AN ABSTRACT OF THE DISSERTATION OF

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Muscle weakness and fatigue are debilitating symptoms of multiple sclerosis (MS). Approximately 50% of muscle weakness and fatigue have been attributed to deficits within the peripheral nervous system, specifically mechanisms residing in the muscle. The goals of this study were to identify the cellular mechanisms of contraction within the muscle cell, which could contribute to the muscle weakness and fatigue in MS. Whole muscle assessment of knee extensor strength revealed that subjects with MS (N = 6) were 48% weaker than subjects without MS (N = 6). Pedometer results revealed that MS subjects were 68% less active on a daily basis than controls. Using an in vitro single fiber preparation obtained from the vastus lateralis, cross-bridge mechanisms of contraction were

tested to understand their role in muscle weakness and fatigue. Peak Ca^{+2}-activated force was 13-44% lower ($p<0.05$) in type I, I/IIa, IIa/IIx, IIx fibers from MS subjects. The force deficit was attributed to the 14-32% smaller ($p<0.05$) cross-sectional area (CSA) of type I, I/IIa, IIa, IIx fibers and to a 6% lower specific force ($p<0.05$) in type I fibers from MS subjects. While there were no differences found between groups for fiber unloaded shortening velocity, peak absolute power in type I fibers was 11% lower ($p<0.05$) in MS subjects. Skinned fiber preparations were also used to test peak Ca^{+2}-activated force at varying concentrations (0–30 mM) of inorganic phosphate (P_i) and at different pH (6.2–7.0). Force declined with increases in P_i concentrations, with a greater reduction of force in type I fibers (66%) versus type IIa fibers (40%) at 30 mM ($p<0.05$). In contrast, reductions in force at pH 6.5 (17%) and 6.2 (24%) were similar for type I and IIa fibers. Assessment of the myosin heavy chains (MHC) revealed that MS subjects had 33% fewer type IIa fiber than controls, and there was a trend towards increased numbers of type IIa/IIx and IIx fibers in MS subjects. The results of this study revealed that a portion of the muscle weakness in individuals with MS is due to deficits at the level of the muscle cell and cross-bridge.

Cellular Mechanisms of Muscle Weakness and Fatigability in Individuals with Multiple Sclerosis

by

Dena J.P. Garner

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CONTRIBUTION OF AUTHORS

Dr. Jeff Widrick was involved with the dissection of muscle samples, preparation of solutions used in the physiological experiments, and in the writing of the manuscript.

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CHAPTER 1

Introduction

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Multiple sclerosis

In the United States there are between 200,000 – 350,000 persons with multiple sclerosis (MS). The risk of developing MS in the United States is 1 in 1000 with two-third's being women who are in their late twenties and early thirties (Joy et al. 2001). MS is more common in people with northern European heritage, affecting 200 in 100,000 people from this region (Kurtzke 1985). Studies have also shown that there is an increased prevalence of MS in those who live in higher latitudes during childhood, whereas, MS is virtually non-existent in populations around the equator (Kurtzke 1985). The increased prevalence of MS in the moderate to colder climates has been attributed to genetic and environmental factors, yet no causal relationship has been established between the disease and these factors (Martin et al. 1997).

Pathophysiology of multiple sclerosis

Although the exact cause of MS has not been determined, it is generally believed to be an autoimmune disease. Normally the brain is protected from foreign bodies and toxins by the blood brain barrier. However, during MS, blood-borne immune cells cross the blood brain barrier and destroy the myelin sheath, resulting in inflammation and lesion formation. The attack of the myelin sheath leads to the formation of active or chronic lesions, or plaques, which are typically located within the central nervous system. Active lesions suggest an area of active inflammation and therefore progressive demyelination, whereas chronic lesions are

considered dormant with no signs of active inflammation (Hallpike 1983; Whitaker et al. 1997; Joy et al. 2001; National MS Society, October 1997).

The destruction of the myelin sheath results in the many symptoms associated with MS. Myelin is a fatty substance that insulates axons and increases the speed of action potentials. If myelin is destroyed, action potentials between and within the central and peripheral nervous systems will be slowed or halted. The slowing or halting of action potentials causes many of the primary, secondary, and tertiary symptoms associated with MS (McAlpine et al. 1972; Hallpike 1983; Whitaker et al. 1997; Joy et al. 2001).

Primary symptoms of MS are directly related to demyelination and include weakness, fatigue, walking difficulties, pain, numbness, and spasticity. Secondary symptoms occur due to complications and recurrences of the primary symptoms. Secondary symptoms include urinary tract infections from repeated bladder dysfunction, and physical inactivity due to increased fatigue and weakness. The tertiary symptoms are a consequence of the primary and secondary symptoms and are most often related to psychological and vocational issues. A tertiary symptom may be depression which can result from the loss of a job, inability to ambulate, and the ineffectiveness to complete activities of daily living (McAlpine et al. 1972; Hallpike 1983; Joy et al. 2001; National MS Society, October 1997)

Impairments in muscle function, which include muscle fatigue and weakness, are predominant symptoms of MS (Joy et al. 2001). Fatigue has been cited as the most prevalent and debilitating symptom of MS, affecting 75-85% of

individuals with MS (Freal et al. 1984; Krupp et al. 1988). Muscle weakness has been cited as affecting 80% of persons with MS (Matthews 1998). Fatigue and weakness in MS affects activities of daily living, making it harder to complete common tasks, such as brushing teeth or making a bed, maintaining physical activity, and meeting daily responsibilities (Krupp et al. 1988; Vercoulen et al. 1996).

Muscle weakness in multiple sclerosis

Researchers have cited that persons with MS, on average, are 25-40% weaker than subjects without MS (Rice et al. 1992; Kent-Braun et al. 1994; Sharma et al. 1995; Schwid et al. 1999; Lambert et al. 2001). Rice and colleagues (Rice et al. 1992) studied the extent of muscle weakness in MS by examining maximal voluntary contractions (MVCs) in subjects with and without MS. They found that the MVCs of the quadriceps from all their subjects with MS ranged from 40-70% below mean normal values. de Haan et al. (de Haan et al. 2000) obtained similar results, in which they found that the average MVCs of the quadriceps in persons with MS were 31% lower than the MVCs of persons without MS. Other studies involving the lower limbs have found that knee extensor strength, knee flexor strength, and the anterior tibialis strength of persons with MS was 26-38% lower than that of controls (Kent-Braun et al. 1994; Sharma et al. 1995; Schwid et al. 1999; Lambert et al. 2001).

To determine how much of that weakness in MS could be contributed to deficits within the muscle, studies have bypassed central nervous mechanisms by using electrical stimulation protocols (Rice et al. 1992; Kent-Braun et al. 1994; Sharma et al. 1995). Electrical stimulation measures the intrinsic force that muscle can produce without the influence of central factors such as motivation or motor unit recruitment (Kent-Braun 1997). Rice et al. (Rice et al. 1992) compared force produced during electrical stimulation to that of the subject's MVC and found that approximately 50% of muscle weakness in persons with MS was due to peripheral deficits, while 50% was due to central mechanisms of fatigue. Sharma et al. (Sharma et al. 1995) attributed 65% of the muscle weakness to peripheral deficits, while Kent-Braun et al. (Kent-Braun et al. 1994) found even greater deficits in peripheral mechanisms that contribute to muscle weakness in MS. These studies reveal that part of the muscle weakness in MS is due to deficits within the central nervous mechanisms such as decreased ability to excite motor neurons, but between one-half to two-thirds of muscle weakness can be attributed to deficits within the peripheral nervous system (Rice et al. 1992; Kent-Braun et al. 1994; Sharma et al. 1995).

Mechanisms of muscle contraction

The peripheral mechanisms of muscle weakness are related to the function of muscle cells, or fibers, which comprise the whole muscle. A single muscle fiber is made of numerous myofibrils which are parallel bundles containing repeating

units of sarcomeres. A Z line, or a Z disk, binds each sarcomere at its end. Between the Z lines of one sarcomere are interdigitating proteins called thick and thin filaments. The thin filament is comprised mostly of the protein actin, whereas the thick filament consists of mostly myosin. The interaction of actin and myosin is the basis of the cross-bridge mechanisms of contraction (Huxley 1974).

The initiation of muscle contraction depends on the neural activation of the muscle cell. Action potentials from the central nervous system (CNS), involving the brain and the spinal cord, travel to the muscle via alpha-motor neurons. Alpha-motor neurons have long projections from the neuron called axons, which are protected by a myelin sheath, a lipid substance that increases the conductivity of the action potentials (Waxman 1998). To transmit the action potential from the alpha-motor neuron to the muscle cell, the action potential must cross a synaptic cleft, an area separating the motor neuron and the muscle cell it innervates. For this transmission to occur synaptic vesicles on the alpha-motor neuron release the neurotransmitter acetylcholine (ACh), which will bind to receptors on the motor end plate of the muscle cell causing a depolarization of the muscle cell (Gandevia 2001).

Excitation-contraction coupling

The processes by which the depolarization of the muscle cell leads to muscle contraction are termed excitation-contraction coupling (ECC). When the muscle is depolarized, the action potential travels down the cell membrane and into

invaginations of the muscle cell called t-tubules. Depolarized t-tubules will activate the sarcoplasmic reticulum (SR) to release calcium (Ca^{+2}). The mechanisms by which the depolarization of t-tubules cause the SR to release Ca^{+2} are still not entirely clear. Chandler et al. (Chandler et al. 1976) suggests that there is a mechanical linkage between the t-tubules and the SR. The dihydropyridine receptors (DHPR), which serve as "charge sensors" on the t-tubules, are mechanically linked to the ryanodine receptors (RyR) on the SR, which function as Ca^{+2} release channels. A depolarization of the t-tubules will cause a conformational change in the DHPR, which is transmitted over a mechanical linkage, "unplugging" the RyR and allowing Ca^{+2} to leave the SR and initiate muscle contraction.

Cross-bridge cycle

The release of Ca^{+2} from the SR enables the myosin heads of the thick filament to strongly bind with actin, termed an actomyosin cross-bridge. Strongly bound cross-bridges may undergo cycles of detachment and re-attachment. Each cross-bridge cycle is associated with a power stroke, whereby myosin pulls actin towards the center of the sarcomere producing force and shortening the sarcomere. The cross-bridge cycle depends on the presence of adenosine triphosphate (ATP), which comes from anaerobic and aerobic metabolism. ATP attaches to the actomyosin cross-bridge and quickly dissociates the actin from the myosin. ATP remains attached to the myosin but quickly hydrolyzes into adenosine diphosphate

(ADP) and inorganic phosphate (P_i). The myosin-ADP-P_i complex reattaches itself to actin and forms a weak bond. P_i release is associated with the actomyosin complex undergoing a transition from a weakly bound, low force producing state to a strongly bound, force producing state. After the power stroke, ADP is released resulting in a rigor cross-bridge. The cross-bridge is now set for another round of the cycle (Cooke 1997).

Muscle fiber types

Muscle fibers within a given muscle express a specific myosin heavy chain isoform (MHC). In human muscle there are three basic MHC isoforms, type I, IIa, and IIx isoforms, with hybrids of these fiber types that include type I/IIa and IIa/IIx isoforms. While muscle fibers, regardless of their MHC isoform, undergo similar cross-bridge mechanisms of contraction, each MHC isoform is associated with variations in the amount of force, shortening velocity and power produced during cross-bridge cycling (Moss et al. 1995).

Differences in force between the MHC isoforms are due to the amount of mitochondria present in the fiber. Type I fibers contain increased concentrations of mitochondria causing this fiber type to be the most oxidative and therefore least fatigue-resistant of any fiber type. However, with increased mitochondria in the muscle fiber there is less room for actin and myosin cross-bridge cycling, resulting in lower force production of the type I fibers. Type IIa and IIx have lesser amounts of mitochondria, with IIx having the least amount of mitochondria, leading to an

increased number of cross-bridge formations, and therefore, greater force production (Tikunov et al. 2001).

MHC isoforms also exhibit different rates of cross-bridge cycling, with the rate of cross-bridge cycling correlated to shortening velocity. Studies have shown that the type I MHC isoform has the lowest shortening velocity, while the type IIa and IIx have faster shortening velocities than the type I fibers, with the MHC type IIx exhibiting the fastest shortening velocity (Fitts 1994). Due to differences in shortening velocity, type I MHC isoforms are typically categorized as slow fiber types, while type IIa and IIx MHC isoforms are labeled as fast fiber types.

Mechanisms of muscle weakness in multiple sclerosis

Rice et al. (Rice et al. 1992), Kent-Braun et al. (Kent-Braun et al. 1994), and Sharma et al. (Sharma et al. 1995) attributed part of the muscle weakness in MS to deficits within the muscle cell resulting from muscle disuse and de-conditioning. Studies have shown that persons with MS are significantly less active than persons without MS (Kent-Braun et al. 1997; Ng et al. 1997; Stuifbergen 1997). Stuifbergen (Stuifbergen 1997) correlated maximal activity score and adjusted activity scores of subjects with MS and found a 26% difference between maximal and adjusted activity scores. This indicates that the average physical activity level in persons with MS was significantly lower than the maximal level of healthy controls. Studies using accelerometers, an instrument that directly measures physical activity by recording motion as body acceleration,

reveals that subjects with MS are 37% less active than controls (Kent-Braun et al. 1997; Ng et al. 1997).

Extreme levels of physical inactivity, termed models of disuse, may help explain the muscle weakness experienced by persons with MS. Models of disuse, which include both short-term models such as spaceflight and bed-rest and long-term models such as spinal cord injury, have found significant changes in muscle cell function and composition. Such changes include reductions in the cross-sectional area (CSA) of fibers, decreases in absolute and specific fiber force (P_o) of the fiber, and a decreased percentage of type I fibers and an increase in type II fibers.

