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QUANTITATIVE ASSESSMENT OF STRENGTH AND FATIGUE IN PATIENTS WITH MYASTHENIA GRAVIS

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**QUANTITATIVE ASSESSMENT OF STRENGTH AND FATIGUE IN PATIENTS
WITH MYASTHENIA GRAVIS**

(Spine title: Strength and Fatigue in Patients with Myasthenia Gravis)

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by

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Graduate Program in Kinesiology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO
FACULTY OF GRADUATE STUDIES

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Quantitative Assessment of Strength and Fatigue in Patients with Myasthenia Gravis

Is accepted in partial fulfilment of the
requirements for the degree of
Master of Science

Date

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ABSTRACT

The objective of this thesis was to quantify, by direct measurement of muscle force, the strength and fatigue of patients with myasthenia gravis (MG). MG is characterized by fatigable muscle weakness resulting from impaired neuromuscular transmission.

A maximal voluntary isometric contraction protocol of shoulder abductors was used in conjunction with conventional fatigue and disease severity instruments. Results from patients with (D-MG) and without decrement (ND-MG) on repetitive nerve stimulation (RNS) were compared to controls.

Patients with MG reported experiencing greater fatigue than controls. Muscle strength was the lowest in the D-MG group followed by the ND-MG group and controls, respectively. Surprisingly, normalized shoulder abduction fatigue and recovery values did not differ between patients with MG and controls.

Greater experienced fatigue in MG may correspond to confounding variables such as physical inactivity. In addition, decrement upon RNS, appears to relate best to disease severity and muscle weakness but not to fatigue in this population.

Keywords: myasthenia gravis, repetitive nerve stimulation, fatigue, weakness, isometric force, deltoid muscle, quantitative myasthenia gravis score, manual muscle testing, myasthenia gravis fatigue scale

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LIST OF ABBREVIATIONS

ACh- acetylcholine
AChE- acetylcholinesterase
AChR- acetylcholine receptor
ADL- activities of daily living
ANOVA- analysis of variance
BMI- body mass index
Ca²⁺ - calcium
CAF - central activation failure
CNS- central nervous system
CMAP- compound muscle action potential
D-MG – decrementing myasthenia gravis
EAMG – experimental autoimmune myasthenia gravis
EPP- end-plate potential
MFAP- muscle fiber action potential
MG- myasthenia gravis
MG-ADL- myasthenia gravis- activities of daily living scale
MGFS- myasthenia gravis fatigue scale
MIR- main immunogenic region
MMT- manual muscle testing
MN- motor neuron
MS- multiple sclerosis
MU- motor unit
MVC – maximal voluntary contraction
MVIC- maximal voluntary isometric contraction
NMD – neuromuscular disorders
ND-MG - non-decrementing myasthenia gravis
NMJ- neuromuscular junction
NMT- neuromuscular transmission
NpAmp- negative peak amplitude
QMG- quantitative myasthenia gravis score
RNS- repetitive nerve stimulation
SD- standard deviation
SFEMG- single fiber electromyography
VAS- visual analogue scale
VGCC- voltage gated calcium channels
VGNa⁺ channels- voltage gated sodium channels

GLOSSARY OF TERMS

Acetylcholine (ACh) – neurotransmitter at the neuromuscular junction

Acetylcholinesterase (AChE) – enzyme responsible for hydrolyzing free acetylcholine within the neuromuscular synapse into choline and acetate

Acetylcholine receptor (AChR) – integral membrane receptor of the muscle end-plate which responds to binding of two acetylcholine molecules by undergoing a conformational change which promotes the entry of cations into the muscle end-plate

Compound muscle action potential (CMAP) – surface detected potential representing the summation of all the muscle fiber action potentials within a muscle following supramaximal stimulation of the appropriate motor nerve

End-plate potential (EPP) – partial post-synaptic cell depolarization produced by opening of cation channels at the muscle end-plate

Fatigue – deficit in a muscle or muscle groups ability to sustain a given or required force over time

Jitter – variability in neuromuscular transmission time across different neuromuscular junctions belonging to a single motor unit

Muscle end-plate – region of muscle, characterized by a high density of neuromuscular junctions, which is responsible for the initiation of muscle fiber action potentials

Muscle fiber action potential (MFAP) – action potential across the muscle fiber sarcolemma and T-tubules which initiates a muscle contraction

Myasthenia gravis (MG) – an autoimmune disease characterized by fatigable skeletal muscle weakness resulting from impaired neuromuscular transmission

Myasthenia gravis- activities of daily living scale (MG-ADL) – a Likert scale that measures the impact of myasthenia gravis on activities of daily living as an indicator of disease severity

Myasthenia gravis fatigue scale (MGFS) – self-report questionnaire examining three subscales of fatigue including perception of fatigue, task avoidance behaviors, and observable motor signs

Manual muscle testing (MMT) – clinical evaluation of muscle strength developed by the medical research council (MRC)

Maximal voluntary isometric contraction (MVIC) – maximal effort voluntary contraction where the joint angle remains constant during the contraction

Negative-peak amplitude (NpAmp) – a measurement relating to the change in recorded millivolts from baseline to the peak of the negative phase of a surface detected compound muscle action potential

Neuromuscular junction (NMJ) – a specialized synapse between a motor neuron and a muscle end-plate

Peak torque – the maximum production of force about a vector (the dynamometer arm) for a single contraction, measured in newton-meters (Nm)

Quantitative myasthenia gravis score (QMG) – a clinical instrument used to assess disease severity by testing the strength of thirteen different muscle groups

Repetitive nerve stimulation (RNS) – an electrodiagnostic test for disorders of neuromuscular transmission which involves recording various parameters of a compound muscle action potential following repeated supramaximal stimulations of the appropriate motor nerve

Sensitivity - the probability of correctly detecting the presence of a given abnormality

Single fiber electromyography (SFEMG) – an electrodiagnostic test for disorders of neuromuscular transmission where a needle electrode is inserted into the muscle to examine for jitter and blocking

Specificity – the probability of correctly detecting the absence of a given abnormality

Visual analogue scale (VAS) – a research instrument designed to measure an attribute that is best illustrated by a continuum of values versus discrete points

Weakness- loss of strength within a given muscle independent of previous work or activity

CHAPTER 1

NEUROMUSCULAR FATIGUE AND WEAKNESS

1.0 GENERAL INTRODUCTION

1.0.1 Myasthenia Gravis

Myasthenia Gravis (MG) is a post-synaptic neuromuscular junction (NMJ) disorder characterized by fatigable skeletal muscle weakness exacerbated with activity and improved with rest^{23,33,25}. The asymmetric and varied presentation of affected muscle groups creates unique challenges in the diagnosis and treatment of MG^{25,33}. Ocular, bulbar, and proximal muscle groups are most commonly involved^{8,33,5} and individuals indiscriminate of race, age, or gender may present with symptoms of MG^{24,33}. Although mortality rates have declined to less than five percent, contemporary prevalence rates continue to advance towards 1 in 5,000 people^{20,28}. With the aid of animal and human experiments, the site and mechanism of fatigue in MG have been identified as neuromuscular transmission (NMT) failure across a specialized synapse called the NMJ^{23,25,20,33}.

1.0.2 The Neuromuscular Junction

Voluntary movement generated by mammalian skeletal muscle is the product of an intricate orchestration of central and peripheral events. The motor unit (MU), comprised of a single α - motor neuron (MN) and the muscle fibers its' axon innervates, is responsible for translating signals from the central nervous system (CNS) into meaningful motor responses^{19,32,8}. Each MN cell body, located in the anterior horn of the spinal column⁸, projects a myelinated motor axon to a specific muscle⁸. Once the motor axon enters the muscle, it loses its myelin sheath and divides into multiple terminal

branches forming a NMJ with each individual muscle fiber³². The NMJ is a specialized synapse within the MU (Figure 1.1).

Action potentials from MNs are conveyed via the neurotransmitter acetylcholine (ACh) across the NMJ to the motor end-plate of the muscle to initiate the series of events that eventually lead to contraction. When an action potential in the MN reaches the nerve terminal, it stimulates voltage gated calcium channels (VGCC) to open^{5,19}. The influx of positive charge results in the exocytosis of ACh from vesicles into the primary synaptic cleft¹⁹. Each ACh vesicle contains a discrete amount of ACh molecules referred to as quanta^{19,21}. In the pre-synaptic terminal, ACh quanta are organized into immediately releasable, mobilizable, and reserve stores^{19,5,8}. Only a modest number of quanta are available for immediate release from the active zones of the pre-synaptic membrane. As the initial stores deplete, the mobilizable stores are engaged to release additional ACh quanta^{5,8}. Once in the synaptic cleft, ACh rapidly diffuses across the 50 nm space to interact with the nicotinic ACh receptors (AChR) located on the crests of the junctional folds of the post-synaptic muscle membrane^{5,19,33}. ACh-AChR interactions open the AChR channel pores allowing an influx of cations, predominately Na⁺, into the post-synaptic muscle cell^{5,33}. In addition to increasing surface area, the narrow junctional folds act to create a high resistance path to current flow facilitating the activation of voltage gated sodium (VG Na⁺) channels concentrated at the base of the folds^{8,19}.

Each ACh quantum released results in approximately a 1 mV change of post-synaptic membrane potential⁵. If the local depolarizations, so called end-plate potentials (EPP), reach a certain threshold an all-or-none muscle fiber action potential (MFAP) occurs^{5,33,32}. Each MFAP travels bi-directionally along the muscle fiber stimulating

subsequent events which generate a muscle contraction⁷. The safety factor responsible for preserving signal transmission across the NMJ is protected by an excess of ACh-AChR interactions above the amount required for a threshold response²³. After closure of the AChR pore, ACh either diffuses away from the NMJ or is hydrolyzed by the enzyme acetylcholinesterase (AChE)^{23,33,5}.

1.0.3 Neuromuscular Junction Pathophysiology in Myasthenia Gravis

Impaired NMT is the result of a T-cell dependent autoimmune attack on various epitopes of the skeletal muscle end-plate region³⁹. One of the putative epitope regions is the main immunogenic region (MIR) of the α -subunit of AChRs embedded in the postsynaptic membrane^{39,18,20}. The binding of two ACh molecules, released from the MN terminal, to AChR α -subunits is required to initiate a cascade of events resulting in muscle contraction^{39,33,23}. When the NMT is blocked at the postsynaptic membrane, as is the case in MG, the corresponding muscle fibers fail to contract resulting in weakness and fatigue³³.

Three principal mechanisms have been proposed to explain how anti-AChR antibodies operate at the NMJ. The first mechanism by which anti-AChR antibodies may compromise NMT is by interfering, either competitively or non-competitively, with the ability of ACh to bind to the α -subunit of the AChR^{39,33}. In healthy NMJs, AChRs are continually being replaced and have a half-life of approximately 8-11 days before they are internalized and degraded⁴¹. However due to clustering of AChRs on the crests of junctional folds, anti-AChR antibodies are able to easily cross-link adjacent AChRs^{39,33}. The second mechanism, cross-linking receptors, results in faster internalization kinetics and lower steady-state AChR concentrations³³. The third proposed mechanism reveals

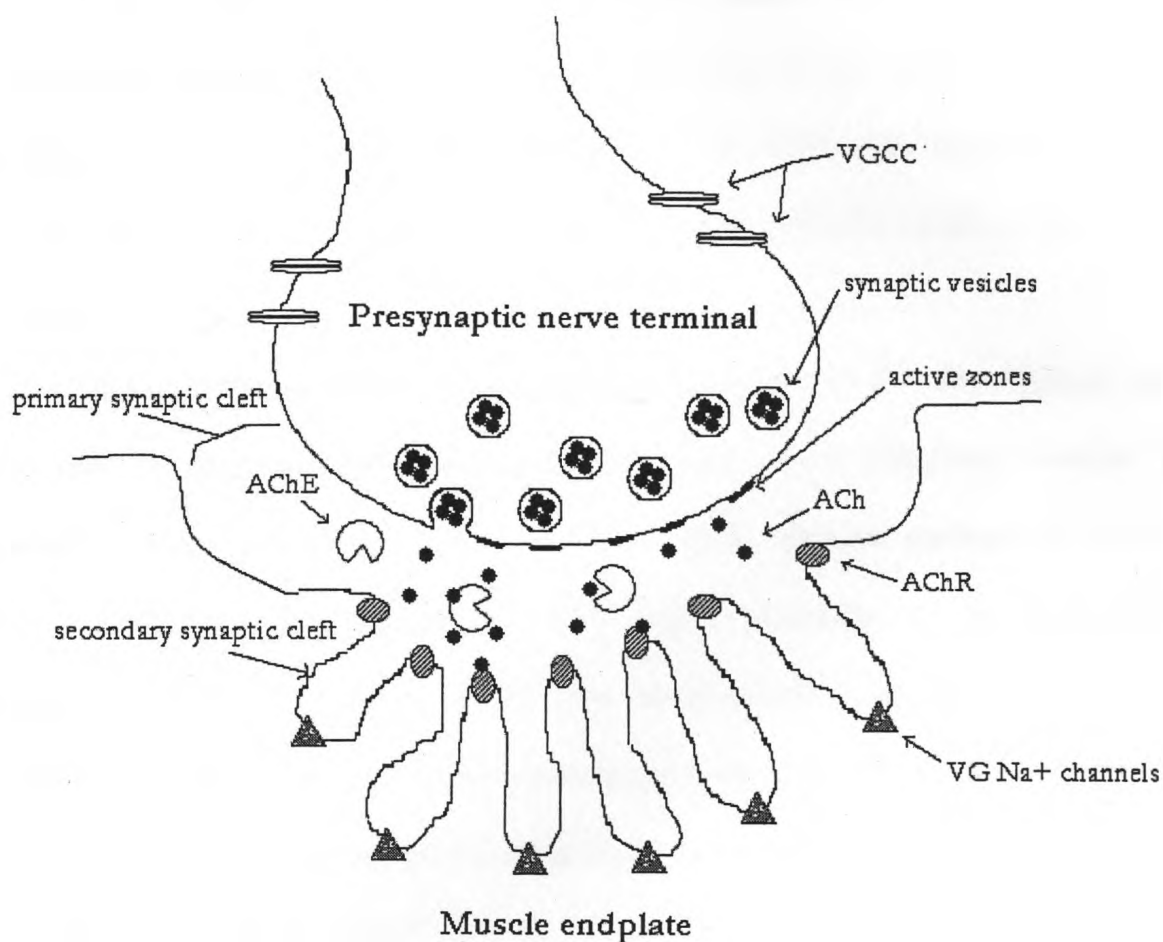


Figure 1.1. Schematic of an intact neuromuscular junction. The pre-synaptic nerve terminal contains synaptic vesicles and voltage-gated calcium channels (VGCC). Free acetylcholine (ACh) and the enzyme acetylcholinesterase (AChE) are depicted in the primary synaptic cleft. Acetylcholine receptors (AChR) are located on the crests of post-synaptic junctional folds of the muscle endplate, where voltage-gated sodium (VG Na⁺) channels are concentrated in the troughs. (Adapted from Nicolle MW: Myasthenia Gravis. *Neurologist* 2002; 8 (1): 2-21.)³³

