor units were also found to be contrary to predicted. In

contrast to whole muscle research, the twitch contraction times of thenar motor units were found to be prolonged (Häger-Ross et al., 2006).

1.8 Summary and Statement of Purpose

Paralyzed muscles undergo many adaptations following chronic SCI. Although the data are limited, changes in contractile properties have been examined in several muscle groups with a focus on twitch response characteristics, fatigue properties and force-frequency response. The most commonly studied muscles include soleus, tibialis anterior, thenar and quadricep muscles. In general, faster contractile properties, including a shorter time to peak torque and shorter half relaxation time, have been observed in paralyzed muscle following chronic SCI (Gerrits et al., 1999; Rochester et al., 1995a; Shields 2002; Shields et al., 1997). There have been, however, some conflicting findings. In paralyzed thenar muscles (Thomas, 1997) and motor units (Häger-Ross et al., 2006), contractile speed has been shown to be either the same or slower than that observed in controls. It is unclear whether certain upper extremity muscles undergo different adaptations following chronic SCI. However the examination of contractile properties of several muscle groups, including soleus, tibialis anterior and thenar muscles, within the same individual may result in a better understanding of the changes following SCI.

In addition, paralyzed muscles are characterized by a reduction in fatigue resistance. However a more in depth analysis of fatigue characteristics is warranted that would allow for a better understanding of the degree of fatigability at various frequencies of stimulation, knowledge that can be applied towards the development of rehabilitation

programs, such as functional electrical stimulation. The FFR has also been examined in several paralyzed muscles, including the quadriceps femoris (Gerrits et al., 1999; 2000; Scott et al., 2006), tibialis anterior (Rochester et al., 1995a) and thenar muscles (Thomas, 1997). Despite the fact that paralyzed muscles exhibit contractile properties of faster muscles compared to controls, the few studies that have examined the force-frequency relationships have not shown the predicted rightward shift of the FFR curve, in fact they are generally shifted to the left (Gerrits et al., 2005).

There has also been very limited research done on twitch potentiation in paralyzed muscle. Due to the adaptations following chronic SCI, one would expect the paralyzed tibialis anterior muscle to contain a higher percentage of type II fibers than normal. Therefore, it would be anticipated that PTP in paralyzed tibialis anterior muscles would be greater than in control muscles. Thus, the main purpose of this investigation was to assess the contractile properties in both upper and lower limb muscles in individuals with SCI and to compare them to those in matched able-bodied controls. The primary objective was to characterize the changes in twitch and M-wave characteristics in the paralyzed soleus, tibialis anterior and thenar muscles. The second portion of this investigation involved an extended evaluation of the paralyzed tibialis anterior muscle, in which fatigue characteristics, the FFR and PTP in individuals with SCI were compared to controls.

Chapter 2
Twitch Contractile Properties of the Soleus, Tibialis Anterior and Thenar Muscles in Individuals with Spinal Cord Injury

2.1 Introduction

Paralyzed muscles undergo various adaptations in the first few years following SCI, including muscle atrophy and transformation of type I to type II muscle fibers (Gordon and Mao, 1994). It is expected that these changes would result in the conversion of muscle contractile properties as well. Decreased torque production and generally faster contractile properties prior to fatigue have been observed in several paralyzed muscles (Gerrits et al., 1999; 2001; Shields, 1995; 2002). The majority of studies have focused on twitch contractile properties in lower extremity muscles, especially the quadriceps femoris, tibialis anterior and soleus muscles.

The quadriceps femoris muscle of able-bodied individuals can have anywhere from an equal number of type I and II fibers (Chilibeck at al., 1999) to a slightly higher proportion of fast twitch fibers (vastus lateralis have approximately 53-68% type II fibers; Johnson et al., 1973). In contrast, it has been shown that the vastus lateralis of individuals with chronic SCI can have an upwards of 91% type II fibers (Chilibeck et al., 1999). In conjunction with this finding, chronically paralyzed quadriceps femoris muscle exhibit faster contractile properties, including faster time to peak torque and half relaxation time (Gerrits et al., 1999; 2001; Scott et al., 2006). Similar observations have

been made with postural muscles that have a higher proportion of slow fibers. Faster contraction and relaxation along with a decrease in force of the paralyzed tibialis anterior muscle compared to controls was observed by Rochester *et al.* (1995a) prior to electrical stimulation training. In a similar study, untrained paralyzed tibialis anterior muscle had faster contraction and half relaxation times compared to controls, though this difference was not significant (Stein et al., 1992). However, Stein *et al.* (1992) did find that the SCI group had significantly smaller M-wave amplitude than controls. In addition, they reported that twitch force of the paralyzed tibialis anterior muscle was similar to those of controls, suggesting that there may be less hypertrophy in the tibialis anterior muscle compared to other paralyzed muscles following SCI (Stein, et al., 1992). The soleus, another well studied postural muscle, is normally made up of almost exclusively type I slow fibers in able-bodied individuals (Johnson et al, 1973) and so should exhibit slower contractile properties compared to other fast twitch muscles. This is in contrast to the reduction in type I fibers and a higher percentage of type IIx fibers found in the paralyzed soleus muscle, 8-10 months post injury (Lotta et al., 1991). Furthermore, chronically paralyzed soleus muscle generally exhibits faster contractile properties compared to acutely paralyzed muscle (Shields et al., 1997), including, on average, a 20-ms shorter time to peak twitch torque and 25% shorter twitch half relaxation time following chronic SCI (Shields, 2002). Thus the chronically paralyzed soleus muscle had become a predominately fast twitch muscle.

Despite the convincing evidence supporting a change to faster contractile properties following SCI, there have been studies of the lower extremity muscles that

have found conflicting results. Jayaraman and colleges (2005) examined muscle function in knee extensor and plantarflexor muscle groups in individuals with incomplete SCI. Although an obvious deficit in voluntary torque production was observed, no differences were found between participants with SCI and able-bodied controls in electrically elicited contractile properties, including rate of rise and relaxation (Jayaraman, et al., 2005). There was also no difference in peak twitch torque of knee extensors or plantarflexor muscles between groups (Jayaraman, et al., 2005). However, the participants in this study were classified as mostly ASIA D and some ASIA C, and some were capable of ambulation over ground, thus they may not have experienced the same degree of adaptations following SCI as individuals with greater levels of impairment.

Although most studies exploring changes in contractile properties after SCI in humans focus on lower limb muscles, there are a few studies that have concentrated on upper limb muscles, namely the thenar muscles. One study found that thenar muscle contractile properties in persons with SCI depended on baseline M-wave amplitude. Those participants with low M-wave amplitudes had significantly lower tetanic forces compared to able-boded controls. Twitch force was only slightly lower while time to peak force and twitch half relaxation times were longer, though not significantly. This was in contrast to the SCI participants with relatively higher M-wave amplitudes, who had similar values to controls in tetanic force, time to peak torque and twitch half relaxation time (Thomas, 1997). Surprisingly significantly stronger twitch forces were also observed in this SCI subgroup compared to controls. In a more recent study assessing twitch properties in thenar motor units of individuals with chronic SCI, a

slowing of conduction velocity and twitch contraction time were also observed (Häger-Ross, Klein and Thomas, 2006). Motor units of paralyzed thenar muscles also displayed smaller EMGs, stronger twitch forces and weaker tetanic forces compared to control (Häger-Ross et al., 2006). Thus it appears that paralyzed thenar muscles undergo different adaptations in twitch contractile properties following SCI compared to muscles from the lower limbs and may even exhibit a transition to slower contractile speeds. The thenar muscles are supplied by the recurrent branch of the median nerve originating from the C8, T1 segments (compared to L4 for tibialis anterior and S1 for soleus). Therefore, while most individuals with tetraplegia will have some resultant paralysis of the thenar muscle group, the degree of impairment may be quite variable between individuals depending on the severity of the injury.

Individuals with SCI form a very heterogeneous population with varying levels of impairment even between individuals with the same injury level. This has hampered the interpretation of studies exploring changes in muscular properties following injury. Not only is there limited data for some muscle groups, but conflicting results have also been presented. Examining contractile properties of several muscle groups, including soleus, tibialis anterior (TA) and thenar muscles, in the same individual may result in a better understanding of the changes following SCI. Thus the purpose of this investigation was to assess the twitch contractile properties in both upper limb and lower limb muscles in individuals with SCI and to compare them to those in matched able-bodied controls. It was hypothesized that paralyzed muscle would exhibit smaller M-wave amplitudes and faster contractile properties, characteristic of muscle atrophy and fiber type

transformation. It was expected that the lower limb muscles, the soleus and TA muscles, would display a greater degree of adaptation compared to the thenar muscles.

2.2 Materials and Methods

2.2.1 Subjects

Nine people with SCI (8 ♂, 1♀) and 9 age- and gender-matched able-bodied controls (AB; 8 ♂, 1♀) participated in the study (Table 1 and 2 respectively). Inclusion criteria for the SCI participants were more than 1 year post injury, upper motor neuron lesion level higher than T2, American Spinal Injury Association (ASIA) classification A-C and wheelchair-dependent. The exclusion criterion for participants with SCI was a significantly reduced ankle range of motion (< 30°). All able-bodied participants were healthy with no history of neuromuscular disorders. The subjects participated with informed, written consent and with the approval of McMaster University's Research Ethics Board.

2.2.2 Apparatus

During all testing procedures, all of the SCI subjects remained in their wheelchairs except for one, who transferred into the same chair used by the AB group. While assessing contractile properties of the lower leg, the right leg was placed in a custom-made isometric ankle torque dynamometer (Marsh et al., 1981). For the first part of the protocol, testing of the soleus muscle, the ankle joint was fixed at 10° of

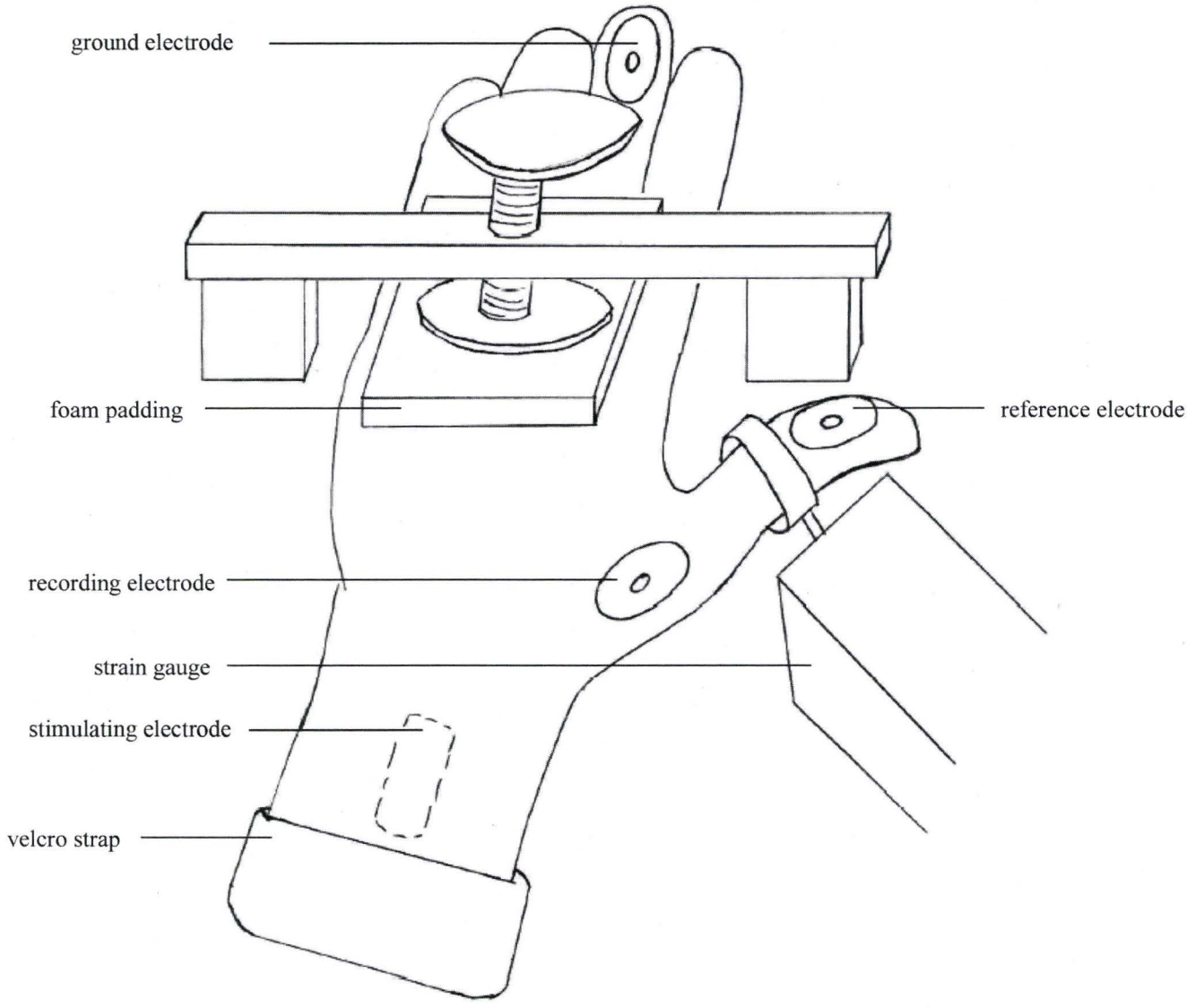
dorsiflexion from the neutral position (neutral position = 90° angle formed by tibia and sole of the foot). During stimulation of the TA muscle, the ankle joint was fixed at 10° of plantarflexion from the neutral position. These joint positions were chosen as they allowed for stretching of the soleus and TA, respectively, since longer muscle length ensure a maximal twitch response, but they were still within the range of ankle motion of the SCI participants. Velcro straps secured the foot to an aluminum plate instrumented with a strain gauge, which measured the torque generated by the contraction of the soleus or TA.

While assessing contractile properties of the thenar muscles, the right hand of each participant was secured in a custom made device (Figure 1). In the supine position, the lower arm was secured to a stabilizing platform with Velcro straps and the hand held in a fixed position by an adjustable padded clamp. Thenar force was measured using a 3.5 cm steel ring and steel cable assembly coupled in line with a 20 kg load cell (OMEGA Engineering, Model LC101-50; Stamford, CT). The steel ring was slipped over the interphlangeal joint of the extended right thumb and the complete arrangement positioned such that "on axis" linear force measurements resulted.

For both experimental set-ups (leg and hand), the stimulator used was a Digitimer (model 3072; Hertfordshire, UK). The signal from the load cells were amplified and low pass filtered at 1 kHz by a custom made DC amplifier. The EMG signal was amplified and band pass filtered 1 Hz- 1kHz, using a UFI device (model 2283f/I; Morro Bay, CA). Calibrated signals from both channels were digitized at 4 kHz using a Measurement & Computing device (Model USB-1608FS; Norton, MA). Data was stored for later

analysis, with custom designed software (Labview, National Instruments; Austin, TX), on an IBM-compatible personal computer.

Figure 1.



2.2.3 Stimulation and EMG Recording

In order to activate the soleus and TA muscles, the tibial and lateral peroneal nerve were stimulated by electrodes saturated with electrode gel, respectively. The skin was prepared by rubbing it with isopropyl alcohol wipes. The tibial nerve was stimulated by a double pronged electrode placed on the skin in the popliteal fossa. EMG disposable electrodes were applied to the skin over the muscle belly of the soleus (stigmatic), on the calcaneal tendon (reference) and on the peroneaus longus muscle region (ground).

For activation of the TA muscle, rubber stimulating electrodes (1" diameter) were placed on the skin over the neck of the fibula and the medial part of the tibia head. The electrodes were secured to the skin with micropore surgical tape. EMG disposable recording electrodes were applied to the skin over the muscle belly of the TA (stigmatic), the inferior extensor retinaculum (reference) and on the peroneaus longus muscle region (ground).

The thenar muscles were activated by stimulating the median nerve with a double prong electrode placed just proximal to the wrist. EMG electrodes were placed on the belly of the thenar muscles (stigmatic), on the distal phalanx of the thumb (reference) and on the distal phalanx of the middle finger (ground).

2.2.4 Protocol

The lower extremity muscles were assessed on the same day and the thenar muscles group was examined at least 3 days later. Upon arriving for the first laboratory visit, AB participants sat at rest for a minimum of 15 minutes before commencing. The

right legs of both the SCI and AB participants were secured in the apparatus. Contractile properties of the lower extremity muscles were determined separately; the soleus was examined first, then the TA. For each muscle, a maximum twitch response was determined by delivering a series of single stimuli (pulse width of 100μs) gradually increasing in intensity until the compound action potential (M-wave) and peak torque reached a plateau. On the second laboratory visit, single electrical stimuli (pulse width of 50μs) were delivered for the determination of maximum twitch and M-wave characteristics of the thenar muscles.

2.2.5 Measurements

Twitch response measurements included peak torque (PT), time to peak torque (TPT), half relaxation time (½RT), rate of torque development (RTD) and rate of torque relaxation (RTR). TPT was calculated as the time it took for the muscle to reach the peak torque once it started to develop force and ½RT was the amount of time for the torque to decline to 50% of the peak torque value. The peak-to-peak M-wave amplitude and M-wave duration corresponding to each twitch response was also measured.

2.2.6 Statistics

Data from SCI and AB participants were tested using an F-max test to determine whether or not the variance in each parameter was equal. On a measurement by measurement basis, if the groups had significantly different amounts of variance then the more conservative 2-tailed t-test for unequal variance was used to compare the values of

SCI and AB groups. For those measures where variance was equal between groups, an independent sample 2-tailed t-test was used to determine if differences exist between groups. Statistical significance was set at $p \leq 0.05$.

2.3 Results

The data from one male subject with SCI was not included in the analysis of soleus and TA muscles due to lack of EMG signal. The data from 3 subjects with SCI (2 male, 1 female) were not included in the thenar muscle analyses due to two subjects not being able to place their right hands in the custom built device and the other participant having an injury level of T1-T2 and thus had full motor function in his right hand.

All M-wave and twitch characteristics of the SCI and AB groups for all three muscle groups are given in Tables 3-5.

2.3.1 Soleus Muscle (n=16; 8 SCI, 8AB)

Unequal variance was found between SCI and AB groups for ½RT and RTD, and thus the t-test for unequal variance was used. Soleus PT of the AB group was 14.2 ± 3.9Nm, compared with 8.9 ± 6.1Nm in the SCI group (Figure 2). This difference, however, was not statistically significant ($p = 0.058$). TPT was significantly greater in the AB group compared to the SCI group (111.5 ± 15.4ms versus 76.7 ± 25.0ms, respectively; $p = 0.002$). This difference in TPT was primarily due to the difference in PT, since RTD was not statistically different between groups. Similarly, ½RT and RTR

were not different between the AB and SCI groups. See Figure 3 for speed contractile properties.

Peak-to-peak M-wave amplitude of the AB was more than double that of the SCI, 13.5 ± 5.3mV and 5.5 ± 4.0mV, respectively ($p = 0.004$). M-wave duration was similar in both groups.