Short-term and long-term disuse studies reveal that while there is atrophy of all fiber types, there is selective atrophy of type II fibers. After 17 days of spaceflight, Widrick et al. found a 26% reduction in CSA of the type IIa fibers with a 15% reduction of the CSA of the type I fibers (Widrick et al. 1999). Castro et al. (Castro et al. 1999) found that six weeks post spinal cord injury resulted in a 40% reduction of CSA of the type IIa and IIax + IIx fibers versus a 31% reduction of CSA of the type I fibers. In a study that assessed CSA of muscle samples from subjects with MS, Kent-Braun et al. (Kent-Braun et al. 1997) found that there was a 25% reduction of CSA of type I fibers from the tibialis anterior in subjects with MS and a 31-36% reduction of CSA of type IIa and IIax fibers. These results reveal greater atrophy of the type II fibers in subjects with MS, with atrophy of both fiber

types exceeding the atrophy noted in short-term disuse and approaching atrophy seen in 6-week post spinal cord injury.

Short-term disuse also affects changes in the absolute force, total force that a fiber can produce, and specific force, force per CSA of a muscle fiber. Widrick et al. (Widrick et al. 1999) found a 21% reduction in absolute force of type I fibers after 17 days of spaceflight, while bed-rest revealed that absolute force in type I fibers dropped 13% after 17 days of disuse (Widrick et al. 1997). Short-term disuse also reveals that the specific force of type I fibers is 6% lower after 17 days of spaceflight (Widrick et al. 1999). Decreases in absolute force can be attributed to decreases in CSA of the fiber. Absolute force is related to the number of cross-bridge formations; a smaller fiber will have fewer cross-bridges, resulting in decreased force production. Specific force accounts for the CSA of the fiber, thus decreases in specific force are attributed to deficits at the level of the cross-bridge. Deficits at the level of the cross-bridge may include shifts from high force-producing states to low force-producing states, thereby reducing the average force per cross-bridge.

Muscle fatigue

Physiological fatigue has been defined as the inability to sustain the required or expected force (Fitts 1994). The identification of fatigue can be a complex issue due to the various factors that elicit the fatigue response in individuals. These factors include the type, duration, and intensity of the activity,

the training status of the individual, and muscle fiber recruitment during the activity (Fitts 1994). The physiological causes of muscle fatigue can occur within the central and peripheral nervous system. These sites within the central nervous system include: 1) supraspinal failure, 2) segmental afferent inhibition, 3) depression of motor neuron excitability, 4) loss of excitation at branch points, 5) pre-synaptic failure, and 6) inability to transmit an action potential from the neuromuscular junction. Within the peripheral nervous system fatigue can occur due to: 1) failure of the muscle membrane to sustain the action potential, 2) loss of coupling between the t-tubules and the sarcoplasmic reticulum, 3) depressed Ca^{+2} release from the sarcoplasmic reticulum, 4) reduced binding affinity of Ca^{+2} to troponin, 5) failure of the cross-bridge cycle, 6) delayed cross-bridge dissociation, and 7) depressed re-accumulation of the Ca^{+2} into the sarcoplasmic reticulum. A failure at any one of these central or peripheral areas will contribute to muscular fatigue (Green 1987; Westerblad et al. 1991; Fitts 1994; Gandevia 2001).

A distinction between peripheral and central fatigue can be made during a fatiguing protocol by comparing voluntary contractions to electrical stimulation of the muscle group or by interposing twitch stimulation to a voluntary contraction (Gibson et al. 1985). Studies have shown that in well-motivated subjects, there is little indication that central mechanisms contribute to fatigue, rendering peripheral mechanisms the most likely source of fatigue (Merton 1954; Bigland-Ritchie et al. 1978). In a classic fatigue study, Merton (Merton 1954) asked subjects to maximally contract the adductor pollicis until the muscle fatigued. With the onset

of fatigue all central mechanisms were bypassed by electrically stimulating the ulnar nerve, a nerve that stimulates the adductor pollicis. Subjects were also asked to complete a maximal voluntary contraction after fatigue. Merton found that the muscle contractions with electrical stimulation and the voluntary maximal contractions were similar, concluding that fatigue was due to deficits within the peripheral nervous system.

Muscle fatigue in multiple sclerosis

Since MS is considered a disease of the central nervous system, researchers have focused on central nervous system mechanisms of fatigue as the most probable cause for the muscle fatigue in those with MS. Studies which involve upper limb muscle groups such as bilateral biceps, adductor pollicis, and flexor carpi radialis have shown that muscle fatigue in these groups was due solely to deficits within the central nervous system (van der Kamp et al. 1991; Djaldetti et al. 1996; Sheean et al. 1997; Petajan et al. 2000; de Ruiter et al. 2001). These studies attributed muscle fatigue to increases in central motor conduction time, decreases in motor unit firing rates, and decreases in motor unit recruitment.

Not all of the muscle fatigue in MS can be explained by deficits within the central nervous system. In studies which involve lower limbs, such as the quadriceps and ankle dorsiflexors, muscle fatigue is partially due to peripheral nervous system deficits (Lenman et al. 1989; Rice et al. 1992; Kent-Braun et al. 1994; Sharma et al. 1995). A study by de Haan and colleagues (de Haan et al.

2000) involved electrically stimulating the quadriceps of subjects with and without MS during a fatiguing protocol. When compared to initial, pre-fatigue force values, the subjects with MS showed significant reduction, 31%, in force after fatigue, whereas, the controls had a smaller reduction, 24%, in force values after fatigue. Sharma et al. (Sharma et al. 1995) found similar results during a fatiguing protocol involving the anterior tibialis of subjects with and without MS. There was a 35% and 14% reduction in force after fatigue as compared to initial force values in subjects with and without MS, respectively. In addition, Sharma and colleagues saw no impairments in compound muscle action potential, a measure of action potential propagation from the neuromuscular junction, in subjects with MS before and after the exercise protocol. This would suggest that there were no impairments of action potential propagations from the central nervous mechanisms to the muscle during and after exercise.

Studies have also shown that the recovery of force after fatigue is significantly prolonged in subjects with MS versus controls (Kent-Braun et al. 1994; Sharma et al. 1995). Results from Kent-Braun et al. (Kent-Braun et al. 1994) reveal that tetanic relaxation both pre- and post-exercise was 35% and 20%, respectively, longer than tetanic relaxation of controls at baseline. Sharma et al. also found that tetanic ½ relaxation time after electrically stimulated exercise was 30% longer in subjects with MS versus controls. The delayed force recovery after exercise suggests impairments within the muscle, specifically at the level of excitation contraction coupling.

Mechanisms of muscle fatigue

Using skinned fiber preparations, researchers have isolated cross-bridge mechanisms of contraction without the influence of excitation-contraction coupling mechanisms. This preparation allows researchers to manipulate the cellular environment to mimic biochemical conditions that occur during muscle fatigue. As fatigue occurs in skeletal muscle, metabolic changes occur in the muscle, resulting in a depression of force. These metabolic changes include: 1) an increase accumulation of P_i due to the breakdown of creatine phosphate and 2) lactic acid accumulation, which results in an increase of hydrogen ions and depressed pH (Fitts 1994; Westerblad et al. 2002). During the cross-bridge cycle, ADP and P_i are split from ATP. P_i is then released from ADP, resulting in ADP and P_i accumulation in the muscle. Though there is an accumulation of ADP, it has been shown to have minor effects on muscle, whereas, accumulation of P_i has major effects on muscle function (Fitts 1994). This increased accumulation of P_i keeps more cross-bridges in a weakly bound state, reducing the number of strongly bound force producing cross-bridges, and thereby reducing the force a fiber can produce (Fitts 1994; Westerblad et al. 2002). During fatigue lactic acid also accumulates and it will dissociate into lactate and hydrogen ions. It is the increase of hydrogen ions which is believed to affect force either by interfering with actomyosin cross-bridge cycling, resulting in a decreased number of cross-bridge formations and/or by a decreased sensitivity of troponin for calcium and an increased sensitivity to

hydrogen ions, thereby, decreasing the force per cross-bridge (Fabiato et al. 1978; Metzger et al. 1987; Renaud et al. 1987; Metzger et al. 1990; Fitts 1994).

During rest, intramuscular P_i values are estimated to be between 1-6 mM, with intramuscular values of P_i exceeding 30 mM during fatigue (Cady et al. 1989). Studies have shown that this increased accumulation of P_i during fatigue has differential effects on fibers expressing slow or fast MHC isoforms (Nosek et al. 1990; Stienen et al. 1992; Fryer et al. 1995; Potma et al. 1995; Widrick 2002). However, it is debatable whether increased concentrations of P_i affect fast or slow fibers to a greater or lesser extent. Stienen and colleagues (Stienen et al. 1992) found that the force of rabbit psoas fibers (fast fibers) was more affected by increased P_i than were rabbit soleus fibers (slow fibers). They noted a 42% drop in force in fast fibers and a 22% reduction in force in slow fibers at a P_i concentration of 15 mM. Potma (Potma et al. 1995) and Nosek (Nosek et al. 1990) found that fast and slow fibers were equally sensitive to increases in P_i. They found a 40-50% reduction in force for both the fast and slow fibers at 30 mM P_i. At 30 mM of P_i, Widrick (Widrick 2002) found a 44% reduction of force in type I rat fibers and a 42% reduction of force in type IIb fibers, similar to the 40-50% reduction of force found by Potma et al. (Potma et al. 1995) and Nosek et al. (Nosek et al. 1990). Others have found that the slow fiber types are more affected than fast fiber types with increases in P_i. Fryer et al. found a 65% and 45% reduction of force in rat slow and fast fibers, correspondingly, at 30 mM of P_i, with significant differences

in force found between slow and fast fibers at P_i concentrations of 1, 5, 25, and 50 mM (Fryer et al. 1995).

In the muscle cells, resting pH values are approximately 7.0. With fatiguing exercise pH values drop to pH 6.5, with this drop in pH being proportional to increased accumulation of hydrogen ions (Cady et al. 1989). Studies have also shown that changes in pH, i.e. increased accumulation of hydrogen ions, have different effects on the force of fast and slow MHC fiber types. With decreasing pH, the decline of force in fast fibers appears to be greater than the decline of force in slow fibers (Donaldson et al. 1978; Metzger et al. 1987; Chase et al. 1988; Lynch et al. 1994; Potma et al. 1994).

Studying rabbit adductor magnus, a fast-twitch skeletal muscle, and the rabbit soleus, a slow-twitch skeletal muscle, Donaldson and Hermansen (Donaldson et al. 1978) and Chase et al. (Chase et al. 1988) found that the force of the soleus fibers was generally unaffected by a decrease in pH, whereas, the force of the adductor magnus fibers was significantly depressed with a decrease in pH. Chase et al. (Chase et al. 1988) found when pH was decreased from 7.1 to 6.0, the force of psoas fibers was reduced by 50%. This was a greater reduction in force as compared to the 35% reduction in force of the soleus fibers that occurred with the same changes in pH. Donaldson and Hermansen (Donaldson et al. 1978) found that fast fibers in the rabbit had a 30% drop in force at pH 6.5 while the slow fibers had a 12% drop in force at pH 6.5. In rats, when pH was decreased from 7.0 to 6.2 the force of slow twitch soleus muscles was reduced 27%, while there was a 35%

reduction of force in the fast twitch superficial and deep vastus lateralis muscles with the same changes in pH (Metzger et al. 1987). In one known human study, Lynch et al. (Lynch et al. 1994) found that changes in pH from 7.1 to 6.6 resulted in 7% force reduction in the type I fibers of the vastus lateralis and a 18-21% reduction in force of the type IIa and IIx fibers with the same changes in pH. Hence, both animal and human studies have shown that with decreases in pH the force of fast fiber types is affected significantly more than the force of slow fiber types.

Mechanisms of increased fatigability in multiple sclerosis

Studies have shown that there is increased muscle fatigue in persons with MS that cannot be solely explained by central nervous system deficits (Kent-Braun et al. 1994; Sharma et al. 1995). These studies have indicated that impairments within the muscle cell are responsible for part of the muscle fatigue in persons with MS. Deficits within the muscle may be partially explained by the exaggerated response of metabolites, specifically inorganic phosphate (P_i), and hydrogen ions (pH), in subjects with MS. Sharma et al. (Sharma et al. 1995) found that after a fatigue protocol there was a 42% increased accumulation of P_i with a 2% decrease in pH in subjects with MS versus controls. Based on the data in this study, Sharma and colleagues concluded that part of the fatigue in subjects with MS could be attributed to a greater accumulation of metabolites.

Shifts in myosin heavy chain isoforms from type I MHC to type II MHC may also explain increased fatigability in individuals with MS. Studying muscle biopsies from the tibialis anterior, Kent-Braun et al. found an increased percentage of faster, more fatigable fiber types in persons with MS as compared to subjects without MS (Kent-Braun et al. 1997). Subjects with MS had 13% fewer of the type I oxidative fibers and 22-32% more type IIa and IIax fibers versus controls.

These findings with isoform shifts in MS are comparable to isoform shifts during short-term and long-term disuse model studies. After spaceflight, studies have also shown there is as much as a 12% decrease in the number of type I fibers and a 9% increase in the number of the faster, more glycolytic type IIa fibers (Thomason et al. 1990; Edgerton et al. 1995; Widrick et al. 1998; Widrick et al. 1999; Fitts et al. 2000). An analysis of 24-week post-spinal cord injury showed no changes in type I fibers, but there was a 56% reduction of type IIa fibers and a 27% increase in the type IIax + IIx fibers as compared to fiber type percentages at 6-weeks post-spinal cord injury (Castro et al. 1999). Thus, the shifts of MHC isoforms in MS are slightly greater than shifts seen in short-term disuse but not as great as those seen in spinal cord injury.

Hypothesis

Previous studies have suggested that not all of the muscle fatigue and weakness is due to deficits within the central nervous system, but may also be attributed to peripheral nervous system deficits, specifically at the muscle level.

The purpose of this project is to investigate the possible deficits within the muscle that could be responsible for muscle fatigue and weakness in persons with MS. The hypothesis to be tested is that cross-bridge mechanisms of contraction and fiber type content and composition in subjects with MS are responsible for part of the muscle fatigue and weakness in this population. In order to test this hypothesis, experiments will be performed with the following specific aims:

1. The effect of MS on cross-bridge mechanisms of contraction will be evaluated by comparing the peak Ca^{+2}-activated force (P_o), maximal shortening velocity (V_o), and peak power of single muscle fibers from individuals with and without MS.
2. Whether fibers from individuals with MS are more sensitive to changes in the metabolites will be examined by comparing the effect of the metabolites, H^+ and P_i, on P_o of single muscle fibers in individuals with and without MS.
3. In order to test whether cross-bridge mechanisms of contraction are affected more in fibers expressing slow versus fast contractile proteins, the MHC isoform content will be determined in all fibers studied in Aims 1 and 2. Fibers will then be grouped by their isoform composition for statistical analysis.
4. In order to determine whether there is a shift in fiber MHC isoform content in vastus lateralis muscle fibers from subjects with MS, the MHC isoform content will be verified on single fibers isolated from muscle biopsies.

