UNIVERSITY OF CALGARY

Voluntary Muscle Activation and Exercise Recovery in Chronic Fatigue Syndrome

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

FACULTY OF KINESIOLOGY

CALGARY, ALBERTA

June, 2010

Your file Votre référence
ISBN:978-0-494-69608-8
Our file Notre référence
ISBN: 978-0-494-69608-8

Abstract

Individuals with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) commonly experience symptom exacerbations after exercise (post-exertional malaise). Interpolated twitch analysis and electromyography were used to quantify central and peripheral fatigue in the right quadriceps femoris muscles of 9 women with ME/CFS and 9 sedentary but otherwise healthy control subjects (CON) before and after 2 incremental cycle ergometer exercise tests to exhaustion 24 hrs apart. Peak O_2 consumption, heart rate and aerobic power were the same in both groups and on both tests. Although baseline MVC was similar (ME/CFS 85.1±24.1 N·m; CON 90.5±19.4 N·m), MVC was significantly decreased in ME/CFS after the 2^{nd} test ($p=0.040$). Voluntary activation ratio was lower in ME/CFS than CON (81.9 vs. 93.2 %, $p=0.012$) but there was no group by time interaction, suggesting that central fatigue did not cause the decreased MVC. Instead, low-frequency fatigue combined with central activation failure may explain the results and may contribute to post-exertional malaise.

Acknowledgements

I would like to thank the following people for their support throughout this process:

Dr. Brian MacIntosh, for all of the teaching, encouragement and patience you have shown me along this journey, and most of all, for allowing me to make this experience my own.

Dr. Eleanor Stein, for serving on my supervisory committee, and also for the endless hours spent recruiting and screening volunteers for the study.

Dr. Patrick Neary, for serving on my supervisory committee and providing me with invaluable advice and guidance along the way.

Dr. Richard Walker, for serving on my examining committee.

Jared Fletcher, for your assistance with data collection.

The dedicated volunteers for this study, who committed themselves 100 %.

The members of the Applied Muscle Physiology Lab, for sharing your knowledge and expertise with me and welcoming me into your family.

All of the faculty, staff and students in the Roger Jackson Center that I have gotten to know and work with over the last 3 years. Your friendship and support have been invaluable.

My family, for encouraging me to follow my dreams and supporting me in every way throughout this process.

And last, but certainly not least, all of my friends who have accompanied me along this journey and reminded me to work hard, play harder and enjoy every moment.
Thank you!

I would also like to acknowledge that chapters 3, 4 and 5 of this thesis are based upon a manuscript to be submitted for publication by myself, Jared R. Fletcher, Eleanor Stein and Brian R. MacIntosh entitled “Reduced voluntary muscle activation and exercise recovery in women with myalgic encephalomyelitis / chronic fatigue syndrome”.

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Symbol	Definition
[…]	Concentration of…
ACTH	Adrenocorticotropic Hormone
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BLa^-	Fingertip Blood Lactate
BP	Blood Pressure
bpm	Beats Per Minute
Ca^{2+}	Calcium Ion
CAF	Central Activation Failure
CDC	US Centers for Disease Control
Cl^-	Chloride Ion
$\dot{V}CO_2$	Rate of Carbon Dioxide Consumption
CON	Control Group
CRH	Corticotrophin Releasing Hormone
DHPR	Dihydropyridine Receptor
H^+	Hydrogen Ion / Proton
HADS	Hospital Anxiety and Depression Scale
HFF	High Frequency Fatigue
HPA Axis	Hypothalamic-Pituitary-Adrenal Axis
HR	Heart Rate
HRV	Heart Rate Variability
HSD	Honestly Significant Difference
IT	Interpolated Twitch
K^+	Potassium Ion
LFF	Low Frequency Fatigue
LT	Lactate Threshold
ME/CFS	Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome
MEP	Motor Evoked Potential
Mg^{2+}	Magnesium ion
MVC	Maximal Voluntary Contraction
NMJ	Neuromuscular Junction
$\dot{V}O_2$	Rate of Oxygen Consumption
^oC	Degrees Celsius
P_i	Inorganic Phosphate
POMS	Profiles of Mood States Questionnaire
P_{peak}	Maximal Aerobic Power
RCT	Randomized Controlled Trial
RER	Respiratory Exchange Ratio
RESTQ-Sport	Recovery-Stress Questionnaire for Athletes
RMS EMG	Root Mean Square Electromyography

RPE	Rating of Perceived Exertion
rpm	Revolutions per minute
RyR	Ryanodine Receptor-Ca^{2+} Release Channel
SA Node	Sinoatrial Node
SD	Standard Deviation
sEMG	Surface Electromyography
SF-36	Medical Outcomes Study Short Form – 36 Questionnaire
SpO_2	Blood Oxygen Saturation
SPSS	Statistical Package for the Social Sciences
SR	Sarcoplasmic Reticulum
TES	Transcranial Electric Stimulation
TMS	Transcranial Magnetic Stimulation
T_{post}	Electrically evoked twitch from rest after MVC
T_{pre}	Electrically evoked twitch from rest prior to MVC
VAR	Voluntary Activation Ratio

Epigraph

There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after.

J.R.R. Tolkien, The Lord of The Rings

Chapter One: Introduction

1.1 Introduction

Myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) is a debilitating condition with complex effects on the nervous, endocrine, immune and musculoskeletal systems. As the name suggests, patients with ME/CFS experience fatigue that is not only disproportionate to the effort that they have exerted, but their fatigue persists longer than that in healthy individuals. Fatigue has both psychological and physical components, although, the relationship between them is poorly understood. Thus, one aim of this thesis is to investigate the specific contribution of skeletal muscle fatigue to the psychological sensation of fatigue in ME/CFS. It is acknowledged that since skeletal muscle fatigue is only one aspect of the overall sensation of fatigue, there are likely to be additional factors contributing to the symptoms of ME/CFS.

The definition of ME/CFS has evolved as our understanding of the disease has progressed. Over the years, ME/CFS has been referred to by a variety of other names by patients, researchers and clinicians, including: *Chronic Fatigue Syndrome*, *Myalgic Encephalomyelitis, Post-infectious Disease Syndrome* and *Chronic Fatigue and Immune Dysfunction Syndrome.* ME/CFS is the term most commonly used in the recent literature, and therefore is the name used in this thesis. "Chronic fatigue syndrome" was first defined in 1988 by the US Centers for Disease Control and Prevention (CDC) as "fatigue lasting at least 6 months and causing at least a 50 % decrease in daily activity" (Holmes *et al.*, 1988). Shortly after, similar definitions were developed for both Australian (Lloyd *et al.*, 1990) and British (Sharpe *et al.*, 1991) populations. The CDC definition was based on the assumption that the symptom of extreme fatigue was unique to chronic fatigue syndrome, which is both incorrect and misleading. In 1994, Fukuda and colleagues revised the CDC definition to exclude any individuals whose fatigue could be caused by known illnesses, psychiatric disorders, a history of alcohol or substance abuse and/or severe obesity. Continued research has suggested that this definition is still not selective or specific enough to ensure a correct ME/CFS diagnosis. As a result, a group of prominent ME/CFS researchers collaborated to publish the Canadian Consensus

Document (Carruthers *et al.*, 2003). This document defines even more specific inclusion criteria and more stringent exclusion criteria for an ME/CFS diagnosis than the Fukuda definition, making it the best case-definition so far (Jason *et al.*, 2004). The Canadian Consensus Document states that an ME/CFS diagnosis requires fatigue lasting at least 6 months, post-exertional malaise, sleep dysfunction, myalgia, neurological and cognitive impairment and involvement of the autonomic, neuroendocrine or immune systems in addition to exclusion of any medical disorders that might explain the symptoms. Recently, it has been suggested that post-exertional malaise, the exacerbation of both physiological and psychological symptoms following a bout of exercise, may be a hallmark symptom of ME/CFS (Nijs *et al.*, 2008a; Nijs *et al.*, 2010; Van Oosterwijck *et al.*, 2010; VanNess *et al.*, 2010).

Despite the evolution of the ME/CFS case-definition, some researchers still use one of the older definitions. In conjunction with the complex diagnostic procedures required to confirm an ME/CFS diagnosis, this poses major problems for compiling and comparing the literature in this field. Since each definition varies in the criteria for an ME/CFS diagnosis, the subjects in 1 study cannot necessarily be compared to those of another if a different definition was used. Furthermore, within each definition, patients may suffer from different comorbidities and have varying levels of disease severity leading to heterogeneous study groups. Until both researchers and physicians agree on a universal definition for ME/CFS, heterogeneous study groups will make it difficult to compare between studies and to identify differences between individuals with ME/CFS and healthy or unhealthy controls.

The pathology of ME/CFS remains elusive, as there have been inconsistent reports (see chapter 2) of statistically significant differences in exercise capacity, endocrine, immune or autonomic nervous system function with respect to healthy individuals. Furthermore, there are varying reports of the primary symptoms of ME/CFS. Despite the uncertain pathology, specific predisposing factors, disease triggers and symptoms have been documented (Salit, 1997; Shephard, 2001; Afari and Buchwald, 2003; Cleare, 2003; Maquet *et al.*, 2006). Although some studies have suggested that ME/CFS may be associated with viral infection and reactivation (Levy, 1994; Afari and Buchwald, 2003;

Maquet *et al.*, 2006), or chronic overtraining (Fry *et al.*, 1991; Derman *et al.*, 1997; Rowbottom *et al.*, 1998), there is often no apparent triggering event. This has led researchers to believe that several interacting factors predispose an individual to ME/CFS, and that these factors subsequently trigger and perpetuate the condition (Maquet *et al.*, 2006).

Estimates of the prevalence of ME/CFS have ranged anywhere from 0.2 – 11.3 % of the population, depending on the stringency of the definition used and whether or not individuals with comorbidities such as major depression and fibromyalgia were included (Wessely, 1995; Wessely *et al.*, 1997; Maquet *et al.*, 2006; van't Leven *et al.*, 2010). ME/CFS is 2-3 times more common in females and symptom onset is usually in the late 30's or early 40's (Jason *et al.*, 1999). An even more concerning statistic is the fact that less than 10 % of individuals diagnosed with ME/CFS report full recovery without medical intervention and only 30-40 % report any improvement in symptom severity (van der Werf *et al.*, 2002; Nisenbaum *et al.*, 2003; Cairns and Hotopf, 2005; van't Leven *et al.*, 2010). Thus, it is imperative that better diagnostic procedures and treatment tools are developed for ME/CFS. In order to do this, a better understanding of what causes the symptoms is required.

A study published in 2007 (VanNess *et al.*, 2007) suggested that women with ME/CFS cannot reach the same $\dot{V}O_{2peak}$ on 2 incremental exercise tests to exhaustion 24 hrs apart while healthy women can. The reasons for this finding remain unclear. Changes in the ability to voluntarily activate skeletal muscle could influence not only the ability to generate force, but also the perceived effort of any given contraction. Since both of these factors could contribute to an inability to reach the same $\dot{V}O_{2peak}$ on the 2nd test, it seems appropriate to investigate whether there are changes in voluntary muscle activation in women with ME/CFS following an incremental exercise test to exhaustion.