potentially one of the most detrimental and long-term consequences of anti-AChR antibodies. Anti-AChR antibodies are able to activate the complement system³⁹. Once activated, the complement system forms a membrane-attack complex through a network of associated proteins which results in the destruction of the muscle end-plate^{39,33}. Overall, each antibody mechanism compromises an important safety factor for NMT by impeding the actions of ACh or reducing the number of available AChRs at the muscle end-plate.

Through elucidating the complex pathophysiology of MG, investigators have improved the prognosis and available treatment options to effectively manage the disease^{25,7,33}. For example, the development of AChE inhibitor medications, such as pyridostigmine bromide (Mestinon®), has proven successful in the symptomatic treatment of MG^{23,33,24}. Mestinon® works by inhibiting AChE and thus increasing the amount or potential of ACh to interact with available receptors^{24,33}. Additional treatment options, such as immunosuppressive medications or thymectomy, are also available for the clinical management of MG³³.

1.0.4 Electromyographic Assessment of Neuromuscular Transmission

Repetitive Nerve Stimulation

Repetitive nerve stimulation (RNS) is a putative electrodiagnostic test for confirming disorders of NMT such as MG^{5,7,23,33}. The theoretical foundation of RNS relies on physiological principles of signal transmission across the NMJ^{19,32,21}. In 1941, Harvey and Masland first noted experiments involving repetitively stimulating a nerve supramaximally and recording the compound muscle action potential (CMAP) over the appropriate muscle end-plate as a method to test for NMT disorders^{12,26}. The CMAP, the

primary outcome measure for RNS, represents the summation of all the MFAPs in a muscle following supramaximal stimulation of the appropriate motor nerve^{5,10}. Although RNS is a straightforward technique, researchers have elucidated important considerations and assumptions that must be understood when using it as an investigational tool. Nerve-muscle selection, limb temperature, recording techniques and stimulation parameters are specific components of RNS that have undergone critical review^{5,7,12}.

A standard low frequency RNS protocol employs a train of 4-10 supramaximal stimuli applied to a nerve at frequencies between 2-3 Hz^{10,31,43}. Stimulation frequencies must be kept low enough to prevent calcium (Ca^{2+}) accumulation⁵. Surface electrodes are positioned over the appropriate muscle of interest to record the resultant CMAP^{26,5,12,10}. During RNS, immediately releasable ACh quanta stores are progressively depleted^{23,5,7,26}. After the 4th stimulation of the RNS protocol, with less ACh available to interact with AChRs, an observable reduction in the EPP is evident^{5,7,26}. However, in healthy subjects the reduction in EPP remains above threshold values and is able to generate a MFAP^{23,5,7}. After 1-2 seconds, secondary ACh stores are mobilized to match the discharge rate resulting in a subsequent recovery of EPP towards the end of the RNS train^{5,7,26}. Therefore, in healthy subjects, the negative peak CMAP amplitude remains constant with RNS^{23,5,7,10}.

However, if enough muscle fibers experience NMT failure, such as in MG, the resultant negative peak CMAP amplitude is reduced^{33,23}. The RNS results of a patient with MG are characterized by a stereotypical U-shaped response pattern of the CMAP negative peak amplitude^{33,26} (Figure 1.2). The initial CMAP response is of normal amplitude and the 2nd to 4th responses show a progressive decrement of amplitude

representing NMT failure at hundreds of muscle fibers²³. As the immediate releasable ACh quanta stores become depleted, in contrast to their healthy counterparts, an increasing number of EPPs in patients with MG become sub-threshold and thus unable to transmit MFAPs^{5,7,26}. As the mobilization stores of ACh quanta are activated, a subsequent improvement in CMAP amplitude may result but these values typically do not return to normal without a period of rest. Decrement is calculated by comparing the lowest negative peak CMAP amplitude with the initial response from the RNS train⁵. Decrement of $\geq 10\%$ change from the CMAP amplitude of the normal response are typically used as the cut-off value for an abnormal result in patients with MG^{33,23,10}. This inability to generate MFAPs with RNS correlates with symptoms of weakness and fatigue in this patient population.

The low-frequency stimulations (2-3 Hz) used in standard RNS protocols are not representative of true physiological firing rates of motor nerves which during maximal voluntary contractions may reach or exceed 30 Hz^{21,5}. This is important because the number of quanta released upon motor nerve stimulation depends both on the number available for immediate release as well as the probability of release⁵. Increased or prolonged Ca^{2+} influx into the motor axon terminal, evident with higher stimulation frequencies, improves the probability of releasing ACh quanta^{5,7,12}. This phenomenon is referred to as Ca^{2+} facilitation^{12,5,7}. Rapid RNS, at stimulation frequencies of 10-50 Hz, can be used to demonstrate Ca^{2+} facilitation^{12,5,7}. However, high stimulation frequency RNS can cause discomfort and pain for the subject and is often not well-tolerated. To improve diagnostic yield, integrating maximum voluntary exercise into a standard low

frequency RNS protocol has been substituted as a less painful alternative for high frequency RNS^{5,31}.

When adding exercise to a standard slow RNS protocol, the subject is asked to exercise the muscle by performing an isometric contraction, for 5-10 s^{7,26}, followed immediately by a RNS train to examine for repair of any decrement⁵. More recently, investigators have also employed a longer exercise period of up to 1 min in addition to examining RNS after the initial brief period of exercise^{43,5}. Immediately after cessation of the 1 min exercise period, slow RNS trains at 30 s – 1 min intervals are applied for a period of approximately 5 minutes¹¹. Healthy individuals may demonstrate a brief increase in negative peak CMAP amplitude, termed pseudo-facilitation^{30,31,12}, following this protocol. Although there is no increase in the number of MFAPs contributing to the CMAP, exercise may cause the MFAPs to fire more synchronously^{26,21,7}. Summation of the MFAP responses will produce an increase in CMAP amplitude, a slight decrease in CMAP duration, with little change in overall CMAP area^{5,30}.

Patients with MG demonstrate an improvement in the CMAP decrement demonstrated during resting RNS at the 3 s and 15 s time intervals post-exercise⁷ of either the brief or 1 min exercise periods. The initial improvement in CMAP amplitude, referred to as post-activation facilitation (Figure 1.2), can be explained by the temporary increase in the number of ACh quanta available for release due to improved Ca^{2+} concentration^{26,7,31,23}. At 3-4 minutes post-exercise, initial repair of CMAP is absent and decrement may worsen compared with values obtained during resting RNS, a phenomenon termed post-activation exhaustion^{26,7,31,23} (Figure 1.2). The exact mechanism for post-exercise exhaustion remains controversial, however researchers have proposed

receptor desensitization and a reduction in the availability of ACh vesicles as potential candidates mediating this response^{7,23,37}. Overall, RNS protocols encompassing an exercise regime currently provide an important diagnostic tool and surrogate measure of fatigue in patients with MG.

Single Fiber Electromyography

In 1964, Professor Erik Stalberg introduced single-fiber electromyography (SFEMG) as an electrodiagnostic test for disorders of NMT^{8,10}. In SFEMG, a concentric needle electrode is inserted into the muscle end-plate region to obtain extracellular recordings of single muscle fiber action potentials (MFAPs)^{7,8}. The needle position is adjusted to achieve responses, with sufficient amplitudes and sharp rise times, from a pair of muscle fibers within the same MU¹¹. The MN can be activated either by electrical stimulation or by voluntary contraction⁷. When a MN is repeatedly activated, a natural variability in NMT time across the corresponding individual NMJs is evident due to fluctuating thresholds required to trigger a MFAP post-synaptically⁸. This variability in transmission is termed jitter^{23,33}. Normal ranges of jitter values differ between muscle groups⁷ and jitter is increased in MG^{16,10}. In pathological conditions, blocking may also occur where an individual muscle fiber within a pair fails to fire altogether⁷. SFEMG is a reliable technique to detect pathologically increased jitter and blocking in disorders of NMT^{6,8,23}.

Specificity and Sensitivity

When considering the advantages and disadvantages of various diagnostic tests available for use in MG, it is valuable to examine the relative specificities and sensitivities of each measure. The sensitivity of a technique represents the probability of

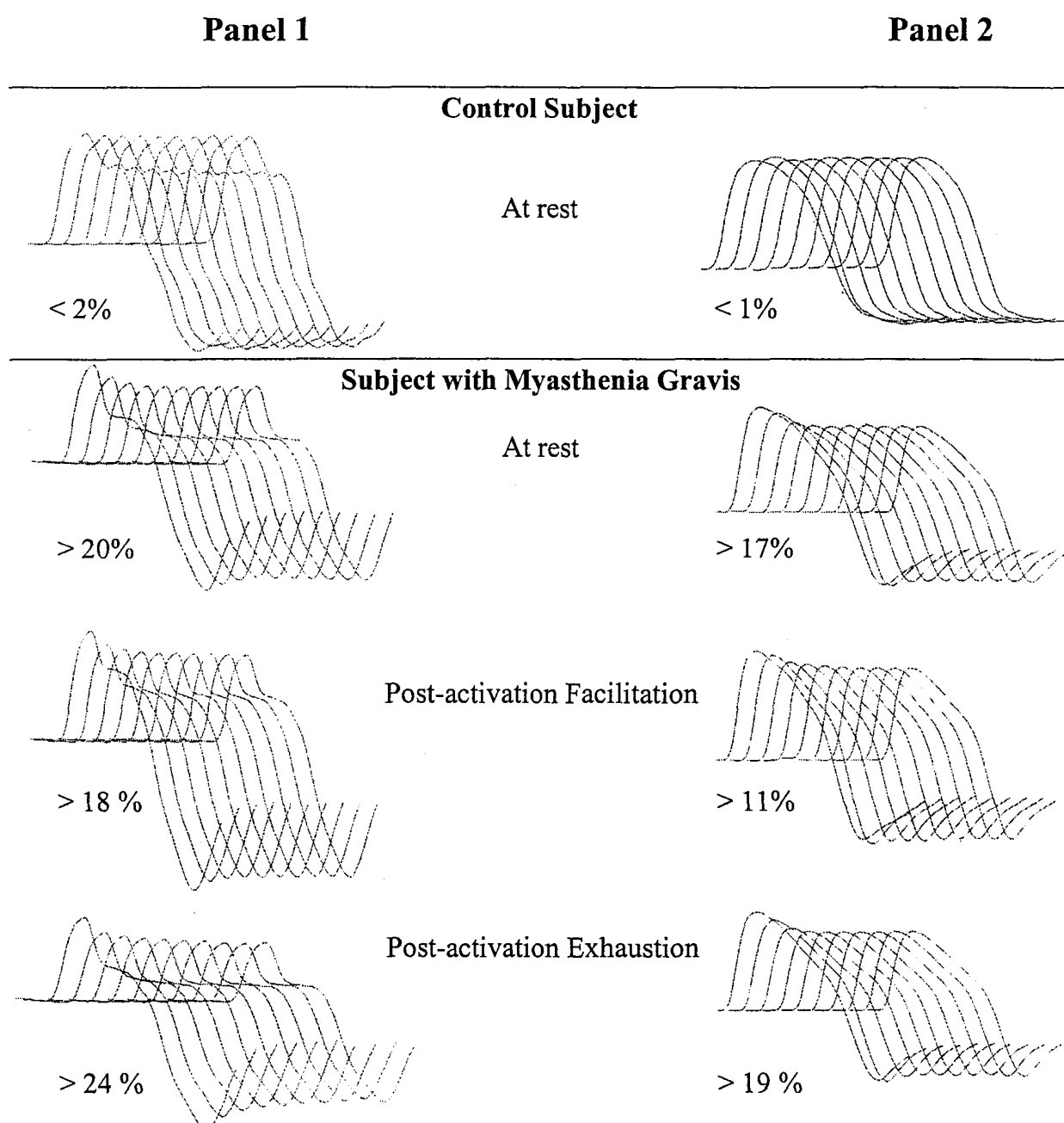


Figure 1.2. Repetitive Nerve Stimulation. Panel 1 (left) represents axillary to deltoid stimulation. Panel 2 (right) represents spinal accessory to trapezius stimulation. Results from a representative control subject and a subject with MG are shown using a scale of 2 mV by 2 ms per a division. An approximate percent decrement of CMAP amplitude for each stimulation train is indicated in the figure. Post-activation facilitation trains were applied immediately following 10 s of exercise. Post-activation exhaustion trains were applied 3 min after 1 min of exercise.

correctly detecting the presence of a given abnormality⁸. In comparison, the specificity of a technique indicates the likelihood of correctly identifying the absence of a given abnormality⁸. Decreasing the potential for a false negative result will increase the sensitivity, whereas decreasing the potential for a false positive result will improve the specificity of a given measure⁸. As it is not possible for a technique to exist with a high probability of detecting both the presence and absence of a given abnormality, sensitivity and specificity share an inverse relationship.