Figure 2. Soleus Muscle

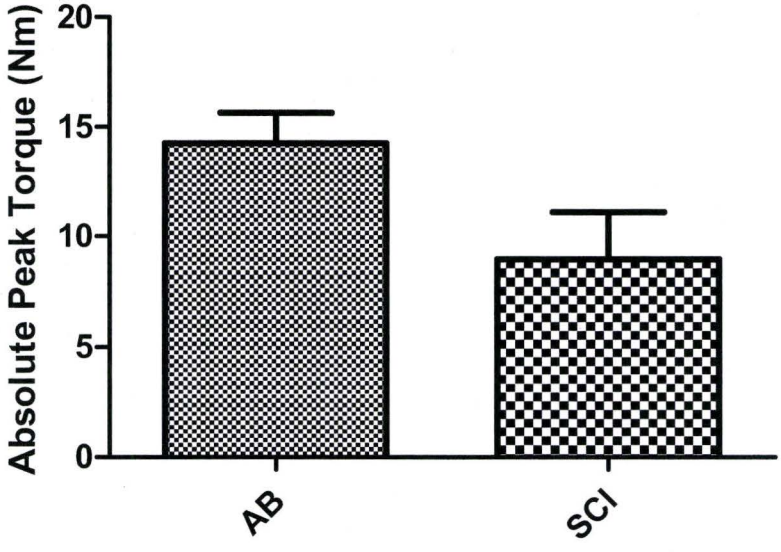


Figure 3a. Soleus Muscle

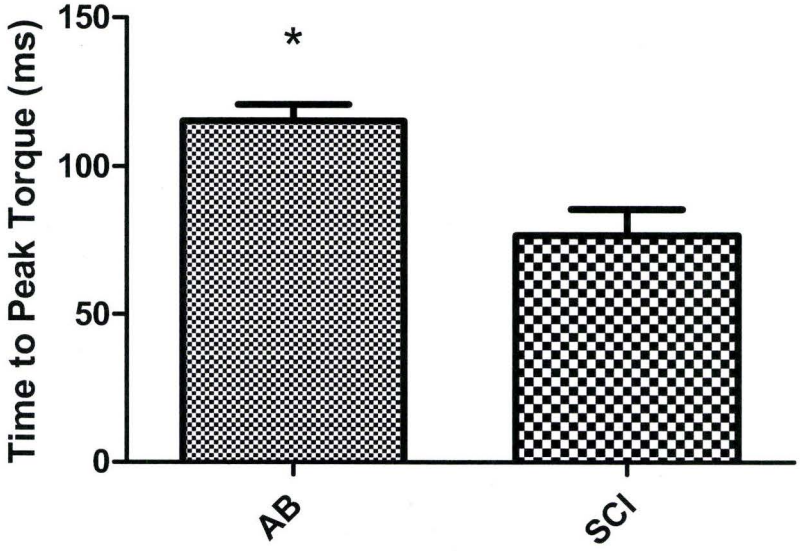


Figure 3b. Soleus Muscle

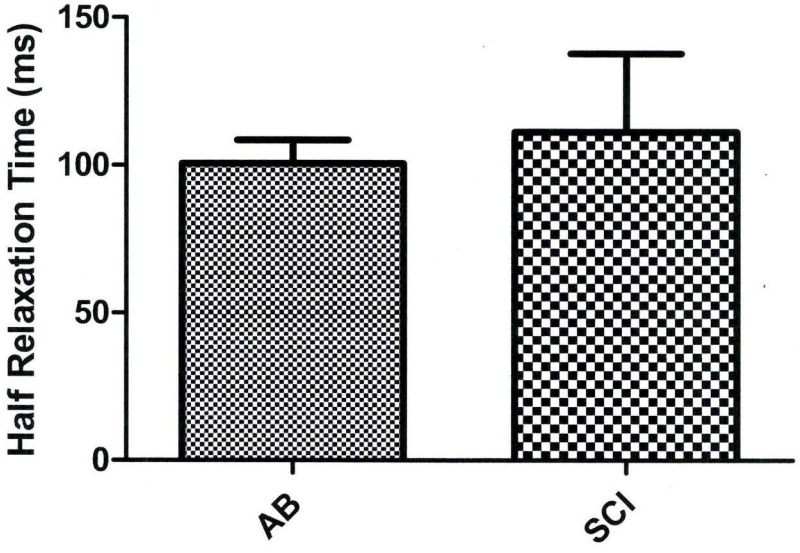


Figure 3c. Soleus Muscle

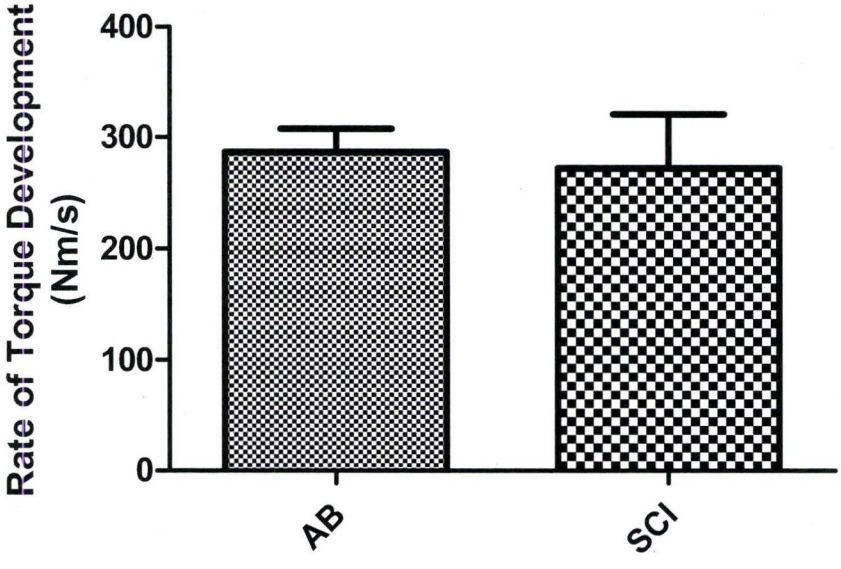
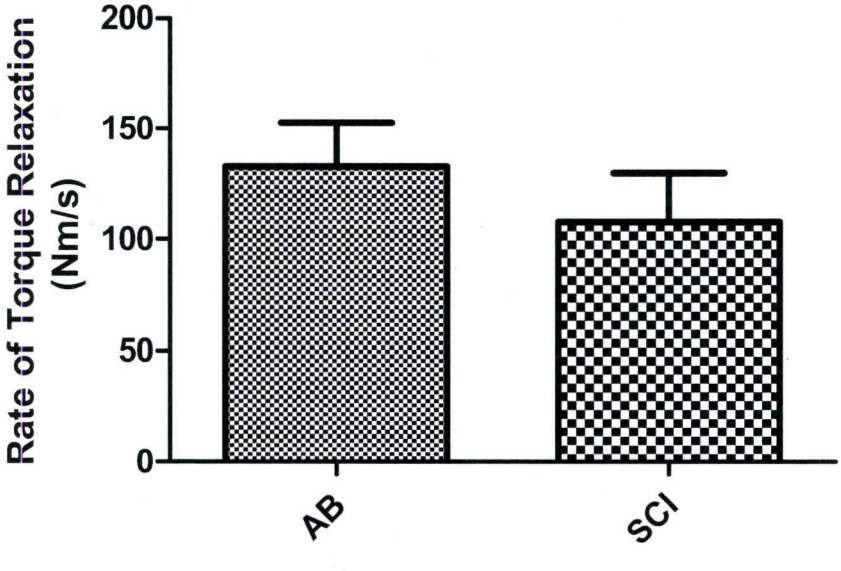


Figure 3d. Soleus Muscle



2.3.2 Tibialis Anterior Muscle (n=16; 8 SCI, 8AB)

Unequal variance was found for PT, TPT and RTD and thus the t-test for unequal variance was used to test for differences between SCI and AB for theses parameters. AB and SCI had similar PT values, 2.8 ± 0.5Nm and 3.2 ± 1.2Nm, respectively ($p = 0.361$; Figure 4). TPT was significantly longer in the AB group compared to the SCI group (78.8 ± 6.6ms versus 63.1 ± 16.6ms, respectively; $p = 0.035$) and RTD was significantly faster in the SCI group than AB group, 108.6 ± 39.7Nm/s compared to 69.3 ± 39.7Nm/s, respectively ($p = 0.027$). Both groups exhibited similar ½RTs. Although not statistically significant, RTR was slightly faster in the SCI group compared to AB (44.5 ± 18.7Nm/s versus 34.7 ± 8.4Nm/s, respectively; $p = 0.196$). For speed contractile properties, see Figure 5.

Peak-to-peak M-wave amplitude was significantly greater in the AB group compared to SCI (8.3 ± 2.6mV versus 4.2 ± 1.7mV, respectively; $p = 0.003$). M-wave duration, however, was similar between groups.

Figure 4. Tibialis Anterior Muscle

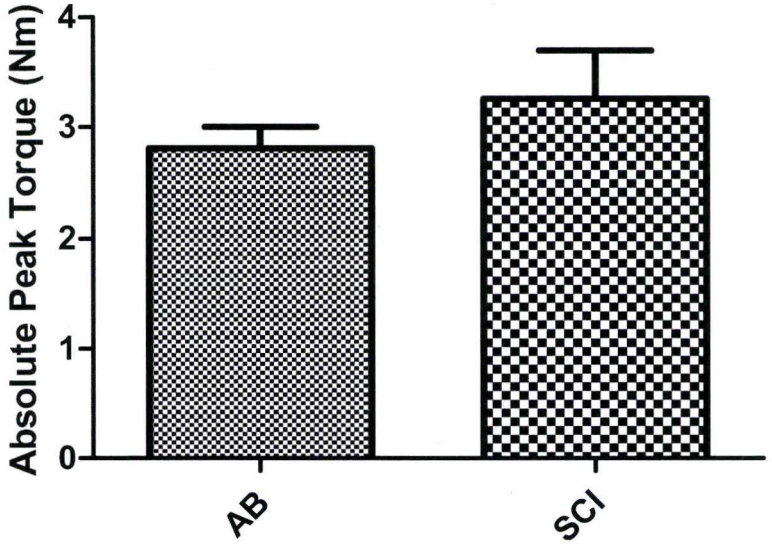


Figure 5a. Tibialis Anterior Muscle

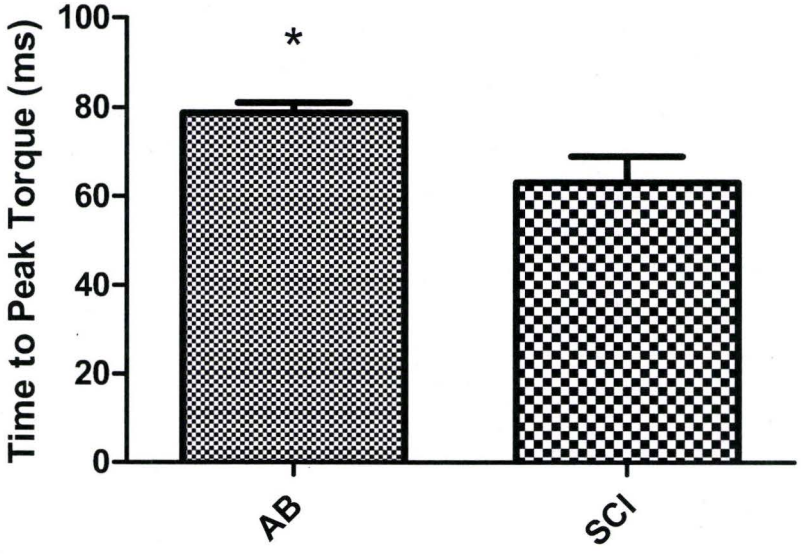


Figure 5b. Tibialis Anterior Muscle

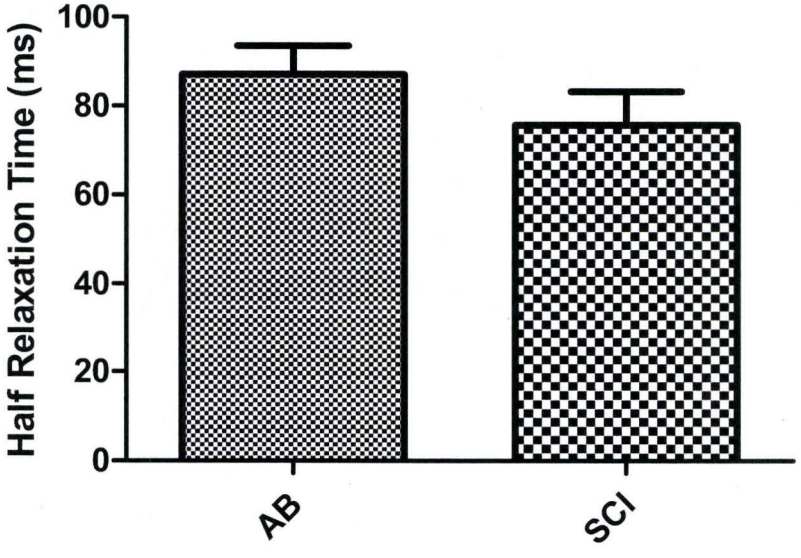


Figure 5c. Tibialis Anterior Muscle

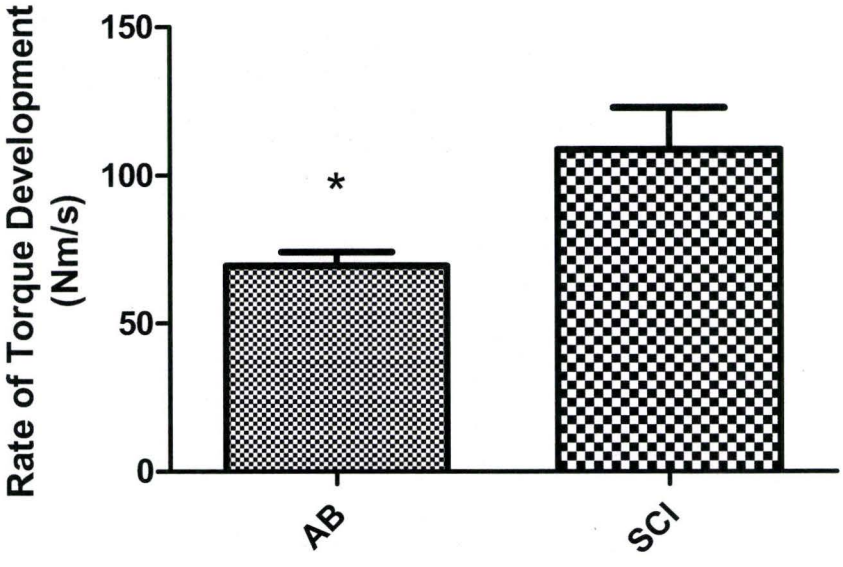
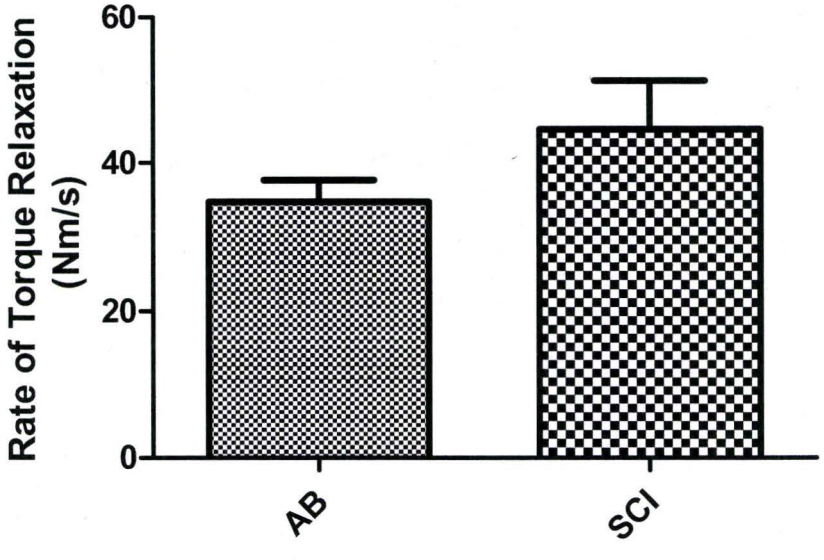


Figure 5d. Tibialis Anterior Muscle



2.3.3 Thenar Muscles (n=12; 6 SCI, 6AB)

Unequal variance between groups was only found for ½RT, but as no significant difference was found between groups (using normal t-test) the more conservative t-test for unequal variance was not performed. Although not statistically significant, PT was greater in the SCI group compared to AB (2.9 ± 1.3Nm versus 1.7 ± 0.8Nm, respectively; $p = 0.094$; Figure 6). Although TPT was similar in both groups, RTD was faster in the SCI group than the AB (96.0 ± 37.7Nm/s versus 54.8 ± 21.4Nm/s, respectively; $p = 0.042$). There was also a trend towards faster relaxation in the SCI group. ½RT of the SCI and AB groups were 80.4 ± 38.2ms and 66.7 ± 5.7ms, respectively ($p = 0.424$) while the RTR was 39.9 ± 15.3Nm/s for the SCI group and 27.6 ± 8.8Nm/s for the AB group ($p = 0.12$). See Figure 7 for speed contractile properties.

Although not significant, peak-to-peak M-wave was larger in the AB group compared to the SCI groups, 9.3 ± 2.6mV and 6.9 ± 2.4mV, respectively ($p = 0.124$). M-wave duration was similar between groups.

Figure 6. Thenar Muscles

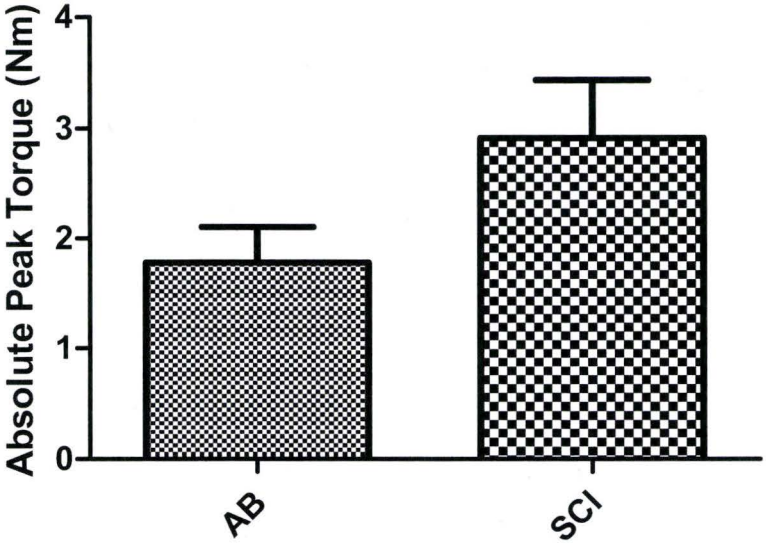


Figure 7a. Thenar Muscles

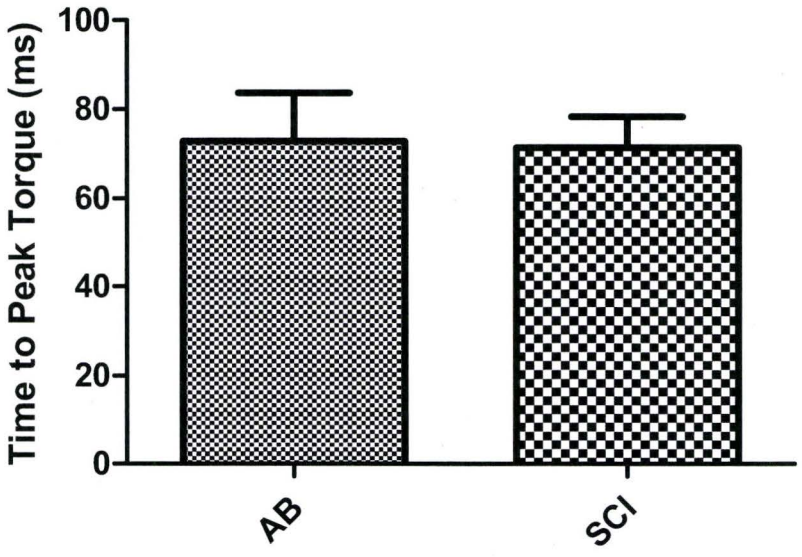


Figure 7b. Thenar Muscles

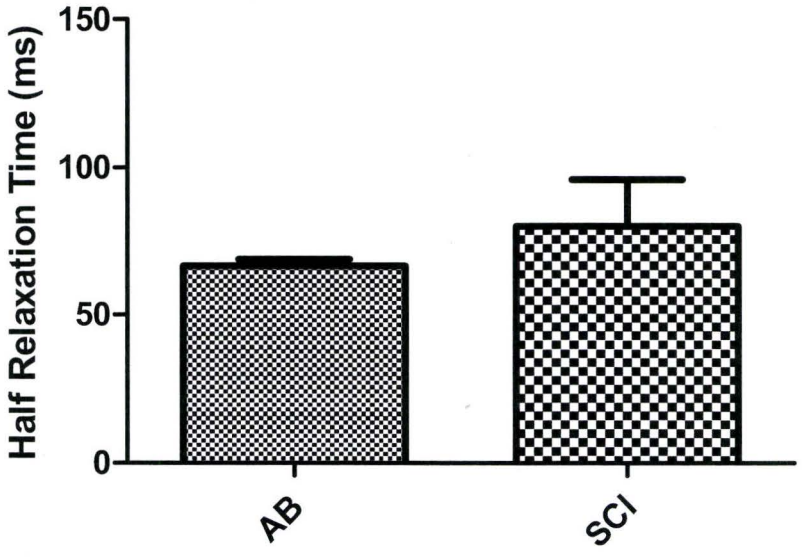


Figure 7c. Thenar Muscles

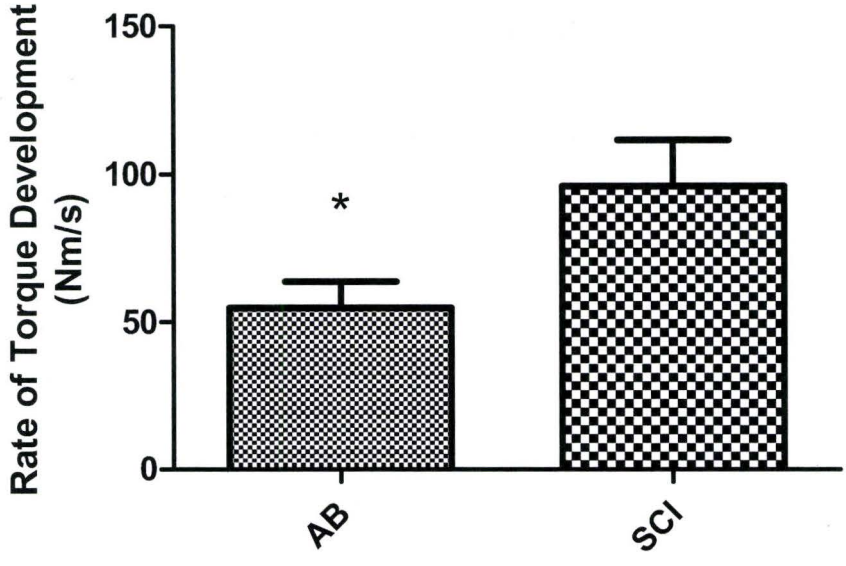
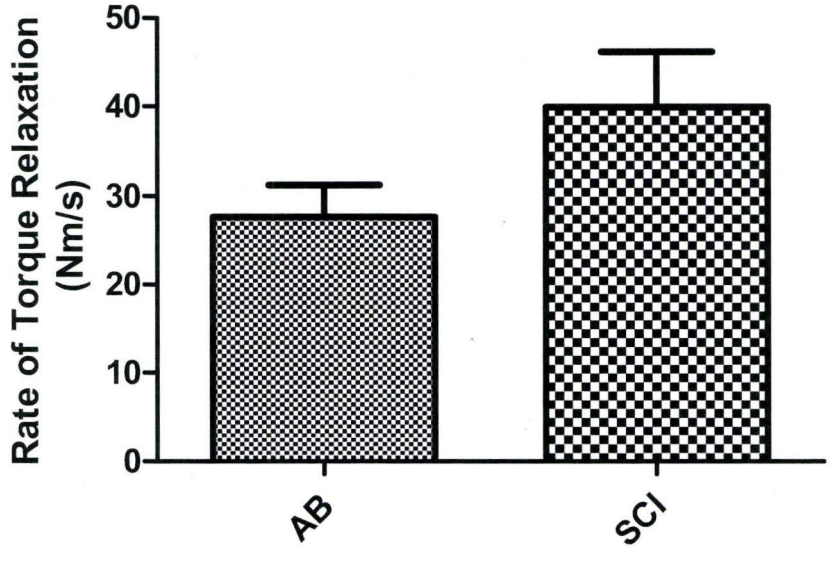


Figure 7d. Thenar Muscles



2.4 Discussion

A predominance of type II fibers and even a complete loss of type I fibers has been reported in paralyzed muscle of individuals with SCI (Burnham et al. 1997; Chilibeck at al., 1999; Malisoux et al., 2007; Round at al., 1993). It would be expected that along with fiber type transformation, changes in contractile properties would be observed as well. Several studies have examined adaptations that follow SCI in both small and large lower limb muscles, with more limited study done on upper limb muscles. The present study evaluated 3 different paralyzed muscles in the same individuals with chronic SCI to provide a more systematic evaluation of twitch contractile properties in this population. The main finding of this study was that changes in contractile properties following SCI differ between muscle groups. Faster contractile properties indicative of fiber type transformation were more evident in the TA and thenar muscle groups, compared with the soleus.