1.2 Purpose

The overwhelming majority of research on ME/CFS has investigated the changes that occur in the endocrine, immune and autonomic nervous systems. There is relatively

little research focusing on changes in skeletal muscle function or in the role that central muscle activation may play in the development of post-exertional malaise in ME/CFS. Thus, this thesis was guided by 3 specific research objectives:

[1] To determine whether women diagnosed with ME/CFS were able to reach the same $\dot{V}O_{2peak}$ as age- and activity-matched but otherwise healthy females on an incremental bicycle exercise test to exhaustion.

[2] To confirm previous findings (VanNess *et al.*, 2007) that women with ME/CFS cannot reach the same $\dot{V}O_{2peak}$ on 2 incremental exercise tests to exhaustion 24 hrs apart while healthy women can.

[3] To investigate whether skeletal muscle fatigue, as evidenced by the presence of central activation failure (CAF), central fatigue and/or peripheral fatigue, is different following repeated incremental exercise tests to exhaustion in women with ME/CFS and in sedentary but otherwise healthy females.

1.3 Thesis Overview

This thesis consists of 6 chapters. The first chapter introduces ME/CFS as well as the purpose of this thesis. Chapter 2 consists of a literature review of skeletal muscle fatigue, the relevant physiological changes in ME/CFS and the rationale for using several questionnaires to evaluate quality of life, mood state and symptom severity in ME/CFS. The third chapter outlines, in detail, the methods employed in this thesis. Chapter 4 presents the results from the study while chapter 5 discusses their relevance for the diagnosis and treatment of ME/CFS. Finally, chapter 6 concludes this thesis with a summary of key findings, limitations and suggested future research.

Chapter Two: Literature Review

As alluded to in the introduction, ME/CFS is a complex disease with a vast number of symptoms that can differ in severity between individuals. Despite an increased research focus in the last 15-20 years, there is still a very limited understanding of how this disease develops and what physiological changes are associated with it. This chapter will begin with a discussion of skeletal muscle fatigue; how it is defined and how it can be measured *in vivo*. After that, the current understanding of the physiological changes associated with ME/CFS will be explored. Since questionnaires are often employed to monitor psychological changes in ME/CFS patients, the final section of this chapter will deal with the rationale behind the questionnaires chosen to evaluate mood state, symptoms and quality of life in this thesis.

2.1 Defining Skeletal Muscle Fatigue

The word "fatigue" is commonly used to describe the sensation of feeling exhausted, worn out or run down. Due to the subjective nature of this definition, fatigue has been further defined in the literature as an inability to maintain performance (Fitts, 1994). The question becomes, what is performance? Performance is some expected outcome, which might refer to a single effort, a series of repeated efforts, or a long term series of training sessions. A decrease in some criterion measure of performance is thus considered to indicate the presence of fatigue. Two examples of fatigue are a decrease in isometric contractile torque during a sustained maximal voluntary contraction (MVC) and a decline in peak power when 20 maximal effort squat jumps are repeated with minimal rest. While this definition of fatigue is correct, it does not provide any insight into how or why performance decreases.

A specific definition of skeletal muscle fatigue is "a decrease in muscle force generation for a given level of stimulation due to prior contractile activity" (MacIntosh and Rassier, 2002). This definition implies that fatigue can be present at all levels of muscle recruitment, not just at maximal effort. In other words, a less than anticipated muscle force at some submaximal stimulation frequency is indicative of fatigue. This

definition also stipulates that the change in muscle force generation is the direct result of prior muscle contraction. Therefore, this definition allows us to distinguish changes in muscle force generation due to fatigue from those due to long-term structural changes in the muscle, such as atrophy. It is also important to acknowledge that fatigue begins to develop almost immediately after a contraction begins. It does not occur abruptly at the moment when performance can no longer be maintained (Gandevia, 2001). The muscle is initially able to counteract the effects of fatigue by changing the pattern of muscle fibre recruitment, thereby maintaining performance. Eventually, the muscle can no longer overcome the effects of fatigue, and performance decreases. Thus, the ability to maintain performance does not necessarily mean that fatigue is not present.

There are many steps required to convert a conscious intent to contract a muscle into an actual contraction (Figure 2-1). The pathway begins with the excitation of neurons within the brain's pre-motor and motor cortex, signifying a conscious intent to contract a muscle. These neurons synapse with α-motor neurons within the spinal cord. The α-motor neuron cell body receives and integrates a multitude of excitatory and inhibitory post-synaptic potentials. If the net signal is excitatory, then a wave of action potentials travels down the axon towards the neuromuscular junction (NMJ). The subsequent release of acetylcholine from the synaptic terminals leads to depolarization of the muscle cell membrane (sarcolemma). Action potentials then spread along the sarcolemma and into the transverse (T)-tubules towards the center of the muscle fibre, ultimately leading to the release of calcium ions (Ca^{2+}) from the terminal cisternae of the sarcoplasmic reticulum (SR). Ca^{2+} then binds to troponin, causing a conformational change resulting in the exposure of a myosin binding site along each actin filament that is normally covered by tropomyosin. This allows actin-myosin crossbridges to form, and force generation to occur. Fatigue can result from the impairment of any of the steps in the excitatory pathway. Two types of fatigue: central and peripheral, can be identified based on the location of the step(s) that are impaired.

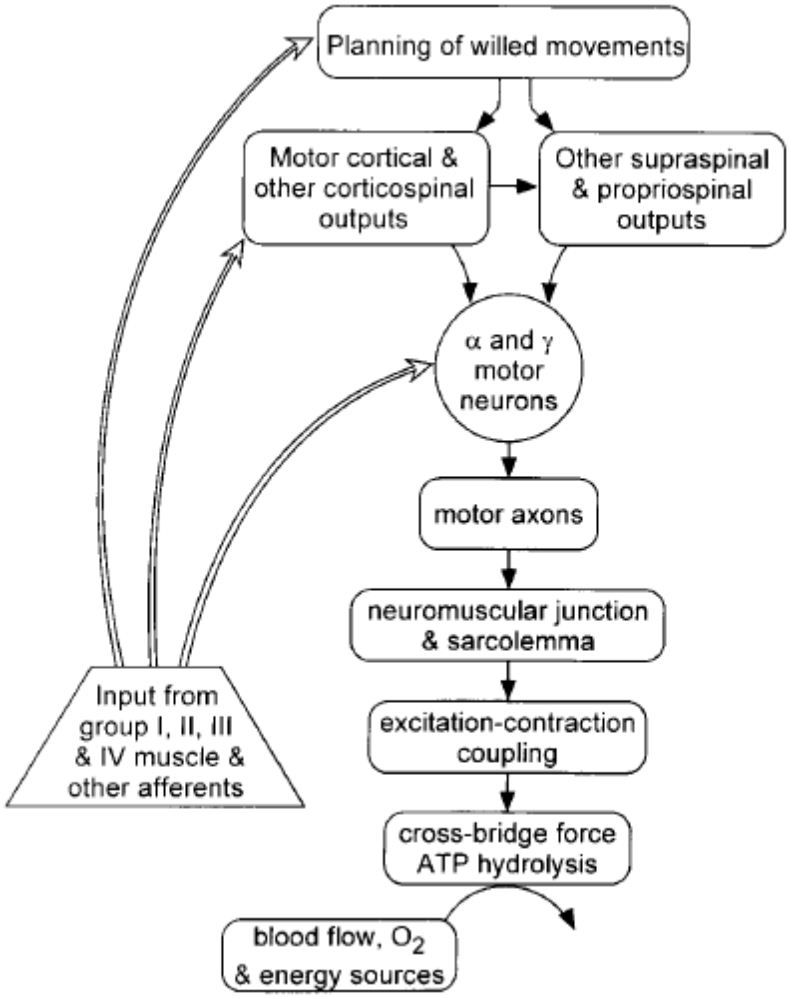

Figure 2-1. Diagram of the steps involved in voluntary muscle activation.

(From Gandevia, 2001, with permission)

2.1.1 Central Fatigue

Central fatigue results from changes in supraspinal and spinal signalling within the central nervous system. As mentioned above, each α-motor neuron cell body receives input from many other neurons, including interneurons, descending motor neurons and afferent sensory neurons. These inputs may be either excitatory or inhibitory in nature. A net increase in inhibitory input or a net decrease in excitatory input leads to decreased α-motor neuron excitability and fewer action potential signals generated on the α-motor neuron. This ultimately results in a reduction in motor unit (α-motor neuron plus all of the muscle fibres that it innervates) activation for a given level of conscious effort or intention, which is known as central fatigue.

Despite the fact that it is hard to demonstrate that changes in central nervous system activity during exercise actually cause a decrease in skeletal muscle force generation, there are several observations from both animal and human studies that support the existence of central fatigue. As far back as 1896, Waller showed that even once an individual could no longer voluntarily reach a target torque output, electrical stimulation of the α-motor neuron or muscle itself resulted in increased force production. This suggests that there is a central component to fatigue, which limits voluntary muscle torque below that which the muscle is actually capable of generating. It has been shown repeatedly in a variety of human skeletal muscles *in vivo* that electrical stimulation superimposed upon a maximal voluntary contraction (MVC) almost always leads to an increase in torque output (Belanger and McComas, 1981; Bigland-Ritchie *et al.*, 1986; Lloyd *et al.*, 1991; Herbert and Gandevia, 1996; Suter *et al.*, 1996; Allen *et al.*, 1998; Kent-Braun, 1999; Babault *et al.*, 2001; Schillings *et al.*, 2004). It is also evident from this research that the extent of voluntary activation varies between individuals and between muscle groups.

Similar increases in torque during sustained MVCs have been elicited using transcranial magnetic stimulation (TMS) and transcranial electric stimulation (TES) (Gandevia *et al.*, 1996; Taylor *et al.*, 2000). It has also been suggested that fluctuations in torque during a sustained MVC indicate central fatigue. It is thought that these fluctuations may be caused by (centrally mediated) changes in motor unit firing patterns

in an attempt to maintain torque output (Gandevia, 2001). In 1971, Marsden and colleagues showed that during a sustained MVC of the 1st dorsal interosseous muscle, motor unit firing rates decreased from 60-80 Hz at the start to only 20 Hz after 30 s. This too suggests that there is a central component of fatigue. Finally, the fact that verbal encouragement or motivation can lead to an increase in MVC torque provides even more evidence that MVC torque is not actually the maximal torque that the muscle is capable of generating (Bigland-Ritchie *et al.*, 1979).