CHAPTER 2

Cellular Mechanisms of Muscle Weakness and Fatigability in Individuals with Multiple Sclerosis

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Abstract

Muscle weakness and fatigue are debilitating symptoms of multiple sclerosis (MS). Approximately 50% of muscle weakness and fatigue have been attributed to deficits within the peripheral nervous system, specifically mechanisms residing in the muscle. The goals of this study were to identify the cellular mechanisms of contraction within the muscle cell that could contribute to the muscle weakness and fatigue in persons with MS. Whole muscle assessment of knee extensor strength revealed that subjects with MS (N = 6) were 48% weaker than subjects without MS (N = 6). Pedometer results revealed that subjects with MS were 68% less active on a daily basis than controls. Using an in vitro single fiber preparation, cross-bridge mechanisms of contraction were tested to understand their role in muscle weakness and fatigue. Vastus lateralis muscle samples were obtained from subjects with and without MS and 530 single skinned muscle fibers were mounted between a force transducer and a servo-controlled motor. Number of fibers were as follows: type I: 134 non-MS, 147 MS; type I/IIa: 3 non-MS, 6 MS; type IIa: 114 non-MS, 67 MS; type IIa/IIx: 22 non-MS, 21 MS; type IIx: 5 non-MS, 11 MS. Peak Ca^{+2}-activated force was 13-44% lower ($p<0.05$) in type I, I/IIa, IIa/IIx, IIx fibers from subjects with MS. The force deficit was attributed to the 14-32% smaller ($p<0.05$) cross-sectional area (CSA) of type I, I/IIa, IIa, IIx fibers and to a 6% lower specific force ($p<0.05$) in type I fibers from subjects with MS. While no differences were found between groups for fiber unloaded shortening velocity, peak absolute power in type I fibers was 11% lower ($p<0.05$)

in subjects with MS. Skinned fiber preparations were also used to test peak Ca^{+2}-activated force at varying concentrations (0–30 mM) of inorganic phosphate (P_i) and at different pH (6.2–7.0). Force declined with increases in P_i concentrations, with a greater reduction of force in type I fibers (66%) versus type IIa fibers (40%) at 30 mM ($p<0.05$). In contrast, reductions in force at pH 6.5 (17%) and 6.2 (24%) were similar for type I and IIa fibers. These results indicated no difference in fiber susceptibility to metabolites P_i and hydrogen ions on single muscle fibers from subjects with or without MS. Assessment of the myosin heavy chains (MHC) revealed that subjects with MS had 33% fewer type IIa fiber than controls, and there was a trend towards an increased number of type IIa/IIx and IIx fibers in subjects with MS. The results of this study revealed that a portion of the muscle weakness in individuals with MS is due to deficits at the level of the muscle cell and cross-bridge.

Introduction

In the United States there are between 200,000 – 350,000 people with multiple sclerosis (MS). The risk of a person developing MS is 1 in 1000, with most developing the disease in their late twenties and early thirties (Joy et al. 2001). The direct and indirect economic costs of MS in the United States range from 6.5 to 11.9 billion dollars a year, averaging 34,000 dollars per person annually. Estimates of the indirect and direct costs associated with MS rank this disease as the second most costly neurological disease behind Alzheimer's Disease (Whetten-Goldstein et al. 1998; National MS Society, November 1998).

Although the exact causes of MS are unknown, it is generally considered to be an autoimmune disease in which the myelin sheath is destroyed resulting in the slowing or halting of action potential propagation. A slowing or halting of action potentials leads to the primary and secondary symptoms associated with MS. Primary symptoms of MS are directly related to demyelination and include weakness, fatigue, walking difficulties, pain, numbness, and spasticity. Secondary symptoms occur due to complications and recurrences of the primary symptoms and include urinary tract infections from repeated bladder dysfunction, and physical inactivity due to increased fatigue and weakness (McAlpine et al. 1972; Hallpike 1983; Joy et al. 2001). Of these symptoms, fatigue and weakness have been cited as two of the most debilitating and prevalent symptoms, affecting 75-85% of persons with MS (Freal et al. 1984; Krupp et al. 1988; Matthews 1998).

Since MS is considered to be a disease principally of the central nervous system, researchers initially assumed that the occurrence of muscular fatigue and weakness in MS was due to problems within the central nervous system. Deficits in the central nervous system such as decreased motor unit firing rates, decreased motor unit recruitment, and increased central motor conduction time have been shown to contribute to muscular fatigue and weakness in persons with MS (van der Kamp et al. 1991; Rice et al. 1992; Sharma et al. 1995; Djaldetti et al. 1996; Sheean et al. 1997; Petajan et al. 2000). Yet not all of the muscular fatigue and weakness have been explained by deficits within the central nervous system.

Rice and colleagues (Rice et al. 1992) studied the extent of muscle weakness in MS by examining maximal voluntary and electrically stimulated contractions. They concluded that approximately 50% of the muscular weakness could be attributed to peripheral mechanisms of fatigue. Subsequent studies by Kent-Braun et al. (Kent-Braun et al. 1994) and Sharma et al. (Sharma et al. 1995) found that approximately 65% of muscle weakness in MS is due to deficits within the peripheral nervous system, specifically within the muscle cell.

Not only are subjects with MS weaker but they also fatigue significantly sooner and to a greater extent during an exercise protocol, with prolonged recovery times versus controls (Kent-Braun et al. 1994; Sharma et al. 1995). Sharma et al. (Sharma et al. 1995) found that the tetanic force during exercise of the anterior tibialis from subjects with MS was 25% of controls, while Kent-Braun et al. (Kent-Braun et al. 1997) found controls exercised 50% longer than subjects with MS

before fatigue developed in the ankle dorsiflexor. Fifteen minutes post exercise, Sharma et al. saw that force recovery in subjects with MS was 23% less complete than subjects with MS (Sharma et al. 1995).

If definitive differences in muscular contractility and composition can be identified as partial causes of muscular fatigue and weakness, then this may enable researchers to design effective treatments to target these debilitating secondary symptoms of MS. The purpose of this study was to test the hypothesis that the muscle fatigue and weakness in MS has a peripheral, cellular component. To test this hypothesis, an in vitro single fiber preparation was used to investigate, a) cross-bridge mechanisms of contraction, including peak Ca^{+2}-activated force (P_o), maximal shortening velocity (V_o), and peak power, b) the effect of metabolites, specifically inorganic phosphate (P_i) and hydrogen ions, on peak Ca^{+2}–activated force, and c) the myosin heavy chain (MHC) composition of single muscle fibers from subjects with and without MS.

Methods

Subject selection

Subjects were recruited from advertisements in the local newspapers and announcements at local MS chapter meetings. Twelve subjects, six subjects with MS and six subjects without MS participated in this study. The Oregon State

University Institutional Review Board approved this project, and all subjects signed an informed consent after they were given a full explanation of the study.

Disability status and physical activity

A physical therapist, with experience evaluating functional measures, used the Expanded Disability Status Scale (EDSS), the modified Ashworth Spasticity Scale, and the Physiotherapy Clinical Outcome Variables (C.O.V.S.) to assess the level of disability of the subjects with MS (Kurtzke 1983; Seaby et al. 1989; Pandyan et al. 1999).

To assess daily physical activity levels, MS and non-MS subjects wore an activity pedometer (Optimal Health Products Clicker) continually throughout the day with the exception of sleeping hours. Subjects were trained in proper use of the pedometers. Stride length for each subject was measured and subsequently recorded in the pedometer. Pedometers were worn for a period of 14 consecutive days, and at the end of each day subjects recorded total miles and steps taken for that day.

Muscle biopsy

Muscle biopsies from the vastus lateralis were obtained using the percutaneous biopsy technique (Bergström 1975). Based on the functional assessment of the physical therapist, the subject's weakest leg was biopsied. The muscle biopsy was immediately placed in dissection solution where it was

dissected into 8-10 separate bundles. A large bundle was frozen and stored in liquid nitrogen for MHC isoform analysis. The other bundles were stored in skinning solution (for composition, see Solutions section). This solution chemically skins the fibers, permeabilizing the t-tubules, sarcoplasmic reticulum, mitochondria, and the sarcolemma, while leaving the cross-bridges and thin filament regulatory proteins intact. Bundles were stored at 4°C for 24 hours and then transferred to fresh skinning solution and stored for up to 30 days at -20°C.

Evaluation of neuromuscular strength

Subjects returned to the laboratory approximately two weeks after the muscle biopsy procedure. Muscle strength in the quadriceps was assessed using an isokinetic dynamometer (Chattecx Corporation, KinCom III; Hixson, TN). The lateral condyle of the knee was aligned with the axis of the dynamometer lever arm with the knee and hip at 90° of flexion. The subject's thigh was then strapped to the seat with a Velcro strap, a seat belt was fit across the lap, and another Velcro strap was fit across the chest to limit extraneous movement and the use of muscles other than the quadriceps. The force transducer was attached to the lever arm and to the lever arm the subject's medial malleolus was secured with a Velcro strap.

Measurements were conducted on the biopsied leg. Each protocol was preceded by two warm-up trials. The first test protocol was an isometric test in which the subjects used the knee extensors and performed three consecutive maximal voluntary isometric contractions. Each contraction lasted for 5 seconds

with approximately one minute separating each trial. The second protocol was isokinetic knee extension in which the subject's maximal voluntary concentric knee extension strength was tested at angular velocities of 30°, 60°, 120°, 180°, and 240° per second, through a range of 10°-90° of knee flexion. Subjects completed three trials at each angular velocity with approximately one-minute rest after each test. The highest value during the three trials of each test was used for statistical analysis.

Solutions

One relaxing solution and nine different activating solutions were used in this study. All solutions contained: 7.0 mM EGTA, 20.0 mM imidazole, 1 mM free Mg^{+2}, 4 mM Mg^{2+}-ATP, 14.5 mM creatine phosphate, and 15 $U \cdot ml^{-1}$ creatine kinase. A $CaCl_2$ standard solution was used to adjust the free Ca^{+2} concentration of the relaxing solution to pCa 9.0 ($pCa = -\log[Ca^{+2}]$) and all of the activating solutions to pCa 4.5. The pH of the standard activating solution and relaxing solution was 7.0 (adjusted with KOH) and neither solution contained any added P_i. A second activating solution contained 30 mM P_i (adjusted with KH_2PO_4), pH 7.0. Five additional activating solutions of varying concentrations of P_i (2 mM, 5 mM, 10mM, 15 mM, and 20 mM), all pH 7.0, were obtained by mixing aliquots of the 0 mM P_i activating solution and the 30 mM P_i activating solution. The final two activating solutions contained no added P_i but had a pH of 6.5 and 6.2, respectively. The total final ionic strength was 180 mM (adjusted with KCl) for all solutions.

The composition of all solutions used in these experiments was determined using the iterative computer program of Fabiato (Fabiato 1988) and apparent stability constants adjusted for total ionic strength, temperature (15°C), and pH (Fabiato 1985).

The dissection solution consisted of protease inhibitors dissolved in relaxing solution (Complete Mini EDTA-Free Protease Inhibitor Tablets, Boehringer Mannheim, Indianapolis, IN). The skinning solution consisted of 50% dissection solution and 50% glycerol.

Experimental instruments

The experimental apparatus used in the single fiber experiments included a force transducer (Aurora Scientific, Aurora Ontario; Model 400), a high-speed servomotor (Aurora Scientific; Model 308B), a stainless steel plate with three chambers, two stainless steel troughs for fiber attachment, an aluminum-cooling block, and a thermocouple. The foundation of the experimental apparatus was a stainless steel, spring-loaded dip-plate into which three stainless steel chambers were milled, with each chamber holding ~250ml of solution. Located on either side of the stainless steel plate were the servomotor and force transducer. Two stainless steel troughs, extending from the servomotor and force transducer, were suspended within a chamber and held the single muscle fiber during the physiological experiments. Peltier cells and an aluminum cooling block worked in conjunction with a circulating water bath to heat or cool the experimental apparatus

so that solution temperature was maintained at 15°C + 0.5°C throughout the experiment.

Mounting of fibers and measurement of fiber CSA and length

Muscle fiber segments (~5 mm in length) were isolated from the muscle bundle in a chamber containing relaxing solution. The ends of the segment (~1 mm of each end) were attached to the stainless steel troughs. To attach the fiber, a 4.0 monofilament post placed on top of each fiber end and 10.0 suture were used to tie the post securely into the trough. Once the fibers were secured to the troughs, the experimental apparatus was mounted to the stage of the inverted microscope (Olympus IX-70). The fiber was observed (600x) through a glass plate that formed the bottom of each chamber.

Sarcomere length was adjusted to 2.5 μm using a calibrated eyepiece micrometer. Fiber length was measured using a digital micrometer. Fiber width was read from the eyepiece micrometer at three points along the length of the fiber, at approximately 25%, 50%, and 75% of fiber length. Width measurements were conducted while the fiber was briefly suspended in air (<5 s). The fiber was quickly returned to the relaxing solution between each of the three measurements. Fiber cross-sectional area (CSA) was calculated from each width measurement with the assumption that the fiber forms a cylinder while in air (Metzger et al. 1987; Widrick et al. 1996). Final fiber CSA was the mean of these three CSA measurements.

Output from the force transducer and position motor was monitored on a digital oscilloscope (Integra 10, Nicolet Technologies, Madison, WI). Amplified signals (Model 300-FC1, Positron Development, Inglewood, CA) were digitized (5Hz) and interfaced to a personal computer by a data acquisition board (Model AT-MIO-16E, National Instruments, Austin, TX). Custom software (LabView, National Instruments) was used to control the experiments and to store and analyze data.