It was surprising that the paralyzed soleus muscle did not have faster contraction times similar to the other muscles examined. This does not support earlier findings of shorter contraction and relaxation in chronically paralyzed soleus muscle (Shields, 2002; Shields et al., 1997). One possible explanation for the lack of differences observed in the paralyzed soleus muscle compared to AB could be the spasticity level in the SCI group. There are studies that suggest the degree of spasticity can influence the muscle dynamics in individuals with SCI. Ditor *et al.* (2004) suggested that spasticity may preserve or enhance the expression of slow twitch fibers. Also the changes in contractile properties after SCI exhibited in paralyzed soleus muscles of individuals with low degrees of

spasticity have been shown to be greater than in those with high degrees of spasticity (Hidler, Harvey and Rymer, 2002). It was suggested that although high levels of spasticity do not sufficiently build muscle, it may allow for adequate maintenance of contractile dynamics (Hidler et al., 2002). Furthermore it was suggested by Hidler and colleagues (2002) that it is not possible to draw general conclusions about contractile properties for the whole SCI population but that it is necessary for individuals with SCI to be grouped according to spasticity level prior to examination of muscle characteristics. In the present study some individuals in the SCI group had high levels of spasticity, although spasticity was not quantified in this study. Also many of the SCI participants were on medication to reduce spasticity (Baclofen), thus it is difficult to account for the possible effect of spasticity in this particular study.

The shift towards faster contractile properties observed in the TA was expected, despite previous mixed reports. Although the study by Rochester *et al.* (1995a) observed significantly faster contraction and relaxation times in the TA of individuals with SCI, Stein *et al.* (1992) did not. The results from this study support the expected increase in contractile speed, most likely due to atrophy of type I fibers and transition from type I to II fibers.

Another surprising finding was the faster contractile speeds in the paralyzed thenar muscles compared to the AB. The significant increase in RTD observed in the paralyzed thenar muscles is in contrast with previous studies. Thomas (1997) found that individuals with chronic SCI exhibited normal or slightly slower contractile speeds, compared with controls. The SCI participants in that study all had cervical injuries with

complete paralysis of the thenar muscles, however adaptations in contractile speeds were still not observed. In a more recent study assessing twitch properties in thenar motor units of individuals with chronic SCI, a slowing of conduction velocity and twitch contraction time were also observed (Häger-Ross, Klein and Thomas, 2006). It was suggested that the slow conduction velocities could be a result of deficits in the axon or muscle while the prolonged contraction times could be due to changes in calcium release or sensitivity of the actin-myosin to Ca^{2+} following chronic SCI (Häger-Ross et al., 2006). It is obvious that the contractile properties of thenar muscles have been under-studied, especially in relation to other muscle groups, and warrant further evaluation.

The significantly smaller M-waves seen in the lower extremities support the predicted muscle atrophy following SCI, while there was only a trend for smaller M-wave amplitude in the thenar muscles. This decreased M-wave amplitude in the lower limb muscles was expected. As M-wave amplitude is often a property used to assess muscle loss and weakness (Thomas, 1997), it can be assumed that the SCI participants had significant muscle atrophy in both the soleus and TA muscles. These observations support earlier findings, such as by Stein *et al.* (1992) who found that the maximum M-waves of paralyzed TA were significantly lower than able-bodied controls.

Another interesting finding was that the average peak torque in the SCI group was comparatively different than in the AB group depending on the particular muscle. While the soleus muscle in the SCI generated less torque than the AB, the paralyzed thenar and TA muscles appeared to generate more torque than the AB. Thomas (1997) observed increased twitch force in paralyzed thenar muscles but only in individuals with SCI who

had normal M-wave amplitudes. Those participants with significantly lower M-wave amplitudes compared to AB had similar twitch forces to AB (Thomas, 1997). It has been suggested that some paralyzed muscles, even after years of disuse, may maintain their strength due to changes in muscle length and loading (Thomas, 1997). Muscles that are maintained at longer lengths while immobilized retain more of their strength than muscle kept at shorter lengths (Simard, Spector and Edgerton, 1982). For example, if spasms regularly occur in the soleus muscle then the TA muscle, its antagonist, will remain in the lengthened position (Stein et al., 1992). However chronic inactivity of the paralyzed muscle could also lead to the shortening of muscle length (Scott et al., 2006).

One obvious limitation of this study was the small sample size. Though this is common for investigations in this population, with a smaller sample size there is less power with which to detect statistically significant differences and less confidence that the findings reflect those of the whole SCI population. It also makes it difficult to split SCI participants into groups based on factors that may influence findings, such as spasticity. In addition, the mean age of the SCI participants in this study was 40.3 ± 11.7 years. This is generally older than the average age (20-40years) of the participants in the majority of studies examining contractile properties following SCI, which could account for some disparity observed between this study and previous ones. Methodological differences, such as joint angle, and other differences in participant characteristics, such as level of impairment, could also play a role in the results obtained in this study compared to others. In order to accommodate for the decreased range of motion in the SCI participants, the ankle joint angles, in this study, were set at 10° of plantarflexion

(PF) and 10° of dorsiflexion (DF) for the evaluation of the TA and soleus muscles, respectively. However, these joint angles do not place the TA and soleus muscles at their optimal length for force production in the AB group. Previous evaluation of the length-tension relationship in human muscles have demonstrated that the maximum peak torque occurred when the plantarflexor muscles are placed at 20° of DF (Winegard, Hicks and Vandervoort, 1997) and the dorsiflexor muscles at 30° of PF (van Schaik, Hicks and McCartney, 1994) in both young and elderly able-bodied individuals. Thus twitch contractile properties examined may have been underestimated in the AB group. As the length-tension relationship of the paralyzed TA and soleus muscles has not been examined, the optimal angles for force production in these muscles are unknown, though it is possible that paralyzed muscles were overly stretched. Future comparisons of optimal angles for force production between AB and individuals with SCI are needed so that the optimal angle for each group can be selected.

Finally, it has been suggested that the normally colder muscle temperatures of individuals with SCI may have lead to an underestimation of contractile speed (Gerrits et al., 2000). Gerrits *et al.* (2000) found that when paralyzed vastus lateralis muscle was heated to that of the average temperature of the AB muscle, the paralyzed muscle exhibited faster contractile speeds. In conclusion, following SCI there are adaptations of contractile properties that differ between muscle groups. Faster contractile properties indicative of fiber type transformation were more evident in TA and thenar muscle groups, compared with the soleus muscle. Smaller M-waves were seen only in the lower extremities, supporting the significant muscle atrophy following SCI.

2.5 Figure Captions

Figure 1. Drawing of hand (in supine position) in the custom-made apparatus

Figure 2. Mean (± SE) absolute peak torque generated by the soleus muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 3a. Mean (± SE) absolute time to peak torque of the soleus muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 3b. Mean (± SE) absolute half relaxation time of the soleus muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 3c. Mean (± SE) rate of torque development of the soleus muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 3d. Mean (± SE) rate of torque relaxation of the soleus muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 4. Mean (± SE) absolute peak torque generated by the tibialis anterior muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 5a. Mean (± SE) absolute time to peak torque of the tibialis anterior muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 5b. Mean (± SE) absolute half relaxation time of the tibialis anterior muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 5c. Mean (± SE) rate of torque development of the tibialis anterior muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 5d. Mean (± SE) rate of torque relaxation of the tibialis anterior muscle in response to single stimuli at maximum intensity of AB (n=8) and SCI (n=8) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 6. Mean (± SE) absolute peak torque generated by the thenar muscles in response to single stimuli at maximum intensity of AB (n=6) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 7a. Mean (± SE) absolute time to peak torque of the thenar muscles in response to single stimuli at maximum intensity of AB (n=6) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 7b. Mean (± SE) absolute half relaxation time of the thenar muscles in response to single stimuli at maximum intensity of AB (n=6) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 7c. Mean (± SE) rate of torque development of the thenar muscles in response to single stimuli at maximum intensity of AB (n=6) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 7d. Mean (± SE) rate of torque relaxation of the thenar muscles in response to single stimuli at maximum intensity of AB (n=6) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Table 1. Characteristics of Subjects with SCI

	Subject No.	Sex	Age at Enrollment	SCI Level	ASIA Class	Time since injury (y)	Medications
	1	M	34	C4	C	9.5	Ba; Ep; Te; Co; Gl
*	2	F	22	C3-4	A	9	Am; Ba; De; Ni; Se; So
*	3	M	30	C4	B	4	Ba; Co; De; Se
*	4	M	47	C5-6 break; T1-2 lesion	B	29	Mo; Ox
	5	M	45	C5-7	C	19	Ac; Ba; Co; Di
	6	M	59	C5-7	C	20	none
	7	M	38	C6	A	15	Ba; Di
#	8	M	35	C5-6	B	13	Ax; Ba; Co; Di
	9	M	53	C4-7	C	21	Ba; Pa; Va; Di; No

* Did not participate in evauation of thenar muscles

Did not participate in evauation of soleus and TA muscles

Medications: Ac - Actonel; Am - Amitriptyline; Ax - Axid; Ba - Baclofen; Co - Colace De - Detrol; Di - Ditropan; Ep - Epival; Gl - Glucosamine; Mo - Monocycline; Ni - Nitrofurantoin; No - Norvasc; Ox - Oxybutynin chloride; Pa - Paxil; Se - Senokot; So - Soflax; Te - Tegretol; Va - Vasotec

Table 2. Characteristics of Able-bodied Subjects

	Subject No.	Sex	Age at Enrollment
	1	M	32
*	2	F	23
*	3	M	29
*	4	M	50
	5	M	42
	6	M	59
	7	M	36
#	8	M	35
	9	M	55

* Did not participate in evauation of thenar muscles

Did not participate in evauation of soleus and TA muscles

Table 3. Soleus - EMG and Torque Parameters

	SCI	AB
M-wave Amplitude, mV	5.5 ± 4.0	13.5 ± 5.3 *
EMG Duration, ms	21.1 ± 5.8	19.4 ± 4.9
Twitch Peak Torque, Nm	8.9 ± 6.1	14.2 ± 3.9
Time to Peak Torque, ms	76.7 ± 25.0	115.5 ± 15.4 *
Half Relaxation Time, ms	111.2 ± 74.3	100.5 ± 22.5
Rate of Torque Development, Nm/s	272.5 ± 136.9	286.8 ± 58.7
Rate of Torque Relaxation, Nm/s	107.5 ± 62.8	132.6 ± 56.7

Values are means ± SD

* Denotes significant difference between groups ($p<0.05$)

Table 4. Tibialis Anterior - EMG and Torque Parameters

	SCI	AB
M-wave Amplitude, mV	4.3 ± 1.7	8.4 ± 2.7 *
EMG Duration, ms	42.7 ± 8.2	42.8 ± 5.2
Twitch Peak Torque, Nm	3.3 ± 1.2	2.8 ± 0.5
Time to Peak Torque, ms	63.2 ± 16.6	78.8 ± 6.6 *
Half Relaxation Time, ms	75.7 ± 21.2	87.1 ± 17.9
Rate of Torque Development, Nm/s	108.7 ± 39.7	69.3 ± 13.2 *
Rate of Torque Relaxation, Nm/s	44.6 ± 18.7	34.7 ± 8.4
Twich to Tetanus Ratio	0.22 ± 0.05	0.099 ± 0.03 *

Values are means ± SD

* Denotes significant difference between groups ($p<0.05$)

Table 5. Thenar - EMG and Torque Parameters

	SCI	AB
M-wave Amplitude, mV	6.9 ± 2.3	9.3 ± 2.6
EMG Duration, ms	28.5 ± 6.4	25.5 ± 4.9
Twitch Peak Torque, Nm	2.9 ± 1.2	1.7 ± 0.7 *
Time to Peak Torque, ms	71.3 ± 17.2	72.7 ± 26.8
Half Relaxation Time, ms	80.4 ± 38.2	66.7 ± 5.6
Rate of Torque Development, Nm/s	96.0 ± 37.6	54.8 ± 21.4 *
Rate of Torque Relaxation, Nm/s	39.9 ± 15.2	27.6 ± 2.7

Values are means ± SD

* Denotes significant difference between groups ($p<0.05$)

Chapter 3
Fatigue and Force-Frequency Relationship of the Tibialis Anterior Muscle in Individuals with Spinal Cord Injury

3.1 Introduction

Following chronic spinal cord injury (SCI) a transition from type I to II fibers has been observed in several paralyzed muscles (Burnham et al., 1997; Chilibeck et al., 1999). With a predominance of type II fibers, a reduction in fatigue resistance would be expected. Several studies have demonstrated that following SCI chronically paralyzed muscle exhibits reduced resistance to fatigue characterized by a greater force decline and an increase in half relaxation time (Gerrits et al., 1999; 2001; Shields, 2002). In the quadriceps muscle, Gerrits *et al.* (1999) demonstrated a faster rate of fatigue in the paralyzed group compared with able-bodied controls. A significant decrease in force was evident after only 2 minutes and greater prolongation of half relaxation time was also observed (Gerrits et al., 1999). Also in the quadriceps femoris, Castro and colleagues (2000) examined acutely injured individuals from 6 weeks to 24 weeks post injury and compared fatigability to able-bodied controls. The drop in force following a fatigue protocol was found to be significantly greater in the SCI group than controls, 27% and 9% decrease, respectively.

Similar observations have been made in the chronically paralyzed tibialis anterior muscle. Rochester *et al.* (1995a) found that the paralyzed tibialis anterior muscles

exhibited reduced fatigue resistance. Prior to this study, Stein *et al.* (1992) observed a similar increase in fatigability in the tibialis anterior muscle. A considerable decrease in force (70% decline in 3 minutes) was observed in the group with chronic SCI, while force was relatively well maintained in the control group (only 20% decline). In the soleus muscle, a decline in fatigue resistance after SCI has also been reported. Following a fatigue paradigm of 20Hz trains delivered for 330ms every second for 4 minutes, chronically paralyzed soleus muscle demonstrated 80% less torque, had 20% reduced M-wave amplitudes and 40% increased M-wave duration compared to individuals with acute SCI (< 6 weeks post-injury; Shields et al., 1998).

In 2006, Shields *et al.* attempted to establish the time course of changes in fatigue resistance in paralyzed human soleus muscles. By evaluating the Fatigue Index (FI; quotient of torque generated at the beginning and end of a series of repetitive contractions) of individuals with SCI varying from 3 weeks to 12 years post injury, a rapid decline in FI was observed immediately following SCI with a break point occurring at 1.7 years (Shields et al., 2006). It was suggested that the increased fatigability that occurs in the first 1.7 years may be due to a predominance of type I fiber atrophy, conversion of type I to II fibers or chronic disuse (Shields et al., 2006).

Although increased fatigability of paralyzed muscle is consistently observed, it is difficult to compare study results because of methodological differences in the fatigue paradigms used. Increased fatigability of paralyzed muscle has been demonstrated using a variety of stimulation pulse patterns and frequencies (20, 30 or 40Hz). Additionally several of these have cited using a variation of the Burke Fatigue Protocol (Burke et al.,

1973), a fatigue paradigm used to assess fatigue sensitivity in motor units of the cat gastrocnemius. It might be predicted that fatigue resistance depends on the frequency of the stimulation, yet few studies have compared different stimulation patterns to evoke fatigue. It is evident that a more in-depth analysis of fatigue characteristics is warranted, especially in some of the less-studied muscle groups, e.g. tibialis anterior. This would allow for a better understanding of the degree of fatigability at various frequencies of stimulation, knowledge that can be applied towards the development of rehabilitation programs.

The nonlinear force-frequency curve depicts the relationship between stimulation rate and resultant mean muscle force (Heckman and Rymer, 2003). Characteristically sigmoidal in shape, the steep portion of the curve occurs at relatively low discharge rates in slow muscles (shift to left) while faster muscles only achieve full tetanus after more frequent neural activation (shift to right; Heckman and Rymer, 2003). Despite the known shift in fiber type distribution from slow to fast fibers following SCI, the FFR in paralyzed muscle does not always shift in the expected direction. It would be expected that paralyzed muscle, associated with a greater percentage of type II fibers, would exhibit a right-ward shift in the FFR. However a recent study by Scott *et al.* (2006) examining contractile properties in the quadriceps femoris muscle, demonstrated that there was no difference in the FFR between individuals with chronic SCI and able-bodied controls in the non-fatigued state. Furthermore, many studies have found an unexpected leftward shift in FFR in the paralyzed quadriceps (Gerrits et al., 1999; 2001), tibialis anterior (Rochester et al., 1995a) and thenar muscles (Thomas, 1997). Although it is

unclear why this leftward shift occurs many explanations have been suggested, such as changes in muscle compliance (Thomas, 1997), an impaired high-frequency response or an augmented low-frequency response (Gerrits et al., 1999). In fact, it has been suggested that the leftward shift in FFR may be an actual property of paralyzed muscle (Gerrits et al., 2005) even though it does not support the conversion from slow to fast muscle fibers following SCI.

Thus the purpose of this study was to characterize the force-frequency relationship and fatigability at 2 different frequencies of the paralyzed tibialis anterior muscle. It was hypothesized that the chronically paralyzed tibialis anterior muscle would be less fatigue resistant, especially at lower stimulation frequencies. Further, similar to previous studies, we expected that paralyzed muscle would exhibit a FFR shifted to the left compared to that of able-bodied controls.

3.2 Materials and Methods

3.2.1 Subjects

Seven people with SCI (6 ♂, 1♀) and 7 age- and gender- matched able-bodied controls (AB; 6 ♂, 1♀) participated in the study (Table 1 and 2 respectively). Inclusion criteria for the SCI participants were more than 1 year post-injury, upper motor neuron lesion level higher than T2, American Spinal Injury Association (ASIA) classification A-C and wheelchair-dependent. The exclusion criterion for participants with SCI was a significantly reduced ankle range of motion (< 30°). All AB participants were healthy

with no history of neuromuscular disorders. The subjects participated with informed, written consent and with the approval of McMaster University's Research Ethics Board.

3.2.2 Apparatus

During all testing procedures, all of the SCI subjects remained in their wheelchairs except for one, who transferred into the same chair used by the AB group. For assessment of FFR and fatigue, the right leg was placed in a custom-made isometric ankle torque dynamometer (Marsh et al., 1981). The ankle joint was fixed at 10° of plantarflexion from the neutral position (neutral position = 90° angle formed by tibia and sole of the foot). This joint position, although not completely optimal for the AB group (van Shaik et al., 1994), was chosen because it was within the range of ankle motion of the SCI participants. Velcro straps secured the foot to an aluminum plate instrumented with a strain gauge, which measured the torque generated by the contraction of the tibialis anterior.

The stimulator used was a Digitimer (model 3072; Hertfordshire, UK). The signal from the torque dynamometer was amplified and low pass filtered at 1 kHz by a custom made DC amplifier. The EMG signal was amplified and band pass filtered 1 Hz-1kHz, using a UFI device (model 2283f/I; Morro Bay, CA). Calibrated signals from both channels were digitized at 4 kHz using a Measurement & Computing device (Model USB-1608FS; Norton, MA). Data was stored for later analysis, with custom designed software (Labview, National Instruments; Austin, TX), on an IBM-compatible personal computer.

3.2.3 Stimulation and EMG Recording

In order to activate the TA muscles, the lateral peroneal nerve was stimulated by electrodes saturated with electrode gel. The skin was prepared by rubbing it with isopropyl alcohol wipes. Rubber stimulating electrodes (1" diameter) were placed on the skin over the neck of the fibula and the medial part of the tibia head. The electrodes were secured to the skin with micropore surgical tape. EMG disposable recording electrodes were applied to the skin over the muscle belly of the TA (stigmatic), the inferior extensor retinaculum (reference) and on the peroneaus longus muscle region (ground).

3.2.4 Protocol

Subjects came to the laboratory on 2 separate days for the assessment of fatigue and FFR. Upon arrival at the laboratory, AB participants sat at rest for a minimum of 15 minutes before commencing. The right legs of both the SCI and AB participants were secured in the apparatus. A maximum twitch response was determined by delivering a series of single stimuli (pulse width of 100μs) gradually increasing in intensity until the compound action potential (M-wave) and peak torque reached a plateau. The stimulation intensity for all procedures was set at the intensity needed to elicit the maximum M-wave.

On day 1, force-frequency relationship was determined for the non-fatigued TA muscle by delivering electrical stimuli in 1s bursts at the following frequencies of 1 (single twitch), 10, 15, 30, 45, 60 and 100Hz. Burst were delivered randomly with 2 minutes of rest in between each frequency. Following a 10-minute rest period, the 15Hz

fatigue was performed consisting of 1 minute of electrical simulation at 15Hz. On the second laboratory visit, following a 15-minute rest period, the 30Hz fatigue was performed, which consisted of 1 minute of electrical simulation at 30Hz.