There are a variety of proposed mechanisms for central fatigue, and it is likely that they all contribute in one instance or another (Gandevia, 2001). Feedback from sensory neurons originating within the muscle can modulate both supraspinal and α-motor neuron excitability. The decrease in α-motor neuron firing rates that has been recorded during sustained contractions, and thought to be indicative of central fatigue, may be the result of decreased activation from descending cortical neurons. On the other hand, this may actually be an intrinsic property of α-motor neurons themselves. Thirdly, there is evidence to suggest that both spinal and supraspinal neurons can modulate α-motor neuron excitability (Taylor *et al.*, 2006).

Muscle spindle inputs (group Ia and II), Golgi tendon organs (group Ib) and small diameter (group III and IV) muscle afferents all have the ability to modulate α-motor neuron excitability. Regardless of the exact site and mechanism of interaction, a net increase in inhibitory post-synaptic potentials in the cell body of an α-motor neuron ultimately leads to decreased excitability (Gandevia, 2001; Amann and Dempsey, 2008). Muscle spindles, which lie along the muscle belly, are the muscle's stretch sensors. There is evidence to suggest that these neurons are activated during an MVC and that their firing rates, like α-motor neurons, decrease over time (Wilson *et al.*, 1997). Several years previously, Gandevia and colleagues (1990) had shown that α-motor neuron discharge rates dropped by as much as 30% during a maximal voluntary contraction of the intrinsic hand muscles when the ulnar nerve was blocked distally, leading the researchers to infer that muscle spindle inputs facilitate α-motor neuron activity.

Despite this evidence, it is important to note that muscle spindle activity has not yet been recorded during naturally occurring muscle fatigue (as opposed to fatigue caused

by electrical stimulations or chemically-induced α-motor neuron blockade), and the extent to which these fibres contribute to central fatigue remains unknown. Amman and Dempsey (2008) inferred that neural feedback from fatiguing muscles modulates central motor drive such that the development of peripheral fatigue is not allowed to rise above a certain level. They asked cyclists to perform 2 standardized constant-load trials at different intensities and quantified the resultant peripheral fatigue by comparing the magnitude of a supramaximal electrical stimulus to the quadriceps femoris muscle prior to and 4 min after each trial. Then, they had the cyclists repeat these standardized trials followed 4 min later by a 5km time trial. They measured central drive to the muscles during the time trials by analyzing sEMG amplitude. They also repeated the supramaximal electrical stimulus following the time trial to estimate peripheral fatigue. They found that after the higher intensity constant-load trial, there was a greater decrease in EMG amplitude during the 5 km time trial, suggesting less central motor drive compared to the easier constant-load trial. However, the magnitude of peripheral fatigue at the end of the time trials was the same. The authors inferred that the greater amount of peripheral fatigue present after the higher intensity constant-load trial (as evidenced by a greater decrease in electrically stimulated twitch amplitude), caused a reduction in central motor drive during the time trial such that peripheral fatigue at the end of the time trial was not allowed to exceed a certain threshold. This, the authors concluded, was evidence of feedback from sensory neurons modulating α-motor neuron activation.

Unlike muscle spindles, Golgi tendon organs lie along the tendon and are sensitive to the tension (and therefore force) generated by the muscle. As tendon tension increases, so does Golgi tendon organ firing rate (Edin and Vallbo, 1990; Horcholle-Bossavit *et al.*, 1990; Gandevia, 2001). When activated, Golgi tendon organs inhibit α-motor neuron excitability. However, as described in a review by Gandevia (2001), Golgi tendon organs are subject to presynaptic modulation, and they have been shown to both stimulate and inhibit various interneurons, therefore it is challenging to predict their contribution to central fatigue.

Finally, group III and IV afferent neurons are widely distributed throughout skeletal muscle and they respond to local mechanical, biochemical and thermal stimuli

including stretch and contraction, hypoxemia and ischemia (Gandevia, 2001). Their discharge has been shown to increase during sustained MVC, especially when tissue perfusion is limited (Rotto and Kaufman, 1988). Like the Golgi tendon organs, these neurons synapse with and can therefore modulate interneurons within the spinal cord, thereby affecting α-motor neuron excitability. Figure 2-2 shows the proposed interactions between these sensory neurons that originate within the muscles, descending corticospinal inputs and the α-motor neurons innervating the muscles.

Considering the evidence from interpolated twitch analysis, TMS and TES, it appears that cortical excitability is submaximal even during maximal voluntary contractions and that descending corticospinal neurons can modulate α-motor neuron excitability. It is likely that a combination of changes in corticospinal activity as well as afferent neuromuscular feedback causes and perpetuates central fatigue.

2.1.1.1 Quantifying Central Fatigue

Central fatigue is a reduced ability to maximally activate all motor units during and following a fatiguing activity. As alluded to above, central fatigue can be measured using interpolated twitch analysis by determining the voluntary activation ratio (VAR) (Gandevia, 2001; Shield and Zhou, 2004). VAR represents the proportion of motor units that are maximally activated during an MVC. This technique involves delivering a supramaximal electrical stimulus to the α-motor neurons innervating the voluntarily activated muscle both during the MVC and 2-5 s after. The reason for a supramaximal stimulus is to ensure that all motor units are being activated. This can be an uncomfortable sensation, with multiple stimuli (doublets, triplets, etc.) being more painful than twitches. If there is any increase in torque amplitude when an electrically stimulated twitch (single stimulus) is superimposed upon an MVC in a fatigued state compared to a rested state, it implies that some motor units were not voluntarily maximally activated and therefore that central fatigue was present.

If, even in a non-fatigued state, there is a measurable increase in MVC torque with a superimposed stimulus, it implies that the individual is not able to voluntarily maximally activate all motor units. This is termed central activation failure (CAF). While

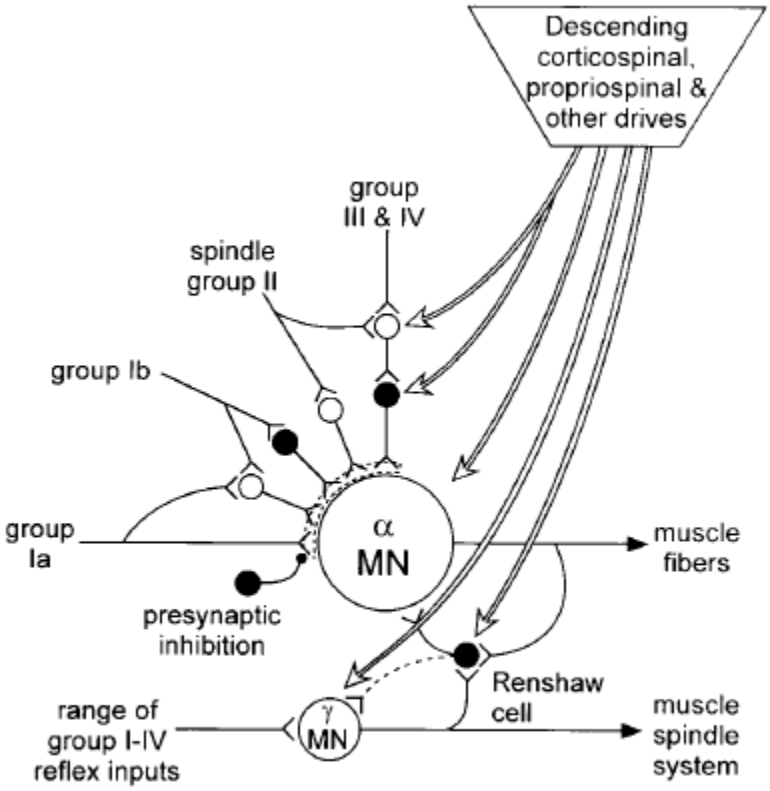

Figure 2-2. Inputs to an α-motor neuron cell body.

An α-motor neuron is shown, along with various inputs from sensory neurons originating in the muscles and descending corticospinal neurons. Open-circle cell bodies indicate an excitatory input while closed-circle cell bodies indicate an inhibitory input. Muscle spindles are group Ia and II, Golgi tendon organs are group Ib, and type III and IV are group III and IV, respectively. (From Gandevia, 2001, with permission)

VAR indicates the proportion of motor units activated during a voluntary contraction, CAF reflects the proportion of motor units not voluntarily activated. Therefore, when both VAR and CAF are expressed as percentages, VAR + CAF = 100. For example, if only 85 % of the motor units comprising the rectus femoris muscle are voluntarily maximally activated during an MVC, VAR = 85 % while CAF = 15 %. It is important not to confuse the decrease in muscle force associated with CAF and central fatigue, with muscle weakness. The decrease in muscle force in the presence of either CAF or central fatigue is due to submaximal motor unit activation, while a weak or atrophied muscle is simply not capable of generating more force. CAF and central fatigue are very similar. Indeed, the only difference between CAF and central fatigue is whether or not a prior fatiguing activity may have caused the reduction in muscle activation. Therefore, performing interpolated twitch analysis on an individual who has been resting evaluates CAF, while the same technique completed after a fatiguing exercise evaluates central fatigue.

Both central fatigue and CAF can also be measured using TMS and TES. TMS uses a precisely positioned magnetic field to induce current in a specific area of the motor cortex, leading to α-motor neuron activation and skeletal muscle contraction. TES, on the other hand, directly stimulates target cortical neurons. When using either TMS or TES, changes in the resultant sEMG response in a specific muscle to a given stimulus (termed a motor evoked potential (MEP)) are used to detect central fatigue. Like interpolated twitch analysis, stimuli are delivered during a sustained MVC and an increase in MEP amplitude suggests that cortical stimulation is suboptimal. The benefit of both TMS and TES over interpolated twitch analysis is that they can measure the level of activation of muscles innervated by nerves that are not superficial or easily accessed for direct stimulation. However, there are several drawbacks to TMS and TES such as the recruitment of both synergist and antagonist muscle groups which can confound voluntary activation measurements. The cortical neuron being stimulated may synapse with a variety of α-motor neurons leading to the activation of a variety of motor units. Also, it is not possible to estimate VAR, as the stimulus required to generate a superimposed twitch is less than a supramaximal stimulus at rest (Todd *et al.*, 2004).

Therefore, it is not possible to compare the magnitude of central fatigue measured by interpolated twitch analysis to that from TMS or TES.

Given the equipment available for this thesis, and the fact that the muscle groups of interest (vastus lateralis, rectus femoris and vastus medialis) are innervated by an easily accessed, superficial nerve (femoral), interpolated twitch analysis will be used to measure central fatigue and CAF. Given that the target population consists of individuals who are likely hypersensitive to painful stimuli and may already suffer from some level of myalgia (Carruthers *et al.*, 2003), electrically evoked twitches will be used instead of doublets or trains. Behm *et al.* (1996) have shown that VAR estimated using superimposed twitches was similar to that estimated using superimposed doublets or quintuplets.