Physiological experiments

The maximal Ca^{+2}-activated force (P_o) and the unloaded shortening velocity (V_o) of the fiber were measured by the slack test procedure. During the slack test procedure, the fiber was placed in an activating solution containing 0 mM of added P_i and pH 7.0. The fiber was subjected to a series of 5 steps, with each slack step greater in length and no step exceeding 20% of the fiber length (Edman 1979) (Figure 1a). The time it took for tension to re-develop after each slack step was plotted versus the slack step length, and the slope of the line fit to these points was V_o (Figures 1b). Since the shortening velocity is proportional to the fiber length, V_o was divided by fiber length and expressed in normalized terms, i.e. as fiber lengths per seconds (Fl/s). The P_o was determined from the difference in the baseline measurement of force in the relaxing solution and the peak force obtained when placed in activating solution.

Fibers were subjected to a series of isotonic contractions in order to obtain a force-velocity and a force-power relationship. The fiber was activated in the standard activating solution (0 mM P_i, pH 7.0). After the fiber reached peak force, it was subjected to three isotonic load steps. The duration of each step varied between 60-100 ms for fast fibers and 100-150 ms for slow fibers. The shortening velocity (slope of the position signal) and force of each isotonic step was assessed over the final one-fourth of the isotonic step. Data were fit to the Hill equation (Hill 1938) (Figure 2). The following parameters which describe the Hill equation were determined: Vmax, the velocity extrapolated to a force of zero; P_o, force attained at a velocity of zero; and a/P_o, a unitless parameter describing the shape or curvature of the force-velocity relationship (Woledge et al. 1985). Peak power was calculated from these three parameters (Woledge et al. 1985). Power was expressed in absolute terms, the product of force (mN) and the shortening velocity (FL/s), and in normalized terms, the product of the specific force (kN/m^2) and shortening velocity (FL/s), which is equivalent to Watts/Liter of fiber (Widrick et al. 1995; Widrick et al. 1996).

Peak Ca^{+2}-activated force was measure at various P_i and H^+ concentrations in a sub-group of fibers. Fibers were placed in activating solutions containing varying pH (7.0, 6.5, and 6.2), or varying concentrations of P_i (0 mM, 2 mM, 5 mM, 10 mM, 15mM, 20 mM, and 30 mM) (Figures 3 and 4). Peak force in each solution was measured as the difference between the force in relaxing solution and

peak force attained during the contraction. Activating solutions were stirred to prevent the formation of P_i of H^+ gradients within the fiber (Kentish 1991).

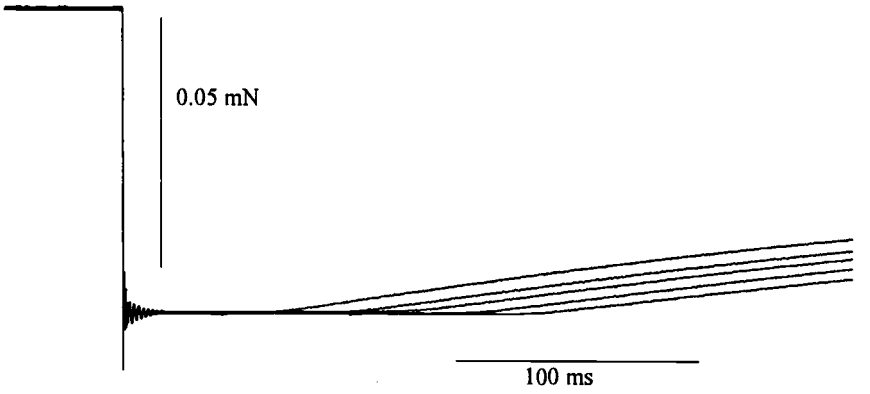

Figure 1a. Superimposed force records following slack steps at 200, 250, 300, 350, and 396. Time required for force redevelopment was determined from each record and plotted in Figure 1b. Calibration bars are 0.05 mN and 100 ms.

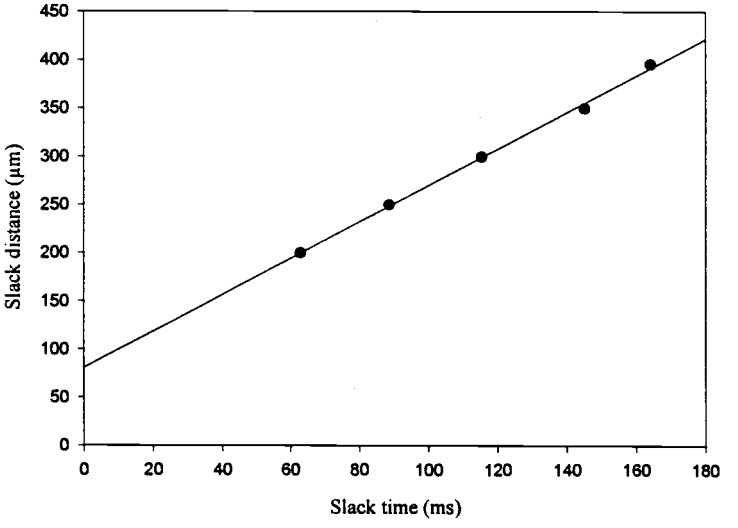

Figure 1b. Time for force development vs. slack distance was fit with a linear regression. The slope of the relationship is the fiber's maximal shortening velocity (V_o). V_o was normalized to fiber length (FL). Experiment #2889: slope = 1.89, r^2 = 0.998, FL = 3.06 mm, V_o = 0.62 FL/s

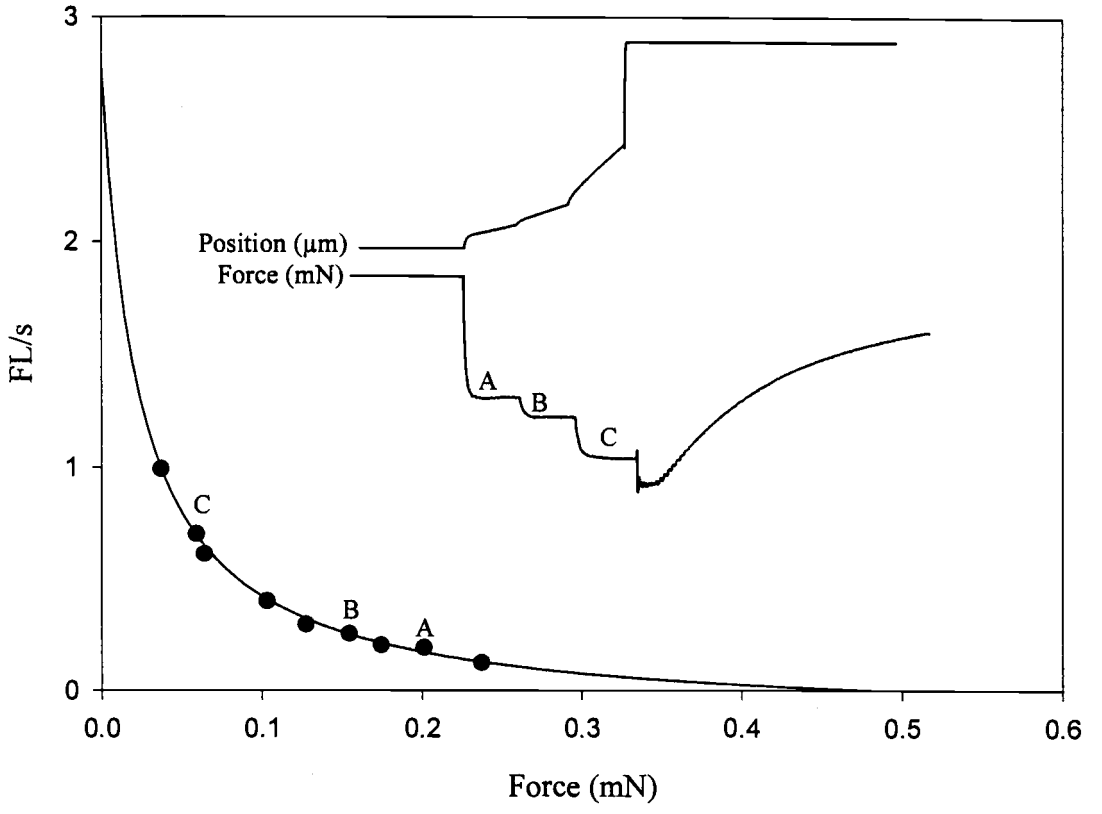

Figure 2. Force-velocity relationship for a single human muscle fiber. Inset: position and force records obtained during 3 isotonic contractions at peak Ca^{+2}-activated force (P_o). Isotonic force for the 3 steps was 42, 32, and 12% of P_o, and corresponding shortening velocities were 0.201, 0.154, and 0.059 FL/s. These data points were plotted and indicated by corresponding points, A, B, and C. This was repeated 3 times resulting in 9 points (as seen on graph), which were fit by the Hill equation.

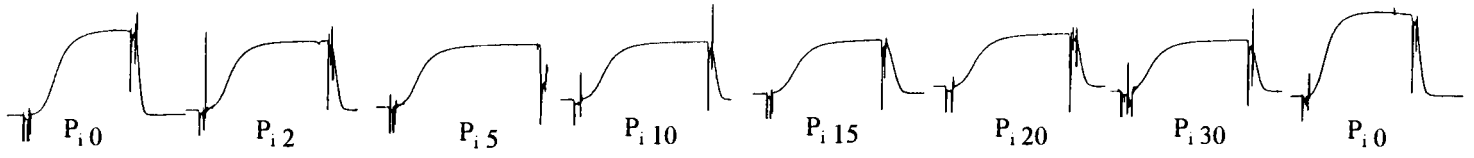

Figure 3. Effect of different concentrations of P_i on peak Ca^{+2}-activated force on a single skinned muscle fiber expressing type IIa MHC. Each trace represents a fiber that rapidly attained peak force after being placed in activating solution after which it was returned to the relaxing solution. Sharp spikes in a trace represent movement artifact.

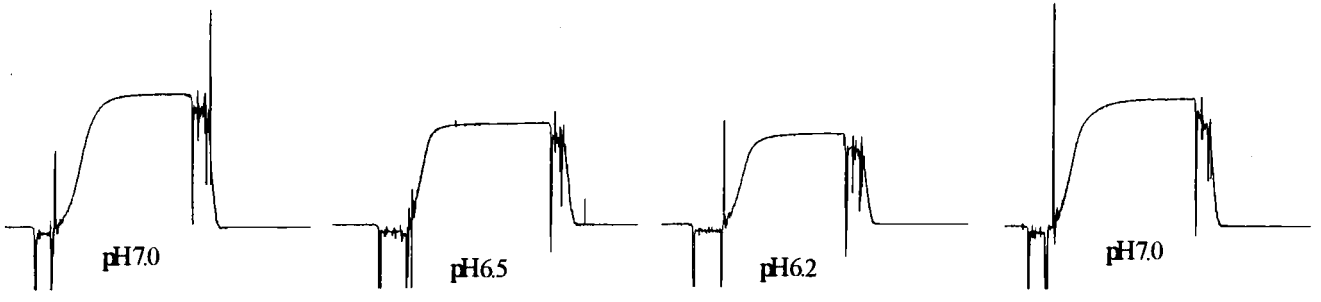

Figure 4. Effect of different pH on peak Ca^{+2}-activated force on a single skinned muscle fiber expressing type IIa MHC. Each trace represents a fiber that rapidly attained peak force after being placed in activating solution after which it was returned to the relaxing solution. Sharp spikes in a trace represent movement artifact.

Gel electrophoresis

After the physiological analysis of the single fiber was completed, the fiber was removed from the stainless steel troughs and stored in 30 µl of SDS sample buffer (containing 62.5 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 5% beta-mercaptoethanol, and 0.001% bromophenol blue). Each fiber was denatured for 4 minutes at 95°C and stored at –80°C. Later, a portion of the denatured solute was electrophoresed on polyacrylamide gels to determine the fiber MHC isoform composition. MHC isoform separation was performed on 7% separating gels with a 3.5% stacking gel. A Bio-Rad mini-Protean 3 electrophoresis system (Bio-Rad Laboratories, Hercules, CA) was used to run the gels at a voltage of 70 for a period of 22 hours (Fauteck et al. 1995). A silver staining procedure was used to visualize the protein bands (Shevchenko et al. 1996). Fiber MHC isoform content was evaluated by comparison to human myosin standards run on one or more lanes of each gel (Figure 5).

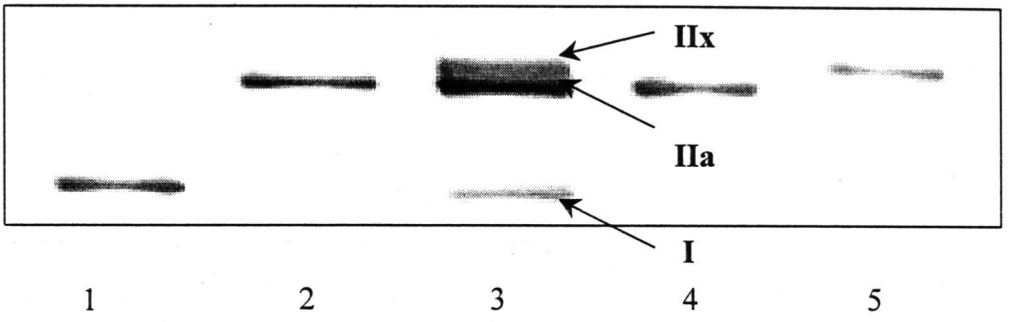

Figure 5. Silver stained 7% polyacrylamide gel electrophoresis of human fibers expressing type I, IIa, and IIx MHC as compared to a human vastus lateralis muscle standard. Lane 1: type I; lane 2: type IIa; lane 3: MHC standard expressing type I, IIa, and IIx; lane 4: type IIa; and lane 5: type IIx. Each lane contains a single muscle fiber.

Statistics

One-way ANOVA was used to compare the physiological properties (P_o, V_o, Vmax, a/P_o, and peak power) of fibers from non-MS and MS subjects. Separate analyses were conducted on MHC type I, I/IIa, IIa, IIa/IIx, and IIx fibers. A repeated measures ANOVA compared the relative force at each P_i and H^+ concentration for slow and fast fibers obtained from MS and non-MS subjects. Significance was set at $p<0.05$ for all statistical tests.