3.2.5 Measurements

To quantify the FFR, the average peak torque (PT) over the 1-second burst was measured for each frequency. Each subject's PT was normalized to the 100Hz response. F50, the frequency required to elicit 50% of maximum PT, was calculated for each group. The peak force response of the single pulse and the 1-second 100Hz burst were used to calculate the twitch-to-tetanus ratio for each of the groups.

To assess fatigue characteristics, PT and peak-to-peak M-wave were measured over the 1 minute protocol. The average torques elicited at 5-s intervals (and corresponding M-waves) were analyzed, for a total of 12 time points over the 1 minute period.

3.2.6 Statistics

For the FFR analysis, a two-factor (between, group; within, frequency) analysis of variance (ANOVA) was used to compare the PT at each frequency. F50 and twitch-to-tetanus ratios were analyzed separately with a 2-tailed independent sample t-test, to determine if differences existed between the SCI and AB groups. F-max tests were performed to determine if the SCI and AB groups had significantly different variances for

both parameters. If the groups had significantly different variance then the more conservative t-test for unequal variance was used.

A two-factor (between, group; within, time) repeated measures ANOVA was used to compare each of the fatigue properties (torque and peak-to-peak M-wave amplitude) over the 1 minute fatigue. Statistical significance was set at $p \leq 0.05$.

3.3 Results

All SCI and AB subjects participated in the assessment of fatigue and FFR. However, the data from one of the subjects in the SCI group was omitted from the FFR analysis due to technical difficulties in achieving torques at frequencies above 45Hz.

3.3.1 FFR and Twitch-to-Tetanus Ratio

Analysis of PT at different frequencies revealed a significant main effect for frequency and a significant interaction between group and frequency. Although higher frequencies elicited greater PT in both groups, this effect was greater in the AB compared to SCI. At 100Hz, the PT was significantly different between the AB and SCI groups, 28.4 ± 11.5Nm and 17.4 ± 3.1Nm, respectively ($p = 0.002$).

When normalized to the 100Hz response, the FFR of the SCI was shifted to the left of the AB (Figure 1). F50 of the SCI was significantly lower than that of the AB (6.7 ± 3.4Hz and 16.7 ± 4.1Hz, respectively; Figure 2). The twitch-to-tetanus ratio was significantly larger in the SCI group compared to the AB (0.218 and 0.098, respectively; Figure 3).

Figure 1

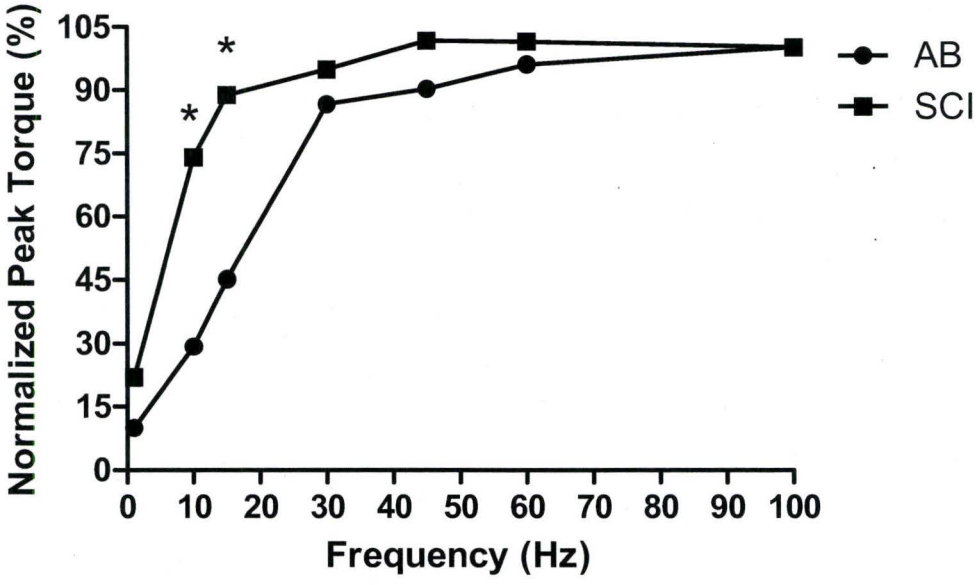


Figure 2

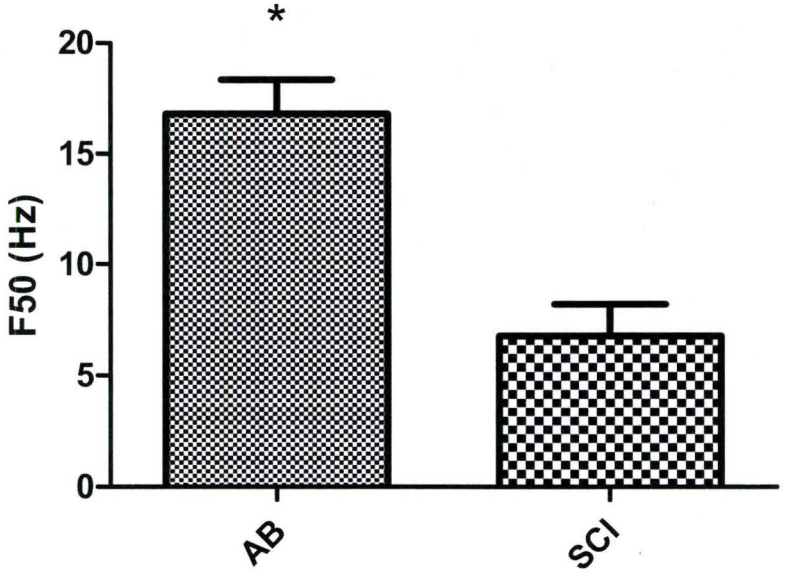
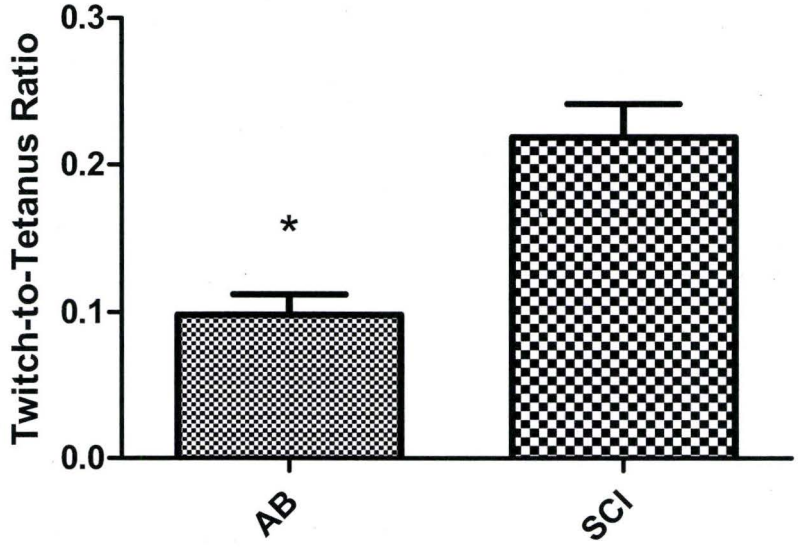


Figure 3



3.3.2 Fatigue - 15Hz

Analysis of PT over the one minute fatigue at 15Hz indicated that there was a significant main effect for time and significant interaction between time and group ($p < 0.05$ for both). The AB group did not experience any significant decline in torque over the one minute, whereas the force output of the SCI group did decrease (Figure 4). Although there was not a significant main effect for group ($p = 0.069$), the SCI group had lower PT throughout the whole minute compared to AB.

Peak-to-peak M-wave amplitude were similar in both groups over the one minute fatigue, though there was a slight trend towards lower M-wave amplitudes in the SCI group compared to AB (7.2 ± 4.0mV versus 10.0 ± 4.4mV, respectively; $p = 0.272$; Figure 5).

Figure 4. 15Hz Fatigue

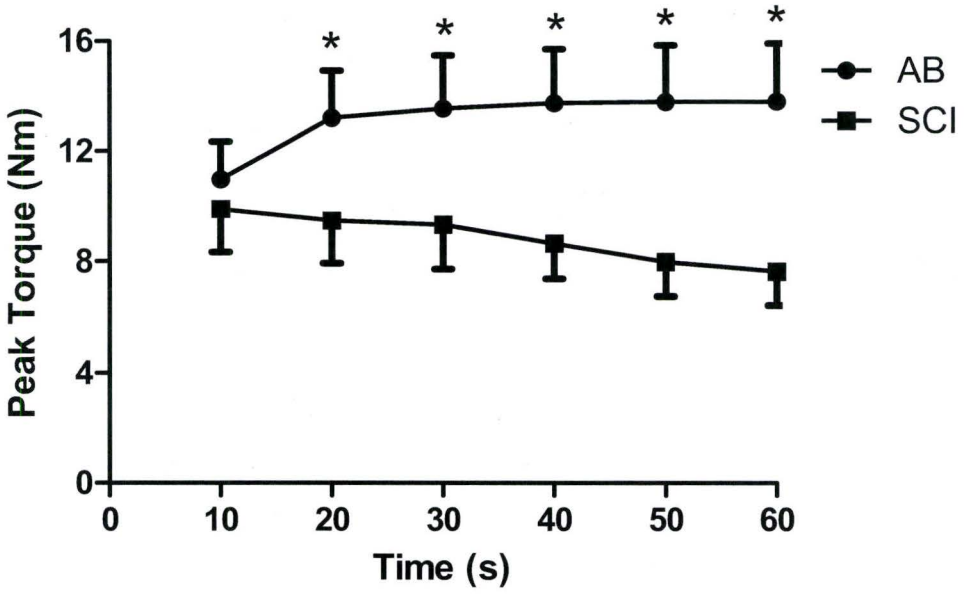
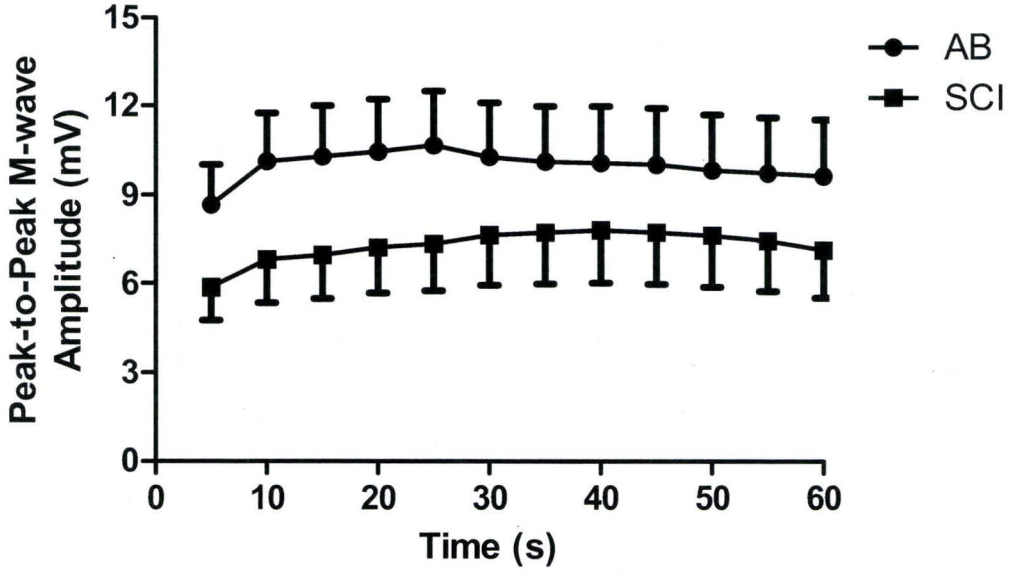


Figure 5. 15Hz Fatigue



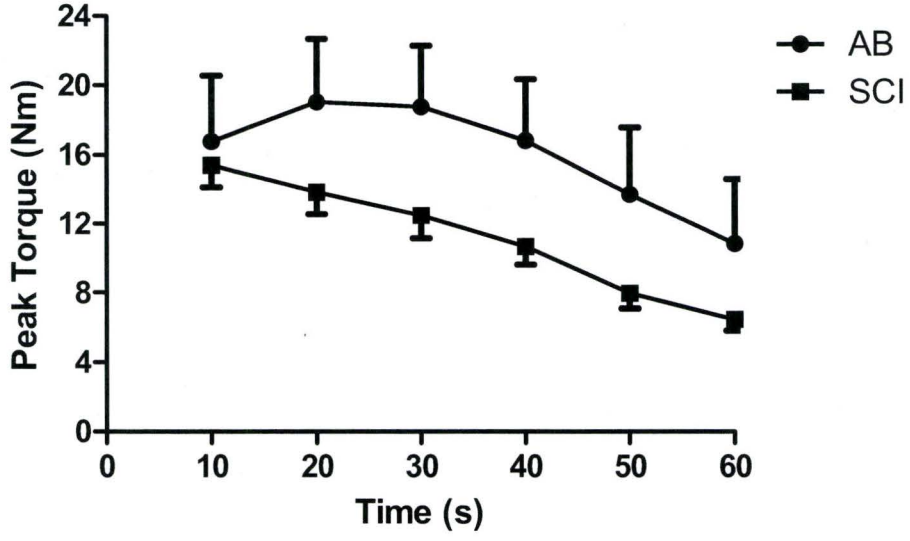
(No significant time x group interaction)

3.3.3 Fatigue – 30Hz

PT analysis revealed a significant main effect for time over the one minute fatigue at 30Hz. The PT declined in both groups over the one minute, but it appeared to decline more rapidly in the SCI group. Though not significant, the average PT over the 1-minute fatigue was lower in the SCI group compared to the AB group (Figure 6).

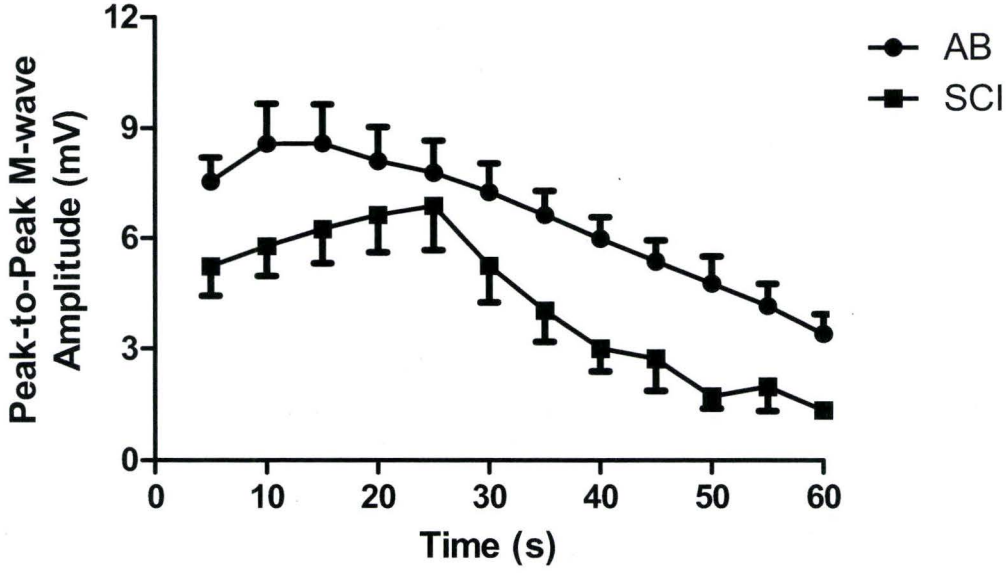
Analysis of peak-to-peak M-wave amplitude indicated a significant main effect for group and time ($p < 0.05$ for both). M-wave amplitude was lower in the SCI group compared to the AB (4.2 ± 2.8mV versus 6.5 ± 2.6mV, respectively). Both groups experienced an early potentiation of the M-wave, but then M-wave amplitude decreased in both groups (Figure 7).

Figure 6. 30Hz Fatigue



(No significant time x group interaction)

Figure 7. 30Hz Fatigue



(No significant time x group interaction)

3.4 Discussion

The force-frequency curve is often used to compare slow versus fast muscles; a curve shifted to the left is representative of a muscle with a greater representation of slow-twitch fibers. Following SCI, there is a shift towards a greater representation of fast-twitch fibers in the paralyzed muscle, and this is accompanied by faster contractile properties (Burnham et al., 1997; Chilibeck et al., 1999; Gerrits et al., 1999; 2001; Shields et al., 1995). Despite these changes, previous studies of the FFR in paralyzed muscle have shown an unexpected leftward shift in FFR (Gerrits et al., 1999; 2001; Rochester et al., 1995a; Thomas, 1997). This leftward shift was also confirmed in the present study. Therefore, in order to generate the same relative torque a lower frequency was required in the SCI group compared to the AB. Also, significantly lower discharge rates (frequency) were required to elicit half the maximal PT (F50) in the paralyzed TA muscle compared to those of AB controls. The FFR exhibited by the TA muscle of the SCI participants in this study might suggest that there is a 'slowing' of the muscle in comparison to what is observed in the AB. However, the twitch contractile properties of the TA of these individuals with SCI displayed significantly faster contractile properties compared to AB (Chapter 2). It has been suggested that there may be inherent properties in paralyzed muscle (e.g. reduced response to higher frequencies, decreased compliance) that may be contributing to this change in FFR (Gerrits et al., 1999; Scott et al., 2006), but further studies are definitely warranted to examine this phenomenon in more detail. Observations from the present study support either of these mechanisms as playing a role. For example, the fact that the SCI group had similar peak twitch torques to the AB, yet

lower tetanic PT above 15Hz, suggests that there may, in fact, be an impaired high-frequency response in paralyzed muscle which contributes to the leftward shift of the FFR. In addition, there is evidence that there may have been reduced compliance in the paralyzed muscle based on the higher twitch-tetanus ratio seen in the SCI group. Elevated twitch-to-tetanus ratios have been observed previously in other chronically paralyzed muscles including the quadriceps femoris (Gerrits et al., 1999; Scott et al., 2006) and thenar muscles (Thomas, 1997), and it has been suggested that it may be due to both increased stiffness of the muscle and muscle shortening following chronic SCI (Scott et al., 2006). To estimate the degree of stiffness in the TA of the SCI and AB, the electro-mechanical delay (time between onset of M-waveand onset of twitch) was examined post hoc. It was expected that the stiffer muscle would have a shorter electro-mechanical delay due to less series elastic elements; however there was no significant difference in the delay between the paralyzed TA muscle and that of the AB.

In contrast to what has been found with the FFR, prior research examining the fatigue characteristics of paralyzed muscles does support the predicted slow-to-fast transition of fiber type distribution after SCI (Castro et al., 2000; Gerrits et al., 1999; 2001; Rochester et al., 1995a; Shields et al., 1998; Stein et al., 1992). In the present study, however, the message was not as clear. As expected, during the 15Hz fatigue protocol, the SCI group exhibited less fatigue resistance than the AB group. However, at 30Hz there were no significant differences in PT over the 1 minute protocol between the AB and SCI groups. This is in contrast to previous studies that have demonstrated a

reduced resistance to fatigue in paralyzed muscle compared to controls following stimulation protocols at 30Hz (Gerrits, et al., 1999; 2001).

It is important, however, to recognize that methodological differences in fatigue stimulation protocols make direct comparisons between studies problematic (Scott et al., 2006). Many studies have used longer fatigue protocols ranging from 2-4 minutes or even longer, until a set percent of declined force is observed. These studies activated muscles by delivering series of short stimulation trains per second so that there were short rest periods in between each train. It is possible that the supposed changes in fatigue resistance after SCI depend on the stimulation pattern of the fatigue produced. There are few studies that have examined the effect of stimulation protocol on fatigability in individuals with SCI. Recently some research has been devoted to quantifying the effect of different stimulation patterns on force response for the purpose of developing functional electrical stimulation programs; however fatigue properties are not often assessed. Probably the most comprehensive examination of fatigue in paralyzed muscles was done by Thomas *et al.* (2003), where fatigability of thenar muscles in individuals with chronic SCI was examined using 4 different fatigue protocols (constant and variable pulse pattern at both 20 and 40Hz). Significant force reductions in both paralyzed and control muscles were observed in all 4 fatigue protocols, however the relative reduction in force was only significantly greater in the SCI group for 3 out of 4 protocols (Thomas et al., 2003). Following the constant high frequency protocol (40Hz), both the control and SCI groups exhibited similar force reductions. All protocols consisted of pulse trains followed by short rest periods (remainder of the second; for example 300-ms train each

second), whereas in the present study a constant stimulation pattern was used. It appears that the constant stimulation protocol used in this study induced greater fatigue in the AB group at 30Hz compared to 15Hz. It is possible that, using the present protocol, a frequency of 30Hz is too high to produce differences in fatigability between the AB and SCI groups, since this high frequency protocol caused similar force reductions in both groups. It is evident that further study of fatigue characteristics in paralyzed muscle is warranted.

An interesting observation made in this study was the potentiation of M-wave amplitude at the onset of fatigue in the 30Hz fatigue protocol. More notably the potentiation occurred earlier in the AB than the SCI group and was more pronounced in the SCI group. A similar finding was demonstrated by Stein *et al.* (1992) in the paralyzed TA muscle. In the first minute of a 4-minute stimulation fatigue protocol, the M-waves of both SCI and AB groups showed some potentiation (Stein et al., 1992); however the amount of potentiation and time it occurred was not noted.

In conclusion, the predicted transformation towards a higher proportion of fast-twitch fibers following paralysis was partly supported by the significant decreased fatigue resistance in the SCI group, but only at the 15Hz stimulation frequency. The leftward shift in the FFR, however, is a phenomenon that does not necessarily support the fiber transformation predicted after SCI; the mechanisms behind this warrant further investigation.

3.5 Figure Captions

Figure 1. Normalized force-frequency relationship of AB (n=7) and SCI (n=6) individuals, where torques at different stimulation frequencies are expressed relative to the 100Hz response. * denotes significant difference between groups ($p<0.05$) at the given time point.