2.1.2 Peripheral Fatigue

In contrast to central fatigue, peripheral fatigue results from changes in excitation-contraction coupling to ultimately cause either a decrease in Ca^{2+} release from the SR or reduced Ca^{2+} sensitivity within the muscle (Allen *et al.*, 2008). It is referred to as peripheral fatigue because the changes occur outside of the central nervous system. Reduced Ca^{2+} release from the SR can be the end result of a failure of action potential propagation down the α-motor neuron axon, along the muscle fibre sarcolemma or into the T-tubules, in addition to impaired transmission of the neural signal across the neuromuscular junction (NMJ). The exact mechanism behind impaired Ca^{2+} release is not known, but there are several proposed theories. Changes in Ca^{2+} sensitivity are thought to be related to the accumulation of several metabolites, particularly inorganic phosphate (P_i) and hydrogen ions (H^+) (Allen *et al.*, 1995).

Failure of action potential propagation down the α-motor neuron axon, along the sarcolemma or through the T-tubules may be the result of extracellular potassium ion (K^+) accumulation following repeated, high frequency stimuli. This could lead to a decrease in resting membrane potential (less negative) and therefore a decrease in action potential amplitude, thereby resulting in a decrease in dihydropyridine receptor (DHPR) activation. DHPR is responsible for opening the ryanodine receptor-Ca^{2+} release channels

(RyR) in the SR, so reduced DHPR activation leads to decreased Ca^{2+} release from the SR (Allen *et al.*, 2008). Although this mechanism has been suggested, there are factors that prevent an accumulation of extracellular K^+ from causing a decrease in the resting membrane potential of motor neuron axons *in vivo,* including the movement of chloride ions (Cl^-) to counteract the depolarizing effects of K^+ efflux from the cell (Allen *et al.*, 2008). Furthermore, the action of the many Na^+-K^+ ATPase pumps along the cell membrane helps to restore the K^+ concentration gradient by pumping K^+ back into the cell. In contrast, there are relatively few Na^+-K^+ ATPase pumps in the T-tubules, and this combined with the small lumen means that large fluctuations in extracellular [K^+] can occur in the T-tubules and may affect action potential propagation during high frequency stimulation (MacIntosh *et al.,* 2006)

Decreased Ca^{2+} release may also be mediated by changes in intracellular adenosine triphosphate (ATP), adenosine diphosphate (ADP) and magnesium ion (Mg^{2+}) levels during skeletal muscle contraction (Allen *et al.*, 2008). Laver *et al.* (2001) described an ATP regulatory site on the RyR that must be bound for optimal Ca^{2+} release. They also showed that ADP competes with ATP for this site, meaning that decreases in intracellular [ATP] and increases in [ADP] as a result of skeletal muscle contraction lead to reduced RyR opening and Ca^{2+} release. In a separate study, Laver and colleagues (Laver *et al.*, 1997) also showed that Mg^{2+} inhibits RyR activity, thereby leading to a decrease in Ca^{2+} release.

The accumulation of intracellular P_i as a result of both creatine phosphate metabolism and ATP hydrolysis by myosin ATPase, Na^+-K^+ ATPase and SR Ca^{2+} ATPase during muscular contraction is thought to be another major contributor to the development of peripheral fatigue (Westerblad *et al.*, 2002). Not only does P_i compete with ATP for binding to the ATP regulatory site on RyR, but it has also been shown to enter the SR and bind to Ca^{2+}, reducing the amount of free Ca^{2+} available for release (Dutka *et al.*, 2005). P_i also decreases Ca^{2+} sensitivity by decreasing the number of strong cross-bridges that can form (Millar and Homsher, 1990), although both Coupland *et al.* (2001) and MacIntosh (2003) have shown that the ability of P_i to decrease Ca^{2+}

sensitivity is mitigated at physiological temperatures compared to the cooler temperatures used in the Millar and Homsher study.

Finally, the increased $[H^+]$ as a result of repeated or prolonged muscle contraction was originally thought to reduce Ca^{2+} sensitivity in 2 ways. First of all, like P_i, it was shown to decrease the number of strong cross-bridges that form (Metzger and Moss, 1990). Secondly, Blanchard and colleagues (1984) showed that H^+ competes with Ca^{2+} for binding sites on troponin C, ultimately leading to a decrease in Ca^{2+} sensitivity. However, these early studies were completed at room temperature and studies completed closer to 37 ^oC, have shown a limited impact of $[H^+]$ on Ca^{2+} sensitivity at physiologic temperatures (Westerblad *et al.*, 1997). As a result, it has been questioned whether or not increasing $[H^+]$ with repeated or prolonged muscular contraction actually contributes to the development of fatigue (Keeton and Binder-Macleod, 2006). Regardless of which mechanism(s) are involved, a decrease in Ca^{2+} release from the SR leads to a less than expected force output given the level of α-motor neuron activation (assuming that Ca^{2+} has not increased), thereby satisfying the definition of fatigue.

2.1.2.1 Quantifying Peripheral Fatigue

The most commonly reported methods for quantifying peripheral fatigue are surface electromyography (sEMG) and electrical twitch stimulation. sEMG involves placing electrodes on the skin to detect the movement of electrical (ionic) signals as they travel down α-motor neuron axons towards the muscle fibres that they innervate. In theory, the amplitude of an sEMG signal is proportional to both the number of motor units recruited in a given contraction and the average frequency of activation of these motor units. At a relatively low level of voluntary activation, the sEMG signal amplitude is quite small. As voluntary activation increases, both the number of motor units recruited and the average frequency at which they are being activated increases. As a result, the amplitude of the sEMG signal also increases. In the presence of peripheral fatigue, the force generated by any specific motor unit for a given frequency of stimulation is decreased. Therefore, either an increase in the frequency of motor unit activation or the recruitment of new motor units, or a combination of both, must occur in order to maintain

a consistent torque output. Thus, an increase in the sEMG signal amplitude for a given torque means that more motor units were recruited and/or are firing at a higher frequency than before, indicating the presence of peripheral fatigue (Gandevia, 2001). It is the change in sEMG amplitude from 1 point in time to another that allows a conclusion to be made about the development of peripheral fatigue. Therefore, no conclusions can be made about the presence of peripheral fatigue at the initial point in time based on sEMG amplitude.

In addition to analyzing the amplitude of an sEMG signal, fast Fourier transformation can be used to analyze the frequency content of the sEMG signal itself. This frequency analysis relates to the oscillation of the sEMG signal and is unrelated to the frequency of motor unit activation. Instead, the frequency of the sEMG signal relates to the rate of signal conduction along the surface of the muscle fibre. A decrease in the median sEMG frequency during sustained muscle contractions indicates a decrease in muscle fibre signal conduction velocity, which has been suggested to be an indicator of fatigue (MacIntosh *et al.*, 2006). However, this frequency depression is short-lived and recovers well before the muscle regains its ability to generate torque; therefore, it is not the best tool to identify the presence of persistent fatigue.

Electrically evoked twitches from rest can also be used to quantify peripheral fatigue (Gandevia, 2001). By directly stimulating the α-motor neuron along its axon, both spinal and supraspinal factors of fatigue are eliminated. This stimulation can be achieved transcutaneously using rubber pads and conducting gel. A smaller twitch amplitude as a result of a given stimulation voltage, amperage and frequency indicates a net presence of peripheral fatigue. Usually, a single electrical stimulus is used to evoke a twitch, as stimulus trains are more painful than single twitches yet they may not be any more sensitive to the factors of fatigue (Behm *et al.*, 1996).

A more rigorous approach to measuring peripheral fatigue would be to electrically stimulate the motor nerve at a variety of frequencies in order to determine a force-frequency relationship for the muscle(s) of interest. This can allow the sub-classification of peripheral fatigue as either high- or low-frequency fatigue. This will now be discussed.

2.1.3 High vs. Low Frequency Fatigue

Depending on the mechanism causing peripheral fatigue, it may be measurable only at certain activation frequencies (Jones, 1996; Rassier and MacIntosh, 2000). Therefore, peripheral fatigue can be sub-classified as either high- or low- frequency fatigue based on the frequency of motor unit activation at which a decrement in skeletal muscle force can be detected. In order to do this, a force-frequency relationship for the muscle(s) of interest must be recorded in both the rested and fatigued state.

Cellular membranes are composed of a phospholipid bilayer which is impermeable to ions, however, protein channels are scattered along the surface of the membrane that allow selective ions to pass in and out of the cell down their electrochemical gradients. As a result, the concentration of various ions differs inside and outside the cell. The action of ionic pumps along the length of neuronal axons, as well as on the muscle cell sarcolemma and T-tubules, helps to maintain an electrochemical gradient across the cell membrane at rest for both Na^+ and K^+. These gradients are what drive the influx of Na^+ and efflux of K^+ when voltage-gated ion channels are opened during an action potential. Repetitive high frequency stimulation can lead to a decrease in the electrochemical gradient of both Na^+ and K^+ if the Na^+-K^+ ATPase pump cannot fully restore the gradient between action potentials (MacIntosh *et al.*, 2006). As mentioned in the above discussion of peripheral fatigue, the accumulation of extracellular K^+ can impair action potential propagation. This leads to a disproportionate decrease in force at high stimulation frequencies (Jones, 1996). Alternatively, a drop in extracellular Na^+ can lead to decreased action potential amplitude, resulting in reduced voltage-dependant activation of DHPR and decreased Ca^{2+} release (Westerblad *et al.*, 1991). Therefore, high frequency fatigue (HFF) is characterized by impaired maximal force production while submaximal force generation is largely unaffected (Jones, 1996; MacIntosh and Rassier, 2002). The observed decrease in maximal force is usually quite drastic, yet it tends to recover quite quickly once the stimulation frequency decreases (or stops altogether) (Jones, 1996). The force-frequency curve in a muscle with HFF is almost identical to that of the muscle in a rested state, except that the flat, upper portion of the curve is lower (Figure 2-3).