Electrodiagnostic evaluations, such as RNS and SFEMG, are sensitive to NMT failure³⁸. Although decrement upon RNS is sensitive for indicating impaired NMT, it is not specific for MG²³. The sensitivity of RNS in MG is enhanced by testing multiple symptomatic proximal muscles where the skin temperature at the recording site has been warmed to approximately 35°C^{10,11}. Even when these considerations are met, only 75-95% of patients with MG have positive RNS^{23,10,35}. Although time-consuming and technically challenging, the improved resolution obtained with SFEMG yields a higher sensitivity (90-99%)³⁵ for detecting NMT abnormalities in comparison with RNS^{33,23}. However, in both techniques the likelihood of detecting false positive results from other neurogenic or myogenic disorders makes improving the specificity of electrodiagnostic testing for MG difficult^{33,8}. Other diagnostic measures, such as serologic testing for anti-AChR antibodies, have high specificity for MG³³. Auto-antibodies are detectable in approximately 70-90% of cases of MG^{39,35,18,7}. Integrating additional evaluations of fatigue and weakness may aid in alleviating the limitations of RNS, SFEMG, and serologic testing in developing a comprehensive understanding of MG.

1.0.4 Neuromuscular Fatigue and Weakness in Myasthenia Gravis

The well established etiology of fatigue in MG creates the opportunity to apply contemporary research techniques to further our understanding of the functional implications of the disease. Neuromuscular fatigue, hereafter referred to as fatigue; and muscle weakness are interrelated but distinct occurrences. Fatigue is described as a deficit in a muscle's ability to sustain a given or required force over time^{15,5,14}. In comparison, muscle weakness is defined by a loss of strength within a given muscle independent of previous physical work or activity¹⁵. Both a subjective evaluation and force measurement are useful for a comprehensive analysis of fatigue and weakness^{27,14}.

A subjective evaluation of fatigue is accomplished through the use of questionnaires and surveys^{27,17,36}. The Myasthenia Gravis Fatigue Scale (MGFS)²⁷ represents one of the few disease-specific fatigue questionnaires available for use in MG. Using a Likert-scale format, the MGFS attempts to quantify the subjective experience of fatigue by including 23 items addressing the constructs of depression, disease severity, as well as cognitive and physical fatigue. In addition, visual analogue scales (VAS) are employed as a complementary method of evaluating subjective perceptions of fatigue in this population.

Strength measurements are important for disease management in MG. Manual muscle testing (MMT)^{2,42,34} is a standard technique used clinically to assess muscular strength. Each muscle tested is graded from 0 to 5, with 0 representing no contraction and 5 indicating normal power¹³. Scores of 4 signify active movement against gravity and resistance and can be further subdivided to 4-, 4, 4+ to correlate with slight, moderate, and strong applied resistance, respectively^{13,1}. The Quantitative Myasthenia Gravis Score

(QMG)⁴, an instrument used to test the strength of 13 different muscle groups has also been validated as a useful investigational tool when assessing weakness and disease severity. Both MMT and the QMG, although requiring specially trained personnel for administration, are efficient and cost-effective ways of assessing muscle strength.

In most cases, results from a subjective analysis of fatigue are consistent with traditional physical strength measurements. As previously described, decrement upon RNS correlates with fatigue and weakness experienced in this population. However, a subset of patients with MG paradoxically express symptoms of weakness and fatigue in affected muscle groups without demonstrating decrement with RNS studies performed on the same muscle groups. Thus, more information is required to illuminate potential justifications for this apparent discrepancy in the fatiguing characteristics of patients with and without decrement upon RNS.

Although useful measures exist to evaluate muscle strength in MG, quantifying fatigue requires directly measuring force production through the use of a dynamometer⁹. Clinically weak muscles work at a higher percentage of their maximal capacity while performing everyday tasks and thus may also exhibit unique fatiguing characteristics¹⁵. Despite presenting as a prototypic fatiguing disorder, limited research has been invested into quantifying fatigue in MG.

Selection of appropriate muscle groups, contraction protocols and intensities are important parameters to consider when designing a fatigue protocol⁹. Protocols involving maximal effort voluntary isometric contractions (MVIC) have been reliably applied to studies examining fatigue in patients with multiple sclerosis (MS)^{44,40}. Isometric contractions are defined by the production of increasing tension with a constant muscle

length. Measuring time to task failure or percentage drop of initial force within a pre-determined time are two fatigue indexes which have been used to facilitate group comparison in studies involving patient population groups^{29,44} and the elderly^{3,22}. Applying a fatigue protocol incorporating changes in force as the primary outcome measure provides a logical approach to objectively compare fatigue and weakness in patients with MG who present with varying RNS results. Quantitative indicators of fatigue and weakness may provide indirect evidence of NMT failure in patients who do not show decrement with RNS. Alternatively, objective measurement of fatigue might show a pattern of differentiating NMT failure from non-NMT failure causes for weakness and fatigue. In addition, the adequacy of RNS as a surrogate measure of fatigue may be further validated.

The objective of this thesis was to evaluate the strength and fatigue of patients with MG who present with and without decrement and to further determine whether the pattern of weakness in MG patients with normal RNS differs from those with decrement and controls.

Objectives

1. To compare shoulder abduction maximum isometric strength in patients with MG in comparison to controls.
2. To compare the strength and fatigue of patients with MG who show decrement with RNS to patients who do not show decrement with RNS and control subjects.
3. To compare the recovery phase following a fatigue protocol of patients who show decrement with RNS with patients who do not show decrement with RNS and control subjects.

4. To measure the subjective perception of fatigue in patients with MG and control subjects with the Myasthenia Gravis Fatigue Survey (MGFS) and a brief visual analogue scale.
5. To measure the strength and disease severity of patients with MG with traditional manual muscle testing and the Quantitative Myasthenia Gravis Score.

Hypotheses

1. Patients with MG who show decrement with RNS will have reduced strength in comparison to patients who do not show decrement and to control subjects.
2. Patients with MG who do not have decrement with RNS will demonstrate strength similar to control subjects.
3. The fatigue index of patients who show decrement upon RNS will be greater than patients who do not show decrement with RNS and control subjects.
4. There will be no difference between the fatigue index of patients who do not show decrement with RNS and control subjects.
5. Patients who do not show decrementing upon RNS, and control subjects will recover their initial strength before patients who show decrement upon RNS.
6. Control subjects will have lower subjective ratings of fatigue compared to patients with MG.
7. There will be no difference in the subjective fatigue rating of patients who show decrement with RNS and patients who do not show decrement with RNS.

1.2 REFERENCES

1. A comparison of muscle strength testing techniques in amyotrophic lateral sclerosis. *Neurology* 2003;61(11):1503-1507.
2. Aitkens S, Lord J, Bernauer E, Fowler WM, Lieberman JS, Berck P. Relationship of Manual Muscle Testing to Objective Strength Measurements. *Muscle Nerve* 1989;12:173-177.
3. Allman BL, Rice CL. An age-related shift in the force-frequency relationship affects quadriceps fatigability in old adults. *J Appl Physiol* 2004;96(3):1026-1032.
4. Barohn RJ. Standards of measurements in myasthenia gravis. *Ann N Y Acad Sci* 2003;998:432-439.
5. Bashar K, Preston DC, Kaminski HJ, Ruff RL, Shapiro BE. *Neuromuscular Disorders in Clinical Practice*. Woburn: Butterworth-Heinemann; 2002. 1432 p.
6. Benatar M, Hammad M, Doss-Riney H. Concentric-needle single-fiber electromyography for the diagnosis of myasthenia gravis. *Muscle Nerve* 2006;34(2):163-168.
7. Brown WF, Bolton CF. *Clinical Electromyography*. Stoneham: Butterworth-Heinemann; 1993. 810 p.
8. Brown WF, Bolton CF, Aminoff MJ. *Neuromuscular Function and Disease: Basic, Clinical, and Electrodiagnostic Aspects*. Philadelphia: W.B. Saunders Company; 2002. 1867 p.
9. Cairns SP, Knicker AJ, Thompson MW, Sjogaard G. Evaluation of models used to study neuromuscular fatigue. *Exerc Sport Sci Rev* 2005;33(1):9-16.
10. Chiou-Tan FY, Tim RW, Gilchrist JM. Literature review of the usefulness of repetitive nerve stimulation and single fiber EMG in the electrodiagnostic evaluation of patients with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome. *Muscle Nerve* 2001;24(9):1239-1247.

11. Chiou-Tan FY, Tim RW, Gilchrist JM. Practice parameter for repetitive nerve stimulation and single fiber EMG evaluation of adults with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome: summary statement. *Muscle Nerve* 2001;24(9):1236-1238.
12. Costa J, Evangelista T, Conceicao I, de Carvalho M. Repetitive nerve stimulation in myasthenia gravis--relative sensitivity of different muscles. *Clin Neurophysiol* 2004;115(12):2776-2782.
13. Council MR. Aids to the Examination of the Peripheral Nervous System. London: W.B. Saunders; 2000. 62 p.
14. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *J Appl Physiol* 1992;72(5):1631-1648.
15. Feasson L, Camdessanche JP, El Mhandi L, Calmels P, Millet GY. Fatigue and neuromuscular diseases. *Ann Readapt Med Phys* 2006;49(6):375-384.
16. Gilchrist JM, Massey JM, Sanders DB. Single fiber EMG and repetitive stimulation of the same muscle in myasthenia gravis. *Muscle Nerve* 1994;17(2):171-175.
17. Grohar-Murray ME, Becker A, Reilly S, Ricci M. Self-care actions to manage fatigue among myasthenia gravis patients. *J Neurosci Nurs* 1998;30(3):191-199.
18. Hughes BW, Moro De Casillas ML, Kaminski HJ. Pathophysiology of myasthenia gravis. *Semin Neurol* 2004;24(1):21-30.
19. Hughes BW, Kusner LL, Kaminski HJ. Molecular architecture of the neuromuscular junction. *Muscle Nerve* 2006;33(4):445-461.
20. Juel VC, Massey JM. Myasthenia gravis. *Orphanet J Rare Dis* 2007;2:44.
21. Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science. Toronto: McGraw-Hill Health Professions Division; 2000. 1414 p.

22. Katsiaras A, Newman AB, Kriska A, Brach J, Krishnaswami S, Feingold E, Kritchevsky SB, Li R, Harris TB, Schwartz A, Goodpaster BH. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. *J Appl Physiol* 2005;99(1):210-216.
23. Keeseey JC. AAEE Minimonograph #33: electrodiagnostic approach to defects of neuromuscular transmission. *Muscle Nerve* 1989;12(8):613-626.
24. Keeseey JC. A history of treatments for myasthenia gravis. *Semin Neurol* 2004;24(1):5-16.
25. Keeseey JC. Clinical evaluation and management of myasthenia gravis. *Muscle Nerve* 2004;29(4):484-505.
26. Kimura J. *Electrodiagnosis in diseases of Nerve and Muscle: Principles and Practice*. Philadelphia: F.A. Davis; 1983. 672 p.
27. Kittiwatanapaisan W, Gauthier DK, Williams AM, Oh SJ. Fatigue in Myasthenia Gravis patients. *J Neurosci Nurs* 2003;35(2):87-93, 106.
28. Kupersmith MJ. Does early immunotherapy reduce the conversion of ocular myasthenia gravis to generalized myasthenia gravis? *J Neuroophthalmol* 2003;23(4):249-250.
29. Lambert CP, Archer RL, Evans WJ. Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis. *Med Sci Sports Exerc* 2001;33(10):1613-1619.
30. Lo YL, Dan YF, Leoh TH, Tan YE, Ratnagopal P. Decrement in area of muscle responses to repetitive nerve stimulation. *Muscle Nerve* 2003;27(4):494-496.
31. Lo YL, Dan YF, Leoh TH, Tan YE, Nurjannah S, Ratnagopal P. Effect of exercise on repetitive nerve stimulation studies: new appraisal of an old technique. *J Clin Neurophysiol* 2004;21(2):110-113.
32. MacIntosh BR, Gardiner PF, McComas AJ. *Skeletal Muscle Form and Function*. Windsor: Human Kinetics; 2006. 423 p.