Figure 2. Mean (± SE) F50 values, frequency required to elicit 50% of maximum PT, of AB (n=7) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 3. Mean (± SE) twitch-to-tetanus ratio of single pulse response to 100Hz response of AB (n=7) and SCI (n=6) individuals. * denotes significant difference between groups ($p<0.05$).

Figure 4. Change in absolute peak torque (values are means ± SE) during the 1-minute 15Hz fatigue protocol for AB (n=7) and SCI (n=7) individuals. * denotes significant difference between groups ($p<0.05$) at the given time point.

Figure 5. Change in M-wave amplitude (values are means ± SE) during the 1-minute 15Hz fatigue protocol for AB (n=7) and SCI (n=7) individuals. No significant time x group interaction was found.

Figure 6. Change in absolute peak torque (values are means ± SE) during the 1-minute 30Hz fatigue protocol for AB (n=7) and SCI (n=7) individuals. No significant time x group interaction was found.

Figure 7. Change in M-wave amplitude (values are means ± SE) during the 1-minute 30Hz fatigue protocol for AB (n=7) and SCI (n=7) individuals. No significant time x group interaction was found.

Table 1. Characteristics of Subjects with SCI

Subject No.	Sex	Age at Enrollment	SCI Level	ASIA Class	Time since injury (y)	Medications
1	M	34	C4	C	9.5	Ba; Ep; Te; Co; Gl
2	F	22	C3-4	A	9	Am; Ba; De; Ni; Se; So
3	M	30	C4	B	4	Ba; Co; De; Se
4	M	47	C5-6 break; T1-2 lesion	B	29	Mo; Ox
5	M	45	C5-7	C	19	Ac; Ba; Co; Di
6	M	59	C5-7	C	20	none
7	M	38	C6	A	15	Ba; Di

Medications: Ac - Actonel; Am - Amitriptyline; Ba - Baclofen; Co - Colace; De - Detrol; Di - Ditropan; Ep - Epival; Gl - Glucosamine; Mo - Monocycline; Ni -

Table 2. Characteristics of Able-bodied Subjects

Subject No.	Sex	Age at Enrollment
1	M	32
2	F	23
3	M	29
4	M	50
5	M	42
6	M	59
7	M	36

Chapter 4
Posttetanic Potentiation Following SCI

4.1 Introduction

Following a brief tetanic stimulation the force of a single twitch is greater than it was before the tetanus (Brown and von Euler, 1938). This enhancement of twitch force is known as posttetanic potentiation (PTP). The most accepted mechanism for PTP is phosphorylation of the regulatory light chains during the conditioning tetanus (Tubman, MacIntosh and Maki, 1996), most likely causing an increase in sensitivity of the actin-myosin to Ca^{2+} in the subsequent twitch. The increase in responsiveness that follows repetitive stimulation is greatest immediately after tetanus. Although there is a rapid decline of PTP over time, it is still evident up to 10 minutes after the conditioning tetanus has ended (Brown and von Euler, 1938; Hughes and Morrell, 1957). Twitch potentiation has been observed in both animals (Brown and von Euler, 1938; Tubman and Rassier, 1997; Wilson and Skirboll, 1974) and humans (O'Leary, Hope and Sale, 1997; Rassier, 2000) in a variety of skeletal muscles.

Several factors influence the degree of PTP, including characteristics of the tetanic stimulation used and those of the muscle being examined. The intensity, frequency and duration of the conditioning tetanus can affect the magnitude of the potentiation. An increase of any of these three variables results in a greater immediate PTP (Hughes and Morrell, 1957). Muscle fiber type can also influence the extent to

which a twitch is potentiated. In animal studies, PTP in muscles with predominately type I slow twitch fibers is smaller or almost non-existent when compared with fast muscles (Buller et al., 1981; Standaert, 1964). In a study by Vandervoort and McComas (1983), it was demonstrated that PTP was greater in the lateral gastrocnemius, with a higher percent of type II fibers, than in the soleus following a 3-second tetanus at 50Hz. Another factor affecting the level of potentiation is muscle length. Vandervoort *et al.* (1983) observed that the degree of potentiation was greatest when the tibialis anterior muscle was in the shortened position (full dorsiflexion) compared to mid-position and lengthened position (full plantarflexion). Upon examination of fatigue and PTP in knee extensor muscles at 3 different lengths, Rassier (2000) also demonstrated that PTP was length-dependent. Although PTP was observed at all knee angles, 39% ± 4, 47% ± 2 and 68% ± 5 at 90°, 60° and 30°, respectively (full extension = 0°), PTP was greater at shorter muscle lengths. Finally, the magnitude of PTP has been shown to be temperature-dependent; PTP increases as the temperature of the muscle increases (Gossen, Allingham and Sale, 2001).

Although the enhancement of a twitch torque observed following a tetanic stimulation has been well documented in able-bodied human muscles, the magnitude of PTP in diseased or paralyzed human muscles has been less studied. Following SCI there are significant muscular changes that occur below the level of lesion (Gerrits et al., 1999). Paralyzed muscle usually displays a decrease in evoked torque production and generally faster contractile properties prior to fatigue, including a shorter time to peak twitch torque and a decrease in half relaxation time, characteristic of faster muscles (Gerrits et al., 1999; 2001; Shields, 1995; 2002). Since PTP has been shown to be more

pronounced in muscles with higher concentrations of fast-twitch fibers, it must be predicted that the magnitude of PTP might be greater in the lower limb muscles after SCI.

In 2006, Shields and colleagues examined the degree of post-fatigue potentiation (PFP) in both acutely and chronically (> 2 years) paralyzed soleus muscle, as well as the effects of long-term electrical stimulation training on PFP of acutely paralyzed muscle. Following repetitive stimulation paralyzed muscle is subject to long lasting fatigue. PFP refers to the potentiation of peak forces exhibited by fatigued muscles following repetitive single-pulse activation or tetanic stimulation (Shields et al., 2006). This is the only study to date that documents potentiation in whole muscle of individuals with SCI. Chronically paralyzed soleus muscle demonstrated post-fatigue potentiation (90%) during repetitive activation, while no potentiation was observed in the acutely paralyzed group. These results provided indirect evidence that there was a switch to more fast-twitch fibers in the soleus of the chronically paralyzed participants, but this transformation had not yet occurred in those with acute injuries. The examination of twitch and tetanic forces in SCI individuals was broadened in a recent study by Häger-Ross and colleagues (2006). In the first study to examine PTP of paralyzed motor units, posttetanic twitches were delivered to thenar motor units of individuals with SCI following 0.5 – 2s stimulation trains at various frequencies. Contrary to what might be expected, however, the thenar motor units from the paralyzed muscle potentiated less than units from able-bodied controls (Häger-Ross et al., 2006). Although this was unexpected, prior research in the thenar muscle group has shown that this muscle may not undergo the predicted slow-to-fast transition

after SCI, in fact, the twitch contraction times of thenar motor units have been shown to be prolonged (Häger-Ross et al., 2006).

It is evident that there are very few studies that have examined PTP in paralyzed muscles after SCI. Thus the purpose of this study was to investigate the degree of twitch potentiation in the tibialis anterior muscle following a brief tetanic stimulation, and to evaluate the changes in twitch contractile properties for 4 minutes post tetanus. Given that paralyzed muscle is predicted to be composed of predominately type II fibers, it was hypothesized that PTP would be greater in individuals with SCI compared to AB controls, and that the SCI group would be characterized by faster contractile properties.

4.2 Materials and Methods

4.2.1 Subjects

Eight people with SCI (7 ♂, 1♀) and 8 age- and gender-matched able-bodied controls (AB; 7 ♂, 1♀) participated in the study (Table 1 and 2, respectively). Inclusion criteria for the SCI participants were more than 1 year post injury, upper motor neuron lesion level higher than T2, American Spinal Injury Association (ASIA) classification A-C and wheelchair-dependent. The exclusion criterion for participants with SCI was a significantly reduced ankle range of motion (< 30°). All AB participants were healthy with no history of neuromuscular disorders. The subjects participated with informed, written consent and with the approval of McMaster University's Research Ethics Board.

4.2.2 Apparatus

All but one of the subjects with SCI remained in their wheelchairs during the testing procedure, the other SCI participant and the AB group sat in an adjustable chair. The tibialis anterior (TA) muscle of the right leg was tested in a custom-made isometric ankle torque dynamometer (Marsh et al., 1981). The ankle joint was fixed at 10° of plantarflexion from the neutral position (neutral position = 90° angle formed by tibia and sole of the foot). This joint position was chosen as it stretches the TA, ensuring a maximal twitch response (Marsh et al., 1981), but it was still within the ankle range of motion of the SCI participants. Velcro straps secured the foot to an aluminum plate instrumented with a strain gauge, which measured the torque generated by the contraction of the TA. The signals from the strain gauge were amplified and low pass filtered at 1 kHz by a custom made DC amplifier. The EMG signal was amplified and band pass filtered 1 Hz- 1kHz, using a UFI device (model 2283f/I; Morro Bay, CA). Calibrated signals from both channels were digitized at 4 kHz using a Measurement & Computing device (Model USB-1608FS; Norton, MA). Data was stored for later analysis, with custom designed software (Labview, National Instruments; Austin, TX), on an IBM-compatible personal computer.

4.2.3 Stimulation and EMG Recording

In order to activate the TA muscle, the lateral peroneal nerve was stimulated through rubber electrodes (1" diameter) saturated with electrode gel. The skin was prepared by rubbing it with isopropyl alcohol wipes. Stimulating electrodes were placed

on the skin over the neck of the fibula and the medial part of the tibia head. The electrodes were secured to the skin with micropore surgical tape. EMG disposable recording electrodes were applied to the skin over the muscle belly of the TA (stigmatic), the inferior extensor retinaculum (reference) and on the peroneaus longus muscle region (ground).

4.2.4 Protocol

Upon arriving to the laboratory, AB participants sat at rest for a minimum of 15 minutes before commencing. The right legs of both the SCI and AB participants were secured in the apparatus. A maximum pretetanic twitch response was determined by delivering a series of single stimuli (pulse width of 100μs), gradually increasing in intensity until the compound action potential (M-wave) and peak torque (PT) reached a plateau. For the tetanic stimulation and the posttetanic twitches, the stimulation intensity was set at the lowest intensity that generated the maximum PT. This was done to ensure the tetanus was not overly uncomfortable for participants with sensation.

Following one minute of rest, tetanic stimuli were applied for 5 seconds at a frequency of 100Hz. The posttetanic stimulation protocol consisted of single twitches delivered at 5 seconds after the tetanus and then at 30-second intervals for a total of 4 minutes following tetanus.

4.2.5 Measurements

Twitch response measurements included PT, time to peak torque (TPT), half relaxation time (½RT), rate of torque development (RTD) and rate of torque relaxation (RTR). The peak-to-peak amplitude of muscle compound action potential (M-wave) corresponding to each twitch response was also measured.

4.2.6 Statistics

A two-factor (between, group; within, time) ANOVA was used to analyze the pretetanus and posttetanus values of each of the dependent variables separately over the 4 minute time period. This ANOVA was done for both measures expressed in absolute units (e.g. torque in Newton meters) and normalized as a percent change from pretetanus values. For the analysis of the normalized values, the pretetanus values (100%) were not entered into the analysis. The ANOVA on normalized values was performed in order to control for any differences between the absolute values of the two groups (SCI and AB). When significant main effects or interactions were found, the Tukey's post-hoc procedure tested the difference between means. Statistical significance was set at $p \leq 0.05$.

4.3 Results

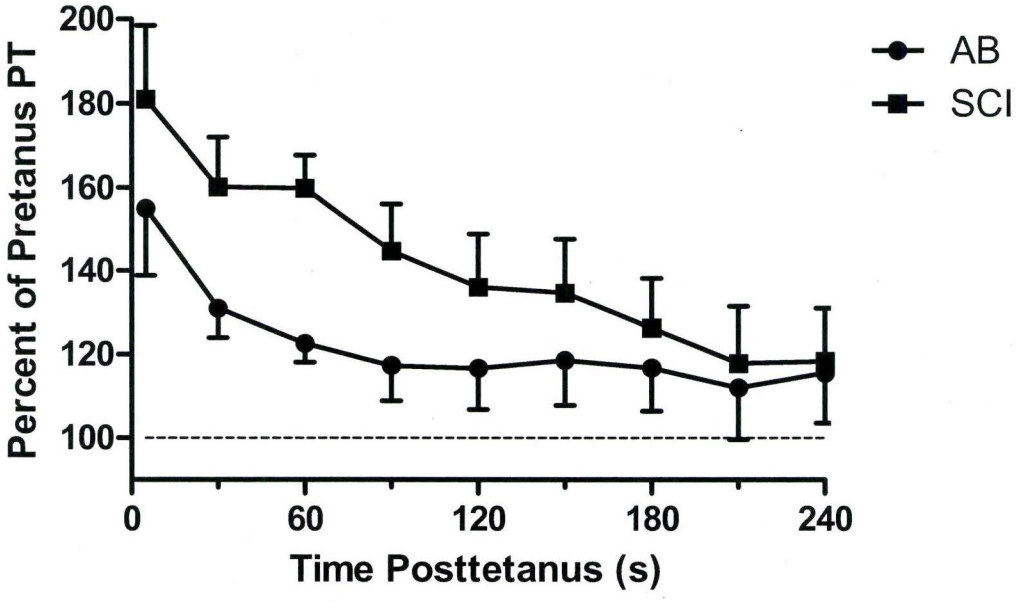
4.3.1 Baseline Characteristics

There was no significant difference in PT at baseline between the AB and SCI groups (2.7 ± 0.3Nm and 2.9 ± 0.8Nm, respectively). Similarly there were no differences in TPT, ½RT, RTD or RTR between groups at baseline. See Table 3 for baseline characteristics. Peak-to-peak M-wave amplitude at baseline was significantly greater in the AB group compared to the SCI group (8.5 ± 2.7mV and 4.4 ± 2.1mV, respectively).

4.3.2 Time Pattern of PTP over 4 minutes Following Tetanus

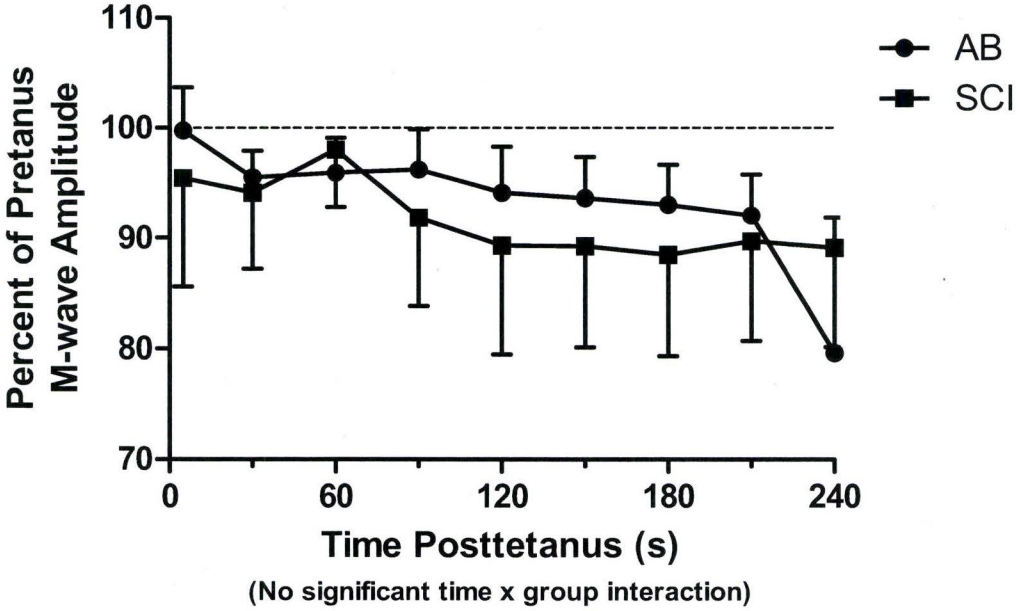
The analysis of PT revealed a significant main effect for time in the 4 minutes following tetanus. At 5 seconds, the PT was significantly greater in both groups then as time progressed, the amount of potentiation diminished until the PT was no longer significantly greater than baseline by 3 minutes 30 seconds. The PT group x time interaction was just short of being statistically significant ($p = 0.058$); as seen in Figure 1 the amount of potentiation was greater in the SCI group compared to the AB at all time points. Peak-to-peak M-wave amplitude did not change with either group immediately after tetanus. However, there was a small and gradual decrease in peak-to-peak M-wave over the 4 minutes in both groups (Figure 2).

Figure 1



(No significant time x group interaction)

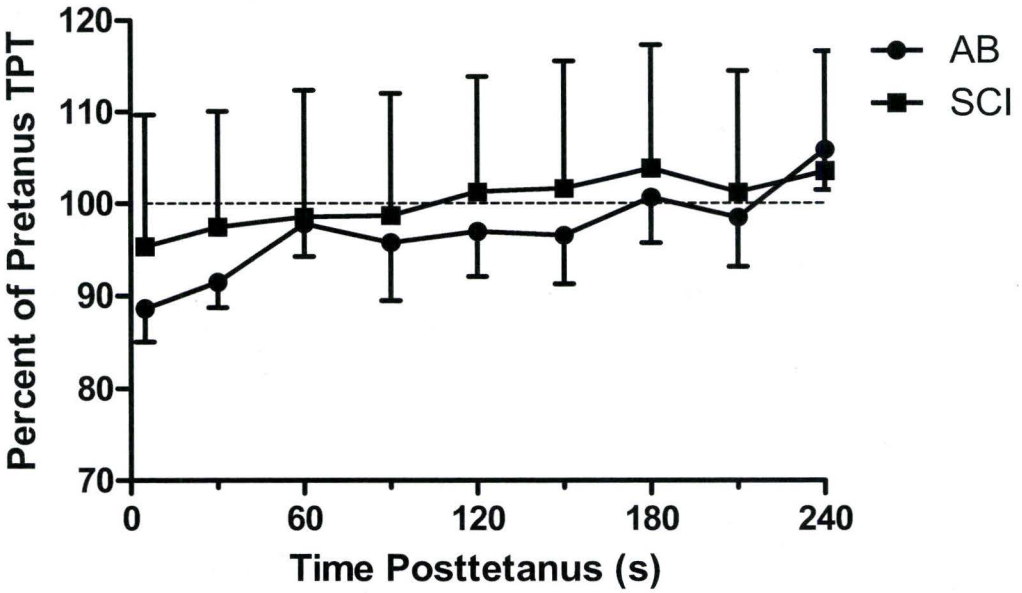
Figure 2



(No significant time x group interaction)

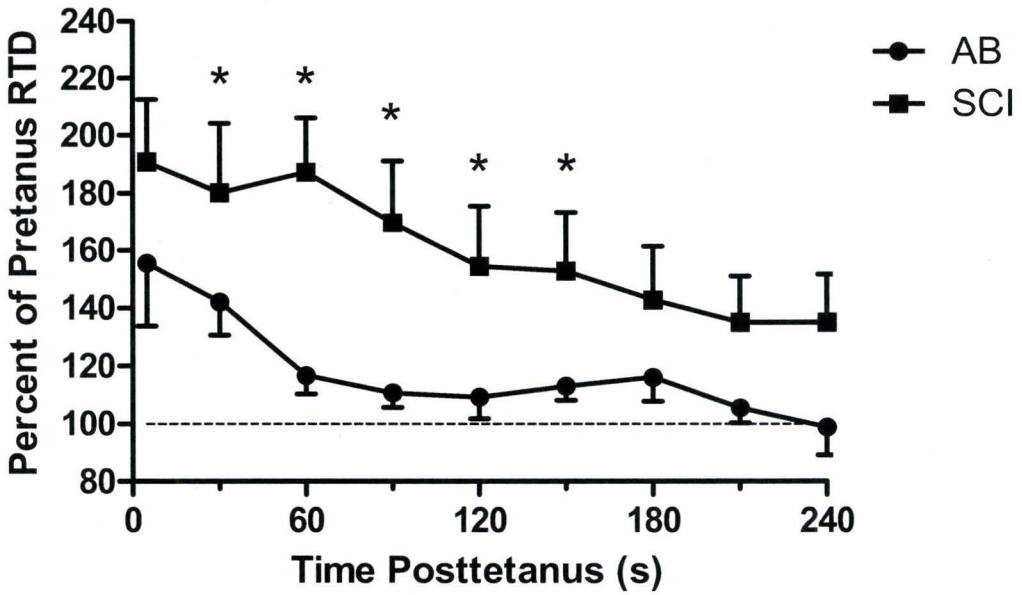
TPT was relatively well maintained over the 4 minutes in both groups (Figure 3). Analysis of absolute RTD indicated a significant group x time interaction such that the AB group had a significantly greater RTD only at 5 seconds following tetanus compared to baseline while the RTD of the SCI group remained significantly elevated above baseline until 3 minutes 30 seconds. Analysis of normalized RTD, however, only revealed a significant main effect for time and almost significant main effect for group and group x time interaction ($p = 0.056$ and $p = 0.072$, respectively). Thus, though RTD increased in both groups immediately post-tetanus, by an average of 56% in the AB group and 91% in the SCI group, the SCI group experienced a more gradual recovery to baseline values compared the AB group (Figure 4).