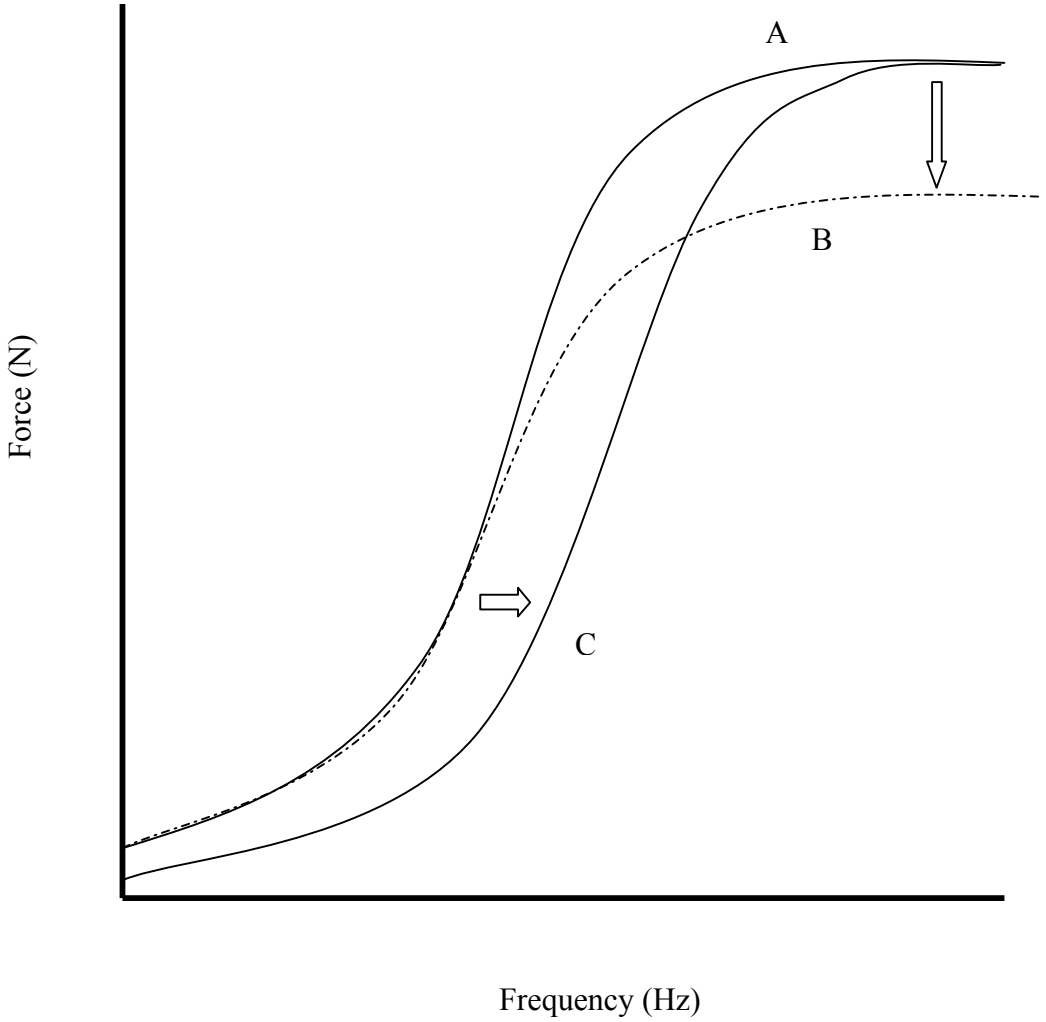

Figure 2-3. Hypothetical force-frequency curves showing HFF and LFF

The force-frequency curve for human skeletal muscle is S-shaped, or sigmoidal. Curve A represents the normal force-frequency curve. Curve B shows the disproportionate decrease in force at high stimulation frequencies characteristic of HFF. Curve C shows the disproportionate decrease in force at low stimulation frequencies characteristic of LFF.

In contrast, disruption of excitation-contraction coupling, specifically impaired release of intracellular Ca^{2+} from the SR, tends to result in a disproportionate drop in force at low stimulation frequencies (Westerblad *et al.*, 1993; Chin *et al.*, 1997; Rassier and MacIntosh, 2000; MacIntosh and Rassier, 2002). Low frequency fatigue (LFF) is characterized by impaired submaximal force generation despite normal maximal force generation, which is evident as a rightward shift in the force-frequency relationship (

Figure 2-3). Unlike HFF which tends to recover quickly, LFF may persist for several hours or even days after fatiguing exercise (Fitts, 1994; Jones, 1996).

Most human motor units function in the range of 10-50 Hz, with frequencies in the 10-20 Hz range considered to be low frequency, while those near 50 Hz are considered to be high frequency (Jones, 1996). It is important to understand that high frequency motor unit stimulation causes both HFF and LFF. However, because of the fast recovery of HFF upon cessation of stimulation, most researchers believe that LFF is the more important factor with respect to prolonged fatigue. If ME/CFS patients experience peripheral fatigue, then it is possible that LFF contributes to the sensation of persistent fatigue and post-exertional malaise.

2.2 Muscle Function in ME/CFS

There is quite a body of research to suggest that motor unit recruitment is submaximal even during an MVC in healthy subjects (see (Gandevia, 2001) for review). Thus, maximal voluntary torque is often less than true maximal muscle torque. Herbert and Gandevia (1996) reported median VAR of the right thumb adductors in 11 healthy subjects to be 90.3 %. Similar findings of VAR < 100 % have been reported by numerous researchers for the quadriceps femoris (Suter *et al.*, 1996; Babault *et al.*, 2001), biceps brachii (Allen *et al.*, 1998), brachioradialis (Allen *et al.*, 1998) and tibialis anterior muscles (Kent-Braun, 1999).

Greater than normal CAF may be a contributing factor towards the sensation of persistent fatigue, post-exertional malaise and altered perception of effort reported by individuals suffering from ME/CFS. These symptoms will be discussed in detail in the

following sections. Reduced central motor drive may mean that for a given conscious effort, fewer α-motor neuron signals reach the skeletal muscle. As a result, the patient may choose to work at the same level of conscious effort (and therefore at a lower intensity than before becoming sick), increase their conscious effort in order to maintain intensity or some combination of both. Thus, changes in CAF could account for both persistent complaints of fatigue and increased perceived effort, in addition to contributing to post-exertional malaise.

Interpolated twitch analysis has been used to show that individuals diagnosed with ME/CFS have greater CAF than healthy control subjects (Kent-Braun *et al.,* 1993; Schillings *et al*., 2004). Schillings and colleagues reported that CAF in the biceps brachii prior to a 2 min MVC was 37 % in ME/CFS subjects compared to just 13 % in controls. In addition, a comparison of electrically evoked twitch amplitude post-MVC (T_{post}) to that pre-MVC (T_{pre}) showed that T_{post} was only 46 % of T_{pre} magnitude in controls, but 77 % in ME/CFS. The smaller magnitude of peripheral fatigue in ME/CFS subjects compared to control subjects (as suggested by the smaller decrease in T_{post} amplitude in the ME/CFS subjects) can be attributed to the reduced motor unit recruitment during the 2 min MVC (as suggested by the greater CAF in the ME/CFS subjects). As in the biceps brachii, greater CAF has been reported in the quadriceps femoris muscles in ME/CFS subjects compared to healthy control subjects (Kent-Braun *et al.*, 1993).

In contrast, an earlier study by Lloyd *et al*. (Lloyd *et al.*, 1991) found no evidence of greater central fatigue in the biceps brachii of ME/CFS subjects compared to healthy subjects during a series of intermittent contractions at 30 % MVC lasting 45 min in total. Interestingly, this study had an inclusion criteria of VAR > 95 %, which might explain why no differences between the ME/CFS and healthy groups were observed. It is also possible that since torque data were only collected at a frequency of 50 Hz, that small changes in torque output were missed. This could have limited the ability to detect changes in IT amplitude and therefore VAR.

In addition to elevated CAF, several researchers have reported changes in skeletal muscle excitability and fatigue in ME/CFS (Samii *et al.*, 1996; Schillings *et al.*, 2004; Jammes *et al.*, 2005). Muscle excitability has been measured using both M-waves and

MEPs. The M-wave represents the collection of EMG signals from the motor units that are activated synchronously by an electrical stimulus. Reduced M-wave amplitude is associated with lower muscle activation (Allen *et al.*, 2008), while longer M-wave duration is associated with slower signal conduction along the surface of the muscle fibre. An MEP, on the other hand, is the sEMG signal from a muscle that results from magnetic stimulation of cortical neuron(s) (see section 2.1.1.1). Samii *et al.* (1996) have shown that MEP amplitude can be either enhanced or depressed following exercise, depending on the intensity and duration of the exercise. Higher intensity activity appears to depress MEP amplitude, while lower intensity activity facilitates it.

Jammes and colleagues (2005) measured M-wave amplitude and duration in both healthy individuals and those with ME/CFS before and after an incremental cycle ergometer test to exhaustion. Although there were no changes during exercise in either group, M-wave amplitude was consistently lower in ME/CFS subjects (4 mV versus 5.5 mV) and M-wave duration increased by 40 % during recovery compared to only 10 % in control subjects. Samii *et al*. (1996) used TMS to measure changes in cortical excitability in ME/CFS patients and healthy control subjects during a series of 30 s isometric wrist extensions at 50 % MVC torque. The exercise was stopped when the subjects could no longer sustain the target torque. The researchers found that net post-exercise MEP facilitation was significantly less in ME/CFS subjects after exercise compared to control subjects. Post-exercise MEP amplitude was 218 % of pre-exercise levels in the control subjects, but only 126 % in ME/CFS subjects. The authors concluded that this was evidence of reduced cortical excitability and an altered time course of MEP facilitation in ME/CFS subjects. It should be noted, however, that while MEP amplitude is clearly influenced by cortical excitability, there are additional factors that may influence MEP amplitude. One such factor is the effect that repeated motor unit activation has on the magnitude of Na^+ and K^+ flux during action potentials in the α-motor neuron (MacIntosh *et al.*, 2006). Reduced ion flux will result in decreased action potential amplitude and therefore decreased MEP amplitude. Thus, the smaller increase in MEP in the ME/CFS subjects after exercise might have been the result of a combination of changes in cortical excitability and action potential propagation.

Based on the available literature, it appears that most studies have reported evidence of reduced central muscle activation in ME/CFS compared to healthy controls. Some studies have also shown greater central fatigue responses to exhaustive exercise. However, the possible effect that this phenomenon might have on subsequent activity 24 hrs or more after the fatiguing event has yet to be investigated. Furthermore, the possibility of a connection between increased CAF, central fatigue and post-exercise depression of cortical excitability, and the symptoms of post-exertional malaise has not been considered either. In contrast, many researchers have investigated the possible connection between endocrine system dysfunction and ME/CFS. This will now be addressed.

2.3 Neuroendocrine Function in ME/CFS

Compared to the relatively small pool of literature regarding skeletal muscle function in ME/CFS, there is a relatively large body of research regarding possible changes in neuroendocrine function and how they might be responsible for the primary symptoms of ME/CFS. Hormones play an essential role in regulating homeostasis, thus, it not surprising that hormone imbalances are responsible for causing many known diseases such as: type I diabetes mellitus (insulin deficiency); acromegaly, gigantism and dwarfism (growth hormone); Addison's disease and Cushing's disease (hypo- and hyper-cortisolism); Hashimoto's disease and Grave's disease (hypo- and hyper-thyroidism) (Gould, 2006). Since many of these diseases are associated with symptoms of abnormal fatigue, sleep, mood and cognitive impairment, it is not surprising that researchers have proposed connections between altered neuroendocrine activity and ME/CFS (Cleare, 2003; Maquet *et al.*, 2006; Meeusen *et al.*, 2006). Cortisol has been the most-studied hormone by far.

In response to a stressful stimulus, corticotrophin releasing hormone (CRH) is released from the hypothalamus, which in turn stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH then stimulates the adrenal cortex to release cortisol. Cortisol then exerts negative feedback on the release of both CRH and ACTH (Gould, 2006). Cortisol, which is synthesized in the

adrenal cortex, stimulates the release of glucagon while inhibiting insulin. This leads to increases in plasma glucose availability, increased liver glycogen content, and upregulation of fat and protein metabolism. Cortisol has also been implicated in impaired immune system function through the prevention of T-cell proliferation. Abnormally low levels of cortisol are associated with both fatigue and depression.