33. Nicolle MW. Myasthenia gravis. *Neurologist* 2002;8(1):2-21.
34. O'Brien MD. *Aids to the Examination of the Peripheral Nervous System*. London: W.B. Saunders; 2000. 62 p.
35. Oh SJ, Kim DE, Kuruoglu R, Bradley RJ, Dwyer D. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. *Muscle Nerve* 1992;15(6):720-724.
36. Paul RH, Cohen RA, Goldstein JM, Gilchrist JM. Fatigue and its impact on patients with myasthenia gravis. *Muscle Nerve* 2000;23(9):1402-1406.
37. Petretska A, Jarrar R, Rubin DI. Radial nerve repetitive stimulation in myasthenia gravis. *Muscle Nerve* 2006;33(6):817-819.
38. Preston DC, Shapiro BE. *Electromyography and Neuromuscular Disorders Clinical-Electrophysiological Correlations* Philadelphia: Elsevier 2005. 685 p.
39. Richman DP, Agius MA, Kirvan CA, Gomez CM, Fairclough RH, Dupont BL, Maselli RA. Antibody effector mechanisms in myasthenia gravis. The complement hypothesis. *Ann N Y Acad Sci* 1998;841:450-465.
40. Romani A, Bergamaschi R, Candeloro E, Alfonsi E, Callieco R, Cosi V. Fatigue in multiple sclerosis: multidimensional assessment and response to symptomatic treatment. *Mult Scler* 2004;10(4):462-468.
41. Ruff RL. Neurophysiology of the neuromuscular junction: overview. *Ann N Y Acad Sci* 2003;998:1-10.
42. Sanders DB, Tucker-Lipscomb B, Massey JM. A simple manual muscle test for myasthenia gravis: validation and comparison with the QMG score. *Ann N Y Acad Sci* 2003;998:440-444.
43. Schumm F, Stohr M. Accessory nerve stimulation in the assessment of myasthenia gravis. *Muscle Nerve* 1984;7(2):147-151.
44. Schwid SR, Thornton CA, Pandya S, Manzur KL, Sanjak M, Petrie MD, McDermott MP, Goodman AD. Quantitative assessment of motor fatigue and strength in MS. *Neurology* 1999;53(4):743-750.

CHAPTER 2

QUANTITATIVE ASSESSMENT OF STRENGTH AND FATIGUE IN PATIENTS WITH MYASTHENIA GRAVIS

2.0 INTRODUCTION

Patients with myasthenia gravis (MG) characteristically present with fatigable muscle weakness resulting from impaired neuromuscular transmission (NMT)^{24,25,36}. NMT failure is demonstrated electrophysiologically by decrement of the compound muscle action potential (CMAP) amplitude in response to repetitive nerve stimulation (RNS)^{6,8,24}. A decrement of $\geq 10\%$ of the CMAP amplitude when comparing the initial response to the fourth response with low frequency repetitive stimulation is typically considered abnormal in this population^{8,9,12,36}. Although decrement upon RNS is sensitive for detecting impaired NMT, it is not specific for MG^{24,12,30}. Additional diagnostic measures, such as serologic testing for anti-acetylcholine receptor (AChR) antibodies, have high specificity for MG^{21,36}. Paradoxically, a subset of seropositive patients with MG fails to show decrement upon RNS despite symptoms of weakness and fatigue.

Quantifying a decline in force over time is another putative approach used to investigate muscle fatigue^{10,28,47,46}. However, despite presenting as a prototypic fatiguing disorder, few studies have directly measured force to evaluate weakness or fatigue in myasthenia^{19,26}. The use of questionnaires^{27,39} and surveys^{49,45}, in addition to manual muscle testing (MMT)^{1,13}, have emerged as preferred tools to assess weakness and fatigue clinically in MG.

Studies of patients with other neuromuscular disorders (NMD) have applied integrative approaches which incorporate direct force measurements. Schillings et al.⁴⁶ reported that patients with NMD experienced a higher level of fatigue but lower peripheral fatigue than controls as determined by self-report questionnaires and a two-minute sustained maximal voluntary contraction (MVC) of the biceps brachii. Investigators attributed the lower peripheral fatigue observed in NMD to their ability to better maintain the intracellular environment of the muscle due to a lower intramuscular pressure produced by smaller MVCs. A study by Garssen et al.¹⁷ examining the residual fatigue of patients with Guillain-Barré syndrome (GBS) supported the aforementioned findings of a higher level of experienced fatigue in the patients versus controls. In addition, for the first minute of a two-minute sustained MVC of the biceps brachii, patients with GBS developed peripheral fatigue more slowly than controls which aligns with results from patients with other NMD. Overall, the multidisciplinary methodology used to study weakness and fatigue in other NMD provides a viable framework to study weakness and fatigue using a myasthenic model.

The aim of the present study was to objectively measure strength and fatigue in a cohort of patients with MG who present with varying RNS results. We hypothesized that quantitative indicators of weakness and fatigue may provide indirect evidence of NMT failure in patients who do not show decrement with RNS. Additionally, objective measurement of fatigue might show a pattern differentiating NMT failure from non-NMT failure causes for weakness and fatigue in myasthenia. Lastly, the adequacy of RNS as a surrogate measure of fatigue may be further validated.

2.1 METHODS

2.1.1 Subjects

Twenty patients with clinical features of generalized MG, followed through the MG clinic at London Health Sciences Center, were invited to participate in the study. Patients were included if they were seropositive for acetylcholine receptor (AChR) antibodies and indicated self-reported symptoms of weakness or fatigue of their shoulder girdle muscles. In addition, a group of 21 healthy control subjects without evidence of neuromuscular or musculoskeletal disorders volunteered to participate. Any individuals presenting with other conditions that could cause weakness or fatigue were excluded from the study. All subjects provided informed written consent and the study was approved by The University of Western Ontario, Health Sciences Research Ethics Board.

2.1.2 Fatigue Questionnaires

All data were collected during a single visit to the Neuromuscular Assessment Laboratory at University Hospital (London, Ontario). At the beginning of the study visit, all participants completed the MG Fatigue Survey (MGFS)²⁷ (Appendix A) and a 2-item visual analogue scale (VAS) (Appendix B). The MGFS is a Likert scale containing 26 items addressing 3 sub-scales of fatigue including perception of fatigue, task avoidance behaviors, and observable motor signs associated with fatigue²⁷. Potential scores range from 25 to 130, with higher scores indicating greater fatigue²⁷. For each item of the VAS, subjects were instructed to quantify their responses by intersecting a 10 centimeter horizontal line anchored with various descriptors at either end. The 2-items of the VAS specifically asked subjects to quantify their current level of fatigue and respond to how their fatigue has impacted their activities of daily living (ADL) within the past month.

Both measures, MGFS and VAS, were included to provide subjects with the opportunity to assess their individual fatigue characteristics.

2.1.3 Clinical Examination

The disease severity of patients with MG was evaluated by an experienced advanced practice nurse using the modified Quantitative MG Score for Disease Severity Assessment (13 muscles tested) (QMG)⁵ (Appendix C) and the MG Activities of Daily Living Scale (MG-ADL)⁴⁹ (Appendix D). The QMG is a Likert scale with a potential score range of 0 to 39, with higher scores indicating greater muscle weakness and disease severity⁵. The grade assigned to each muscle tested by the QMG ranges from 0 to 3, where 0 = no muscle weakness and 3 = severe muscle weakness. The QMG score associated with shoulder muscle weakness was evaluated independently within the groups. The MG-ADL is also a Likert scale, with potential scores ranging from 0 to 24, with higher scores suggesting greater disease severity⁴⁹.

In addition, a neurologist (Dr. M. W. Nicolle, director of MG Clinic, London Health Sciences Centre) evaluated the strength of 7 upper extremity muscle groups (neck flexors, neck extensors, deltoid, biceps, triceps, wrist flexion, and wrist extension) of each patient using the Medical Research Council (MRC) Scale¹³ for manual muscle testing (MMT)^{37,15}. Baseline and fatigue MMT strength measurements were determined for each muscle group. Raw scores for each muscle group from the MMT were converted to a 10 point scale¹⁵ and summed to determine an overall Megascoring ranging from 0 – 70 points¹⁵. The converted deltoid muscle baseline and fatigue MMT scores were compared within each patient group.

2.1.4 Spinal Accessory and Axillary Nerve Repetitive Nerve Stimulation

Proximal nerve-muscle combinations are more commonly and typically more severely affected in patients with MG in comparison to more distal muscles⁴¹. Thus, repetitive stimulation of the spinal accessory nerve to trapezius and the axillary nerve to deltoid were performed on the dominant side of all participants. Patients withheld taking anticholinesterase medications (e.g. Mestinon) for at least 5 h prior to their study visit. For each nerve studied, subjects were in a supine position with their arm at their side. Limb muscles under investigation were warmed with the aid of a heat lamp. After preparing the skin with 70% v/v isopropyl alcohol, disposable surface recording electrodes (Ag-AgCl Mactrode Electrodes; GE Medical Systems, Milwaukee, Wisconsin), cut into 10 mm by 30 mm strips, were secured with tape to the appropriate recording sites for the muscle being studied. The spinal accessory nerve was stimulated at the posterior border of the sternocleidomastoid muscle. The active electrode was positioned over the motor point of the trapezius muscle, with the reference electrode positioned over the acromion. The axillary nerve was stimulated at Erb's point, with the active electrode positioned over the motor point of the middle deltoid and the reference electrode was positioned distally over the lateral epicondyle of the humerus. A full sized 27 mm by 22 mm electrode was used as a ground. Electrodiagnostic set-up and technique were consistent with parameters previously described by Preston and Shapiro (2005)⁴¹.

Single cutaneous electrical stimulations of each nerve began at very low stimulation intensities and were gradually increased until the maximal compound muscle action potential (CMAP) amplitude response was obtained. The stimulation intensity was increased an additional 15-20 % to ensure a supramaximal stimulation was delivered

during the entire RNS protocol. All studies were completed on a standard EMG machine (Advantage Medical, London, Ontario, Canada).

The RNS protocol was identical for both nerves being studied. A total of three trains of 10 stimulations, with intensities of 5 – 60 mA, were administered at a frequency of 3 Hz with 0.1 - 0.2 ms square pulse durations. The first train was applied with the subject at rest. Post-exercise facilitation was examined by applying a second train immediately following 10 s of a maximal voluntary isometric contraction (MVIC). A final train, to examine post exercise exhaustion, was administered 3 minutes after 1 minute of intermittent MVICs. The intermittent exercise for both muscles involved performing 12 cycles of 4 s on, 1 s off repeats. Auditory feedback and strong verbal encouragement were provided during the MVICs.

Negative peak amplitude (mV), negative peak area (mV·ms), and the % change of both amplitude and area in comparing the compound muscle action potential evoked by the first and the fourth stimulation of each train were determined. A negative % change (decrement) of CMAP amplitude greater than 10% during any train of the RNS protocol of either nerve-muscle combination was considered indicative of significant decrement^{36,24,8}. Subjects were divided into three groups (controls, decrementing MG (D-MG), non-decrementing MG (ND-MG)) according to the results of RNS of the spinal accessory and axillary nerves.

2.1.5 Force Measurement

Experimental Set-up. A Biodex System 3 dynamometer (Biodex Medical Systems, Shirley, New York) was used to assess the shoulder abduction torque of the dominant side of all participants. Subjects were seated in the adjustable Biodex chair

facing away from the dynamometer with a seat rotation of 90° and a hip joint angle of 85° . Two diagonal shoulder straps and a waist strap were used to stabilize the subject's position in the chair. The dynamometer, with a tilt of 10° , was adjusted so that the axis of rotation of the shoulder (glenohumeral joint) aligned with the axis of rotation of the dynamometer arm. The subject's dominant arm, with 90° of elbow flexion, was secured with wide Velcro straps in a custom-made attachment positioned over the deltoid tuberosity.

The isometric exercise mode for 30° of shoulder abduction was selected using the Biodex Advantage software (version 3.14). The raw isometric shoulder abduction force, obtained from the Biodex, was converted from analog to digital format by a 12-bit converter (CED model 1401 Plus, Cambridge Electronic Design, Cambridge, UK) with a torque scaling of 347 N·m and a sampling rate of 100 Hz. Torque production, measured in N·m, was displayed visually to the subject, in real-time, on a computer screen using Spike 2 software (Spike 2, version 5.14; Cambridge Electronic Design, Cambridge, United Kingdom).

Experimental Protocol. The experimental contraction protocol consisted of three phases of force measurement: baseline, fatigue, recovery (Figure 2.1). Following an explanation of the proper contraction technique, subjects completed a warm-up of 15 submaximal isometric shoulder abduction contractions consisting of 3 s on, 1 s off repeats. Visual feedback and strong verbal encouragement were provided during all contractions. After the warm-up, subjects were instructed to perform 3, 3 s MVICs separated by a 2 min rest period. Maximal voluntary muscle tension has been shown to have a definite and repeatable value compared with tetanic stimulation³⁴. For each

subject, the MVIC with the largest peak torque value was selected for subsequent comparison against fatigue and recovery contractions. A target line, normalized to the subject's highest peak torque produced during the baseline contractions, was drawn on the computer screen. Subjects were instructed to use the target line for guidance to continue to exert a maximal effort during each of the remaining contractions. Fifteen minutes of rest were provided following the baseline measurements. In order to determine fatigue, the subjects then performed a series of 12 intermittent MVICs consisting of 4 s on, 1 s off repeats. Fatigue was calculated by recording and comparing changes in peak torque (N·m) values across all contractions. The recovery phase consisted of single 3 s MVICs at 0.5, 1, 3, 5, 10, and 15 minutes following cessation of the fatiguing phase of contractions. All MVICs during the fatigue protocol were analyzed offline, using Spike 2 software, for peak torque values.