Figure 3



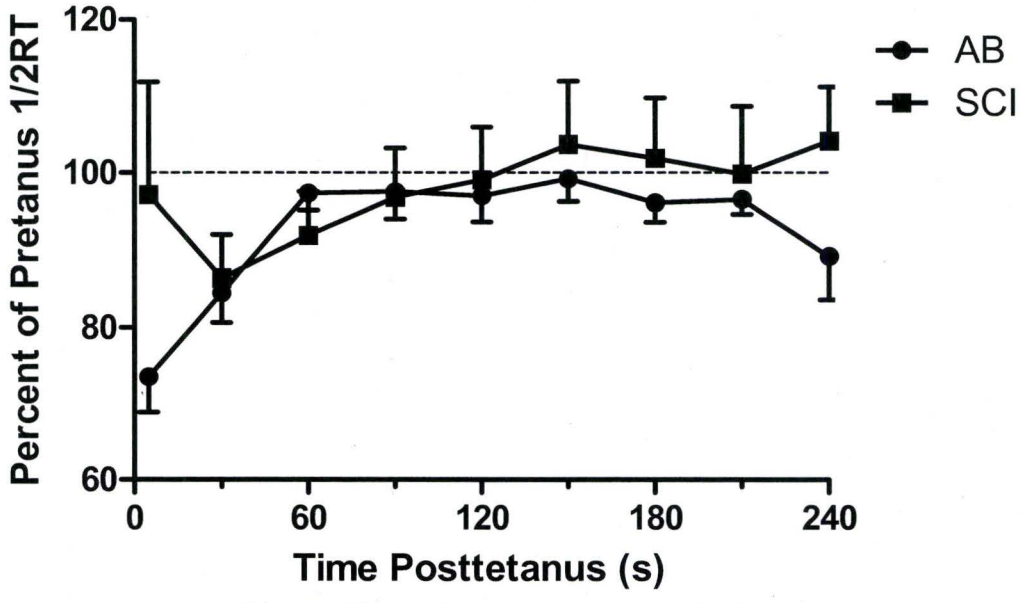
(No significant time x group interaction)

Figure 4



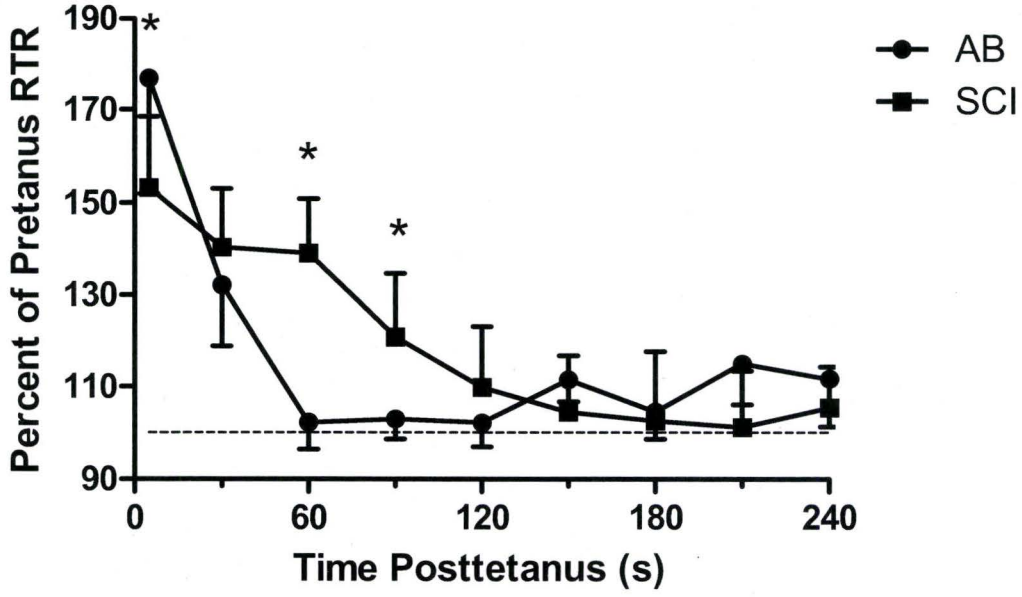
Analysis of ½RT revealed a significant main effect for time. The ½RT decreased immediately following tetanus in both the AB and SCI groups then returned to baseline values (Figure 5). Analysis of RTR indicated there was also a main effect for time and significant group x time interaction ($p < 0.05$). The RTR became faster in both groups following tetanus, with the AB group having a significantly faster RTR (compared to the SCI) immediately post-tetanus; this value then returned to baseline within 1 minute. Alternatively, the SCI group experienced a significantly greater increase in RTR (compared with the AB group) at 60-90 seconds post-tetanus; this value returning to baseline by 2 minutes (Figure 6).

Figure 5



(No significant time x group interaction)

Figure 6



4.4 Discussion

Several studies examining the effect of fiber type on twitch potentiation have shown that a higher percentage of type II fast twitch fibers is associated with a greater enhancement of potentiation (Hamada et al, 2000; O'Leary et al, 1997; Vandervoort and McComas, 1983). Due to the adaptations following SCI, chronically paralyzed muscles consist of primarily fast twitch fibers (Shields, 1995). Thus, a greater degree of potentiation would be expected from paralyzed muscle compared to those from AB controls.

In the present study, both AB participants and those with SCI experienced posttetanic potentiation of their dorsiflexor twitch. The degree of PTP observed in the AB group immediately after tetanus (55%) is consistent with earlier studies examining PTP in dorsiflexors in healthy individuals. In a study by O'Leary and colleagues (1997), PT of the TA muscle was also potentiated by 50% five seconds after tetanic stimulation. Of interest in the present study was the observation that the SCI group displayed a significantly greater enhancement of twitch PT immediately after tetanus compared to the AB group, which supports the predicted transformation of the TA into a faster muscle following SCI. The time course of PTP was also different between the groups over the 4 minutes. Although both groups experienced their peak potentiation at 5 seconds on average, the PT decreased gradually in the SCI group while remaining relatively stable in the AB group following a significant drop at 1 minute.

The peak-to-peak amplitude of the M-wave did not significantly change following tetanic stimulation. Generally very little change in M-wave has been observed in the

posttetanic period. Following a 10-second MVC of the knee extensor muscles, Hamada and colleagues (2000) reported a small but significant increase in M-wave amplitude, which did not remain significantly elevated past 1 minute. O'Leary et al (1998) found that following a 7-second tetanus at 100Hz of the dorsiflexor muscles, M-wave amplitude did not significantly change. Thus, in this study, the M-wave response to tetanus was consistent with previous findings.

Although there were no significant changes in TPT and only a significant decrease in ½RT observed in the AB group following tetanus, both groups exhibited increases in RTD and RTR. This increase in both contraction and relaxation speeds seen in both groups is consistent with previous studies of PTP in human dorsiflexors (O'Leary et al., 1997). At baseline RTD and RTR, though faster in the SCI group, were not significantly different between groups. However, immediately following tetanus, the increase in RTD was greater in the SCI group than AB, while the increase in RTR was greater in the AB group. Interestingly, over the 4 minutes following tetanus, the contraction and relaxation rates of the SCI group took longer to return to baseline than did the AB group. It has been shown that following chronic SCI, paralyzed muscle generally exhibits faster contractile properties prior to fatigue (Gerrits et al., 1999; 2001; Shields, 1995; 2002) and a predominance of type II fibers (Burnham et al., 1997; Chilibeck et al., 1999). Thus faster contraction and relaxation times would be expected from the twitch response of the SCI participants. Without assessing the muscle fiber characteristics of the study participants, we can not be certain that the SCI group had a significantly greater II to I fiber ratio or a greater type II fiber area. However the faster

RTD seen in the SCI group following tetanus compared to the AB group suggests that the paralyzed muscles of the SCI individuals were faster muscles than those of the AB group.

The SCI group was very heterogeneous; the difference in degree of motor and sensory function varied greatly between the participants with SCI. These differences may have contributed to some of the variability observed in this study. Furthermore, as PTP has not yet been investigated in the SCI population, it can only be assumed that properties affecting PTP in AB individuals, namely ankle joint angle, duration and frequency of tetanus, similarly affect PTP in individuals with SCI. Further study is needed to quantify the optimal joint angle for force production after SCI. For twitch responses of the quadriceps muscle, optimal knee joint angle was not found to be significantly different between SCI and AB groups (Gerrits et al., 2005), however this observation may not be the same for other muscle groups. The present study used the same joint angle (10^{o} of plantarflexion) in both groups. This angle is slightly lower than the optimal angle for force production in AB individuals (van Schaik, Hicks and McCartney, 1994), which may have augmented the PTP in the AB group. PTP has been shown to be greatest at shorter muscle lengths in both the dosiflexor (Vandervoot et al., 1983) and knee extensor muscles (Rassier, 2000). The optimal joint angle for dorsiflexor torque is unknown in the SCI population, so we can not comment on whether the PTP was being assessed in a 'shortened' or 'lengthened' state. It has been suggested that ankles of individuals with SCI are maintained in a slightly plantarflexed position due to the seated position in their wheelchairs (McDonald, Garrison and Schmit, 2005), and this might contribute to the relative maintenance of dorsiflexor force generating capacity. Immobilized muscles that

are kept at longer lengths retain more of their strength than those kept at shorter lengths (Simard, Spector and Edgerton, 1982). The fact that the paralyzed TA muscles had a similar PT to those of the AB at baseline supports the possibility that the TA muscle was maintained in a constant lengthened position following SCI. If this in fact is true, then the PTP might have been underestimated in the SCI group (due to their relatively lengthened muscle), suggesting that the difference between groups in PTP might be even greater if muscle length was accurately controlled.

In addition, it has been observed that the temperature of the paralyzed vastus lateralis muscle is on average 5° lower than control muscles (Gerrits et al., 2000). Though muscle temperature was not assessed in the current study, it is possible that a similar temperature difference was present in the TA muscle between the AB and SCI groups. As PTP has been shown to increase as muscle temperature is raised (Gossen et al., 2001), it can only be assumed that the increase in PT after tetanus in the SCI group would be greater still if the paralyzed muscle had been warmed to the same temperature as those of the AB group.

In conclusion a greater potentiation of twitch torque following tetanic stimulation was observed in individuals with SCI compared with AB controls. Greater PTP is expected from muscles with shorter twitch contraction times, a property also displayed by the SCI group in this study. Future studies are needed to determine the optimal conditions for PTP in the SCI population and to compare the degree of PTP in different muscle groups following SCI.

4.5 Figure Captions

Figure 1. Changes in peak torque following tetanus, relative to pretetanus values for AB (n=8) and SCI (n=8) individuals (values are means ± SE). No significant time x group interaction was found.

Figure 2. Changes in peak-to-peak M-wave amplitude following tetanus, relative to pretetanus values for AB (n=8) and SCI (n=8) individuals (values are means ± SE). No significant time x group interaction was found.

Figure 3. Changes in time to peak torque following tetanus, relative to pretetanus values for AB (n=8) and SCI (n=8) individuals (values are means ± SE). No significant time x group interaction was found.

Figure 4. Changes in rate of torque development following tetanus, relative to pretetanus values for AB (n=8) and SCI (n=8) individuals (values are means ± SE). * denotes significant difference between groups ($p<0.05$) at the given time point.

Figure 5. Changes in half relaxation time following tetanus, relative to pretetanus values for AB (n=8) and SCI (n=8) individuals (values are means ± SE). No significant time x group interaction was found.

Figure 6. Changes in rate of torque relaxation following tetanus, relative to pretetanus values for AB(n=8) and SCI (n=8) individuals (values are means ± SE).

* denotes significant difference between groups ($p<0.05$) at the given time point.

Table 1. Characteristics of Subjects with SCI

Subject No.	Sex	Age at Enrollment	SCI Level	ASIA Class	Time since injury (y)	Medications
1	M	34	C4	C	9.5	Ba; Ep; Te; Co; Gl
2	F	22	C3-4	A	9	Am; Ba; De; Ni; Se; So
3	M	30	C4	B	4	Ba; Co; De; Se
4	M	47	C5-6 break; T1-2 lesion	B	29	Mo; Ox
5	M	45	C5-7	C	19	Ac; Ba; Co; Di
6	M	59	C5-7	C	20	none
7	M	38	C6	A	15	Ba; Di
8	M	53	C4-7	C	21	Ba; Pa; Va; Di; No

Medications: Ac - Actonel; Am - Amitriptyline; Ax - Axid; Ba - Baclofen; Co - Colace; De - Detrol; Di - Ditropan; Ep - Epival; Gl - Glucosamine; Mo - Monocycline; Ni - Nitrofurantoin; No - Norvasc; Ox - Oxybutynin chloride; Pa - Paxil; Se - Senokot; So - Soflax; Te - Tegretol; Va - Vasotec

Table 2. Characteristics of Able-bodied Subjects

Subject No.	Sex	Age at Enrollment
1	M	32
2	F	23
3	M	29
4	M	50
5	M	42
6	M	59
7	M	36
8	M	55

Table 3. Pretetanus EMG and Torque Parameters

	SCI	AB
M-wave Amplitude, mV	4.4 ± 2.2	8.5 ± 2.7 *
Twitch Peak Torque, Nm	2.9 ± 0.9	2.7 ± 0.3
Time to Peak Torque, ms	72.8 ± 21.8	79.2 ± 10.9
Half Relaxation Time, ms	77.7 ± 22.9	83.2 ± 15.5
Rate of Torque Development, Nm/s	90.7 ± 17.4	78.2 ± 30.5
Rate of Torque Relaxation, Nm/s	43.2 ± 15.3	36.2 ± 10.3

Values are means ± SD

* Denotes significant difference between groups ($p<0.05$)

Chapter 5
Summary

5.1 Summary

The purpose of this thesis was to examine the contractile properties of the soleus, tibialis anterior (TA) and thenar muscles of individuals with SCI. Although there have been some conflicting findings, previous research has demonstrated that paralyzed quadriceps femoris (Gerrits et al., 1999, 2001; Scott et al., 2006), TA (Rochester et al., 1995a) and soleus (Shields, 1995; Shields et al., 1997) muscles display a decrease in torque production and generally faster contractile properties, while paralyzed whole thenar muscles (Thomas, 1997) and motor units (Häger-Ross, Klein and Thomas, 2006) exhibit similar or slower contractile properties compared to controls. The results from the present study both confirmed and countered previous work. Although the TA of the SCI participants had faster contraction times than controls, faster contractile properties were not reproduced in the paralyzed soleus muscle. Contrary to work by others (Thomas, 1997; Häger-Ross et al., 2006), the paralyzed thenar muscles in our subjects with SCI had faster rates of torque development compared to controls. Although this has not been previously reported, faster contractile properties are expected from muscles that have undergone chronic paralysis due to fiber type transformation. In the study by Thomas (1997), the SCI participants all had cervical injuries (C5-C7) with complete paralysis of the thenar muscles in at least one hand (unspecified ASIA classification); however

adaptations in contractile speeds were still not observed. In the current study, the injury level of the SCI participants ranged from C3-C7 (ASIA A-C). Although, the thenar muscles are supplied by the recurrent branch of the median nerve originating from the C8 and T1 segments, the degree of impairment observed in the hand muscles can vary depending on the severity of the injury. It is possible that differences in the SCI subject's injury level and type may have accounted for some of the differences observed between studies. Finally, similar to previous studies, smaller M-wave amplitudes were seen in the lower extremity muscles of the SCI participants, supporting the significant muscle atrophy following chronic SCI.

In addition to the analysis of the twitch contractile properties of these muscles, the TA muscle was further examined for its force-frequency relationship, fatigue characteristics and posttetanic potentiation. As would be expected with a transformation to fast twitch fibers following SCI, paralyzed muscle has been previously shown to exhibit reduced resistance to fatigue characterized by a greater force decline compared to controls (Castro et al., 2000; Gerrits et al., 1999; 2001; Shields, 2002; Thomas et al., 2005). In this investigation a greater force decline following repeated activation was observed in the SCI participants compared to controls (especially at 15Hz), though the between-group differences were not statistically significant. In contrast both Stein *et al.* (1992) and Rochester *et al.* (1995a) found that paralyzed TA muscles exhibited a significant increase in fatigability compared to controls. The discrepancy between this study and previous ones could be due to methodological differences, such as stimulation frequency and duration, which may have caused more fatigue in the AB subjects.

The known shift in fiber type distribution from slow to fast fibers has been suggested to account for the increased fatigability observed following SCI, however the FFR in paralyzed muscle does not seem to agree with the change in fiber type distribution. Paralyzed muscle, associated with a greater percentage of type IIB fibers, should exhibit a right-ward shift in the FFR. Yet, a leftward shift in FFR was observed in the paralyzed TA muscle of the SCI participants compared to controls. Although this is uncharacteristic of faster muscle, it is a property of paralyzed muscle that has been reproduced in quadriceps femoris (Gerrits et al., 1999; 2001), TA (Rochester et al., 1995a) and thenar (Thomas, 1997) muscles of individuals with SCI, and is likely due to inherent characteristics of paralyzed muscle that may overshadow the effects of fiber type transformation.

To my knowledge, this has been the first investigation of PTP in paralyzed whole muscle to date. Previously, a significant potentiation of twitch torque has been observed in the TA of healthy, able-bodied individuals following brief tetanic stimulation (O'Leary et al., 1997; Vandervoort et al., 1983). It has also been demonstrated that muscle fiber type can influence the extent to which a twitch is potentiated; PTP is greater in muscles with a higher percentage of fast fibers (Buller et al., 1981; Standaert, 1964; Vandervoort and McComas, 1983). If we are to assume that the TA muscle of our SCI participants had undergone fiber type transformation towards a greater proportion of fast twitch fibers, the findings of this study support these previous reports. Despite similar baseline values for PT, the SCI group had a significantly larger PTP immediately after tetanus compared to

the controls. The potentiation also lasted longer in the SCI group; over the 4-min recovery period, PTP declined more rapidly in AB compared to SCI.

It is important to have an understanding of changes in paralyzed muscle following chronic SCI, especially in postural muscles that play important roles in standing and locomotion. This study has provided further insight into the adaptations in muscle contractile properties following SCI and has explored the differences between different muscle groups in the same individuals. As the participants with SCI were classified as ASIA A-C, the findings of this study can contribute to the characterizing and quantifying of the contractile properties of different paralyzed muscles in individuals with different severities of impairment. This information may be helpful for the development of rehabilitation strategies. One method used to restore functional movements in paralyzed muscle is functional electrical stimulation (FES). In order to counter the effects of chronic SCI, FES activates paralyzed muscle to induce improvements in muscle strength and endurance (Scott et al., 2006). For FES to be effective, it is important to assess factors such as muscle fatigue and optimal stimulation frequencies. Thus, the understanding of muscle contractile properties, including fatigability and FFR, is crucial in developing optimal rehabilitation strategies, since adaptations in muscle characteristics following SCI may affect the response to electrical stimulation. To restore functional movements to paralyzed muscle with FES it is necessary to have a clear understanding of muscle strength and fatigue and how they are affected by different stimulation frequencies and durations. Furthermore, the findings of this study can contribute to the construction of muscle models for the study of neuromuscular disorders.

Although this investigation has provided both useful and novel information to the understanding of contractile properties of paralyzed muscle, there are still many questions that remain unanswered. First, further research that focuses on several muscles in the same individual with SCI is warranted. It would also be important to test the same muscle on both sides of the body as it has been suggested that the rate-limiting factors of rehabilitation may be limb specific rather than subject specific, with the paralyzed muscle of one limb exhibiting a different degree of impairment than the same muscle in the other limb (Jayaraman et al., 2005).

Secondly, there must also be more research devoted to the assessment of optimal joint angles, stimulation frequencies and duration, muscle temperature and other conditions needed for the evaluation of muscle contractile properties in SCI. This is especially true for fatigue and PTP. Factors affecting PTP in muscles of healthy, able-bodied individuals have been previously reported, however, it is unclear how these factors will affect PTP in paralyzed muscles. Certain paralyzed muscles, such as the TA muscle, may be maintained at longer muscle lengths due to their position in wheelchairs. The ankle joint used for assessment of the TA (10° of plantarflexion) was chosen to accommodate the somewhat reduced range of motion observed in the SCI subjects. However, the slightly plantarflexed position is not the optimal ankle angle for force production in the AB group. Tetanic stimulation of the TA muscle at frequencies ranging from 20-80Hz revealed that the optimal length of the muscle is at 30° of plantarflexion in both young and elderly able-bodied individuals (van Schaik, Hicks and McCartney, 1994). Future comparisons of optimal angles for force production between AB and

individuals with SCI are needed so that the optimal angle for each group can be selected. Furthermore it is possible that due to different injury levels and variations in impairment, this optimal angle may differ between individuals within the SCI population. It is essential though for future studies to evaluate the length-tension relationship in both dosiflexor and plantarflexor muscles in individuals with SCI.

Thirdly, it would be useful to take muscle biopsies as it would provide information on fiber type distribution of the muscles examined that could be used to explain changes in muscle contractile properties or lack thereof following SCI. In the lower limbs, muscle biopsies are commonly taken from the quadriceps femoris, gastrocnemius and TA muscles (Edwards et al., 1996). Finally the paradoxical leftward shift in FFR of paralyzed muscles remains unexplained. Given that paralyzed muscle is characterized by faster contractile properties and a predominance of fast twitch fibers, it is expected that they would exhibit a rightward shift in FFR compared to AB, however this is not the case. It has been suggested that the leftward shift may be associated with the abnormally large twitch force often observed in paralyzed muscle (Gerrits et al., 2005), however further research is needed to determine the cause of the shift in FFR. Factors, such as changes in muscle length and stiffness, need to be examined as well as they may contribute to this change in FFR.

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Appendix A

Subject Information Letter and Consent Form

McMaster University
Inspiring Innovation and Discovery

Participant Information Letter

A Study of contractile properties of 1 hand muscle and 2 muscles of the lower leg

You are invited to participate in a research study conducted by:

Student Investigator: **Lisa Rodrigues**
Graduate Student
Department of Kinesiology
McMaster University
(905) 525-9140 ext. 24086
rodril@mcmaster.ca

Principal Investigator: **Dr. Audrey Hicks**
Department of Kinesiology
McMaster University
Hamilton, Ontario, Canada
(905) 525-9140 ext. 24643

What is the purpose of this study?

In this study, we are hoping to get a better understanding of the changes in muscle function following spinal cord injury. We are going to measure different properties of muscle contraction in three different muscles (1 in the hand and 2 in the lower leg).

What will my responsibilities be if I participate in this study?