In addition to stimulating cortisol release, stressful stimuli activate the sympathetic nervous system and its "fight or flight" response. This leads to the release of epinephrine from the adrenal medulla and the overflow of norepinephrine from the nervous system into the systemic circulation (Urhausen *et al.*, 1995). Norepinephrine is the main neurotransmitter in the sympathetic nervous system. Epinephrine is a non-specific adrenergic receptor agonist. As a result, it has widespread effects including: skeletal muscle vasodilation, visceral organ vasoconstriction, chronotropic and inotropic effects on cardiac muscle, increased alertness, and increased plasma glucose concentrations. Reduced synthesis and release of epinephrine and/or norepinephrine or decreased adrenergic receptor sensitivity could thereby contribute to the fatigue, mood and cognitive symptoms associated with ME/CFS.

Most ME/CFS research has focused on the hypothalamic-pituitary-adrenal (HPA) axis because of correlations between hyposecretion of adrenal hormones such as cortisol, and fatigue, as in Addison's disease (Brosnan and Gowing, 1996; Streeten and Anderson, 1998). Cleare *et al*. (1995) reported lower plasma free cortisol levels in ME/CFS patients relative to healthy control subjects, while depressed individuals actually showed significantly increased plasma cortisol levels. It is important to understand, however, that cortisol is released in a diurnal pattern. This means that cortisol release naturally varies over the course of the day and night, such that there is a spike in hormone release shortly after awakening in the morning. Generalizing the results of a single hormone sample to total cortisol release may be misleading, especially if there is a shift in the diurnal rhythm. Indeed, a study in 2005 by Jerjes *et al*. (2005) found that there was a shift in the diurnal release of salivary cortisol over a 15 hr period in ME/CFS subjects compared to healthy subjects. Both Cleare *et al.* (2001) and Jerjes *et al.* (2006) have also reported

reduced 24 and 15 hr urinary cortisol release, respectively, in ME/CFS, although no shifts in diurnal pattern were found in either study.

Not all studies, however, have found reduced cortisol levels in ME/CFS (see (Cleare, 2003) for review). Di Giorgio *et al.* (2005) found abnormally low plasma ACTH levels and an altered diurnal ACTH release over a 24 hr period in ME/CFS subjects yet cortisol release was normal. Ottenweller *et al*. (2001) reported normal plasma ACTH, cortisol, epinephrine and norepinephrine levels in ME/CFS subjects prior to a treadmill exercise test, although, this was a single sample. Previously, Wood and colleagues (2000) actually reported greater salivary cortisol levels in ME/CFS compared to controls over a 16 hr sample.

Considering the inconsistent findings amongst the literature, it seems highly unlikely that altered HPA axis function is always the primary cause of symptoms in ME/CFS, although it may play a significant role in some cases. Furthermore, it remains unknown whether impaired HPA axis activity is a cause or effect of ME/CFS. The autonomic nervous system, and its role in regulating both heart rate (HR) and blood pressure (BP) has also been implicated in ME/CFS. This will be discussed in the following section.

2.4 Autonomic Nervous System Function in ME/CFS

Both HR and BP are maintained by the autonomic nervous system. Activation of the sympathetic nervous system leads to increases in heart rate, ejection fraction and blood pressure, while parasympathetic activity leads to decreases in heart rate, ejection fraction and blood pressure. HR is regulated by a group of cells called the sinoatrial (SA) node within the heart that spontaneously depolarize 90-100 times each minute. Since normal resting HR is around 60-70 beats per min (bpm), there is a constant level of parasympathetic innervation to the SA node, via the vagus nerve, to slow HR at rest. Abnormally high resting HR is associated with jitters, tremors and palpitations, while a low HR is usually associated with low BP, weakness and light-headedness. A surprising number of ME/CFS patients complain of dizziness upon standing, walking or exercising

(Komaroff and Buchwald, 1991) leading researchers to investigate whether there is a disruption in autonomic control (in the form of orthostatic hypotension) in ME/CFS.

Instead of directly measuring neural signals in sympathetic or parasympathetic nerves, several surrogates are used to assess autonomic nervous system function. Firstly, simple changes in resting HR and BP have been used to infer changes in autonomic function. The problem with this method, is that resting HR and BP can be influenced by a variety of factors outside of the autonomic nervous system such as blood volume, electrolyte balance and body position. Furthermore, neither HR nor BP is ever constant, even at rest. There are always small fluctuations that must be recognized and taken into account. Secondly, tilt tests have been used to assess the autonomic nervous system's ability to respond to stressful stimuli. An inability to maintain BP when transitioning from a supine to upright position indicates reduced autonomic nervous system sensitivity or function. Thirdly, heart rate variability (HRV), the variation in heart rate from 1 beat to the next, can be used to infer the relative sympathetic and parasympathetic balance as well as overall autonomic system responsiveness (Achten and Jeukendrup, 2003). HRV can be thought of as either the variation in instantaneous HR from one beat to the next, or as the variation in the beat-to-beat interval over time. Parasympathetic activity slows the heart rate, but is associated with greater variability in the beat-to-beat interval, while sympathetic activity raises heart rate and is associated with a reduced variability in the beat-to-beat interval. The magnitude and direction of change in HRV as a result of a postural challenge, for example, is indicative of autonomic system responsiveness. HRV is calculated by using mathematical algorithms to analyze the rate at which the interval between heart beats (and therefore HR itself) changes. Several key ranges have been identified along the resultant frequency spectrum, each of which corresponds to either sympathetic or parasympathetic tone. High frequency variability is associated with parasympathetic dominance while the ratio of low to high frequency variability is indicative of the balance between sympathetic and parasympathetic activity. Since low frequency variability is indicative of sympathetic dominance, the higher the ratio, the greater the sympathetic activity (Achten and Jeukendrup, 2003).

Freeman and colleagues (1997) reported significantly higher systolic BP and HR in the supine position in ME/CFS subjects relative to healthy controls. This may suggest higher than normal sympathetic tone at rest in ME/CFS. When these subjects were submitted to a tilt-table test, heart rate increased significantly more in the ME/CFS patients, despite a greater drop in BP. This orthostatic intolerance has been shown to be caused by both deconditioning as well as autonomic neuropathy. With deconditioning, there is a decrease in plasma volume as fluid redistributes away from the periphery towards the visceral organs leading to natriuresis and diuresis (Greenleaf, 1986). There also appears to be a decrease in baroreceptor sensitivity with deconditioning, as well as leg muscle atrophy that can lead to venous pooling of blood in the legs (Eckberg and Fritsch, 1991). All of these factors lead to decreased cardiac preload thereby causing orthostatic tachycardia upon standing. Given the sedentary lifestyle of those individuals suffering from ME/CFS, it is certainly possible that deconditioning is the mechanism behind the symptoms of orthostatic intolerance. If this is the case, a carefully designed exercise program may help to ameliorate the symptoms. Although less likely, it is also possible that reduced sympathetic nervous system sensitivity results in reduced vasoconstriction of peripheral blood vessels upon standing leading to blood pooling and subsequent orthostatic tachycardia. This would suggest that although ME/CFS patients appear to have higher than normal sympathetic output at rest, there is an impaired sympathetic response to a postural challenge.

The above findings support previous evidence published by Streeten and Anderson (1992; 1998) whereby symptoms of fatigue were associated with evidence of orthostatic hypotension during a tilt table test. They are also confirmed by some HRV studies in ME/CFS subjects both at rest (Boneva *et al.*, 2007) and during tilt-table tests (De Becker *et al.*, 1998) which have shown a skewed power distribution towards low frequency HRV, suggesting abnormal sympathetic dominance. In contrast, Yataco *et al.* (1997) reported no differences in HRV between ME/CFS patients and healthy controls either in the supine position or during a tilt-table test.

Despite the majority of evidence pointing to increased sympathetic nervous system tone (or impaired parasympathetic nervous system function), autonomic

imbalance can only account for some of the symptoms of ME/CFS (De Becker *et al.*, 1998). There does not appear to be a link between autonomic nervous system function and myalgia, mood disruption or cognitive impairment. Thus, it seems unlikely that the primary mechanism of pathogenesis in ME/CFS involves the autonomic nervous system.

Considering the impact that fatigue has on subsequent exercise performance, the literature regarding both submaximal and maximal exercise performance in subjects with ME/CFS will now be discussed.

2.5 Exercise and ME/CFS

Given that overwhelming fatigue is the hallmark symptom of ME/CFS, many studies have aimed to determine whether or not individuals with ME/CFS have a reduced ability to perform and tolerate exercise. In 2000, De Becker and colleagues reported that, using an incremental cycle ergometer exercise test to exhaustion, maximal power output and oxygen consumption were nearly half that of sedentary but otherwise healthy women. Similar findings have been reported elsewhere as well (Riley *et al.*, 1990; Sisto *et al.*, 1996). However, De Becker *et al.* (2000) used the following criteria to determine maximal effort: respiratory exchange ratio (RER) > 1.0 and maximal HR within 85% of age predicted maximum. These criteria are less stringent than those suggested by the American College of Sports Medicine for determining maximal effort during an incremental exercise test (Heyward, 2006) (see section 3.2). Therefore, it is unclear whether or not these subjects truly gave a maximal effort. Indeed, the findings of a significantly reduced $\dot{V}O_{2peak}$ could be the result of decreased effort from the ME/CFS subjects, rather than a decreased ability. In contrast to earlier findings, VanNess *et al.* (2007) reported that both $\dot{V}O_2$ at anaerobic threshold and at the end of a ramp cycle test to exhaustion were the same in ME/CFS patients and sedentary controls.

Given the inherent difficulties in determining whether or not maximal effort has been achieved during incremental exercise tests, Wallman and colleagues compared the response to submaximal cycling in both subjects with ME/CFS and age-, gender- and activity- matched controls (Wallman *et al.*, 2004). HR, RER and $\dot{V}O_2$ were the same in

both groups at each submaximal cycling intensity, despite the fact that rating of perceived exertion (RPE) was significantly higher in the ME/CFS group at each stage. It cannot be known for sure whether these ME/CFS individuals would be able to reach the same maximal power output and oxygen uptake as controls had this study continued to volitional fatigue (and satisfied the appropriate criteria for reaching maximal oxygen uptake).

Wallman *et al.* (2004) used the findings of their study to propose a theory of why $\dot{V}O_{2peak}$ has appeared to be lower in ME/CFS subjects compared to controls in some studies. They postulated that greater RPE observed in ME/CFS subjects during each stage of submaximal exercise was due to a reluctance to exercise at intensities that might exacerbate symptoms. Thus, they proposed that individuals with ME/CFS would stop exercising prior to reaching true maximal oxygen uptake out of fear of exacerbating symptoms. However, a study by Gallagher *et al.* (2005) has since reported that anxiety levels were not elevated by exercise in ME/CFS, which argues against this theory.