Results

Disability status

The mean age (49 ± 2), height (177 ± 4 cm), and weight (77 ± 7 kg) of subjects with MS were not statistically different from the mean age (45 ± 3), height (175 ± 6 cm), and weight (83 ± 7 kg) of the subjects without MS. All MS subjects were ambulatory, with 2 subjects using canes. The mean EDSS rating of the subjects with MS was 4.75 ± 0.28, their mean C.O.V.S. rating was 81/90, and their modified Ashworth Spasticity Scale ratings ranged from 0 to +1.

Physical activity levels

The pedometer results showed that subjects with MS walked significantly fewer steps (MS: 2657 ± 517 steps, n = 4; non-MS: 8317.22 ± 1153 steps, n = 4;

$p<0.05$) and significantly fewer miles (MS: 0.96 ± 0.21 miles, $n = 4$; non-MS: 3.84 $\pm$ 0.34 miles, $n = 4$; $p<0.05$) during a day than subjects without MS.

Neuromuscular strength

All neuromuscular measurements were expressed relative to body mass to normalize for differences in body sizes. There were significant differences in peak isometric torque (Nm/kg), isokinetic force (Nm/kg), and power (Watts/kg) between subjects with and without MS. At all angular velocities tested, subjects with MS had a 44% deficit in peak isometric force as compared to the subjects without MS (Figure 6). Isokinetic force averaged 41% less in the subjects with MS (Figure 6), while peak power at 240°/second was 48% lower in subjects with MS versus subjects without MS (Figure 7).

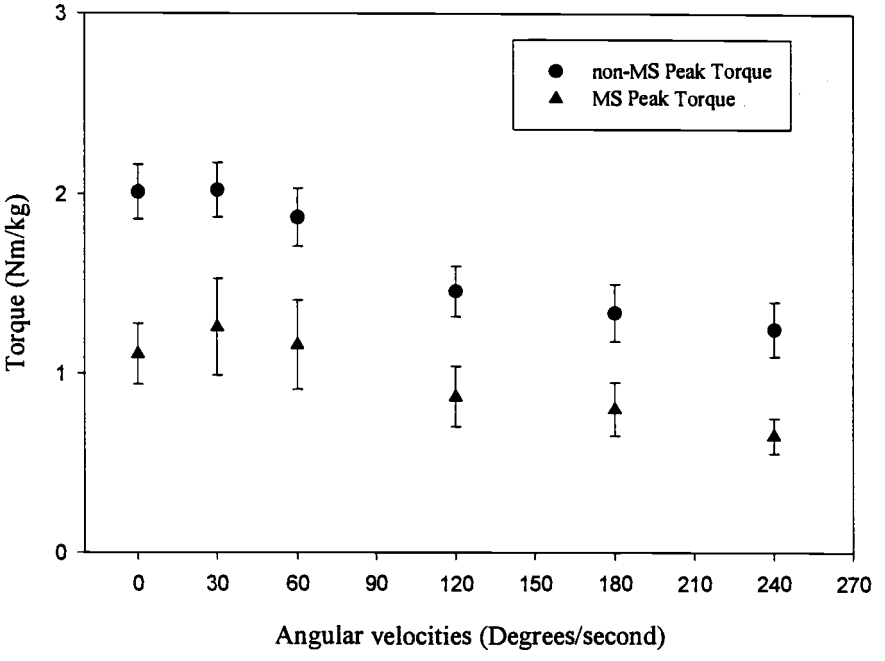

Figure 6. Isometric force and torque at angular velocities of 30, 60, 120, 180, 240. Subjects with MS (triangles) and without MS (circles). Isometric peak force is indicated at 0 degrees/second. All values are mean ± SE, and measurements are expressed relative to body mass (Nm/kg: Newton meters/kilogram).

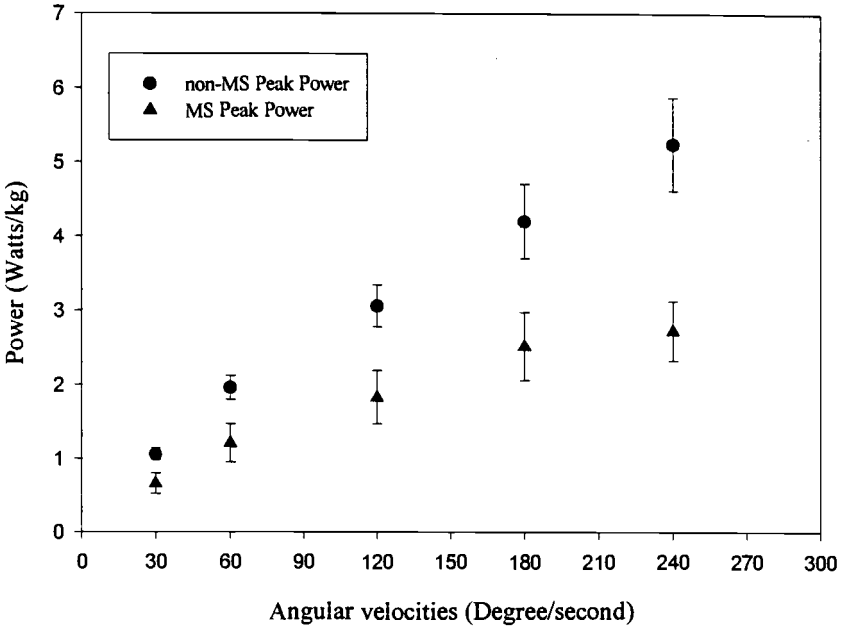

Figure 7. Peak power at angular velocities of 30, 60, 120, 180, 240. Subjects with MS (triangles) and without MS (circles). All values are mean ± SE, and measurements are made relative to body mass (Nm/kg: Newton meters/kilogram).

Fiber CSA

The CSA of all fibers expressing type I, I/IIa, IIa, and IIx MHC isoforms from subjects with MS were 8-32% smaller than the CSA of similar fibers obtained from subjects without MS (Table 1). Type I and IIa fibers from subjects with MS were 8% and 14% smaller, respectively, than the same fiber types from subjects without MS. Type I/IIa, IIa/IIx, and IIx fibers from subjects with MS showed the greatest deficit in CSA at 31%, 29%, and 32%, respectively, as compared to these same fiber types in subjects without MS. Although there was no significant difference found in the IIa/IIx fiber types between groups, CSA approached statistical significance with a p-value of 0.0598.

Fiber force

The absolute force (mN) of fiber types I, I/IIa, IIa/IIx, and IIx was significantly lower in fibers from subjects with MS as compared to non-MS fibers. The absolute force across all fiber types in subjects with MS was on average 25% lower compared to fibers from subjects without MS. MS type I/IIa showed a 45% reduction in absolute force, while type I, IIa, IIa/IIx, and IIx showed reductions ranging from 13-30% versus those same fiber types from subjects without MS (Table 1).

Specific force (kN/m^2) was greater in fast fiber types (IIa, IIa/IIx, and IIx) versus slow fiber types (I and I/IIa) regardless if the fiber was from subjects with or without MS. The specific force (kN/m^2) was significantly lower, 6%, in the type I

fibers from subjects with MS. Though there were no significant differences found in other fiber types, type I/IIa from subjects with MS showed a 21% deficit in specific force versus controls (Table 1).

Unloaded shortening velocity and force-velocity-power relationships

Unloaded shortening velocity was slowest in type I fibers, with incremental increases in V_0 in type I/IIa, IIa, IIa/IIx, and IIx (Table 2). There were no differences in unloaded shortening velocity between fibers from subjects with and without MS. Similar to unloaded shortening velocity from slack tests results, there were no differences in maximal shortening velocity (Vmax) between subjects with and without MS (Table 3). However unlike slack tests, extrapolation of the force-velocity relationship failed to differentiate between fibers expressing fast isoforms (Table 3).

Absolute power is the total power that a fiber can produce, whereas, normalized power accounts for CSA of fiber. Power data revealed that absolute and normalized power was greatest in fibers expressing type IIx MHC (IIx or IIa/IIx), with IIa MHC expressing intermediate power and type I MHC having the lowest power of all fiber types. Type I fibers from subjects with MS had significantly less, 11%, absolute power than subjects without MS, yet there were no differences in absolute power in type IIa, IIa/IIx, or IIx . There were also no significant differences in normalized power between groups (Table 3 and Figure 8).

Table 1. Cross-sectional area, absolute and specific force values for fibers expressing type I, I/IIa, IIa, IIa/IIx, and IIx MHC

MHC	Treatment	Number of fibers	CSA (μm^2)	Po (mN)	Po/CSA (kN/m^2)
Type I	Non-MS	134	6153 ± 165	0.68 ± 0.02	112 ± 1
	MS	147	5672 ± 175*	0.59 ± 0.02*	105 ± 2*
Type I/IIa	Non-MS	3	6980 ± 745	0.83 ± 0.05	121 ± 6
	MS	6	4826 ± 515*	0.46 ± 0.10*	95 ± 14
Type IIa	Non-MS	114	5906 ± 217	0.81 ± 0.03	139 ± 2
	MS	67	5084 ± 333*	0.71 ± 0.04+	143 ± 2
Type IIa/IIx	Non-MS	22	5686 ± 664	0.79 ± 0.08	147 ± 5
	MS	21	4066 ± 499+	0.58 ± 0.07*	143 ± 5
Type IIx	Non-MS	5	6333 ± 846	0.90 ± 0.12	143 ± 6
	MS	11	4332 ± 502*	0.63 ± 0.07*	153 ± 9

Values are mean ± SE. Abbreviations: MHC, myosin heavy chain isoform; CSA, cross-sectional area; P_o, peak Ca^{+2}-activated force; μm^2, micrometers squared; mN, milli-newtons; kN/m^2, kilo-newtons per square meter of fiber. * indicates a significant difference ($p<0.05$) between MS and non-MS fibers and + indicates ($0.05<p<0.10$).

Table 2. Unloaded shortening velocity of fibers expressing type I, I/IIa, IIa, IIa/IIx, and IIx MHC

MHC	Treatment	Number of fibers	Vo (FL/s)
Type I	Non-MS	105	0.61 ± 0.01
	MS	130	0.61 ± 0.01
Type I/IIa	Non-MS	3	1.57 ± 0.30
	MS	2	1.81 ± 0.11
Type IIa	Non-MS	90	2.55 ± 0.09
	MS	54	2.80 ± 0.13
Type IIa/IIx	Non-MS	18	4.19 ± 0.29
	MS	14	4.54 ± 0.37
Type IIx	Non-MS	5	5.27 ± 0.43
	MS	9	5.07 ± 0.46

Unloaded shortening velocity determined by slack test. Values are mean ± SE. Abbreviations: MHC, myosin heavy chain isoform; CSA, cross-sectional area; FL/s, fiber lengths per second.

Table 3. Maximal shortening velocity, absolute power and normalized power for fibers expressing type I, IIa, IIa/IIx, and IIx MHC

MHC	Treatment	Number of fibers	Vmax (FL/s)	Absolute power	Normalized power
Type I	Non-MS	99	0.78 ± 0.03	9.67 ± 0.36	1.57 ± 0.03
	MS	113	0.79 ± 0.03	8.59 ± 0.35*	1.51 ± 0.04
Type IIa	Non-MS	86	1.87 ± 0.06	47.70 ± 1.88	8.50 ± 0.18
	MS	43	1.89 ± 0.07	44.91 ± 3.30	8.92 ± 0.37
Type IIa/IIx	Non-MS	17	2.22 ± 0.16	52.91 ± 4.16	12.39 ± 1.27
	MS	14	2.83 ± 0.39	47.77 ± 7.23	12.57 ± 1.27
Type IIx	Non-MS	5	1.50 ± 0.19	68.90 ± 7.32	11.40 ± 1.59
	MS	7	1.63 ± 0.35	51.20 ± 12.39	13.20 ± 2.81

Values determined by force-velocity relationship. Values are mean ± SE. Abbreviations: MHC, myosin heavy chain isoform; Vmax, maximal shortening velocity; FL/s, fiber lengths per second.
* indicates a significant difference ($p<0.05$) between MS and non-MS fibers.

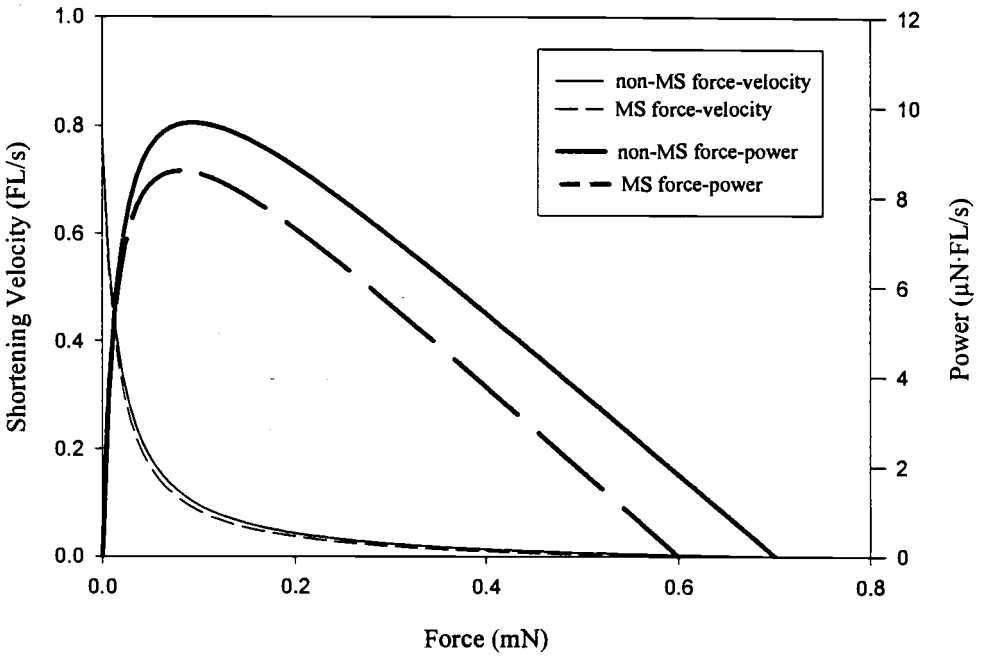

Figure 8. Force-velocity and force-power curves for type I fibers from subjects with and without MS. Dotted lines indicate subjects with MS and solid lines are subjects without MS. Darker lines indicate force-power relationships and lighter lines are force-velocity relationships.