2.1.6 Statistical Methods

Mean values and standard deviations (SD) are presented for all parameters. Group variances were not equal, thus primarily non-parametric statistics were used to analyze the data. To compare the subjective perception of fatigue in patients with MG and control subjects, results from the MGFS and VAS were analyzed using a Kruskal-Wallis test followed by Dunn's Multiple Comparison post-hoc test to determine individual group differences. A Mann-Whitney test was used to compare disease severity between D-MG and ND-MG patient groups for both the QMG and MG-ADL scales. Baseline and fatigue MMT strength measurements for the deltoid muscle were compared separately for D-MG and ND-MG patient groups using a two-tailed paired t-test.

Normalized initial MVIC peak torque values were compared across groups using a Kruskal-Wallis test. Within each group, change in peak torque over time was analyzed using a repeated measures ANOVA across all 12 contractions. Peak torque values normalized to individual MVICs of contraction numbers 1, 6, and 12 of the fatigue protocol were further compared between groups using a Kruskal-Wallis test. A Bonferonni correction, with an α level set at 0.017, was applied to determine individual group differences. A repeated measures ANOVA, with a square root transformation to improve equality of variance, was used to compare the recovery peak torque values between groups. An analysis of covariance, adjusting for MVIC, was used to compare the 0.5 and 15 minute recovery contractions between groups.

Data were analyzed using GraphPad Prism version 4.00 for Windows (GraphPad software, San Diego, California).

2.2 RESULTS

2.2.1 Subject Characteristics

Subject characteristics are presented in Table 2.1. Participants ranged in age from 22 to 81 years, with an average age of 53.61 years (SD = 18.37 years). The majority of subjects were of Caucasian descent, with the exception of one Egyptian subject belonging to the D-MG group. According to international classification standards for body mass index ($\text{height (m)} / \text{weight (kg)}^2$), on average both patient groups were overweight (BMI ≥ 25.00) whereas control subjects were within normal ranges (BMI, 18.50 – 24.99).

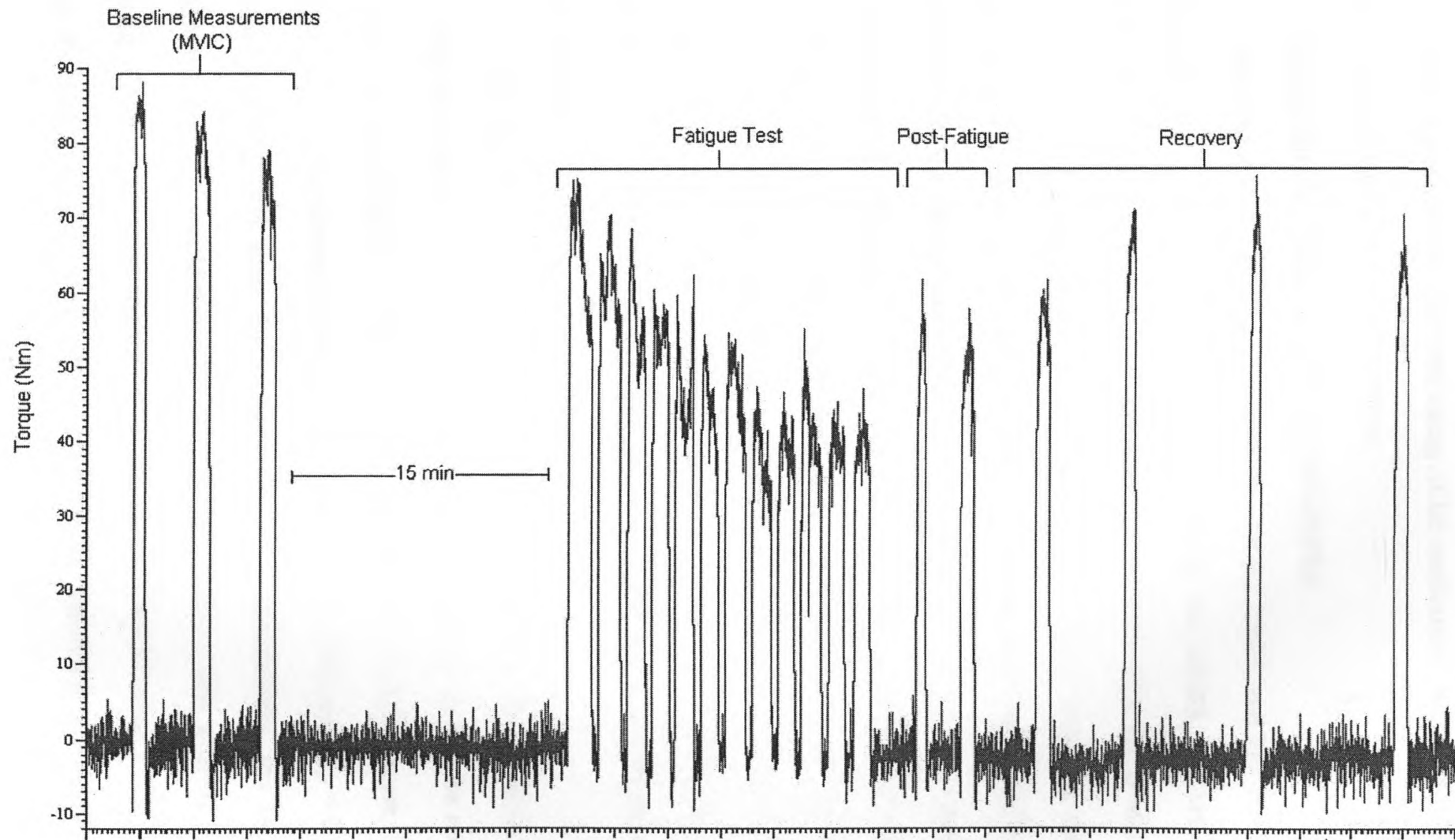


Figure 2.1. Example of experimental protocol. Data is representative of a male control subject. The x-axis, time (min), is not drawn to scale. See text for details. (MVIC = maximal voluntary isometric contraction).

The majority of patients were currently taking AChE medications, such as ®Mestinon, as a part of their symptomatic treatment regime.

2.2.2 Fatigue Questionnaires and Clinical Examination

An overview of scores resulting from the MGFS, QMG, and MG-ADL clinical instruments are included in Table 2.2. According to the MGFS, ND-MG patients experience more fatigue compared with control subjects ($p < 0.05$). The D-MG patients also describe greater fatigue than control subjects ($p < 0.001$). However, the fatigue scores between patient groups were not different. Results from the 2-item VAS were consistent with the MGFS scores. When subjects were asked to quantify their current level of fatigue, D-MG patients indicated a higher level of fatigue compared with control subjects ($p < 0.01$). In the past month relative to testing, fatigue also had a greater impact on ADL in the ND-MG and D-MG groups compared with controls ($p < 0.01$ and $p < 0.001$, respectively). In summary, subjective appraisal of the degree of fatigue experienced between ND-MG and D-MG groups appears similar and both patient groups describe greater fatigue compared with controls.

The clinical evaluation of patients with MG, using the QMG and MG-ADL scales in addition to MMT, yielded valuable information concerning the disease severity and muscle strength differences between groups. According to the QMG score, the D-MG group had a greater disease severity compared with the ND-MG group ($p < 0.05$). When examining the QMG score for shoulder weakness independently, the D-MG group demonstrated mild shoulder weakness (1.25 ± 0.86 , mean \pm SD) whereas the ND-MG group did not demonstrate any shoulder weakness (0.33 ± 0.50 , mean \pm SD). The scores on the MG-ADL scale provided consistent results, indicating that the D-MG group

experienced more severe fatigue compared with the ND-MG group ($p < 0.01$). All of the patients within the ND-MG group had normal strength at the baseline MMT assessment of each of the included muscle groups. In comparison, the D-MG group achieved a mean overall MMT Megascoring of 67.53 (SD = 2.43), with scores ranging from 61 to 70. The deltoid muscle of both patient groups had reduced strength at the fatigue MMT assessment compared with baseline MMT scores (D-MG group, $p < 0.0001$; ND-MG group, $p < 0.05$). In summary, a clinical evaluation of both patient groups indicated that the D-MG group had greater disease severity, but similar baseline upper extremity strength as the ND-MG group.

2.2.3 Repetitive Nerve Stimulation

A summary of the percent decrement values of the recorded CMAP response upon RNS are included for both spinal accessory and axillary nerve stimulations in Table 2.3 and Table 2.4, respectively. Values indicate a comparison of negative peak amplitude (NpAmp) between the first and the fourth CMAP response⁸.

2.2.4 Force Measurement

Peak torque (N·m) and peak torque normalized to MVIC torque values for the shoulder abduction fatigue test contractions are displayed in Figure 2.2. Controls had greater MVIC force compared with the patient groups ($p < 0.001$). Further, the ND-MG group had greater MVIC force compared with the D-MG group. Within all three groups force decreased with time ($p < 0.001$). Between groups analysis revealed that peak torque values normalized to MVIC were different between controls and the D-MG group at contraction #6 ($p = 0.016$). No between group differences were found at contraction #1

Table 2.1. Subject Characteristics

	Age (years) mean \pm SD	Male : Female	BMI mean \pm SD	Currently taking AChE inhibitor medications (n)
Controls (n=21)	53.81 \pm 20.30	10 : 11	24.89 \pm 4.21	
Non-Decrementing MG (n=8)	54.25 \pm 16.80	6 : 2	28.75 \pm 5.36	4
Decrementing MG (n=12)	52.83 \pm 17.24	5 : 7	27.53 \pm 5.22	10

BMI= body mass index, AChE = acetylcholinesterase

Table 2.2. Clinical Scales

	MGFS		QMG		MG-ADL	
	total range(25-130)		total range (0-39)		total range (0-24)	
	mean \pm SD	range	mean \pm SD	range	mean \pm SD	range
Controls	40.86 \pm 9.88	26 to 68				
Non-Decrementing MG	57.38 \pm 13.44	46 to 82	4.00 \pm 3.16	0 to 8	1.13 \pm 1.55	0 to 4
Decrementing MG	70.92 \pm 23.20	37 to 121	10.50 \pm 5.57	4 to 21	4.75 \pm 2.96	1 to 10

MGFS = myasthenia gravis fatigue survey, QMG = quantitative myasthenia gravis score,
MG-ADL = myasthenia gravis activities of daily living scale

Table 2.3. Repetitive Nerve Stimulation of Spinal Accessory Nerve

		Controls	ND-MG	D-MG
Rest	%NpAmp of CMAP	-0.8	-3.4	-14.8
	range	-7.2 to 8.2	-7.0 to -0.7	-37.8 to -1.3
	n	21	8	12
Post 10 s of exercise	%NpAmp of CMAP	0.4	-1.9	-13.5
	range	-7.3 to 14.6	-4.8 to 0.7	-32.1 to -1.3
	n	21	8	11
3 min post 1 min exercise	% NpAmp of CMAP	-1.1	-3.6	-15.5
	range	-7.1 to 5.3	-7.2 to -0.5	-39.3 to 0.9
	n	21	8	12

ND-MG = non-decrementing myasthenia gravis, D-MG = decrementing myasthenia gravis, NpAmp = negative peak amplitude, CMAP = compound muscle action potential, n = number of subjects

Table 2.4. Repetitive Nerve Stimulation of Axillary Nerve

		Controls	ND-MG	D-MG
Rest	%NpAmp of CMAP	-2.5	-2.9	-25.7
	range	-9.9 to 5.2	-6.5 to 2.5	-47.9 to -7.1
	n	21	7	12
Post 10 s of exercise	%NpAmp of CMAP	0.3	0.5	-20.0
	range	-4.9 to 7.2	-2.8 to 5.8	-37.3 to -5.1
	n	20	7	12
3 min post 1 min exercise	% NpAmp of CMAP	0.8	-4.0	-31.6
	range	-5.1 to 11.1	-8.8 to -0.4	-56.3 to -14.0
	n	18	7	12

ND-MG = non-decrementing myasthenia gravis, D-MG = decrementing myasthenia gravis, NpAmp = negative peak amplitude, CMAP = compound muscle action potential, n = number of subjects