In addition to comparing the HR response to incremental treadmill exercise in ME/CFS and healthy controls, Ottenweller *et al.* (2001) monitored both the immediate (4 min post) and long term (24 hr post) hormone responses. Both groups exercised for the same amount of time at the same treadmill speeds and inclines, and reached the same $\dot{V}O_{2peak}$. Despite this, HR, RER and blood lactate concentration ($[BLa^-]$) at the end of exercise were lower in ME/CFS subjects than controls. Four min post exercise, plasma ACTH and epinephrine levels were lower in ME/CFS while cortisol levels were unchanged in both groups. By 24 hr after exercise, all hormone levels had returned to baseline levels. The authors also noted a lower baseline level of catecholamine metabolites in the ME/CFS subjects, suggesting a lower rate of catecholamine synthesis and/or metabolism. Thus, it seems likely that the lower post exercise epinephrine levels in ME/CFS were due to less release rather than accelerated metabolism. Furthermore, lower epinephrine release in response to maximal effort exercise could explain the lower HR, RER and $[BLa^-]$ in ME/CFS as epinephrine is known to stimulate both HR and glycogenolysis (Mazzeo and Marshall, 1989).

The different findings amongst the various studies investigating exercise tolerance and capacity in ME/CFS are likely due to a variety of factors: criteria for determining maximal effort in maximal exercise tests, inclusion and exclusion criteria for both ME/CFS and control groups, as well as the exercise testing protocol itself. Including ME/CFS subjects with comorbidities such as fibromyalgia could confound study findings, as the presence of fibromyalgia may contribute to the observed decrease in exercise capacity (Cook *et al.,* 2006). Conversely, the use of a control group that is equally as sedentary as the ME/CFS subjects is essential for comparison, yet very hard to achieve. Most studies that attempt to use a sedentary control group do so by setting unique restrictions on self-reported physical activity. The inherent biases and error associated with self reported physical activity need to be acknowledged. Finally, given the very low fitness levels of the individuals being tested, it is essential that the tests begin at a low enough workload and that the increments are not too large as to result in premature discontinuation of the exercise test.

Another defining characteristic of ME/CFS is the symptom of post-exertional malaise, the exacerbation of both physical and psychological symptoms following a bout of exercise (Komaroff and Buchwald, 1991; Nijs *et al.*, 2010; VanNess *et al.*, 2010). Black *et al.* (2005; 2005) reported that after 4 – 10 days of participating in a 15-25 min walking program, individuals with ME/CFS could no longer maintain their daily activity levels (as determined by an accelerometer worn on the hip), and overall mood worsened while muscle pain, daily fatigue and time spent each day with fatigue increased (as assessed using 0-100 visual analog scales and the Profiles of Mood States questionnaire). Bazelmans *et al.* (2005) found that self-reported fatigue was elevated in ME/CFS patients for 2 days after an incremental bicycle test to exhaustion while reports were back to baseline within 2 hrs of the test in healthy subjects. Blackwood *et al.* (1998) also reported a greater reduction in cognitive function after submaximal treadmill exercise than either healthy controls or individuals diagnosed with major depression.

Considering the symptoms associated with post-exertional malaise, VanNess *et al.* (2007) tested whether or not individuals with ME/CFS demonstrated a reduced ability to exercise the day after an incremental exercise test to exhaustion. They investigated

whether both women with ME/CFS and sedentary but otherwise healthy females were capable of reaching the same $\dot{V}O_{2peak}$ on 2 incremental cycle ergometer tests to exhaustion 24 hrs apart. They found that although $\dot{V}O_{2peak}$ was similar between groups on the 1st test, it was significantly reduced in the ME/CFS group on the 2nd test. In contrast, the control group was able to reach the same $\dot{V}O_{2peak}$ on the 2nd test as on the 1st. The researchers suggested that the reduced $\dot{V}O_{2peak}$ on the 2nd test in the ME/CFS subjects confirmed the presence of post-exertional malaise and that perhaps a dual exercise testing protocol would be useful for the diagnosis of this disease.

There is overwhelming support in the literature to suggest that regular exercise both improves quality of life and reduces morbidity in previously sedentary populations as well as in those diagnosed with chronic diseases such as diabetes, hypertension, chronic obstructive pulmonary disease and heart disease (see Pedersen and Saltin, 2006 for review). Not only does regular activity improve the symptoms of many chronic diseases, but improvements in fitness can reduce risk factors for developing the disease in the first place. Despite the risk of post-exertional malaise, several randomized controlled trials (RCT) have tried to determine whether exercise can be used to improve the symptoms of ME/CFS. Fulcher and White (1997) published the first RCT looking at the benefits of a 12-week graded exercise therapy program on ME/CFS symptoms. They found that 52 % of ME/CFS patients reported "feeling better" 3 months after the program, which consisted of 30 min of physical activity 5 times per week. The exercise intensity gradually increased up to 60 % of $\dot{V}O_{2peak}$. They also reported that only 3 patients dropped out of the study. At least 2 systematic review articles have been published regarding the use of exercise programs as therapy for ME/CFS (Edmonds *et al.*, 2004; Chambers *et al.*, 2006). Both noted that although there is significant evidence to suggest that exercise can be beneficial for improving fitness and reducing symptom severity in ME/CFS, a large number of study participants experience a worsening of symptoms with exercise.

Several recent studies have monitored various aspects of the response to both maximal and submaximal exercise in individuals with ME/CFS, hoping to determine

what types of exercise cause post-exertional malaise (LaManca *et al.*, 1998; Cook *et al.*, 2005; Nijs *et al.*, 2008a; Nijs *et al.*, 2008b; Nijs *et al.*, 2010). At this point, there is no consensus in the literature regarding a threshold below which no symptom exacerbations occur or what the characteristics of a "safe" exercise program might be. Therefore, it seems that more research is required to identify appropriate exercise programs and to determine methods of individualization such that post-exertional malaise is minimized (or eliminated).

The most common method used both clinically and in research to monitor changes in post-exertional malaise in ME/CFS patients is through a variety of questionnaires. The use of such questionnaires will now be discussed.

2.6 Questionnaires Assessing Mood State and Quality of Life in ME/CFS

Changes in mood, sleep and cognitive function are part of the definition of ME/CFS, thus subjective assessments of mood state and quality of life are part of the diagnostic process. In combination with the objective measures described above, subjective assessments may be useful in improving our understanding of this disease. There are, however, limitations to this type of analysis due to its subjectivity. Interestingly, research with overtrained athletes, who suffer from surprisingly similar symptoms to those with ME/CFS (Fry *et al.*, 1991; Derman *et al.*, 1997; Rowbottom *et al.*, 1998), has shown that the findings on such questionnaires often correlate with objective measures. Furthermore, subjective changes in mood state and quality of life may be one of the earliest signs of overtraining (Jürimäe *et al.*, 2002; Jürimäe *et al.*, 2004), and it is possible that they may also be early indicators of ME/CFS.

A wide variety of questionnaires have been developed to assess specific aspects of mood and quality of life. The most commonly used mood state questionnaires are the Profiles of Mood States (POMS), the Hospital Anxiety and Depression Scale (HADS) and the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport). The Medical Outcomes Short Form-36 (SF-36) questionnaire is a commonly used tool for evaluating quality of life in both healthy and various disease states. Specific questionnaires have also been developed to focus on the symptoms associated with specific diseases, in order to

get a better understanding of how each disease affects quality of life. The review that follows will be limited to those questionnaires used in this thesis.

2.6.1 Medical Outcomes Study Short Form-36 (SF-36) Questionnaire

The SF-36 questionnaire was initially developed in 1990 as an improved version of the SF-18 and SF-20 questionnaires (Ware Jr and Sherbourne, 1992; Ware Jr, 2000). In 1996, a 2nd version was released with a few minor adjustments to the content and layout. Furthermore, the 2nd version introduced norm-based scoring, whereby scores were transformed using T-scores with a normal distribution, mean of 50 and standard deviation (SD) of 10. "Normal" values have been established for a variety of populations (Ware Jr, 2000). The rationale behind normalized scoring is that it makes it much easier to compare scores across studies, as well as to determine the effects of various diseases on quality of life.

The SF-36 was designed for use in both clinical and research settings to assess 8 "health concepts": [1] physical functioning; [2] role limitations due to physical health problems; [3] pain; [4] social functioning; [5] general mental health; [6] role limitations due to emotional problems; [7] vitality; and [8] general health perception. In addition to standing alone, these 8 scales are also grouped into either physical or mental categories to create 2 additional "component" scales, the combined physical components and combined mental components scales (Appendix B).

The SF-36 is a commonly used diagnostic tool for measuring quality of life and has been previously validated in both healthy and ME/CFS populations as well as many other chronic diseases (McHorney *et al.*, 1994; Ware Jr, 2000; White *et al.*, 2007). A 2002 comparison of scores on the SF-36 questionnaire and the HADS questionnaire in individuals surviving testicular cancer showed that both the HADS anxiety and depression subscales correlated to the combined mental components scale but not the combined physical components scale of the SF-36 questionnaire (Fosså and Dahl, 2002). The authors concluded with the suggestion that the 2 questionnaires should be used together in order to fully assess quality of life.

2.6.2 Centers for Disease Control (CDC) CFS Questionnaire

The CDC CFS Questionnaire was validated by Wagner *et al.* in 2005 to facilitate the diagnosis of ME/CFS according to the 1994 Fukuda definition (Fukuda *et al.*, 1994). This "CDC Symptom Inventory" assesses the frequency and severity of 19 fatigue and illness-related symptoms associated with ME/CFS in the 1 month period prior to answering the questionnaire (Appendix C). Of the 19 symptoms, 8 are considered to be defining characteristics of ME/CFS: [1] post-exertional malaise, [2] unrefreshing sleep, [3] problems remembering or concentrating, [4] muscle aches and pains, [5] joint pain, [6] sore throat, [7] tender lymph nodes and swollen glands and [8] headaches. The additional symptoms are diarrhea, fever, chills, sleeping problems, nausea, stomach or abdominal pain, sinus or nasal problems, shortness of breath, sensitivity to light and depression. Symptom frequency is scored on a 4-point scale ranging from 1, being "a little of the time" to 4, being "all of the time". Symptom severity is scored on a 3 point scale where 1 = mild, 2 = moderate and 3 = severe.

Individual symptom scores are determined by multiplying the symptom frequency and severity, after adjusting severity scores such that 0 = not reported, 1 = mild, 2.5 = moderate and 4 = severe. A CFS Case Definition score is calculated as the sum of the scores from the 8 CFS symptoms while an Other Symptoms score is calculated as the sum of the remaining 11 symptoms. A total score out of 304 (19 scales x 16 points each) can also be calculated.