Effect of P_i on peak force

Peak Ca^{+2}-activated force declined rapidly as P_i concentration increased from 0 to 10 mM P_i. Smaller decreases in peak Ca^{+2}-activated force occurred as P_i increased from 10 mM to 30 mM. This relationship held for both fiber types (I and IIa), although the magnitude of change was greater in the type I fibers, resulting in a significant difference between fiber types with varying concentrations of P_i. At 30 mM of P_i, type I fibers on average produced 66% less force than at 0 mM of P_i while type IIa fibers produced 40% less force at 30 mM of P_i as compared to initial force at 0 mM of P_i. No significant differences between groups within the same fiber type were found with various concentrations of P_i (Figure 9). These effects of P_i were not due to run-down of the fibers because force at 0 P_i following the 30 mM of P_i trial was 101 % and 99% of the original 0 mM P_i force for type I and type IIa, respectively.

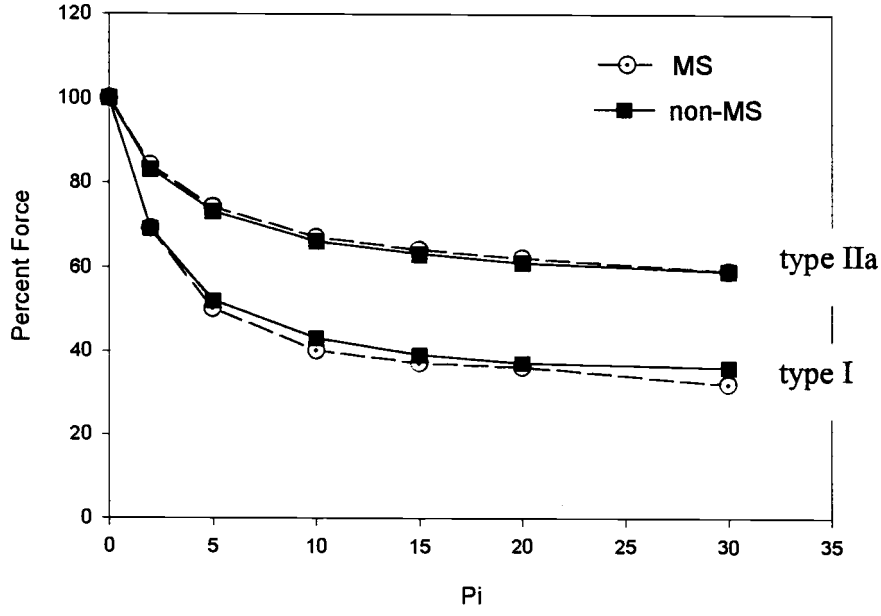

Figure 9. Effects of P_i on peak Ca^{+2}-activated force in type I and IIa skinned single fibers from subjects with and without MS. Circles indicate subjects with MS and squares indicate subjects without MS. Each point represents mean values from each fiber type. Number of fibers studied used to obtain mean values included: type I: non-MS, N = 33; MS, N = 34, type IIa: non-MS, N = 25, MS, N = 24. Significant main effects were found between fiber types ($p<0.05$), but there were no significant differences between groups.

Effect of pH on peak force

Peak Ca^{+2}-activated force in both type I and IIa fibers decreased with increasing concentrations of pH. At pH 6.5, there was an average force reduction of 17% in both fiber types, while at pH 6.2 there was an average force reduction of 24% in both fiber types. There were no significant differences between groups at different pH, nor were there differences between fiber types (I and IIa) at different pH. The effects of pH were not due to fiber run-down because force at the last pH 7.0 in type I fibers was 100% of the initial force value at pH 7.0, and the force of type IIa fibers was 98% of the initial force value at pH 7.0 (Table 4).

Table 4. Effects of different pH on peak Ca^{+2}-activated force in fibers expressing type I and IIa MHC

MHC	Treatment	No. fibers	pH 7.0	pH 6.5	pH 6.2	pH 7.0
Type I	Non-MS	20	103 ± 4	85 ± 3	78 ± 2	102 ± 4
	MS	20	103 ± 4	87 ± 3	80 ± 3	104 ± 4
Type IIa	Non-MS	24	148 ± 3	123 ± 3	112 ± 2	146 ± 3
	MS	21	155 ± 5	128 ± 4	116 ± 4	153 ± 5

Values are mean ± SE. Abbreviations: MHC, myosin heavy chain; No. fibers, number of fibers. All force measurements were made in kilo-newtons per squared meter of fiber (kN/m^2) and values are mean ± SE.

Myosin heavy chain isoforms

There was wide variation in the relative MHC isoform expression in muscle fibers of MS and non-MS subjects. For example MS-1 had 63% type I fibers, 33% type IIa fibers, 3% type IIa/IIx fibers, and 1% type IIx while MS-6 had 28% type I fibers, 28% type IIa fibers, 27% type IIa/IIx fibers and 10% type IIx fibers (Figure 10). Average percentages for MHC expression in type I, I/IIa, IIa, IIa/IIx, and IIx fibers of controls were 40, 3, 46, 10 and 2%, respectively, while average percentages for these same fiber types in subjects with MS were 46, 3, 31, 13, and 8%, respectively. A significant difference was found in the relative number expressing type IIa fibers between groups, with subjects with MS expressing 33% fewer type IIa fibers than subjects without MS. While there were no significant differences between groups in comparing the expression of faster fiber types (IIa/IIx and IIx), the data showed that there was a trend towards increased percentage of these fiber types in MS versus controls. The data also revealed that there was a trend towards a decreased percentage of type I fibers as the EDSS score rose in subjects with MS.

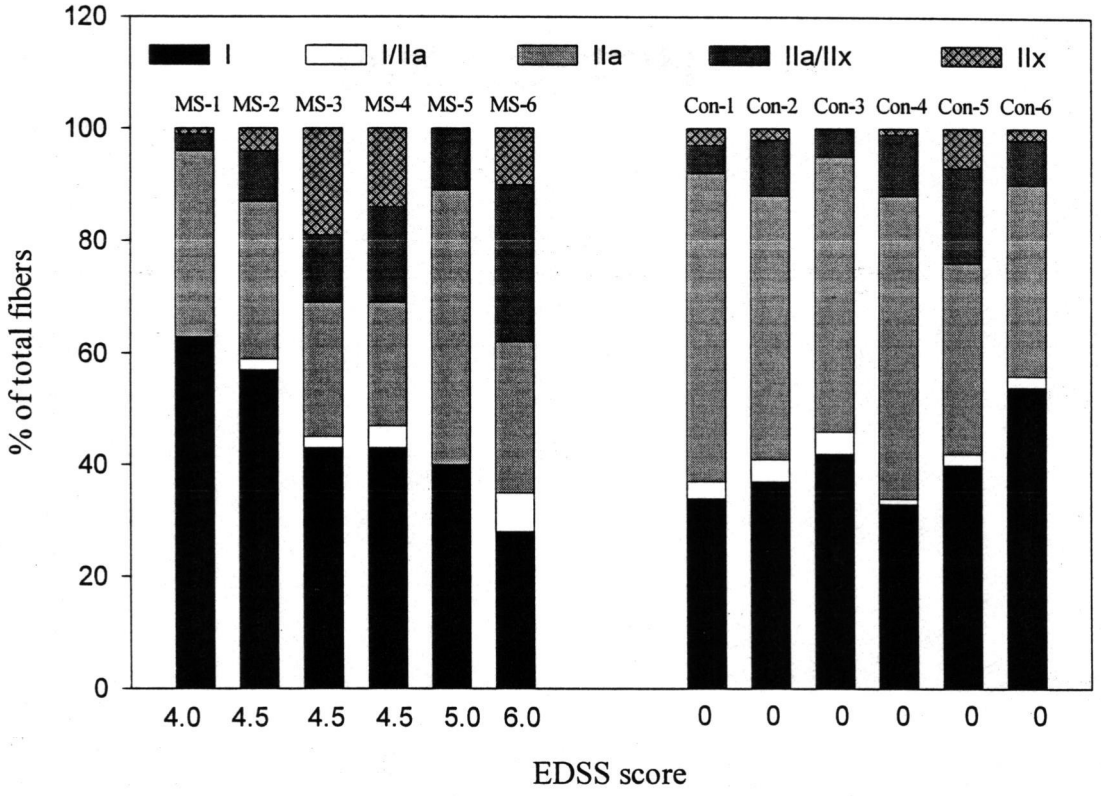

Figure 10. Percentage of myosin heavy chain (MHC) isoform expression from single fibers in subjects with and without MS. The number of fibers analyzed from each subject with or without MS ranged from 87-140. Each bar represents the MHC composition for one subject. Individual MS subjects are indicated by MS-1, MS-2, etc. while individual subjects without MS (controls) are indicated by Con-1, Con-2, etc. EDSS scores for each subject are listed below bars.

Discussion

All of the subjects with MS in this study were ambulatory, with two of the subjects using walking aids for assistance. On average, subjects with MS had been diagnosed for 13.5 years. The level of disability as described by the EDSS scores, ranged from 4.0 to 6.0. A score of 4.0 indicates that subjects were able to walk 500 meters without a walking aid, while subjects with a score of 6.0 walked a maximum of 200 meters (Kurtzke 1983). The C.O.V.S. score was a measure of quality of gait and movement, and these scores ranged from 75/91 to 89/91, with the lowest C.O.V.S. score indicating greater deficits in movement and gait quality (Seaby et al. 1989). The modified Ashworth Spasticity Scores were +1 or less which indicates that subjects had minimal or no spasticity (Pandyan et al. 1999). The present MS subjects are similar in disability status to MS populations used in previous studies which assessed muscle weakness and fatigability (Kent-Braun et al. 1994; Sharma et al. 1995; Lambert et al. 2001).

The results of this study show that subjects with MS are significantly weaker at the whole muscle level than subjects without MS. Peak power and isokinetic torque of the knee extensors were 48% and 41% lower, respectively, while peak isometric torque was 44% lower in subjects with MS as compared to controls. This compares favorably to previous studies which found that subjects with MS were 26-39% weaker in lower limb muscle strength as compared to controls (Kent-Braun et al. 1994; Sharma et al. 1995; Schwid et al. 1999; Lambert et al. 2001). This study tested the novel hypothesis that these changes in muscle

strength in persons with MS are due to deficits in cellular mechanisms of contraction. Specifically, this study tested the hypotheses that muscle weakness in MS may be due to changes in CSA of muscle fibers or deficits at the level of the cross-bridge as indicated by changes in Ca^{+2}-activated P_o, V_o, and peak power.

The CSA of fibers from subjects with MS was significantly smaller than CSA of fibers from subjects without MS. Type I and IIa fibers were 8% and 14% smaller, respectively, while type I/IIa, IIa/IIx, and IIx were 29-32% smaller than those from the subjects without MS. These findings are similar to those reported by Kent-Braun et al. who found 25-36% atrophy of type I, IIa and IIa/x fibers in subjects with MS (Kent-Braun et al. 1997). These authors also saw a greater atrophy in the faster fiber types, which follows the trend in the present study.

This study also found significant reductions in absolute and specific force and absolute power in subjects with MS. There was a 23% decrease in absolute force in all fiber types from subjects with MS. Absolute force in the type I fibers was 13% lower and the specific force was 6% lower than the absolute and specific force of type I fibers in controls. Absolute power in the type I fibers from subjects with MS was 11% lower than the absolute power of type I fibers from controls. While the significant reductions in absolute force and absolute power could be attributed to atrophy of the muscle fibers, the significant difference in specific force of the type I fibers is attributed to deficits at the level of the cross-bridge. Deficits in cross-bridge cycling may include shifts from high force producing states to low

force producing states or loss of cross-bridges due to atrophy of thick and thin filaments.

This study found that subjects with MS are significantly weaker at the whole muscle level than subjects without MS. Results from in vitro preparation indicate that muscle fiber atrophy and cross-bridge mechanisms can account for approximately half of the observed peripheral weakness. Since deficits at the level of cross-bridge cannot account for all of the whole muscle weakness seen in the MS subjects, the remainder of whole muscle deficit must be due to other cellular mechanisms of contraction. One possibility is that there are impairments in excitation-contraction coupling. Sharma et al. (Sharma et al. 1995) and Kent-Braun et al. (Kent-Braun et al. 1994; Sharma et al. 1995) suggested that in MS the lower tetanic force and increased time to recover after exercise may be a result of decreased amounts of calcium being released from the sarcoplasmic reticulum and/or by abnormalities in the calcium pump, both mechanisms involved in excitation-contraction coupling.

While muscle weakness was evident in the present study and in previous studies that investigated whole muscle strength in subjects with MS, the explanations for this increased muscle weakness are not clear. One possible explanation for increased muscle weakness in these subjects with MS may be due to the lower physical activity levels observed in these subjects with MS. Models of disuse, spaceflight, bed-rest and spinal cord injury, have cited changes in muscle CSA and absolute and specific force after disuse. Changes in CSA after spaceflight

and bed-rest have shown a 15% and 5% decrease in CSA of type I fibers, respectively. Type II fibers have shown a 26% and 24% reduction in CSA after spaceflight, while spinal cord injury reports a 40% reduction in CSA of the type IIax fibers (Edgerton et al. 1995; Castro et al. 1999; Widrick et al. 1999). As a result of muscle atrophy, absolute force of type I fibers is reduced 21% and 13% after spaceflight and bed-rest, correspondingly (Widrick et al. 1997; Widrick et al. 1999). When cross-sectional area was taken into account, Widrick et al. reported a 6% loss of specific force in type I fibers after 17-days of spaceflight (Widrick et al. 1999), suggesting impairments in cross-bridge mechanisms of contraction.