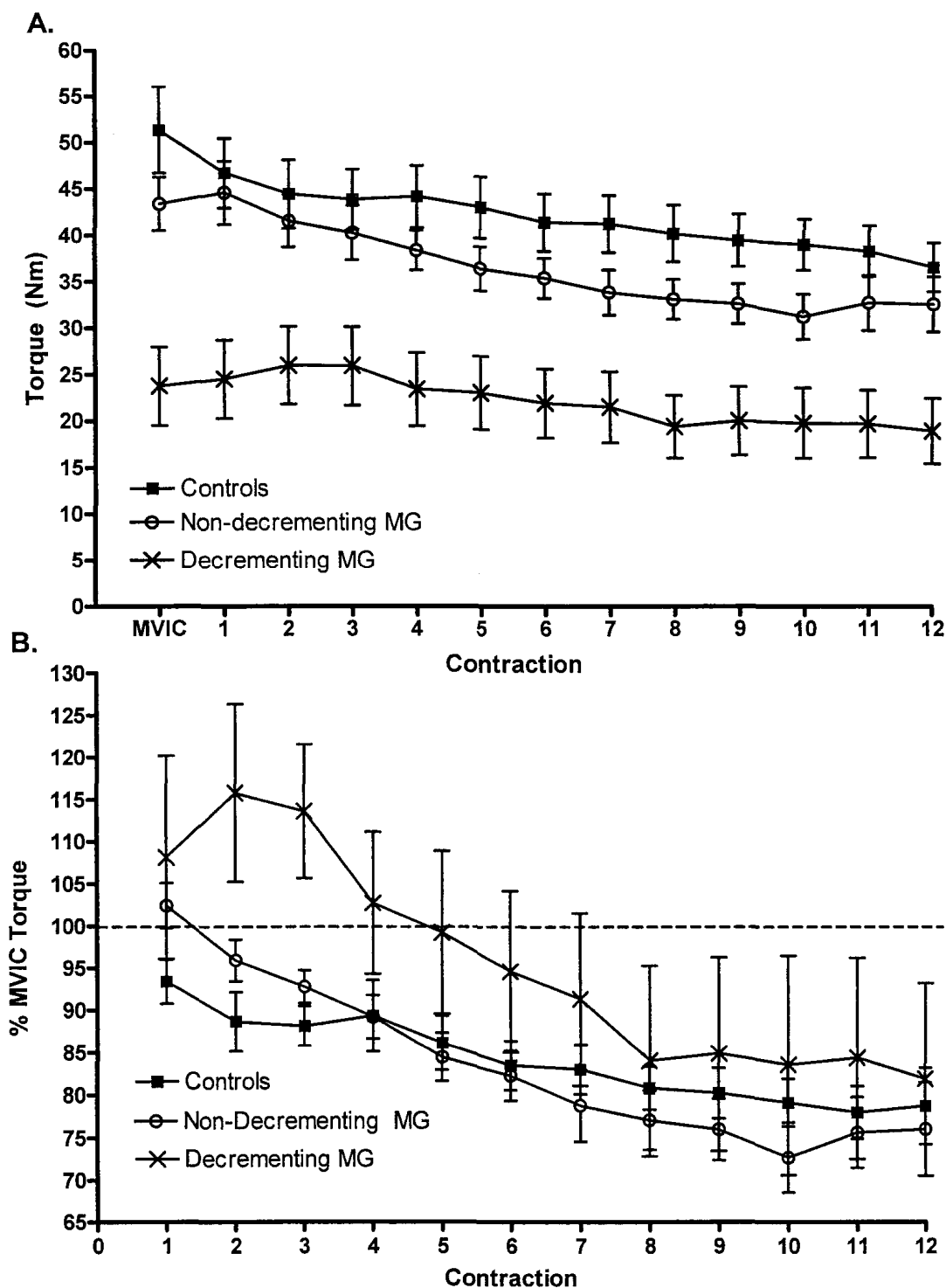


Figure 2.2. Fatigue test. *A*: Peak torque (Nm) values of shoulder abduction fatigue test contractions. *B*: Peak torque normalized to MVIC torque values for fatigue test contractions. Dashed line indicates one hundred percent of initial MVIC. Data are presented as means \pm SEM. (MVIC = maximal voluntary isometric contraction).

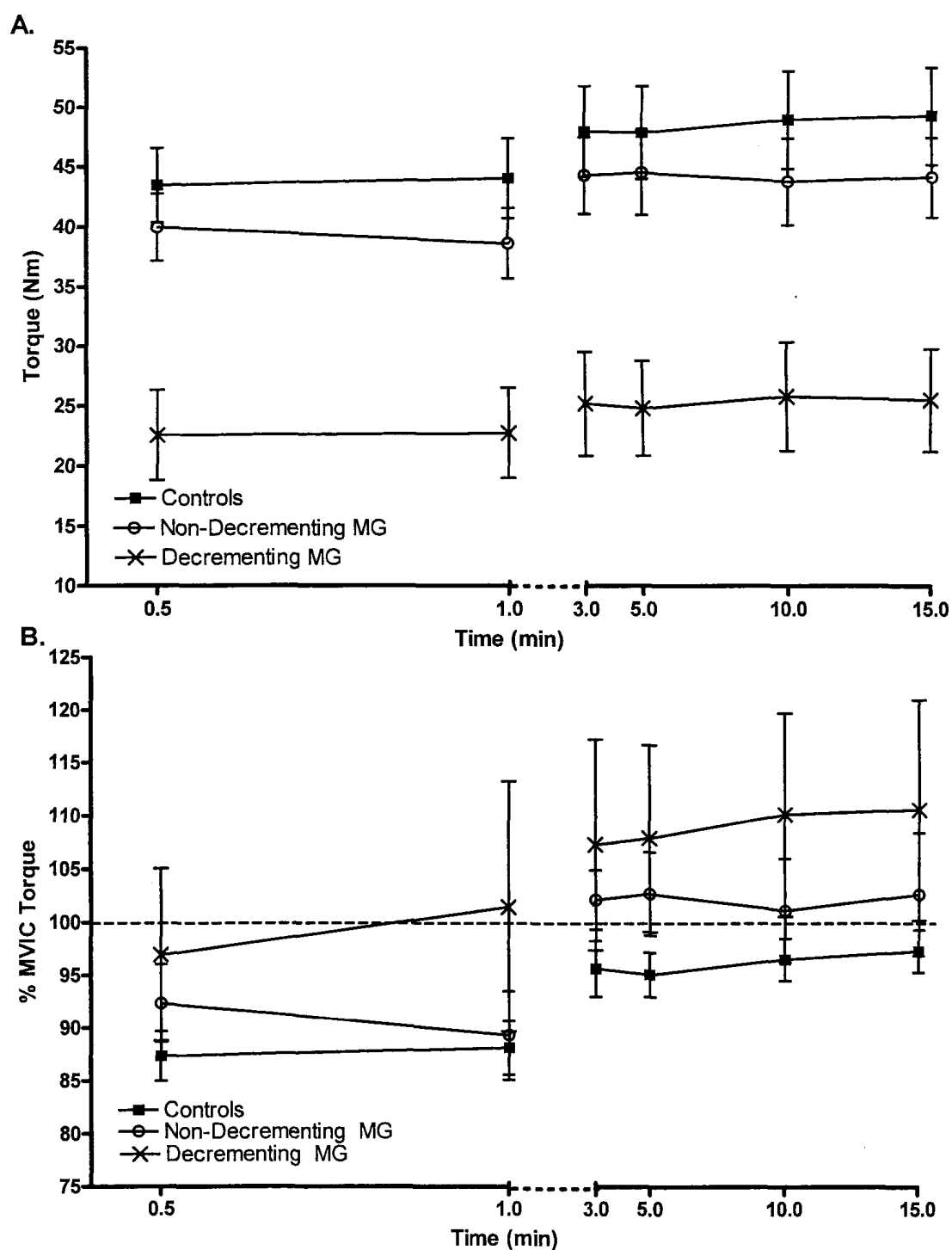


Figure 2.3. Recovery Phase. *A*: Recovery phase peak torque (Nm) values for shoulder abduction contractions. *B*: Peak torque normalized to MVIC torque values for recovery phase contractions. Dashed line indicates one hundred percent of initial MVIC. Data are presented as means \pm SEM. (MVIC = maximal voluntary isometric contraction).

and #12 ($p > 0.017$). Although each group had different initial MVIC force values, the fatigue index between groups was not different.

Recovery phase peak torque (N·m) and peak torque normalized to MVIC torque values are shown in Figure 2.3. Adjusting for MVIC values, there is no evidence that the behavior during recovery differs between groups ($p > 0.05$). In addition, there is no evidence of a difference in recovery between groups at the 0.5 min post-fatigue time ($p = 0.496$) and 15 minute recovery times ($p = 0.823$). Overall, there is no difference in recovery between groups.

2.3 DISCUSSION

The major finding of this study was that fatigue and recovery of normalized peak torque values for shoulder abduction did not differ between patients with MG and healthy controls. However, the baseline maximum voluntary isometric (MVIC) strength differed between groups. The D-MG group produced the lowest MVIC force, followed by the ND-MG group and controls, respectively. All of the ND-MG patients demonstrated normal baseline strength of the upper extremity muscles examined with MMT. The D-MG group exhibited mild weakness according to the overall Megascor obtained from all the upper extremity muscles examined with MMT. Additionally, patients with MG reported experiencing greater fatigue compared with controls. However, self-report fatigue did not differ between D-MG and ND-MG patient groups. Clinical measures, such as the QMG and MG-ADL, indicated that the D-MG group had greater disease severity relative to the ND-MG group.

As weakness and fatigue are predominant characteristics of MG^{20,24,36}, the observation that patients with MG report greater fatigue than healthy control subjects is well supported by clinical reports^{36,24} and research which has utilized self-report fatigue questionnaires in this population^{40,27,18}. In the present study, however, higher levels of experienced fatigue in patients with MG did not translate into greater muscle fatigue, as directly measured by a force dynamometer, as compared to healthy controls. Similar findings have been reported in studies examining patients with Chronic Fatigue syndrome⁴⁸. Patients with Chronic Fatigue Syndrome complain of weakness and fatigue and experience high levels of effort associated with activity with no apparent differences in measured muscle fatigue compared to healthy controls⁴⁸. In addition, a study examining patients with MS reported no difference in muscle fatigue between patients who reported experiencing high (75th percentile) versus low (25th percentile) fatigue using a multidimensional fatigue assessment tool⁴². In patients with MG, one possible explanation of higher experienced fatigue is that confounding variables, such as physical inactivity, sleep disturbances, and psychological factors, contributed to an increased perception of fatigue. A relationship between the pathological decrease in muscle strength and a subsequent decrease in physical activity, increase in sleep disturbances, and increase in pain has been determined using other NMD models^{22,40,18}.

The consequence of the maladaptive responses to reduced muscle strength have been further linked to higher levels of experienced fatigue in a longitudinal research study using three unique NMD models²². In addition, patients with NMD in the longitudinal research study demonstrated greater central activation failure (CAF) which may indicate reduced motivation or effort related to their higher reported level of

experienced fatigue⁴⁶. In a study by Kittiwatanapaisan et al.²⁷, a negative correlation between physical activity and fatigue scores was found in patients with MG. The majority of the participants with MG in the Kittiwatanapaisan et al.²⁷ study were overweight (B.M.I ≥ 25.00) or obese which is a potential side effect of therapeutic steroid medications. The higher B.M.I scores in this group may also be an indirect cause or effect of reduced physical activity, subsequently leading to an increased perception of fatigue. Patients with MG who engage in low-impact exercise have reported lower fatigue severity scores and improved functional capacity compared to their inactive counterparts¹⁸. In the present study although the physical activity status of patients was not measured, the majority of patients with MG were overweight or obese. The interaction between experienced fatigue and physiological muscle fatigue merits further investigation. In addition, determining the impact of confounding variables such as physical inactivity, sleep disturbances, and psychological variables on experienced fatigue in patients with MG will offer the opportunity to implement new strategies to reduce experienced fatigue in this population.

The D-MG and ND-MG patient groups did not differ in their reported level of experienced fatigue. The origin of experienced fatigue in both patient groups may be related to the same aforementioned confounding factors which have the potential to arise in addition to the fundamental pathophysiology of MG, that is, NMT failure. Alternatively, as the sensitivity of RNS for detecting NMT failure is not as high as other techniques such as SFEMG^{8,36}, RNS results may not accurately reflect experienced fatigue. A study by Rostedt et al.⁴³ found no correlation between a global functional fatigue questionnaire score and RNS results of the deltoid muscle in patients with MG.

The global fatigue questionnaire score was derived from individual scores on the myasthenia gravis questionnaire developed by Padua et al.³⁹ and the short form questionnaire (SF-36). However, a positive correlation was found between the global questionnaire score and jitter and blocking on SFEMG results. The increased time and patient co-operation required for SFEMG, made RNS a more preferable alternative for differentiating patient groups in the present study.

According to clinical indicators, the D-MG patient group had more severe MG relative to the ND-MG group. Early animal studies have supported a relationship between disease severity and positive RNS results. For example, an experiment by Berman et al.⁷ using a murine model of experimental autoimmune myasthenia gravis (EAMG) found decrement upon RNS with all moderately affected mice. In comparison, weakly myasthenic mice did not always demonstrate decrement⁷. Rat and guinea pig models with EAMG have also revealed that some animals demonstrate muscle weakness with no electrophysiological correlates²⁹. In the current study, both patient groups with MG were weaker in comparison to healthy control subjects. Further, D-MG patients were weaker than ND-MG patients. Upper extremity weakness detected by MMT was found to relate to decrement upon RNS, but was not able to detect the mild weakness found by direct force measurement in the ND-MG group. The greater reduction in muscle strength evident in the D-MG patient group may be explained by the corresponding greater disease severity of the group.

No overall difference in normalized fatigue or recovery force of shoulder abduction MVIC was evident between groups. A difference in normalized fatigue was found at contraction #6 between the D-MG group and controls, but that difference was

not apparent by contraction #12. Thus, in the first half of the contraction protocol fatigue developed more rapidly in the D-MG group compared to controls.

The absence of a difference in recovery between groups is somewhat surprising. Electrophysiological techniques such as RNS which employ exercise have demonstrated that decrement worsens 3-5 minutes post-exercise, a phenomenon referred to as post-activation exhaustion^{44,6}. Post-activation exhaustion has been attributed to desensitization of AChR coupled with the reduction of available ACh following exercise^{6,8,9}. With worsening decrement, a subsequent reduction in the capability of the muscle to recover during the 3-5 minute time epoch following the fatigue test may be expected. Thus, the absence of a difference in recovery between patients with MG and controls has yet to be explained.