If you participate in this study, you will be asked to come to McMaster University for three visits. During the first visit, you will be shown the equipment that will be needed to measure muscle contractile properties. You will also become familiar with the sensations felt during muscle stimulation. While sitting in a chair, your right foot will be secured in a Velcro strap. Electrodes will be placed on your knee and lower leg. The electrodes will cause your lower leg muscles to twitch. After a few practice twitches, the electrodes will be moved to a hand muscle. The electrodes will then cause your hand muscle to twitch. This first visit will allow us to find the ideal placement of the electrodes and some general neuromuscular characteristics.

The second and third visits will be the experimental session. The electrodes will be placed in the same locations, first on the leg then the hand. We will do a series of twitches in order to obtain the necessary measurements for each of the 3 muscles. The electrical activity caused by the twitch will be recorded by one of the electrodes. All visits will take place in the Ivor Wynne Centre (IWC), McMaster University. Each visit will take up to an hour.

What are the possible risks or discomforts?

If you have sensation, you may feel some temporary discomfort when your muscles are stimulated. One of the leg muscles will be stimulated with some longer series of twitches (up to a minute). During these series of twitches you may feel temporary discomfort while experiencing continuous muscle contraction and electrical pulses in the muscle area stimulated. However, any discomfort will end once the stimulation period is over. The gel placed on the electrodes might feel cold when applied to your skin. There is also a chance you might experience a muscle spasm during the stimulation. While there is no danger with these spasms, the test will be stopped and it will be your decision whether to resume the testing once the spasm has passed or to discontinue the testing.

What are the possible benefits for me or society?

This research will not benefit you directly. However, when the study is over, you will have access to your results. Your results may provide you with information on your muscle function. We hope that this study will help us to understand more about muscle function potential and limitations after a spinal cord injury. This information could help with future rehabilitation programs.

Will there be any payment or reimbursement if I participate in this study?

If you agree to take part in this study, we will reimburse you for parking or transportation costs for the 3 visits.

What information will be kept private?

Your data will not be shared with anyone, except with your consent or as required by law. The information obtained by me will be kept in a locked cabinet. All personal information such as your name, address and phone number will be kept separate from the data. Your data will be linked to a number. The list linking your number and name, with your personal information, will be kept separate from the data in a secure place. If the

results are published, no names or identifying information will be released or published without your specific consent to the disclosure.

What if I change my mind about participating in the study?

Your participation in this study is voluntary. If you volunteer to be in this study, you may withdraw at any time, even after signing the consent form or part-way through the study. In cases of withdrawal your data will be destroyed unless you indicate otherwise. The investigator may withdraw you from this research if circumstances arise which warrant doing so. Your decision whether or not to participate will not affect your continuing access to services at the McMaster Centre for Health Promotion and Rehabilitation.

Will I find out information about the study results?

You may obtain information about the results of the study by contacting Lisa Rodrigues by phone, email or in person. The results will be presented to you in the form of a written summary.

Can I get more information about participating as a study subject?

If you have questions or require more information about the study itself, please contact Lisa Rodrigues by phone or email. The information mentioned above will be discussed and all questions clarified prior to any involvement in the study.

This study has been reviewed and approved by the McMaster Research Ethics Board. If you have concerns or questions about your rights as a participant or about the way the study is conducted, you may contact:

McMaster Research Ethics Board Secretariat
Telephone: (905) 525-9140 ext. 23142
c/o Office of Research Services
E-mail: ethicsoffice@mcmaster.ca

CONSENT

I have read the information presented in the information letter about a study being conducted by Lisa Rodrigues of McMaster University. I have had the opportunity to ask questions about my involvement in this study, and to receive any additional details I wanted to know about the study. I understand that I may withdraw from the study at any time, if I choose to do so, and I agree to participate in this study. I have been given a copy of this form.

_____

Name of Participant

_____

Signature of Participant

In my opinion, the person who has signed above is agreeing to participate in this study voluntarily, and understands the nature of the study and the consequences of participation in it.

_____

Signature of Researcher or Witness

Appendix B

ANOVA Tables

Chapter II. Twitch Contractile Properties of the Soleus, Tibialis Anterior and Thenar Muscles in Individuals with Spinal Cord Injury

T-test for Peak Torque of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
14.25	8.935	3.927	6.124	2.066589	14	0.057789	2.432078	0.263833

T-test for Time to Peak Torque of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
115.56	76.75	15.35	25.01	3.741323	14	0.002191*	2.652626	0.221393

T-test for Half Relaxation Time of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
100.53	111.22	22.54	74.36	-0.389036	14	0.703103	10.99191	0.005442*

T-test for M-wave Amplitude of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
13.542	5.532	5.312	3.989	3.410275	14	0.004226*	1.772983	0.467623

T-test for Rate of Torque Dev. of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
286.82	272.53	58.74	136.9	0.271323	14	0.790102	5.43374	0.040034*

T-test for Rate of Torque Relax. of Soleus Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
132.65	107.52	56.71	62.8	-0.8339849	14	0.415106	1.226345	0.794662

T-test for Peak Torque of TA Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
2.803	3.258	0.547	1.246	-0.945302	14	0.360535	5.183373	0.045371*

T-test for Time to Peak Torque of TA Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
78.81	63.19	6.595	16.55	2.480551	14	0.026441*	6.297752	0.026806*

T-test for Unequal Variance - Time to Peak Torque of TA Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p 2-sided	F-ratio	p-level
78.81	63.19	6.595	16.55	2.480551	14	0.034507*	6.297752	0.026806*

T-test for Half Relaxation Time of TA Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
87.125	75.718	17.93	21.24	1.16057	14	0.265225	1.403012	0.666249

T-test for M-wave Amplitude of TA Muscle
Group 1 = AB (n=8) Group 2 = SCI (n=8)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
8.383	4.27	2.662	1.722	3.670004	14	0.002523*	2.391502	0.272714

T-test for Rate of Torque Dev. of TA Muscle Group 1 = AB (n=8) Group 2 = SCI (n=8)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
69.313	108.68	13.15	39.69	-2.66338	14	0.018538*	9.108236	0.009293*

T-test for Unequal Variance - Rate of Torque Dev. of TA Muscle Group 1 = AB (n=8) Group 2 = SCI (n=8)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p 2-sided	F-ratio Variance	p-level variance
69.313	108.68	13.15	39.69	-2.66338	14	0.027136*	9.108236	0.009293*

T-test for Rate of Torque Relax. of TA Muscle Group 1 = AB (n=8) Group 2 = SCI (n=8)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
34.704	44.556	8.442	18.73	1.356514	14	0.196409	4.921073	0.051977*

T-test for Peak Torque of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
1.7866	2.9133	0.783	1.271	-1.8482	10	0.094323	2.640989	0.310151

T-test for Time to Peak Torque of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
72.75	71.33	26.89	17.2	0.10872	10	0.915575	2.444119	0.349062

T-test for Half Relaxation Time of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
66.79	80.458	5.66	38.21	-0.866744	10	0.406399	45.56552	0.000717*

T-test for M-wave Amplitude of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	**Mean 2**	**SD 1**	**SD 2**	**t-value**	**df**	**p-level**	**F-ratio Variance**	**p-level variance**
9.325	6.9	2.628	2.364	1.680093	10	0.12386	1.236319	0.821603

T-test for Rate of Torque Dev. of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	**Mean 2**	**SD 1**	**SD 2**	**t-value**	**df**	**p-level**	**F-ratio Variance**	**p-level variance**
54.845	96.041	21.44	37.67	-2.32813	10	0.042185*	3.087113	0.241545

T-test for Rate of Torque Relax. of Thenar Muscle Group 1 = AB (n=6) Group 2 = SCI (n=6)								
Mean 1	**Mean 2**	**SD 1**	**SD 2**	**t-value**	**df**	**p-level**	**F-ratio Variance**	**p-level variance**
27.677	39.922	8.782	15.29	1.700723	10	0.11983	3.032788	0.248706

Chapter III. Fatigue and Force-Frequency Relationship of the Tibialis Anterior Muscle in Individuals with Spinal Cord Injury

Absolute Peak Torque At Different Frequencies (SCI, n=7; AB, n=7)
1 = Group 2 = Frequency

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	495.3153	12	229.2161	2.16091	0.167287
2	6	745.6932	72	17.7005	42.1284	0.000000*
1 x 2	6	185.3257	72	17.7005	10.47009	0.000000*

Absolute Peak Torque At Different Frequencies (SCI, n=6; AB, n=7)
1 = Group 2 = Frequency

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	272.8864	11	222.102	1.22865	0.291316
2	6	722.3217	66	18.2912	39.49003	0.000000*
1 x 2	6	156.1605	66	18.2912	8.53745	0.000001*

-one SCI participant removed due to difficulties achieving torques at higher frequencies

Normalized Peak Torque (to 100Hz Response) At Diff. Freq. (SCI, n=7; AB, n=7)
1 = Group 2 = Frequency

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	18945.77	12	2744.635	6.90284	0.022084*
2	6	15335.63	72	413.039	37.12881	0.000000*
1 x 2	6	1869.73	72	413.039	4.52678	0.000602*

Normalized Peak Torque (to 100Hz Response) At Diff. Freq. (SCI, n=6; AB, n=7)
1 = Group 2 = Frequency

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	7288.42	11	297.7692	18.32324	0.001297*
2	6	12854.24	66	133.4302	96.33685	0.000000*
1 x 2	6	1096.7	66	133.4302	8.21927	0.000001*

-one SCI participant removed due to difficulties achieving torques at higher frequencies

T-test for F50 Values
Group 1 = AB (n=7) Group 2 = SCI (n=6)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
16.785	6.7833	4.13	3.452	4.685757	11	0.000665*	1.431328	0.710928

T-test for Twitch-to-Tetanus Ratios
Group 1 = AB (n=7) Group 2 = SCI (n=6)

Mean 1	Mean 2	SD 1	SD 2	t-value	df	p-level	F-ratio Variance	p-level variance
0.0986	0.2188	0.035	0.055	-4.79255	11	0.000560*	2.431335	0.309793

Peak Torque During 15Hz Fatigue (SCI, n=7; AB, n=7)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	736.0286	12	184.7151	3.98467	0.069119
2	11	50.4445	132	3.0511	16.53323	0.000000*
1 x 2	11	10.914	132	3.0511	3.57707	0.000199*

Peak-to-Peak M-wave During 15Hz Fatigue (SCI, n=7; AB, n=7)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	312.3179	12	235.7082	1.325019	0.272114
2	11	3.1777	132	0.5129	6.196191	0.000000*
1 x 2	11	0.7554	132	0.5129	1.47287	0.148967

Peak Torque During 30Hz Fatigue (SCI, n=7; AB, n=7)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	903.9741	12	502.5179	1.79889	0.204681
2	11	218.6464	132	7.1977	30.3774	0.000000*
1 x 2	11	11.1992	132	7.1977	1.55595	0.119241

Peak-to-Peak M-wave During 30Hz Fatigue (SCI, n=7; AB, n=7)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	216.7147	12	36.86208	5.87907	0.032042*
2	11	48.7082	132	1.52977	31.84112	0.000000*
1 x 2	11	1.3592	132	1.52977	0.88848	0.553423

Chapter IV. Posttetanic Potentiation Following SCI

Pretetanic and Posttetanic Absolute Peak Torque (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	19.044	14	7.503609	2.53798	0.133457
2	9	3.93787	126	0.270098	14.57942	0.000000*
1 x 2	9	0.51214	126	0.270098	1.89611	0.058227

Pretetanic and Posttetanic Absolute Time to Peak Torque (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	2644.77	14	2534.895	1.043345	0.324372
2	9	132.173	126	105.625	1.251344	0.270112
1 x 2	9	17.143	126	105.625	0.162304	0.997203

Pretetanic and Posttetanic Absolute 1/2 Relaxation Time (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	480.7689	14	3871.273	0.124189	0.729784
2	9	462.9425	126	90.944	5.090395	0.000007*
1 x 2	9	155.5609	126	90.944	1.270677	0.259208

Pretetanic and Posttetanic Absolute M-wave Amp. (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	667.8159	14	57.82836	11.54824	0.004328*
2	9	0.7922	126	0.28389	2.79039	0.005124*
1 x 2	9	0.0933	126	0.28389	0.32849	0.964187

Normalized Posttetanic Peak Torque (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	13420.18	14	6611.638	2.02978	0.176152
2	8	4522.39	112	411.159	10.99912	0.000000*
1 x 2	8	547.06	112	411.159	1.33052	0.235707

Normalized Posttetanic Time to Peak Torque (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	382.1179	14	6589.629	0.057988	0.813196
2	8	231.0508	112	81.476	2.835814	0.006636*
1 x 2	8	31.0278	112	81.476	0.380821	0.92887

Normalized Posttetanic 1/2 Relaxation Time (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	1105.207	14	1431.689	0.77196	0.394441
2	8	539.152	112	189.928	2.838717	0.006587*
1 x 2	8	304.817	112	189.928	1.604906	0.131219

Normalized Posttetanic M-wave Amp. (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	300.4241	14	2669.016	0.11256	0.742223
2	8	125.7196	112	57.912	2.170867	0.034919*
1 x 2	8	20.4261	112	57.912	0.352708	0.942796

Pretetanic and Posttetanic Absolute Rate of Torque Dev. (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	98917.45	14	17117.42	5.77876	0.030639*
2	9	5245.31	126	413.37	12.68898	0.000000*
1 x 2	9	1334.29	126	413.37	3.22781	0.001481*

Pretetanic and Posttetanic Absolute Rate of Torque Relax. (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	3920.796	14	3302.619	1.18718	0.294299
2	9	819.362	126	66.107	12.39452	0.000000*
1 x 2	9	178.553	126	66.107	2.70098	0.006586*

Normalized Posttetanic Rate of Torque Dev. (SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	64232.63	14	12785.92	4.34417	0.055936
2	8	5692.21	112	427.97	13.30059	0.000000*
1 x 2	8	800.21	112	427.97	1.86977	0.071694

Normalized Posttetanic Rate of Torque Relax. -(SCI, n=8; AB, n=8)
1 = Group 2 = Time

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	139.781	14	6470.542	0.0216	0.885244
2	8	6453.697	112	509.639	12.66328	0.000000*
1 x 2	8	1307.862	112	509.639	2.56625	0.013120*

Appendix C

Raw Data

Soleus - Peak Twitch Response

AB	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	14.65	107.00	105.25	340.40	130.40	10.30	16.00
2	11.28	127.75	139.25	259.31	76.97	4.69	13.75
3	21.30	103.50	91.50	380.00	185.33	15.73	20.75
4	12.97	119.75	116.50	247.36	85.94	14.13	16.75
5	16.00	122.50	60.50	334.79	238.76	18.95	16.75
6	9.10	115.75	91.00	205.13	90.05	10.78	29.75
7	11.35	89.50	98.50	275.01	101.26	21.59	19.25
8	17.35	138.75	101.75	252.59	152.45	12.17	22.25
MEAN	14.25	115.56	100.53	286.82	132.65	13.54	19.41
SD	3.93	15.35	22.54	58.74	56.71	5.31	4.99

SCI	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	20.79	89.50	88.75	361.69	161.79	3.71	19.25
2	2.32	69.75	30.50	78.47	84.45	0.66	15.25
3	7.96	62.25	85.75	414.75	141.61	11.19	22.25
4	10.74	51.00	104.50	363.56	196.54	3.18	23.25
5	8.05	60.00	240.25	265.29	45.59	9.24	16.00
6	4.80	56.50	210.75	131.90	36.62	7.57	20.00
7	13.68	117.25	71.50	418.49	151.33	7.97	33.75
8	3.14	107.75	57.75	146.10	42.22	0.74	19.00
MEAN	8.94	76.75	111.22	272.53	107.52	5.53	21.09
SD	6.12	25.00	74.36	136.93	62.80	3.99	5.80

Tibialis Anterior - Peak Twitch Response

AB	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	2.39	70.25	96.75	62.77	28.40	12.59	24.75
2	3.23	87.50	126.75	63.52	28.40	11.27	25.25
3	2.55	76.75	79.50	54.93	33.25	9.69	28.25
4	2.67	76.00	81.75	97.90	53.81	8.07	33.00
5	3.97	85.50	84.25	75.85	37.74	7.59	27.25
6	2.68	76.75	80.75	66.14	28.40	6.39	38.50
7	2.29	72.00	67.00	71.37	32.88	6.99	37.25
8	2.65	85.75	80.25	62.03	34.75	4.48	28.00
MEAN	2.80	78.81	87.13	69.31	34.70	8.38	30.28
SD	0.55	6.60	17.93	13.15	8.44	2.66	5.32

SCI	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	2.14	69.25	74.75	58.66	34.00	3.81	23.75
2	3.11	40.50	28.75	166.27	78.09	3.22	39.25
3	2.13	59.25	84.75	93.41	25.41	6.88	24.75
4	5.34	67.75	72.50	164.41	61.65	1.30	32.50
5	2.51	54.00	82.25	78.84	31.01	5.22	25.50
6	3.06	97.50	72.00	87.06	33.63	5.01	26.50
7	2.75	55.00	99.25	94.53	35.12	5.44	47.25
8	5.03	62.25	91.50	126.29	57.54	3.28	38.50
MEAN	3.26	63.19	75.72	108.68	44.56	4.27	32.25
SD	1.25	16.55	21.24	39.69	18.73	1.72	8.62

Thenar - Peak Twitch Response

AB	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	0.90	48.75	72.50	33.16	15.56	8.03	21.00
2	3.09	72.00	68.25	84.18	38.52	9.68	18.25
3	2.04	69.00	65.25	72.96	31.63	14.17	26.75
4	1.97	87.00	71.50	51.78	32.14	9.52	30.75
5	1.60	43.00	56.75	57.14	29.59	7.92	26.50
6	1.12	116.75	66.50	29.85	18.62	6.63	30.00
MEAN	1.79	72.75	66.79	54.85	27.68	9.33	25.54
SD	0.78	26.89	5.66	21.44	8.78	2.63	4.96

SCI	PT (Nm)	TPT (ms)	1/2RT (ms)	RTD (Nm/s)	RTR (Nm/s)	M-wave (mV)	EMG dur (ms)
1	2.99	54.50	44.50	128.82	55.10	6.65	31.75
2	1.22	94.75	148.25	45.41	20.92	7.47	28.50
3	1.97	49.00	47.00	73.21	37.75	4.44	21.75
4	2.79	79.25	69.50	71.17	33.42	10.20	19.75
5	4.85	80.50	80.00	119.64	61.22	4.09	35.25
6	3.66	70.00	93.50	138.00	31.12	8.55	34.00
MEAN	2.91	71.33	80.46	96.04	39.92	6.90	28.50
SD	1.27	17.20	38.21	37.67	15.29	2.36	6.46

Force-Frequency Response - Peak Torque

AB	1Hz	10Hz	15Hz	30Hz	45Hz	60Hz	100Hz
1	3.32	9.86	11.88	17.55	19.28	23.88	22.99
2	2.39	7.31	6.56	23.89	27.41	22.76	30.02
3	1.97	4.90	7.75	12.13	12.90	12.16	13.52
4	1.25	4.74	10.78	18.02	17.31	16.45	16.37
5	4.07	11.90	18.59	38.51	35.23	37.81	36.55
6	3.03	8.59	16.34	35.35	40.79	45.46	45.77
7	2.26	7.15	10.86	22.88	23.58	33.12	33.74
MEAN	2.61	7.78	11.82	24.05	25.21	27.38	28.42
SD	0.93	2.59	4.33	9.65	10.00	11.93	11.51

SCI	1Hz	10Hz	15Hz	30Hz	45Hz	60Hz	100Hz
1	3.22	12.63	18.09	20.92	22.20	21.36	19.48
2	4.92	10.46	11.07	16.28	16.65	17.63	17.23
3	2.13	3.83	7.01	10.12	10.88	11.15	11.30
4	3.03	19.43	21.74	17.58	19.51	19.50	19.01
5	4.88	11.24	15.49	16.72	18.16	17.45	18.18
6	4.80	23.61	22.30	18.15	19.55	19.35	19.52
7	1.19	8.22	8.90	8.24	14.22	7.12	4.96
MEAN	3.45	12.77	14.94	15.43	17.31	16.22	15.67
SD	1.48	6.71	6.12	4.55	3.78	5.15	5.52

Twitch-to-Tetanus Ratio

AB	1 Hz	100 Hz	Ratio
1	3.32	22.99	0.144
2	2.39	30.02	0.080
3	1.97	13.52	0.146
4	1.25	16.37	0.076
5	4.07	36.55	0.111
6	3.03	45.77	0.066
7	2.26	33.74	0.067
MEAN	2.61	28.42	0.099
SD	0.93	11.51	0.035

SCI	1 Hz	100 Hz	Ratio
1	3.22	19.48	0.165
2	4.92	17.23	0.286
3	2.13	11.30	0.188
4	3.03	19.01	0.159
5	4.88	18.18	0.268
6	4.80	19.52	0.246
7	1.19	4.96	0.240
MEAN	3.45	15.67	0.222
SD	1.48	5.52	0.051

Fatigue Index

AB	FI - 15Hz	FI - 30Hz
1	7.23	0.00
2	3.26	52.44
3	4.64	68.66
4	16.38	21.51
5	0.00	28.46
6	0.22	81.67
7	29.08	66.30
MEAN	8.69	45.58
SD	10.57	29.61

SCI	FI - 15Hz	FI - 30Hz
1	21.42	61.24
2	6.13	58.10
3	0.00	56.36
4	52.54	53.61
5	25.35	56.46
6	52.20	66.50
7	10.94	55.40
MEAN	24.08	58.24
SD	21.15	4.35