Results from this study reveal that the changes in MS are comparable to changes occurring from short-term disuse. Thus, increases in physical activity may alleviate some of the muscle weakness in individuals with MS. Strength training studies have been shown to increase muscle CSA with a corresponding increase in absolute force in young male subjects without MS, with no effects seen in V_o (Widrick et al. in press). Endurance training studies have shown no effects on CSA and force in muscle fibers in elite older athletes (Widrick et al. 1996). Therefore, strength training exercise interventions rather then endurance training may result in increased CSA and absolute force in persons with MS.

In studying the mechanisms of fatigue in subjects with MS, there were no significant differences with varying concentrations of P_i or changes in pH. However, there were significant differences between fiber types with varying concentrations of P_i. This finding is significant in that it is not known if there are

any other studies that have assessed the effect of P_i on human single muscle fibers. At P_i of 30 mM, the force of slow fibers was 66% lower than at the 0 mM P_i condition, while in fast fibers there was a 40% deficit at 30 mM of P_i from 0 mM P_i condition. Using solutions and methodology identical to this present study, Widrick (Widrick 2002) found that the force of type I rat fibers was depressed 44% and the force of the type IIb rat fibers was depressed 42% at 30 mM P_i. Thus, it appears that the peak Ca^{+2}-activated force of human type I fibers are more sensitive than the rat type I fibers to increases in P_i concentration.

Although there were no significant differences between groups and fiber types at different levels of pH, this finding is important in understanding the effect of pH in human single muscle fibers. The results of this study showed an average 17% and 24% deficit in force at pH 6.5 and 6.2, correspondingly. Lynch et al. (Lynch et al. 1994) assessed the effect of pH in human fibers and found that there was a 7% and 21% loss of force at pH 6.6 in type I and type IIa fibers, respectively. The type I fiber discrepancies between this present study and Lynch et al. may be due to differences in fiber typing techniques and in the functional assays utilized by Lynch and colleagues.

Muscle fatigue in MS may also be attributed to shifts from slower oxidative fibers to faster glycolytic fibers. The myosin heavy chain data revealed that there were differences in fiber MHC isoforms between groups. Controls had significantly greater relative numbers, 33%, of IIa fibers than subjects with MS. While there were no significant differences found in IIa/IIx and IIx fiber types

between groups, there was a trend towards an increased percentage of these fiber types in subjects with MS. In addition, there were no differences in the relative number of type I fibers between groups, yet there was a trend towards a decreased percentage of type I fibers as the level of disability increased, as indicated by the EDSS scores. These data also suggest a shift from the type IIa to type IIx MHC expression in MS. The larger number of fibers co-expressing IIa/IIx MHC in subjects with MS is consistent with this interpretation, since fiber type shifts are thought to progress from IIa to IIa/IIx to IIx. This could explain the increased fatigability in subjects with MS since type IIa/IIx and IIx fibers are the most fatigable fiber types, with type IIx being the most fatigable of these two.

In comparing these MHC isoform results to a previous study by Kent-Braun et al. (Kent-Braun et al. 1997) there were notable similarities and differences between the two studies. The results in this study found an increased percentage, 28%, of type IIa/IIx fibers in subjects with MS as compared to subjects without MS. This finding is similar to that of Kent-Braun et al. (Kent-Braun et al. 1997) who found a 22% increase in type IIa/IIx fibers in subjects with MS. In contrast to the present findings, Kent-Braun et al. (Kent-Braun et al. 1997) found a 13% decrease in type I fibers and a 32% increase in type IIa fibers in subjects with MS. The differences between this present study and that of Kent-Braun et al. could be explained by differences in the muscles studied. This study investigated the vastus lateralis, while Kent-Braun et al. analyzed the anterior tibialis of subjects with MS. The vastus lateralis of control subjects expressed 40% type I, 45% type IIa, and 3%

type IIx fibers versus the tibialis anterior of controls which expressed 76% type I, 28% type IIa, and 6% type IIax fiber types. Thus, a pre-dominantly slow muscle like the anterior tibialis may respond differently to disuse than a mixed muscle like the vastus lateralis.

Conclusions

Previous studies have shown that part of muscle weakness in MS is due to deficits within the central nervous system, while approximately half of the muscle weakness may be attributed to peripheral deficits (Rice et al. 1992; Kent-Braun et al. 1994; Sharma et al. 1995). This study found that subjects with MS are significantly weaker at the whole muscle level than subjects without MS. Results from an in vitro preparation indicate that muscle fiber atrophy and cross-bridge mechanisms of contraction can account for approximately half of the observed peripheral weakness. Fibers from subjects with MS were significantly smaller and produced significantly less absolute force than controls. In addition, the lower specific force in type I fibers from subjects with MS suggests deficits with cross-bridge mechanisms of contraction. There was no evidence at the level of the cross-bridge to suggest that increased muscle fatigability in MS is due to a greater sensitivity to changes in the metabolites P_i and H^+. This study found that approximately half of the peripheral weakness could be explained by cross-bridge mechanisms of contraction and muscle atrophy, while muscle fatigue could not be explained at the level of the cross-bridge. Thus, future studies should examine

other cellular mechanisms, such as excitation contraction coupling, that would contribute to the increased peripheral weakness and fatigability in persons with MS.

BIBLIOGRAPHY

Bergström, J. (1975). "Percutaneous needle biopsy of skeletal muscle in physiological and clinical research." Scandinavian Journal of Clinical Laboratory Investigation **35**: 609-616.

Bigland-Ritchie, B., D. A. Jones, G. P. Hosking and R. H. T. Edwards (1978). "Central and peripheral fatigue in maximum voluntary contractions of the human quadriceps muscle." Clinical Science and Molecular Medicine **54**: 604-614.

Cady, E. B., D. A. Jones, J. Lynn and D. J. Newham (1989). "Changes in force and intracellular metabolites during fatigue of human skeletal muscle." Journal of Physiology (London) **418**: 311-325.

Castro, M. J., D. F. Apple, R. S. Staron, G. E. R. Campos and G. A. Dudley (1999). "Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury." Journal of Applied Physiology **86**(1): 350-358.

Chandler, W. K., R. F. Rakowski and M. F. Schneider (1976). "Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle." Journal of Physiology **254**: 285-316.

Chase, P. B. and M. J. Kushmerick (1988). "Effects of pH on contraction of rabbit fast and slow skeletal muscle fibers." Biophysical Journal **53**: 935-946.

Cooke, R. (1997). "Actomyosin interaction in striated muscle." Physiological Reviews **77**: 671-697.

de Haan, A., C. J. de Ruiter, L. H. V. van der Woude and P. J. H. Jongen (2000). "Contractile properties and fatigue of quadriceps muscles in multiple sclerosis." Muscle and Nerve **23**: 1534-1541.

de Ruiter, C. J., P. J. H. Jongen, L. H. V. van der Woude and A. de Haan (2001). "Contractile speed and fatigue of adductor pollicis muscle in multiple sclerosis." Muscle and Nerve **24**: 1173-1180.

Djaldetti, R., I. Ziv, A. Achiron and E. Melamed (1996). "Fatigue in multiple sclerosis compared with chronic fatigue syndrome." Neurology **46**(3): 632-635.

Donaldson, S. K. B. and L. Hermansen (1978). "Differential, direct effects of H^+ on Ca^{2+}-activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits." Pflügers Archiv European Journal of Physiology **376**: 55-65.

Edgerton, V. R., M.-Y. Zhou, Y. Ohira, H. Kiltgaard, B. Jiang, G. Bell, B. Harris, B. Saltin, P. D. Gollnick, R. R. Roy, M. K. Day and M. Greenisen (1995). "Human fiber size and enzymatic properties after 5 and 11 days of spaceflight." Journal of Applied Physiology **78**: 1733-1739.

Edman, K. A. P. (1979). "The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres." Journal of Physiology (London) **291**: 143-159.

Fabiato, A. (1985). "Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac purkinje cell." Journal of General Physiology **85**: 247-289.

Fabiato, A. (1988). Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods in Enzymology, Academic Press. **157:** 378-417.

Fabiato, A. and F. Fabiato (1978). "Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles." Journal of Physiology (London) **276**: 233-255.

Fauteck, S. P. and S. C. Kandarian (1995). "Sensitive detection of myosin heavy chain composition in skeletal muscle under different loading conditions." American Journal of Physiology **268 (Cell Physiol. 37)**: C419-C424.

Fitts, R. H. (1994). "Cellular mechanisms of muscle fatigue." Physiological Reviews **74**(1): 49-94.

Fitts, R. H., D. R. Riley and J. J. Widrick (2000). "Invited Review: Microgravity and skeletal muscle." Journal of Applied Physiology **89**: 823-839.

Freal, J. E., G. H. Kraft and J. K. Coryell (1984). "Symptomatic fatigue in multiple sclerosis." Archives of Physical Medicine and Rehabilitation **65**: 135-138.

Fryer, M. W., V. J. Owen, G. D. Lamb and D. G. Stephenson (1995). "Effects of creatine phosphate and P_i on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres." Journal of Physiology (London) **482**: 123-140.

Gandevia, S. C. (2001). "Spinal and supraspinal factors in human muscle fatigue." Physiological Reviews **81**(4): 1725-1789.

Gibson, H. and R. H. T. Edwards (1985). "Muscular exercise and fatigue." Sports Medicine **2**: 120-132.

Green, H. J. (1987). "Neuromuscular aspects of fatigue." Canadian Journal of Sports Science **12**(Suppl. 1): 7S-19S.

Hallpike, J. F. (1983). Clinical aspects of multiple sclerosis. Multiple sclerosis. J. F. Hallpike, C. W. M. Adams and W. W. Tourtellotte. Baltimore, Williams and Wilkins: 129-161.

Hill, A. V. (1938). "The heat of shortening and the dynamic constants of muscle." Proc. R. Soc. London Ser. B **126**: 136-195.

Huxley, A. F. (1974). "Muscle contraction." Journal of Physiology (London) **243**: 1-43.

Joy, J. E. and R. B. Johnston, Eds. (2001). Multiple Sclerosis: Current Status and Strategies for the Future. Washington, D.C., National Academy Press.

Kent-Braun, J. A. (1997). "Noninvasive measures of central and peripheral activation in human muscle fatigue." Muscle and Nerve **Suppl. 5**: S98-S101.

Kent-Braun, J. A., A. V. Ng, M. Castro, M. W. Weiner, D. Gelinas, G. A. Dudley and R. G. Miller (1997). "Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis." Journal of Applied Physiology **83**(6): 1998-2004.

Kent-Braun, J. A., K. R. Sharma, M. W. Weiner and R. G. Miller (1994). "Effects of exercise on muscle activation and metabolism in multiple sclerosis." Muscle and Nerve **17**: 1162-1169.

Kentish, J. C. (1991). "Combined inhibitory actions of acidosis and phosphate on maximum force production in rat skinned cardiac muscle." Pflügers Arch. **419**: 310-318.

Krupp, L. B., L. A. Alvarez, N. G. LaRocca and L. C. Scheinberg (1988). "Fatigue in multiple sclerosis." Archives of Neurology **45**: 435-437.

Kurtzke, J. F. (1983). "Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS)." Neurology **33**: 1444-1452.

Kurtzke, J. F. (1985). Epidemiology of multiple sclerosis. Handbook of Clinical Neurology 3 (revised series). P. J. Vinken, H. L. Bruyn, H. L. Klawans and J. C. Koetsier. Amsterdam/New York, Elsevier: 259-287.

Lambert, C. P., R. L. Archer and W. J. Evans (2001). "Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis." Medicine and Science in Sports and Exercise **33**(10): 1613-1619.

Lenman, A. J. R., F. M. Tulley, G. Vrbova, M. R. Dimitrijevic and J. A. Towle (1989). "Muscle fatigue in some neurological disorders." Muscle and Nerve **12**: 938-942.

Lynch, G. S., M. J. McKenna and D. A. William (1994). "Sprint-training effects on some contractile properties of single skinned human muscle fibres." Acta Physiol. Scand. **152**: 295-306.

Martin, R. and H. F. McFarland (1997). Immunology of multiple sclerosis and experimental allergic encephalomyelitis. Multiple Sclerosis: Clinical and Pathogenetic Basis. C. S. Raine. London, Chapman & Hall: 221-242.

Matthews, B. (1998). Symptoms and signs of multiple sclerosis. McAlpine's Multiple Sclerosis. A. Compston, G. C. Ebers, H. Lassmanet al. London, Churchill Livingstone: 145-190.

McAlpine, D., C. E. Lumsden and E. D. Acheson (1972). Multiple Sclerosis: A Reappraisal. Baltimore, Williams and Wilkins Company.

Merton, P. A. (1954). "Voluntary strength and fatigue." Journal of Physiology (London) **123**: 553-564.

Metzger, J. M. and R. H. Fitts (1987). "Role of intracellular pH in muscle fatigue." Journal of Applied Physiology **62**(4): 1392-1397.

Metzger, J. M. and R. L. Moss (1987). "Shortening velocity in skinned single muscle fibers. Influence of filament lattice spacing." Biophysical Journal **52**: 127-131.

Metzger, J. M. and R. L. Moss (1990). "Effects on tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres." Journal of Physiology (London) **428**: 737-750.

Metzger, J. M. and M. R.L (1987). "Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles." Journal of Physiology (London) **393**: 727-742.

Moss, R. L., G. M. Diffee and M. L. Greaser (1995). "Contractile properties of skeletal muscle fibers in relation to myofibrillar protein isoforms." Review of Physiology, Biochemistry, and Pharmacology **126**: 1-63.

National Multiple Sclerosis Society (November 1998). Summary of MS research progress - 1998. http://www.nmss.org/publications/p-893282996/1998/nov/a-910819385.html. N. M. S. Society. 08 May, 1999.

National Multiple Sclerosis Society (October 1997). MS Information [Online]. http://www.nmaa.org/msinfo. N. M. S. Society. 29 Apr, 1999.

Ng, A. V. and J. A. Kent-Braun (1997). "Quantitation of lower physical activity in persons with multiple sclerosis." Medicine and Science in Sports and Exercise **29**(4): 517-523.

Nosek, T. M., J. H. Leal-Cardoso, M. McLaughlin and R. E. Godt (1990). "Inhibitory influence of phosphate and arsenate on contraction of skinned skeletal and cardiac muscle." American Journal of Physiology **250 (Cell Physiol. 28)**: C933-C939.