Few studies have examined muscle fatigue in proximal muscle groups using a myasthenic model. Herbelin et al.¹⁹ used 30 s of maximal effort isometric exercise of hand grip, elbow flexion, elbow extension, knee extension, and ankle dorsiflexion to examine the relationship between isometric fatigue and decrement upon RNS, QMG scores, and MG-ADL scores in MG. Isometric fatigue testing was shown to relate best to decrement upon RNS and was found to account for ten percent of the variability of the QMG and MG-ADL scores. In the present study, as there was no difference in muscle fatigue between D-MG and ND-MG at either 30 s time period or at 60 s, fatigue scores of shoulder abduction contractions in MG do not reflect differences in RNS results.

An earlier study by Kimura et al.²⁶ suggested that MVC contractions may be used to reduce the potential for a false negative diagnosis resulting from routine electromyographic assessments in cases of ocular MG. By comparing changes in

parameters, such as EMG pulses and the mean amplitude of the CMAP response, during a voluntary MVC protocol, greater muscle fatigue of the orbicularis oculi muscle was found in patients with MG compared to controls. An injection of tensilon, a short-acting AChE inhibitor, significantly improved muscle fatigue and recovery in MG compared to controls. However when examining muscle fatigue of proximal limb muscles in the present study, no difference was found between the fatigability or recovery of patients with MG with varying RNS results and healthy controls subjects. Rest following activity has been shown to relieve fatigue in patients with MG^{36,24,25}. One possible explanation for the fatigue results in the current study is that adaptations in the muscle distal to the NMJ may occur in patients with MG that favor fatigue resistance with subsequently similar recovery relative to controls.

Shoulder muscle strength of patients with MG was significantly reduced with no difference in muscle fatigue compared to healthy controls. Studies examining fatigue in elderly subjects provide similar findings of preserved endurance accompanying reduced strength^{35,3,32,23}. Elderly subjects may experience adaptations in the muscle due to ageing and deconditioning^{2,16}. Changes in the fiber composition of the muscle, impairment of high threshold fatigable motor units, and muscle atrophy have all been proposed as potential mechanisms mediating lower muscle fatigue recorded in the elderly^{35,3,32}. A decrease in muscle relaxation rate represents another candidate modulating the preservation of force in fatiguing muscles^{14,3}. In addition, afferent feedback from peripheral sources during fatigue may impact changes in descending drive from the CNS affecting recruitment and rate coding patterns of MNs^{4,14}. Although the pathophysiology

of MG is understood as impairment of NMT, the possibility of further adaptations of the muscle or the CNS to accommodate a disturbance in NMT demands further exploration.

A study by McKenzie and Gandevia³³ provides evidence of a relationship between absolute muscle force and fatigability. Subjects performed intermittent MVIC of the elbow flexors or inspiratory muscles at two different muscle lengths, an optimal length and a length that is shown to decrease the absolute MVIC by 25%. Although absolute force was different, all contractions were performed with maximal effort relative to each muscle length. Each muscle tested was less fatigued at the shorter length and thus the lower absolute force level. The force-fatigability relationship suggests that the greater the absolute force the more rapidly the muscle fatigues¹⁴. In patients with MG, the higher anticipated muscle fatigue due to the underlying pathophysiology of the disease may be curtailed by a significantly reduced absolute force compared to controls.

Studies examining other NMD models⁴⁶, including GBS¹⁷, have implicated the ability of patients to maintain the integrity of the intracellular state of the muscle due to reduced occlusion of blood flow from lower MVIC torques as a potential mechanism to explain reduced fatigue in patients relative to controls. In both studies^{46,17}, sustained MVIC contractions were used in comparison to the intermittent MVIC contractions used in the present study. Thus, potential improvement in blood flow in the control subjects with intermittent versus sustained contractions may explain the absence of a difference in fatigue between patients and controls found in the current study. In addition, both Schillings et al.⁴⁶ and Garssen et al.¹⁷ found higher central activation failure (CAF) in patients with NMD compared to controls. Higher CAF may account for higher levels of experienced fatigue in these populations^{46,17}. Measuring central fatigue, potentially

manifested as higher experienced fatigue, in a myasthenia model may provide further insight into physiological adaptations that occur in response to pathological perturbations of the NMJ.

2.4 LIMITATIONS

It is important to consider the limitations of the present study before attempting to apply the findings to a broader population of patients with MG. One limitation of the study was that the sample size of each subject group was small. Approximately 20-25% of patients with MG do not demonstrate decrement upon RNS³⁸. Of that population, finding patients who reported experiencing fatigue of the shoulder made recruitment of the ND-MG group challenging. In addition, a large variance existed within and between each group. Efforts were made to achieve a similar distribution of both age and gender within groups. However, results from the experimental contraction protocol and the QMG encompassed a wide dispersal of values, possibly reflecting the inclusion of both genders and a wide age range of participants within the present study. Particularly within the D-MG group, definite differences in strength were evident. Interestingly, only in some cases did the subjects with greater recorded strength deficits correspond to greater disease severity or level of experienced fatigue in comparison to other group members. Small sample sizes and a wide variance within and between each group suggest that results from the present investigation should be applied with caution to the broader myasthenic population.

As only the shoulder muscles were tested, extrapolating the study results broadly to other muscle groups in MG may not be appropriate. Although the shoulder muscles

were a very reasonable selection given that MG is known to frequently affect proximal limb muscles^{36,21}, differences in muscle fatigability which may be attributed to fiber type composition of various muscles^{31,10}, may also differ between affected muscles in MG.

Performing intermittent MVICs of shoulder abduction is not customary in the day to day activities of most individuals. However, MG is a peripheral NMJ disorder and in the absence of central fatigue, individuals have been shown to be able to voluntarily maximally activate their deltoid muscle³⁴. Central fatigue is not acknowledged as a contributing mechanism of the primary pathological weakness and fatigue in MG. In addition, due to inherent challenges of measuring central fatigue in the given experimental set-up, it was not measured in the present study. In addition, contemporary methods used to measure voluntary drive rely on the assumption that there is no defect in NMT, an assumption which is clearly violated in patients with MG. Although recently, studies have begun to implicate CAF as an important variable to examine when studying weakness and fatigue in other NMD models^{46,17}. An increase in CAF in patient populations may act as a protective mechanism to prevent any redundant activation of abnormal NMJs. Thus, although muscle fatigue did not differ between patient groups including an indicator of CAF in the present study may have provided valuable information potentially further differentiating patients with MG who present with varying RNS results.

As an electrodiagnostic technique, RNS is also subject to inherent limitations. For example, as previously mentioned, the sensitivity of RNS is not as high as SFEMG. Thus, the potential for false-negative detections of impaired NMT is higher in RNS compared to SFEMG. The sensitivity of RNS was improved in the present study by testing two

symptomatic shoulder girdle muscles and by warming the muscles. In addition, all RNS trials were performed by an experienced EMG technologist. The aforementioned increased patient co-operation and time required to perform SFEMG, made RNS a preferred electrodiagnostic technique for differentiating patient groups in the present investigation.

Lastly, fatigue is a broad and multidimensional construct. The ability to directly link NMJ failure, as represented by decrement upon RNS, to muscle fatigability in the present study is somewhat limited. Adaptive responses to the underlying NMJ pathophysiology of MG may mask the true impact of NMJ failure on muscle weakness and fatigue in this population. A multidisciplinary approach was used in the present study to limit this problem, however in a clinical population group it is challenging to avoid all potentially confounding variables.

2.5 FUTURE STUDIES

The present study has succeeded in providing an introductory contribution to research in directly quantifying muscle weakness and fatigue in MG and has thus provided a framework to direct future investigations. A natural progression from the current findings would be to quantify the fatigue of more muscle groups within a myasthenic population. Developing a database of force and torque values for the strength and fatigue of different muscle groups would be useful in future clinical trials with MG. Repeating the present study using SFEMG instead of RNS to detect NMT failure and establishing a sub-group analysis based on disease severity, may also yield unique findings.

As experienced fatigue does not correspond to recorded shoulder muscle fatigue, exploring potential confounding variables such as physical inactivity, psychological factors, and sleep disturbances in subsequent investigations is important. Including a measurement of experienced fatigue, perhaps by the use of a VAS, at various time points during fatiguing and recovery contractions would provide a more complete understanding of any interactive effects between experienced fatigue and muscle fatigue and recovery within groups. In addition, including an indication of CAF may also shed light on potential differences between patients with MG who present with varying RNS results.

Examining the possibility of physiological adaptations to NMJ failure on muscle weakness, fatigue, and recovery represents an alternative arena for future investigations. A plethora of techniques are available to explore the contributions of various elements to muscle fatigue. For example, twitch interpolation is a putative technique used to examine central activation^{11,9}. Comparisons between the cross-sectional areas of muscles to examine for muscle atrophy, is possible with magnetic resonance imaging¹⁶. Biochemical analysis of muscle tissue obtained by a needle biopsy may reveal any metabolic adaptations to NMJ failure¹⁶. The feasibility or necessity of invasive research procedures, such as muscle biopsy, has yet to be determined in this clinical population. The influence of central fatigue in MG provides a logical starting point to elucidate other contributing mechanisms to weakness and fatigue following NMT failure.

2.6 CONCLUSION

The present investigation has achieved the objective of quantifying strength and fatigue in the shoulder muscles of patients with MG who present with varying RNS

results. Additional information pertaining to disease severity and experienced fatigue in this population, established from the use of multidisciplinary tools, have aligned with findings from previous investigations. No differences, with the exception of disease severity and muscle weakness, were found between the D-MG and ND-MG group. Differences in RNS results appear to relate most closely with disease severity. Obtaining quantitative measures of weakness, fatigue, and recovery in the muscles of patients with MG have implications in the potential re-evaluation of outcome measures used in clinical trials or in examining the effectiveness of non-pharmacological therapeutic interventions. Future investigations examining different muscle groups, in addition to the contribution of CAF to muscle fatigue, are required. The apparent discrepancy between experienced fatigue and measured muscle fatigue also needs to be resolved. The contributions of the present investigation to the understanding of weakness and fatigue in patients with MG who present with varying RNS results have provided a foundation for prospective growth in this area of research.

2.7 REFERENCES

1. Aitkens S, Lord J, Bernauer E, Fowler WM, Lieberman JS, Berck P. Relationship of Manual Muscle Testing to Objective Strength Measurements. *Muscle Nerve* 1989;12:173-177.
2. Allman BL, Rice CL. Neuromuscular fatigue and aging: central and peripheral factors. *Muscle Nerve* 2002;25(6):785-796.
3. Allman BL, Rice CL. An age-related shift in the force-frequency relationship affects quadriceps fatigability in old adults. *J Appl Physiol* 2004;96(3):1026-1032.
4. Asmussen E. Muscle Fatigue. *Medicine and Science in Sports* 1979;11(4):313-321.
5. Barohn RJ, McIntire D, Herbelin L, Wolfe GI, Nations S, Bryan WW. Reliability testing of the quantitative myasthenia gravis score. *Ann N Y Acad Sci* 1998;841:769-772.
6. Bashar K, Preston DC, Kaminski HJ, Ruff RL, Shapiro BE. *Neuromuscular Disorders in Clinical Practice*. Woburn: Butterworth-Heinemann; 2002. 1432 p.
7. Berman PW, Patrick J. Experimental myasthenia gravis. A murine system. *J Exp Med* 1980;151(1):204-223.
8. Brown WF, Bolton CF. *Clinical Electromyography*. Stoneham: Butterworth-Heinemann; 1993. 810 p.
9. Brown WF, Bolton CF, Aminoff MJ. *Neuromuscular Function and Disease: Basic, Clinical, and Electrodiagnostic Aspects*. Philadelphia: W.B. Saunders Company; 2002. 1867 p.
10. Cairns SP, Knicker AJ, Thompson MW, Sjogaard G. Evaluation of models used to study neuromuscular fatigue. *Exerc Sport Sci Rev* 2005;33(1):9-16.
11. Cheng AJ, Rice CL. Fatigue and recovery of power and isometric torque following isotonic knee extensions. *J Appl Physiol* 2005;99(4):1446-1452.

12. Chiou-Tan FY, Tim RW, Gilchrist JM. Literature review of the usefulness of repetitive nerve stimulation and single fiber EMG in the electrodiagnostic evaluation of patients with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome. *Muscle Nerve* 2001;24(9):1239-1247.
13. Council MR. *Aids to the Examination of the Peripheral Nervous System*. London: W.B. Saunders; 2000. 62 p.
14. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *J Appl Physiol* 1992;72(5):1631-1648.
15. Fenichel GM, Florence JM, Pestronk A, Mendell JR, Moxley RT, 3rd, Griggs RC, Brooke MH, Miller JP, Robison J, King W, et al. Long-term benefit from prednisone therapy in Duchenne muscular dystrophy. *Neurology* 1991;41(12):1874-1877.
16. Gardiner PF. *Neuromuscular aspects of physical activity*. Windsor: Human Kinetics; 2001. 236 p.
17. Garssen MP, Schillings ML, Van Doorn PA, Van Engelen BG, Zwarts MJ. Contribution of central and peripheral factors to residual fatigue in Guillain-Barre syndrome. *Muscle Nerve* 2007;36(1):93-99.
18. Grohar-Murray ME, Becker A, Reilly S, Ricci M. Self-care actions to manage fatigue among myasthenia gravis patients. *J Neurosci Nurs* 1998;30(3):191-199.
19. Herbelin LL, Nations SP, Wolfe GI, Foster B, Bryan WW, Barohn RJ. The correlation between static fatigue testing and the quantitative myasthenia gravis score and activities of daily living profile. *Neurology* 2001;56:A63.
20. Hughes BW, Moro De Casillas ML, Kaminski HJ. Pathophysiology of myasthenia gravis. *Semin Neurol* 2004;24(1):21-30.
21. Juel VC, Massey JM. Myasthenia gravis. *Orphanet J Rare Dis* 2007;2:44.
22. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. The development of a model of fatigue in neuromuscular disorders: a longitudinal study. *J Psychosom Res* 2007;62(5):571-579.