15 Hz Fatigue - Peak Torque

AB	1	2	3	4	5	6	7	8	9	10	11	12
1	6.03	15.60	17.41	18.66	19.40	19.02	18.94	18.51	18.13	18.21	17.78	18.00
2	6.12	9.24	10.44	12.57	14.16	13.21	13.54	14.28	13.09	12.81	13.81	13.81
3	3.59	7.35	7.15	7.38	7.39	7.36	6.94	6.89	6.85	7.02	6.70	6.60
4	3.06	12.81	15.44	17.11	16.27	15.14	15.17	15.08	14.98	14.95	15.30	14.70
5	7.71	14.91	15.18	17.55	19.31	20.63	21.33	21.15	22.51	22.53	22.04	22.71
6	4.92	6.38	7.97	9.80	10.86	11.77	12.35	12.39	13.03	13.19	13.09	13.16
7	4.91	10.60	10.27	9.30	8.88	7.55	7.30	7.76	8.30	7.65	7.63	7.52
MEAN	5.19	10.98	11.98	13.19	13.75	13.52	13.65	13.72	13.84	13.76	13.76	13.79
SD	1.59	3.60	4.01	4.56	4.86	5.17	5.42	5.23	5.41	5.51	5.40	5.62

SCI	1	2	3	4	5	6	7	8	9	10	11	12
1	3.71	15.84	16.59	16.29	15.89	16.69	15.17	14.54	14.34	13.66	13.82	13.12
2	1.46	9.31	9.88	10.21	10.29	10.04	10.22	10.48	10.54	10.39	10.23	9.89
3	2.82	7.98	7.49	7.35	7.50	7.61	7.73	8.01	8.01	7.96	8.21	8.36
4	5.68	8.13	6.99	6.66	6.11	5.52	5.54	5.39	4.98	4.70	4.11	3.86
5	4.89	7.53	6.57	6.46	6.44	6.76	6.30	5.91	5.63	5.34	5.09	5.62
6	5.13	15.43	14.49	13.64	13.27	13.05	12.34	10.43	9.21	8.43	7.84	7.37
7	3.90	5.10	5.43	5.67	5.49	5.57	5.51	5.81	5.65	5.39	5.31	5.18
MEAN	3.94	9.90	9.64	9.47	9.28	9.32	8.97	8.65	8.34	7.98	7.80	7.63
SD	1.46	4.12	4.30	4.09	4.01	4.22	3.74	3.36	3.35	3.23	3.40	3.17

15 Hz Fatigue - M-wave Amp.

AB	1	2	3	4	5	6	7	8	9	10	11	12
1	12.58	13.53	14.01	14.70	14.95	14.57	14.45	14.79	14.89	14.50	14.46	14.91
2	7.27	6.38	5.98	6.33	6.56	6.08	5.52	5.47	5.47	5.14	5.03	4.78
3	14.14	15.91	16.24	16.49	16.71	16.83	16.91	16.89	16.76	16.56	16.36	16.03
4	9.15	13.27	13.23	12.93	13.66	12.03	11.78	11.60	11.49	11.38	11.28	11.09
5	6.09	9.05	9.44	9.48	9.42	9.12	8.84	8.50	8.23	7.95	7.78	7.53
6	7.38	8.85	9.43	9.91	10.22	10.41	10.49	10.62	10.63	10.61	10.59	10.48
7	3.96	3.91	3.71	3.35	3.19	2.98	2.86	2.70	2.66	2.65	2.65	2.60
MEAN	8.65	10.13	10.29	10.46	10.67	10.29	10.12	10.08	10.02	9.83	9.73	9.63
SD	3.60	4.29	4.50	4.64	4.80	4.77	4.88	4.99	5.00	4.95	4.92	4.98

SCI	1	2	3	4	5	6	7	8	9	10	11	12
1	4.51	4.82	4.71	4.75	4.52	4.31	4.04	4.08	3.97	3.68	3.53	3.25
2	4.02	4.66	4.93	5.20	5.23	5.39	5.46	5.42	5.49	5.55	5.63	5.69
3	8.79	10.99	11.44	12.28	12.22	13.11	13.25	13.59	13.10	13.19	13.10	12.46
4	1.24	0.77	0.80	0.62	0.66	0.62	0.69	0.69	0.69	0.68	0.67	0.70
5	5.35	5.94	6.31	6.81	7.18	7.64	7.61	7.53	7.49	7.31	7.13	7.14
6	8.18	11.04	10.76	10.50	10.62	10.91	11.17	11.27	11.40	11.13	10.60	9.92
7	8.99	9.40	9.71	10.29	10.85	11.41	11.84	11.94	11.89	11.78	11.39	10.68
MEAN	5.87	6.80	6.95	7.21	7.33	7.63	7.72	7.79	7.72	7.62	7.44	7.12
SD	2.90	3.83	3.87	4.08	4.16	4.48	4.61	4.71	4.63	4.63	4.51	4.23

30 Hz Fatigue - Peak Torque

AB	1	2	3	4	5	6	7	8	9	10	11	12
1	9.76	10.45	12.08	17.04	16.39	17.02	15.97	15.46	16.45	17.34	16.44	17.98
2	2.61	16.58	17.77	18.76	18.33	17.69	15.30	16.11	12.69	9.09	9.59	8.92
3	0.08	4.27	4.72	5.17	4.89	5.00	4.93	4.45	3.61	1.62	-1.01	-2.89
4	2.21	12.76	15.01	16.89	18.70	19.04	18.14	16.95	15.76	16.92	16.29	14.95
5	6.05	36.11	37.62	37.53	37.60	36.47	36.31	35.83	34.86	33.26	30.36	26.91
6	5.02	19.43	21.75	22.02	22.39	21.33	17.75	15.83	12.66	8.67	5.13	4.11
7	4.93	17.99	16.25	15.76	14.70	14.75	13.77	13.13	11.18	9.28	7.60	6.06
MEAN	4.38	16.80	17.89	19.03	19.00	18.76	17.45	16.82	15.31	13.74	12.06	10.86
SD	3.13	9.96	10.18	9.68	9.85	9.40	9.43	9.41	9.59	10.15	10.14	9.89

SCI	1	2	3	4	5	6	7	8	9	10	11	12
1	2.90	19.04	18.09	16.96	16.42	15.51	14.45	13.15	11.38	9.79	8.49	7.38
2	2.39	11.66	10.50	9.78	9.28	9.44	8.58	8.22	7.35	6.31	5.59	4.89
3	0.87	14.77	13.77	13.87	14.16	13.46	12.71	11.62	9.33	7.87	7.17	6.44
4	6.82	20.48	19.74	18.63	18.61	17.68	16.12	14.62	13.21	11.03	9.77	9.50
5	4.25	16.53	16.02	15.48	14.60	13.32	12.15	10.80	9.82	8.95	8.01	7.20
6	1.98	12.63	12.64	10.30	8.52	7.71	7.55	6.43	5.70	3.96	5.18	4.23
7	2.98	12.80	12.39	11.85	11.14	10.37	10.39	9.97	8.92	7.92	6.79	5.71
MEAN	3.17	15.41	14.73	13.84	13.25	12.50	11.71	10.69	9.39	7.98	7.29	6.48
SD	1.91	3.39	3.33	3.37	3.74	3.52	3.08	2.80	2.47	2.33	1.62	1.76

30 Hz Fatigue - M-wave Amp.

AB	1	2	3	4	5	6	7	8	9	10	11	12
1	8.22	5.89	6.49	6.34	6.34	5.99	6.07	6.02	5.85	7.80	6.13	4.82
2	10.17	12.58	12.59	10.87	9.65	8.35	6.88	6.27	6.06	3.70	4.10	2.53
3	8.12	10.07	9.62	9.18	8.55	8.01	7.07	5.73	5.00	3.72	3.06	2.57
4	7.75	8.30	8.50	8.34	8.19	8.05	7.81	7.58	7.20	6.63	6.08	5.43
5	6.84	10.22	9.63	8.77	8.19	7.64	7.06	6.59	5.68	4.82	3.57	2.77
6	7.26	9.10	9.61	9.77	10.24	9.54	8.39	6.92	5.54	4.64	4.40	4.09
7	4.39	3.86	3.64	3.41	3.26	3.11	3.14	2.80	2.24	2.04	1.78	1.55
MEAN	7.54	8.57	8.58	8.10	7.77	7.24	6.63	5.99	5.37	4.76	4.16	3.39
SD	1.75	2.91	2.83	2.49	2.35	2.10	1.70	1.53	1.53	1.93	1.57	1.41

SCI	1	2	3	4	5	6	7	8	9	10	11	12
1	8.58	9.68	10.07	11.05	10.91	9.72	7.63	5.48	7.45	3.06	2.43	2.10
2	4.40	5.38	6.18	6.76	6.66	6.45	5.76	4.63	3.43	2.50	1.93	1.72
3	4.39	5.36	5.24	5.79	5.58	4.60	3.54	2.69	1.89	1.35	1.11	1.05
4	1.70	2.47	2.24	2.57	2.53	1.93	1.30	1.14	0.92	0.62	0.61	0.71
5	5.85	7.05	8.01	8.54	8.06	6.74	4.89	3.41	2.58	2.16	5.66	1.94
6	6.34	5.13	5.39	4.90	3.89	2.86	2.03	1.52	1.18	1.06	0.94	0.89
7	5.36	5.44	6.56	6.78	10.50	4.41	3.00	2.18	1.66	1.37	1.27	1.10
MEAN	5.23	5.79	6.24	6.63	6.87	5.24	4.02	3.01	2.73	1.73	1.99	1.36
SD	2.11	2.19	2.44	2.69	3.17	2.63	2.22	1.60	2.25	0.87	1.73	0.55

PTP - Peak Torque

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	2.39	4.87	3.57	3.20	3.36	3.39	3.41	3.38	3.48	3.37
2	3.09	2.92	2.82	3.74	2.64	2.79	2.75	2.87	2.62	2.73
3	2.43	3.52	3.14	2.87	2.47	2.43	2.75	2.70	2.87	2.76
4	2.99	4.91	3.99	4.31	4.64	4.87	5.15	5.05	4.88	4.96
5	2.86	3.89	3.45	3.40	3.44	3.55	3.38	3.28	3.30	3.29
6	2.52	5.94	3.65	2.54	2.31	2.14	2.01	2.12	2.01	2.86
7	2.29	3.40	3.49	2.89	2.70	2.24	2.26	2.07	1.37	1.24
8	2.65	3.00	3.34	3.12	3.32	3.47	3.57	3.45	3.41	3.52
MEAN	2.65	4.06	3.43	3.26	3.11	3.11	3.16	3.12	2.99	3.09
SD	0.30	1.08	0.35	0.56	0.76	0.91	0.98	0.95	1.06	1.04

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	2.14	2.99	3.71	3.73	3.64	3.45	3.73	3.63	3.59	3.58
2	2.82	6.00	5.33	4.89	4.44	4.31	4.10	3.87	3.62	3.56
3	1.78	3.05	2.14	2.74	1.96	1.63	1.40	1.17	0.79	0.91
4	3.35	5.66	5.17	5.12	4.95	4.65	4.53	4.60	4.60	4.61
5	2.51	7.12	5.37	5.06	5.05	4.92	4.77	3.95	3.66	3.61
6	2.92	5.52	4.68	4.34	4.17	4.12	4.03	3.92	3.79	3.65
7	2.73	4.26	4.26	3.64	3.42	3.32	3.20	3.05	2.68	2.70
8	4.62	5.85	5.28	6.54	4.77	3.91	4.54	4.49	4.21	4.45
MEAN	2.86	5.06	4.49	4.51	4.05	3.79	3.79	3.59	3.37	3.38
SD	0.86	1.48	1.12	1.16	1.03	1.03	1.09	1.09	1.18	1.16

PTP - TPT

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	70.25	63.76	68.75	75.50	78.75	78.50	81.50	78.25	86.50	84.50
2	103.25	113.00	101.75	93.25	65.50	71.00	76.00	88.25	95.50	95.75
3	78.50	74.00	75.00	79.00	71.75	73.00	77.50	78.25	80.25	79.75
4	77.75	65.25	66.50	82.00	79.25	83.00	83.25	90.50	85.75	87.75
5	74.50	64.00	64.50	72.50	72.25	72.25	70.50	73.00	71.25	72.00
6	71.75	54.25	55.50	54.75	57.25	62.75	54.00	54.25	54.25	66.00
7	72.00	62.50	67.25	75.00	73.25	77.00	73.50	78.75	59.75	90.25
8	85.75	70.75	83.00	86.25	101.50	89.00	89.75	93.75	90.50	90.50
MEAN	79.22	70.94	72.78	77.28	74.94	75.81	75.75	79.38	77.97	83.31
SD	10.91	17.97	14.15	11.34	12.88	8.01	10.65	12.44	14.84	10.11

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	69.25	39.00	66.75	70.00	72.00	82.50	75.00	86.50	86.00	89.25
2	115.25	32.75	35.00	35.75	34.50	37.00	37.50	38.50	37.25	38.25
3	60.00	71.50	62.75	62.50	65.25	65.50	62.75	64.75	61.00	64.00
4	68.75	58.00	54.75	53.25	54.50	55.75	55.50	56.00	56.75	59.00
5	54.00	66.25	58.25	58.00	57.25	61.50	64.50	62.50	58.75	62.75
6	96.50	91.25	100.25	92.25	92.75	92.50	95.50	98.00	98.00	95.50
7	54.50	53.00	53.00	53.75	53.50	55.25	52.00	53.50	51.00	52.25
8	63.75	101.75	101.75	110.25	106.50	100.25	110.50	106.75	105.25	103.50
MEAN	72.75	64.19	66.56	66.97	67.03	68.78	69.16	70.81	69.25	70.56
SD	21.80	23.87	23.23	23.78	23.12	21.27	23.85	23.74	24.19	22.91

PTP - 1/2 RT

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	96.75	56.00	85.25	102.75	96.75	91.50	86.50	86.75	83.50	82.75
2	114.75	116.00	120.75	114.25	134.00	132.35	131.75	125.50	117.00	122.50
3	72.00	54.25	61.00	64.50	66.50	64.50	64.50	68.75	69.25	70.75
4	80.25	66.00	66.75	79.50	82.25	77.00	80.00	72.50	78.25	76.50
5	73.50	49.50	62.50	69.75	70.00	71.00	70.50	69.75	68.00	69.00
6	81.00	51.75	53.00	71.00	67.75	67.50	80.50	84.00	84.25	46.25
7	67.00	47.25	53.75	68.50	67.75	64.25	70.25	64.25	66.00	51.75
8	80.25	56.25	67.50	79.75	71.25	83.75	80.25	71.50	76.50	79.25
MEAN	83.19	62.13	71.31	81.25	82.03	81.48	83.03	80.38	80.34	74.84
SD	15.51	22.49	22.37	17.88	23.38	22.69	20.95	19.78	16.34	23.17

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	74.75	52.75	63.00	88.25	100.00	101.50	111.75	105.50	100.50	98.50
2	26.00	47.75	26.50	25.25	23.75	24.00	25.00	25.25	26.25	26.50
3	89.75	77.50	92.50	80.50	87.25	76.00	75.50	67.75	64.50	70.75
4	88.50	80.25	74.75	75.75	77.50	71.50	74.25	75.50	75.50	76.50
5	82.25	68.50	64.75	76.25	81.25	88.00	98.50	102.25	106.50	110.25
6	70.00	93.50	73.75	72.00	74.75	77.25	80.50	77.75	77.00	75.00
7	98.75	62.25	62.25	60.75	70.75	76.75	81.50	83.25	65.25	97.75
8	91.75	60.50	63.75	79.75	80.00	95.50	90.50	88.25	92.25	86.25
MEAN	77.72	67.88	65.16	69.81	74.41	76.31	79.69	78.19	75.97	80.19
SD	22.88	15.23	18.61	19.65	22.32	23.60	25.47	24.99	25.41	25.62

PTP - M-wave Amp.

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	12.59	13.31	12.34	12.37	12.22	12.25	12.23	12.24	12.28	12.25
2	9.85	9.00	8.78	8.43	7.91	7.98	7.91	7.71	7.73	7.31
3	11.86	12.40	12.13	12.03	12.03	11.94	11.87	11.92	11.92	11.80
4	8.12	8.61	8.19	8.15	8.42	8.60	8.58	8.75	8.73	8.72
5	7.86	6.60	6.99	7.30	7.32	7.33	7.35	7.35	7.18	7.20
6	6.55	7.21	6.56	5.76	5.98	5.04	5.31	5.54	5.50	5.60
7	6.99	5.95	5.93	6.19	6.19	6.11	5.92	5.76	5.56	5.09
8	4.48	4.98	4.47	5.03	5.12	4.95	4.77	4.47	4.37	4.50
MEAN	8.54	8.51	8.17	8.16	8.15	8.03	7.99	7.97	7.91	7.81
SD	2.74	2.99	2.83	2.75	2.68	2.83	2.82	2.88	2.93	2.94

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	3.81	3.54	3.83	3.82	3.88	3.76	3.78	3.81	3.83	3.81
2	2.88	3.60	3.39	3.38	3.42	3.42	3.42	3.42	3.43	3.40
3	8.17	8.01	6.30	7.90	6.15	5.35	5.04	4.84	4.69	4.72
4	1.04	1.28	0.97	0.97	0.97	1.02	0.93	0.99	0.99	0.99
5	5.22	6.03	5.92	6.09	6.09	6.11	6.10	5.60	5.66	5.65
6	5.75	5.75	5.48	5.11	4.73	5.05	5.09	5.17	5.23	5.27
7	5.54	3.10	5.49	5.47	5.36	5.23	5.32	5.37	5.62	5.42
8	3.15	1.67	1.78	2.27	1.57	1.08	1.37	1.28	1.41	1.39
MEAN	4.45	4.12	4.15	4.38	4.02	3.88	3.88	3.81	3.86	3.83
SD	2.19	2.30	1.99	2.22	1.96	1.95	1.89	1.81	1.83	1.81

PTP - RTD

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	62.77	130.78	97.90	83.70	76.60	77.35	79.96	76.22	72.86	74.73
2	65.02	53.43	57.54	86.69	81.46	85.94	64.64	78.84	67.63	57.17
3	72.86	97.52	89.30	72.11	66.14	60.53	70.99	76.22	71.37	67.63
4	152.45	140.87	162.54	138.25	161.42	141.24	177.48	128.54	166.65	106.86
5	63.15	122.93	96.78	73.61	76.22	80.34	72.86	76.22	72.86	72.86
6	75.85	197.29	136.38	93.79	78.09	87.06	72.49	91.54	88.56	106.12
7	71.37	108.73	121.06	70.99	66.51	56.80	90.05	66.88	51.94	39.98
8	62.03	74.73	99.02	85.57	77.35	75.10	78.47	100.14	69.13	68.38
MEAN	78.19	115.79	107.57	88.09	85.47	83.05	88.37	86.83	82.63	74.22
SD	30.46	43.92	31.97	21.82	31.17	25.94	36.78	19.78	35.38	22.76

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	58.66	116.95	140.87	122.56	123.31	111.72	121.44	124.43	106.49	106.86
2	107.99	320.97	309.76	301.16	288.09	272.77	258.19	220.83	198.78	205.51
3	94.91	103.50	89.68	116.95	84.07	76.22	64.64	60.16	50.82	56.42
4	104.62	207.00	192.80	200.28	192.80	161.79	186.45	176.74	164.03	170.76
5	78.84	199.53	184.96	181.97	169.64	156.56	139.37	122.56	122.56	126.29
6	82.20	126.29	119.57	154.69	130.78	127.79	111.35	111.72	114.34	109.48
7	88.56	158.43	123.31	122.56	113.22	110.60	112.10	98.27	106.12	93.04
8	109.48	151.33	127.42	151.70	114.71	86.31	98.64	99.02	98.27	94.16
MEAN	90.66	173.00	161.05	168.98	152.08	137.97	136.52	126.72	120.18	120.32
SD	17.35	70.30	69.15	61.11	64.67	62.23	60.19	50.16	44.43	47.28

PTP - RTR

AB	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	28.40	66.51	36.99	28.77	32.13	35.50	38.49	36.62	39.23	37.37
2	29.14	31.76	29.89	33.63	26.53	27.65	29.89	28.40	32.60	30.64
3	34.00	52.31	42.60	38.49	37.74	34.38	38.49	36.62	34.75	34.00
4	60.53	60.16	47.45	41.10	50.07	48.95	57.17	54.93	53.43	56.05
5	37.74	67.26	47.45	43.34	42.22	42.60	42.60	44.09	38.86	38.86
6	31.76	100.14	60.91	28.02	29.14	27.28	38.86	25.03	51.94	53.43
7	32.88	60.16	59.41	35.50	35.87	34.00	31.39	31.39	30.27	23.17
8	34.75	49.32	42.60	37.74	39.23	39.23	40.35	41.85	42.22	42.60
MEAN	36.15	60.95	45.91	35.82	36.62	36.20	39.66	37.37	40.41	39.52
SD	10.30	19.53	10.49	5.49	7.55	7.31	8.31	9.59	8.50	11.07

SCI	Pre	5 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
1	34.00	60.91	45.59	38.86	34.00	32.13	34.38	30.27	31.39	35.87
2	76.60	120.69	124.80	130.03	122.18	114.71	107.99	102.75	100.89	95.65
3	41.85	36.99	34.75	34.38	22.79	22.79	18.68	16.44	20.55	32.88
4	33.63	53.81	56.05	60.91	54.55	49.32	49.32	60.91	53.81	50.07
5	31.01	73.24	61.65	50.07	47.45	41.10	38.11	32.51	31.01	31.01
6	35.87	50.82	49.32	53.43	48.95	47.08	39.98	39.98	41.10	39.98
7	38.49	51.56	50.44	54.18	44.84	42.60	38.86	36.62	35.50	41.10
8	53.81	69.50	58.29	61.28	45.59	31.76	35.87	35.12	37.37	36.24
MEAN	43.16	64.69	60.11	60.39	52.54	47.69	45.40	44.33	43.95	45.35
SD	15.26	25.35	27.44	29.72	29.83	28.49	26.68	26.64	24.89	21.16