Pandyan, A. D., G. R. Johnson, C. I. M. Price, R. H. Curless, M. P. Barnes and H. Rodgers (1999). "A review of the properties and limitations of the Ashworth and modified Ashworth Scales as measures of spasticity." Clinical Rehabilitation **13**: 373-383.

Petajan, J. H. and A. T. White (2000). "Motor-evoked potentials in response to fatiguing grip exercise in multiple sclerosis patients." Clinical Neurophysiology **111**: 2188-2195.

Potma, E. J., I. A. van Graas and G. J. M. Stienen (1994). "Effects of pH on myofibrillar ATPase activity in fast and slow skeletal muscle fibers of the rabbit." Biophysical Journal **67**: 2404-2410.

Potma, E. J., I. A. van Graas and G. J. M. Stienen (1995). "Influence of inorganic phosphate and pH on ATP utilization in fast and slow skeletal muscle fibers." Biophysical Journal **69**: 2580-2589.

Renaud, J. M., R. B. Stein and T. Gordon (1987). "The effects of pH on force and stiffness development in mouse muscles." Canadian Journal of Physiology and Pharmacology **65**: 1798-1801.

Rice, C. L., T. L. Vollmer and B. Bigland-Ritchie (1992). "Neuromuscular responses of patients with multiple sclerosis." Muscle and Nerve **15**: 1123-1132.

Schwid, S. R., C. A. Thornton, S. Pandya, K. L. Manzur, M. Sanjak, M. D. Petrie, M. P. McDermott and A. D. Goodman (1999). "Quantitative assessment of motor fatigue and strength in MS." Neurology **53**: 743-750.

Seaby, L. and G. Torrance (1989). "Reliability of a physiotherapy functional assessment used in a rehabilitation setting." Physiotherapy Canada **41**(5): 264-271.

Sharma, K. R., J. Kent-Braun, M. A. Mynhier, M. W. Weiner and R. G. Miller (1995). "Evidence of an abnormal intramuscular component of fatigue in multiple sclerosis." Muscle and Nerve **18**: 1403-1411.

Sheean, G. L., N. M. F. Murray, J. C. Rothwell, D. H. Miller and A. J. Thompson (1997). "An electrophysiological study of the mechanism of fatigue in multiple sclerosis." Brain **120**: 299-315.

Shevchenko, A., M. Wilm, O. Vorm and M. Mann (1996). "Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels." Analytical Chemistry **68**: 850-858.

Stienen, G. J. M., P. G. A. Versteeg, Z. Papp and G. Elizinga (1992). "Mechanical properties of skinned rabbit psoas and soleus muscle fibers during lengthening: Effects of phosphate and Ca^{2+}." Journal of Physiology (London) **451**: 503-523.

Stuifbergen, A. K. (1997). "Physical activity and perceived health status in persons with multiple sclerosis." Journal of Neuroscience Nursing **29**(4): 238-243.

Thomason, D. B. and F. W. Booth (1990). "Atrophy of the soleus muscle by hindlimb unweighting." Journal of Applied Physiology **68**: 1-12.

Tikunov, B. A., H. L. Sweeney and L. C. Rome (2001). "Quantitative electrophoretic analysis of myosin heavy chains in single muscle fibers." Journal of Applied Physiology **90**: 1927-1935.

van der Kamp, W., A. M. de Noordhout, P. D. Thompson, J. C. Rothwell, B. L. DPhil and C. D. Marsden (1991). "Correlation of phasic muscle strength and corticomotoneuron conduction time in multiple sclerosis." Annals of Neurology **29**: 6-12.

Vercoulen, J. H., O. R. Hommes, C. M. Swanink, P. J. Jongen, J. F. Fennis, J. M. Galama, J. W. van der Meer and G. Bleijenberg (1996). "The measurement of fatigue in patients with multiple sclerosis." Archives of Neurology **53**: 642-649.

Waxman, S. G. (1998). "Demyelinating diseases: New pathological insights, new therapeutic targets." The New England Journal of Medicine **338**(5): 323-325.

Westerblad, H., D. A. Allen and J. Lannergren (2002). "Muscle fatigue: Lactic acid or inorganic phosphate the major cause?" News in Physiological Science **17**: 17-21.

Westerblad, H., J. A. Lee, J. Lännergren and D. G. Allen (1991). "Cellular mechanisms of fatigue in skeletal muscle." American Journal of Physiology **261 (Cell Physiol. 30)**: C195-C209.

Whetten-Goldstein, K., F. A. Sloan, L. B. Goldstein and E. D. Kulas (1998). "A comprehensive assessment of the cost of multiple sclerosis in the United States." Multiple Sclerosis **4**: 419-425.

Whitaker, J. N. and G. W. Mitchell (1997). Clinical features of multiple sclerosis. Multiple Sclerosis: Clinical and Pathogenic Basis. C. S. Raine, H. F. McFarland and W. W. Tourtellotte. London, Chapman & Hall: 3-19.

Widrick, J. J. (2002). "Effect of Pi on unloaded shortening velocity of slow and fast mammalian muscle fibers." American Journal of Physiology Cell Physiology **282**: C647-C653.

Widrick, J. J., S. K. Knuth, K. M. Norenberg, J. G. Romatowski, J. L. W. Bain, D. A. Riley, M. Karhanek, S. W. Trappe, T. A. Trappe, D. L. Costill and R. H. Fitts (1999). "Effect of a 17 day spaceflight on contractile properties of human soleus fibers." Journal of Physiology (London) **516**: 915-930.

Widrick, J. J., K. M. Norenberg, J. G. Romatowski, C. A. Blaser, M. Karhanek, J. Sherwood, S. W. Trappe, T. A. Trappe, D. L. Costill and R. H. Fitts (1998). "Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest." Journal of Applied Physiology **85**: 1949-1956.

Widrick, J. J., J. G. Romatowski, J. L. W. Bain, S. W. Trappe, T. A. Trappe, J. L. Thompson, D. L. Costill, D. A. Riley and R. H. Fitts (1997). "Effect of 17 days of bed rest on peak isometric force and unloaded shortening velocity of human soleus fibers." American Journal of Physiology **273 (Cell Physiol. 42)**: C1690-C1699.

Widrick, J. J., J. G. Romatowski, J. Sherwood, D. L. Costill and R. H. Fitts (1995). "Force-velocity-power relationships of human muscle fibers after unilateral lower leg suspension." American Society of Gravitational and Space Biology Bulletin **9**: 44.

Widrick, J. J., J. E. Stelzer, T. C. Shoepe and D. P. Garner (in press). "Functional properties of human fibers after short-term resistance exercise training." American Journal of Physiology Regulatory Integrative Comparative Physiology.

Widrick, J. J., S. W. Trappe, C. A. Blaser, D. L. Costill and R. H. Fitts (1996). "Isometric force and maximal shortening velocity of single muscle fibers from elite master runners." American Journal of Physiology **271 (Cell Physiol. 40)**: C666-C675.

Widrick, J. J., S. W. Trappe, D. L. Costill and R. H. Fitts (1996). "Force-velocity and force-power properties of single muscle fibers from elite master runners and sedentary men." American Journal of Physiology **271 (Cell Physiol. 40)**: C676-C683.

Woledge, R. C., N. A. Curtin and E. Homsher (1985). Energetic Aspects of Muscle Contraction. Orlando, Academic Press.

APPENDICES

APPENDIX A

Department of Exercise and Sport Science

INFORMED CONSENT DOCUMENT

A. Title of the Research Project: Cellular mechanisms of muscle contraction in individuals with multiple sclerosis

B. Principal investigator:

Dena Garner, M.S.
Doctoral student
Dept. of Exercise and Sport Science
541-737-6619

Co-investigators:

Jeffrey Widrick, Ph.D.
Assistant Professor
Dept. of Exercise and Sport Science
541-737-5923

Jeffrey McCubbin, Ph.D.
Director, Special Motor Fitness Clinic
Professor and Associate Dean
Dept. of Exercise and Sport Science
541-737-5921

C. Purpose of the Research Project.

The purpose of this project is to study processes within individual muscle cells (muscle fibers) that could contribute to the muscle weakness and fatigue associated with Multiple Sclerosis. To gain an understanding of muscle cell processes, a comparison of muscle strength and muscle fiber composition will be made between subjects with and without Multiple Sclerosis.

D. Procedures. I have received an oral and a written explanation of this study and I understand that as a participant in this study the following things will happen:

1. Pre-study screening. I will complete an interview pertaining to my level of disability and physical activity levels in order to determine whether I am a suitable candidate for this study. After completion of this pre-screening, I may or may not be asked to participate in the remainder of the study.

2. What I will be asked to do during the study. If I am chosen to participate in this study, I will be asked to do the following:

   Day 1:

   a) My weight and height will be determined.
   b) *Disability Status.* A physical therapist will evaluate the level of my disability using the Expanded Disability Status Scale (EDSS). During this assessment, the level of my functional impairment along with gait analysis will be assessed. The modified Ashworth Spasticity Scale will also used to evaluate spasticity levels, while the Physiotherapy Clinical Outcomes Variables (C.O.V.S.) assessment will be used to determine the level of my physical mobility.
   c) *Muscle biopsy.* A physician will obtain a muscle sample from the thigh muscle (vastus lateralis) of my dominant leg. The method used to obtain this sample (percutaneous needle biopsy technique) is routinely used by the medical community to obtain small muscle samples for the assessment of neuromuscular diseases. Muscle samples obtained in this way typically weight between 2-4 one-thousandths of an ounce (0.002-0.004 ounce), about the size of a match head. A local anesthetic will be injected at the sample site on my thigh. I may experience a stinging sensation as the anesthetic is injected. A small incision (~3/8 inch long) will be made through the skin and the sheath that surrounds the muscle. I understand that I will have a small scar as a result of the incision. To obtain the muscle sample, a biopsy "needle" (~ 2/10 of an inch in diameter) is inserted through the incision and into the muscle. Most people report feeling an odd, "pressure," sensation as this is done.
   d) *Physical activity levels.* In order to determine my physical activity levels, I will complete the Yale Physical Activity Questionnaire. This test includes questions pertaining to the activities of my daily living such as yard and home activities. This questionnaire also includes questions concerning my level of recreational physical activity such a walking, swimming, or participation in team sports. My physical activity levels will also be monitored with an Optimal "Clicker" Pedometer. This pedometer records steps, miles, and calories burned during a day. I will put on the pedometer as soon as I get up each morning and take it off when I am going to bed for the evening. At the end of the day, I will record the number of steps, miles, and calories that I burned during that day on a data sheet, which will be supplied for me. I will wear the pedometer for a period of 14 days.

Day 14:

a) *Muscular strength.* The strength of my leg muscles will be evaluated using a Kin-Com isokinetic strength testing apparatus. This test involves making maximal contractions with each leg against a pivoting or stationary lever. Each leg will perform ~ 24 contractions or until it is fatigued, whichever comes first.

3. Foreseeable risks or discomforts.
   a) *Muscular strength testing.* I may experience some muscle soreness 2-3 days after this testing. This is a normal response to unaccustomed exercise and will subside within another 1-3 days.
   b) *Muscle biopsy.* Following the biopsy procedure, I may experience muscle soreness or tenderness near the biopsy site. This soreness usually disappears within 24-48 hours. Like any minor surgical procedure, there are certain risks that I am aware of. These include the risk of infection and the development of a hematoma. The following precautions will be taken to minimize these risks:
      1) The biopsies will be performed by a physician using sterile techniques.
      2) I will be given directions as to how to care for the incision. I understand that I must follow these instructions to minimize the above risks.
      3) I will periodically examine the incision and immediately report any unusual response to the principal investigator.

4. Benefits to be expected from the research. It is expected that the results from this research will improve our understanding of muscle function in MS. My participation may thereby contribute to improving the health and quality of life for myself and/or others. I will also benefit by learning about my muscular strength, power, and fatigability and the muscle fiber type composition of my thigh muscle (% slow and % fast fibers).

E. Confidentiality. Any information collected from me will be kept confidential. The only persons who will have access to this information will be the investigators. A code number will be used to identify test results or other information provided by the investigators. My name will never be used in any data summaries or publications.

F. Compensation for injury. I understand that the University does not provide a research subject with compensation or medical treatment in the event that the subject is injured as a result of participation in the research project.

G. Voluntary participation statement. I understand that my participation in this study is completely voluntary and that I may either refuse to participate or withdraw from the study at any time without penalty or loss of benefits to which I am otherwise entitled.

H. If I have questions. I understand that any questions I have about the research study and/or specific procedures should be directed to Jeffrey Widrick, 105 Women's Bldg., Oregon State University, (541) 737-5923. If I have questions about my rights as a research subject, I should contact the IRB Coordinator, OSU Research Office, (541) 737- 8008.

I. Understanding and compliance. My signature below indicates that I have read and I understand the procedures described above and give my informed and voluntary consent to participate in this study. I understand that I will receive a signed copy of this consent form.

Signature of subject	Name of subject
Date signed	
Subjects present address	Subjects phone number
Signature of Principal Investigator	Date signed

APPENDIX B

OREGON STATE UNIVERSITY

COPY

Report of Review by the Institutional Review Board for the Protection of Human Subjects

TO: Jeffrey Widrick, ExSS

COPY: Dena Garner, ExSS

RE: Cellular mechanisms of muscle contraction in individuals with multiple sclerosis.

The referenced project was reviewed under the guidelines of Oregon State University's Committee for the Protection of Human Subjects and the U.S. Department of Health and Human Services. The committee has **approved** your application. The approval of this application expires upon the completion of the project or one year from the approval date, whichever is sooner. The informed consent form obtained from each subject should be retained in program/project's files for three years beyond the end date of the project.

Any proposed change to the protocol or informed consent form that is not included in the approved application must be submitted to the IRB for review and must be approved by the committee before it can be implemented. Immediate action may be taken where necessary to eliminate apparent hazards to subjects, but this modification to the approved project must be reported immediately to the IRB.

Warren N. Suzuki, Chair
Committee for the Protection of Human Subjects
(Education, 7-6393, suzukiw@orst.edu)

Date: 05/09/99