23. Katsiaras A, Newman AB, Kriska A, Brach J, Krishnaswami S, Feingold E, Kritchevsky SB, Li R, Harris TB, Schwartz A, Goodpaster BH. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. *J Appl Physiol* 2005;99(1):210-216.
24. Keeseey JC. AAEE Minimonograph #33: electrodiagnostic approach to defects of neuromuscular transmission. *Muscle Nerve* 1989;12(8):613-626.
25. Keeseey JC. Clinical evaluation and management of myasthenia gravis. *Muscle Nerve* 2004;29(4):484-505.
26. Kimura I, Ayyar DR, Sato G, Kogure K. Electromyographic assessment of fatiguability and recovery of orbicularis oculi in myasthenia gravis. *Tohoku J Exp Med* 1983;141(3):351-358.
27. Kittiwatanapaisan W, Gauthier DK, Williams AM, Oh SJ. Fatigue in Myasthenia Gravis patients. *J Neurosci Nurs* 2003;35(2):87-93, 106.
28. Lambert CP, Archer RL, Evans WJ. Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis. *Med Sci Sports Exerc* 2001;33(10):1613-1619.
29. Lennon VA, Lindstrom JM, Seybold ME. Experimental autoimmune myasthenia: A model of myasthenia gravis in rats and guinea pigs. *J Exp Med* 1975;141(6):1365-1375.
30. Lo YL, Dan YF, Leoh TH, Tan YE, Nurjannah S, Ratnagopal P. Effect of exercise on repetitive nerve stimulation studies: new appraisal of an old technique. *J Clin Neurophysiol* 2004;21(2):110-113.
31. MacIntosh BR, Gardiner PF, McComas AJ. *Skeletal Muscle Form and Function*. Windsor: Human Kinetics; 2006. 423 p.
32. Mademli L, Arampatzis A. Effect of voluntary activation on age-related muscle fatigue resistance. *J Biomech* 2008;41(6):1229-1235.
33. McKenzie DK, Gandevia SC. Influence of muscle length on human inspiratory and limb muscle endurance. *Respir Physiol* 1987;67(2):171-182.

34. Merton PA. Voluntary strength and fatigue. *J Physiol* 1954;123(3):553-564.
35. Muller F, Dehail P, Bestaven E, Petit J, Joseph PA, Barat M, Mazaux JM, Bourdel-Marchasson I. Maximal and sustained isokinetic lower-limb muscle strength in hospitalized older people. *Muscle Nerve* 2007;35(6):739-744.
36. Nicolle MW. Myasthenia gravis. *Neurologist* 2002;8(1):2-21.
37. O'Brien MD. Aids to the Examination of the Peripheral Nervous System. London: W.B. Saunders; 2000. 62 p.
38. Oh SJ, Kim DE, Kuruoglu R, Bradley RJ, Dwyer D. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. *Muscle Nerve* 1992;15(6):720-724.
39. Padua L, Evoli A, Aprile I, Caliandro P, Batocchi AP, Punzi C, Mazza S, Padua R, Tonali P. Myasthenia gravis outcome measure: development and validation of a disease-specific self-administered questionnaire. *Neurol Sci* 2002;23(2):59-68.
40. Paul RH, Cohen RA, Goldstein JM, Gilchrist JM. Fatigue and its impact on patients with myasthenia gravis. *Muscle Nerve* 2000;23(9):1402-1406.
41. Preston DC, Shapiro BE. Electromyography and Neuromuscular Disorders Clinical-Electrophysiological Correlations Philadelphia: Elsevier 2005. 685 p.
42. Romani A, Bergamaschi R, Candeloro E, Alfonsi E, Callieco R, Cosi V. Fatigue in multiple sclerosis: multidimensional assessment and response to symptomatic treatment. *Mult Scler* 2004;10(4):462-468.
43. Rostedt A, Padua L, Stalberg EV. Correlation between a patient-derived functional questionnaire and abnormal neuromuscular transmission in Myasthenia Gravis patients. *Clin Neurophysiol* 2005;116(9):2058-2064.
44. Rubin DI, Hentschel K. Is exercise necessary with repetitive nerve stimulation in evaluating patients with suspected myasthenia gravis? *Muscle Nerve* 2007;35(1):103-106.

45. Sanders DB, Tucker-Lipscomb B, Massey JM. A simple manual muscle test for myasthenia gravis: validation and comparison with the QMG score. *Ann N Y Acad Sci* 2003;998:440-444.
46. Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. *Clin Neurophysiol* 2007;118(2):292-300.
47. Schwid SR, Thornton CA, Pandya S, Manzur KL, Sanjak M, Petrie MD, McDermott MP, Goodman AD. Quantitative assessment of motor fatigue and strength in MS. *Neurology* 1999;53(4):743-750.
48. Stokes MJ, Cooper RG, Edwards RH. Normal muscle strength and fatigability in patients with effort syndromes. *Bmj* 1988;297(6655):1014-1017.
49. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. *Neurology* 1999;52(7):1487-1489.

APPENDIX A

Myasthenia Gravis Fatigue Scale (MGFS)

Following are a number of statements about fatigue related to Myasthenia Gravis. Respond to each statement by indicating how you generally feel when you experience fatigue. The following definition may help you: Fatigue is:

“a subjective experience ranging from tiredness to exhaustion and affects an individual’s ability to perform physical activity, mental activity, or both.”

DIRECTIONS: For the statement below, **circle** the number which best describes how you feel about the item.

1 = Never, 2 = Sometimes, 3 = Frequently, 4 = Usually, 5 = Always

1.	*Fatigue makes me feel weak.	1	2	3	4	5
2.	Fatigue makes me feel “worn out”.	1	2	3	4	5
3.	Fatigue always feels the same.	1	2	3	4	5
4.	I can’t make decisions when I am fatigued.	1	2	3	4	5
5.	I don’t have interest in life when I am fatigued.	1	2	3	4	5
6.	Fatigue makes me feel blue.	1	2	3	4	5
7.	Fatigue keeps me from enjoying usual things.	1	2	3	4	5
8.	*I get “tired” for no reason.	1	2	3	4	5
9.	I feel “tired” all the time.	1	2	3	4	5
10.	Fatigue prevents me from being as productive as I usually am.	1	2	3	4	5
11.	Fatigue prevents me from working.	1	2	3	4	5
12.	Fatigue forces me to stop what I am doing and rest.	1	2	3	4	5
13.	*When I am fatigued it takes me twice as long to do anything.	1	2	3	4	5
14.	Rest relieves my fatigue.	1	2	3	4	5
15.	*Family or co-workers must help me when I am fatigued.	1	2	3	4	5
16.	I plan my day around my pattern of fatigue.	1	2	3	4	5
17.	*When I am fatigued, I avoid unnecessary stress.	1	2	3	4	5
When I am fatigued, I have problems with:						
18.	swallowing	1	2	3	4	5
19.	breathing	1	2	3	4	5
20.	seeing (double vision)	1	2	3	4	5
21.	talking	1	2	3	4	5
22.	personal hygiene	1	2	3	4	5
23.	walking	1	2	3	4	5
24.	using my arms	1	2	3	4	5
25.	Once I become fatigued, it seems to become steadily worse.	1	2	3	4	5
26.	I am most fatigued: (choose only one)					
	upon waking					5
	late morning					4
	early afternoon					3
	early evening					2
	late evening					1

*Adapted from Eileen Hubsky and Jenean Sears.

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APPENDIX B**Visual Analogue Scale (VAS)**

Directions: For the statement below, **provide a mark on the line** which best describes how you feel about the item.

1. My current level of fatigue is:

None at all

Extreme

2. In the past month, fatigue has affected my activities of daily living:

Not at all

Always

APPENDIX C

Quantitative Myasthenia Gravis Score (QMG)

Subject Name: _____

Date of assessment: ____ / ____ / ____
day month year

Test Item	None	Mild	Moderate	Severe	Score
Grade	0	1	2	3	
Double vision on lateral Gaze <input type="checkbox"/> right or <input type="checkbox"/> left (tick one) for _____ seconds	61	11-60	1-10	Spontaneous	
Ptosis (upward gaze) for _____ seconds	61	11-60	1-10	Spontaneous	
Facial muscles	Normal lid closure	Complete, weak, some resistance	Complete, without resistance	Incomplete	
Swallowing 4 oz. water (1/2 cup)	Normal	Minimal coughing or throat clearing	Severe coughing/ choking or nasal regurgitation	Cannot swallow (test not attempted)	
Speech after counting aloud from 1 to 50 (onset of dysarthria) at _____	None at 50	Dysarthria at 30-49	Dysarthria at 10-29	Dysarthria at 9	
Right arm outstretched (90 degrees sitting) for _____ seconds	240	90-239	10-89	0-9	
Left arm outstretched (90 degrees sitting) for _____ seconds	240	90-239	10-89	0-9	
Vital capacity, % predicted _____	≥ 80	65-79	50-64	< 50	
Right hand grip, kgw Men Women	≥ 45 ≥ 30	15-44 10-29	5-14 5-9	0-4 0-4	
Lt-hand grip, kgw _____ Men Women	≥ 35 ≥ 25	15-34 10-24	5-14 5-9	0-4 0-4	
Head lifted (45 degrees supine) for _____ seconds	120	30-119	1-29	0	
Right leg outstretched (45 degrees supine) for _____ seconds	100	31-99	1-30	0	
Left leg outstretched (45 degrees supine) for _____ seconds	100	31-99	1-30	0	
Total QMG score (range, 0-39)					

Completed by: _____ Date: _____

APPENDIX D

Myasthenia Gravis- Activities of Daily Living Scale (MG-ADL)

NAME: _____ HANDEDNESS (circle): RIGHT LEFT

Date of Examination: _____ Time of Examination: _____

Test Items/Weakness	None	Mild	Moderate	Severe	Score
Grade	0	1	2	3	
Talking	Normal	Intermittent slurring or nasal speech.	Constant slurring or nasal, but can be understood.	Difficult to understand speech.	
Chewing	Normal	Fatigue with solid food.	Fatigue with soft food.	Gastric tube	
Swallowing	Normal	Rare episode of choking.	Frequent choking necessitating changes in diet.	Gastric tube	
Breathing	Normal	Shortness of breath with exertion.	Shortness of breath at rest.	Ventilator dependent	
Impairment of ability to brush teeth or comb hair	None	Extra effort, but no rest periods needed.	Rest periods needed.	Cannot do one of these functions.	
Impairment of ability to arise from chair.	None	Mild, sometimes uses arms.	Moderate, always uses arms.	Severe, requires assistance.	
Double Vision	None	Occurs, but not daily.	Daily, but not constant	Constant	
Eyelid Droop	None	Occurs, but not daily.	Daily, but not constant	Constant	
TOTAL SCORE (range 0 - 24)					

APPENDIX E



Office of Research Ethics

MAR 05 2007

The University of Western Ontario
 Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
 Telephone: (519) 861-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
 Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. T.J. Doherty

Review Number: 13059

Review Date: February 6, 2007

Revision Number:

Protocol Title: Quantitative evaluation of peripheral fatigue in myasthenia gravis patients

Department and Institution: Neurology, London Health Sciences Centre

Sponsor:

Ethics Approval Date: March 2, 2007

Expiry Date: June 30, 2008

Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent -Control Subjects, Letter of Information and Consent -Subjects with Myasthenia Gravis, study advertisement

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted full board approval to the above named research study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. John W. McDonald

Deputy Chair: Susan Hoddinott

Ethics Officer to Contact for Further Information

☐ Janice Sutherland (jsuther@uwo.ca) ☒ Jennifer McEwen (jmcewen4@uwo.ca) ☐ Denise Grafton (dgrafton@uwo.ca)

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MAY 23 2007

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. T.J. Doherty

Review Number: 13059

Revision Number: 2

Review Date: May 09, 2007

Review Level: Expedited

Protocol Title: Quantitative evaluation of peripheral fatigue in myasthenia gravis patients.

Department and Institution: Neurology, London Health Sciences Centre

Sponsor:

Ethics Approval Date: May 17, 2007

Expiry Date: June 30, 2008

Documents Reviewed and Approved: revised study methodology, revised study advertisement, revised Letter of Information and Consent, patient recruitment letter.

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

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Deputy Chair: Susan Hoddinott

Ethics Officer to Contact for Further Information

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. T.J. Doherty

Review Number: 13059

Revision Number: 3

Review Date: July 12, 2007

Review Level: Expedited

Protocol Title: Quantitative evaluation of peripheral fatigue in myasthenia gravis patients

Department and Institution: Neurology, London Health Sciences Centre

Sponsor:

Ethics Approval Date: July 31, 2007

Expiry Date: June 30, 2008

Documents Reviewed and Approved: revised inclusion/exclusion criteria, revised sample size, revised Letter of Information and Consent (Subjects with Myasthenia Gravis), revised Letter of Information and Consent (Control Subjects)

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

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<input checked="" type="checkbox"/> Jennifer McEwen (jmcewen4@uwo.ca)	<input type="checkbox"/> Denise Grafton (dgrafton@uwo.ca)	<input type="checkbox"/> Ethics Officer (ethics@uwo.ca)

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