In order to validate the CDC CFS questionnaire, Wagner and colleagues compared the scores on this questionnaire to scores on the SF-36 questionnaire, the Multidimensional Fatigue Inventory and the Chalder Fatigue Scale, all of which had been previously validated for use in ME/CFS diagnoses (Wagner *et al.*, 2005). The CDC CFS Questionnaire proved to be both valid and reliable when used to identify symptoms in a group of 164 individuals diagnosed with ME/CFS. Furthermore, the questionnaire was able to distinguish between individuals with ME/CFS, those with unexplained fatigue but who did not meet criteria for an ME/CFS diagnosis and those without fatigue.

In a separate publication by the same group of researchers, it was stated that the presence of 4 or more of the 8 CFS-defining symptoms and a score of at least 25 on the

Case Definition subscale supported an ME/CFS diagnosis (Reeves *et al.*, 2005). A recent report published by Jason and colleagues (Jason *et al.*, 2010) criticized these cut-off scores for being too low, saying that an individual who reports only 2 of the CFS-defining symptoms all of the time, one with a moderate severity and the other severe would score 25 of the Case Definition subscale. Unfortunately, these researchers did not make any recommendations towards a more appropriate cut-off score. Clearly, additional research is required to determine the best way to use this questionnaire in the diagnosis of ME/CFS.

2.6.3 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale was developed in 1983 by Zigmond and Snaith to identify anxiety disorders and depression (Zigmond and Snaith, 2007). The questionnaire consists of 14 questions, alternating between the anxiety and depression subscale. Each question has 4 possible answers ranging from the least to the most severe expression of the statement. The options are allocated a "score" from 0 (least severe) to 3 (most severe), and the total for each subscale (out of 21) is calculated.

This questionnaire has been validated in a variety of patient populations as well as in healthy individuals, as described in 2 comprehensive review articles, 1 by Herrmann in 1997 and the other by Bjelland *et al.,* in 2002 . The HADS questionnaire was found to be both sensitive and specific with respect to both anxiety and depression despite being much shorter than its predecessors. Since the questionnaire is short and easy to complete, it is commonly used in clinical settings. Although there are many different questionnaires that assess the symptoms of both anxiety and depression, the HADS questionnaire was chosen because it is shorter and easier to complete, while still being able to differentiate between the symptoms of anxiety and depression in ME/CFS subjects.

2.6.4 Karnofsky Performance Status

The Karnofsky Performance Status was originally designed to monitor changes in functional ability in cancer patients undergoing chemotherapy, to help evaluate the

efficacy of the treatment (Karnofsky and Burchenal, 1949). A knowledgeable individual, usually a physician, rates the patient's ability to complete activities of daily living, to work and to care for his/her self on a scale from 0 – 100 %. Every 10 % increment is associated with a comment to guide the rater. Grieco and Long validated the use of this scale as a measure of functional ability in 1984. It is now used in a variety of clinical and research settings to measure the impact of various diseases on functional performance and quality of life.

As part of the validation study, Grieco and Long (1984) compared the scores on the Karnofsky Performance Status in 100 people; either healthy subjects, clinic outpatients, chronic pain sufferers, dialysis patients or stroke patients. Not only did they find the inter-rater reliability to be 0.96 using the Spearman Rank Correlation, but they were able to differentiate between the healthy subjects (who scored a mean of 99.0) and the sick subjects (outpatient = 75.0; pain = 62.0; dialysis = 51.0 and stroke = 39.5). Unfortunately, these authors do not provide the standard deviation around these means nor any statistical analysis, so it is uncertain whether these scores are truly different from one another. Another validation study published in 2006 (Mor *et al.*) confirmed both the reliability and validity of the Karnofsky Performance Scale for use in research.

Bell (1993) adapted the standard Karnofsky Performance Status by expanding the middle ranges of the scale. This adapted Karnofsky Scale is also known as the Bell Ability Scale (Appendix D. By doing so, he found the scale more useful with respect to assessing functional status in ME/CFS patients (Bell, 1997).

2.7 Summary

As should be clear by now, compared to the amount of literature on ME/CFS, there is very little consensus regarding what causes, perpetuates and can improve the symptoms of this disease. This emphasizes the fact that ME/CFS is a complex disease that manifests itself differently in each individual. It is also evident that few researchers have investigated the role that CAF could play in contributing to post-exertional malaise. This thesis aims to shed light on this issue and hopefully improve our understanding of ME/CFS as a disease.

2.8 Hypotheses

Based on previous research, with respect to the 3 research objectives stated in section 1.2, it is hypothesized that:

[1] Women with ME/CFS will be able to reach the same $\dot{V}O_{2peak}$ as age- and activity-matched, but otherwise healthy female controls on the 1st incremental bicycle exercise test to exhaustion.

[2] Women with ME/CFS will not be able to reach the same $\dot{V}O_{2peak}$ on the 2nd exercise test as they could on the 1st while the healthy subjects can.

[3] Women with ME/CFS will have a greater central and more persistent peripheral fatigue response to the incremental exercise tests than the control subjects. This will result in greater central activation failure in the ME/CFS subjects prior to the 2nd exercise test.

Chapter Three: Methods

The procedure outlined below was approved by the Conjoint Health and Research Ethics Board at the University of Calgary. Prior to participating in the study, written informed consent was obtained from 9 females with ME/CFS and 9 activity and age-matched healthy female control subjects (CON). No previous studies reported VAR in ME/CFS subjects using an identical protocol to the one selected for this thesis. However, based on a study by Schillings *et al.* (2004) who reported that CAF was 24 % higher in ME/CFS subjects than control subjects with a SD of 13 %, a sample size of 6 would give adequate power to detect differences in VAR between groups. Given the protocol selected for this thesis, it was hypothesized that there would be at least a 7 % difference in VAR between groups, and a SD of 5%. Based on this estimate, a sample size of 9 (in each group) powered this study at 80 % to identify differences in VAR. Subjects visited the Human Performance Laboratory at the University of Calgary on 2 occasions separated by 24 hrs. They were instructed to refrain from eating or drinking (except water) for 2 hrs prior to testing, and to avoid alcohol, caffeine and vigorous exercise for at least 6 hrs. They were also asked to maintain a similar pattern of activity before both testing sessions.

3.1 Subject Recruitment

All subjects were screened by a physician (ES) specializing in the diagnosis and treatment of ME/CFS and completed the following questionnaires: Short Form (SF)-36 health survey questionnaire, US Centers for Disease Control (CDC) CFS questionnaire, Hospital Anxiety and Depression Scale (HADS) and the adapted Karnofsky scale. As mentioned in chapter 2, the SF-36 is a commonly used diagnostic tool for measuring quality of life and has been previously validated in both healthy and ME/CFS populations (McHorney *et al.*, 1994; White *et al.*, 2007). The CDC CFS questionnaire was used to identify the severity and frequency of 8 key criteria needed for an ME/CFS diagnosis according to the definition of Fukuda *et al.* (1994). The HADS questionnaire was used to

screen for depression and elevated levels of anxiety in the volunteers, while the adapted Karnofsky scale evaluated perceived daily energy levels and functioning.

Inclusion criteria for the ME/CFS group were: [1] satisfied Canadian Consensus Criteria for ME/CFS (Carruthers *et al.*, 2003), [2] met all 8 Fukuda criteria on the CDC CFS questionnaire, [3] had a documented acute onset of ME/CFS and [4] had an adapted Karnofsky scale score ≤ 70 (Bell, 1993).

Inclusion criteria for the CON group were: [1] self reported physical activity of < 30 min of vigorous exercise per week and < 30 min of moderate exercise (ex: leisurely walking) per day, [2] satisfy no more than 1 Fukuda criterion with a moderate frequency on the CDC CFS questionnaire and [3] an adapted Karnofsky scale score of ≥ 80 (Bell, 1993). CON subjects were matched to ME/CFS subjects according to both age and activity level (using the above mentioned exercise restrictions).

Exclusion criteria for all subjects were: [1] presence of any major, confounding medical conditions such as autoimmune disorders or unresolved major depression, [2] taking any medications that could affect or inhibit one's ability to complete the required exercise testing, [3] current smoking habit, and [4] a positive response to the Physical Activity Readiness Questionnaire (CSEP, 2002).

The CDC CFS questionnaire, HADS questionnaire and adapted Karnofsky scale were only used to describe subjects and determine eligibility for participation in the study, thus, the scores on these questionnaires will not be reported as part of this thesis. Only the scores on the SF-36 questionnaire will be reported and discussed.

3.2 Incremental Exercise Tests

All subjects completed 2 incremental exercise tests to volitional exhaustion, 24 hrs apart, on an electrically-braked cycle ergometer (Ergoselect 200, Ergoline, Bitz, Germany). Subjects self-selected their cadence and strong verbal encouragement was given throughout the test. Following an unloaded 3 min warm-up, power output was increased 15 W each min until exhaustion. Maximal aerobic power (P_{peak}) was considered to be the power output corresponding to the last completed 1 –min stage. RPE was measured using the Borg 6-20 scale (Borg, 1982) in the last 15 s of each stage. Fingertip

blood samples for lactate ([BLa^-]) analysis (Lactate Pro, Arkray, Japan) were taken to determine each subject's lactate response to the incremental test. Lactate threshold (LT) was considered to be the workload below the one for which there was an increase of >1 mM [BLa^-] relative to the previous stage (Fletcher *et al.,* 2009; Svedahl and MacIntosh, 2003). Blood samples were taken at rest, following warm-up and every 2 min thereafter, in the last 15 s of the corresponding stage. A final sample was taken immediately at the end of exercise. Heart rate (HR) was recorded telemetrically (Polar s610i, Polar Electro, Oy, Finland) and blood O_2 saturation (SpO_2) was monitored using a temporal pulse oximeter (Nellcor Puritain Bennett, California, USA). To obtain a better understanding of perceived exertion at a specific intensity, the HR:RPE ratio (Neary *et al.,* 2008) was also calculated at both LT and end exercise.

Subjects were fitted with a nose clip and expired gases were measured for O_2 uptake ($\dot{V}O_2$) and CO_2 output ($\dot{V}CO_2$) by a metabolic cart (TrueMax 2400, Parvo Medics, Salt Lake City, UT, USA), with data averaged every 15 s. $\dot{V}O_{2peak}$ was considered to be the highest $\dot{V}O_2$ value attained during the test. The metabolic cart was calibrated before and after each test session by means of a two-point calibration using room air and a gas mixture of known composition (4 % CO_2, 16 % O_2, balance N_2). The flow sensor (Hans Rudolf 3813 heated pneumotachometer) was calibrated with a manual 3 L syringe. The accuracy values provided by the manufacturer were 0.03 % and 0.111 % for O_2 and CO_2 and ± 2 % for volume.

All tests were terminated when the subject was unwilling to continue, or if the cadence dropped below 60 rpm despite encouragement to increase it. SpO_2 never dropped below 85 %. A plateau in $\dot{V}O_2$ was assumed to have occurred if $\dot{V}O_2$ increased by less than 100 $mL \cdot min^{-1}$ with an increase in workload (Figure 3-1). It should be noted that in this study, all subjects who reached a $\dot{V}O_2$ plateau were either unwilling to continue beyond that stage on their own, or were unable to maintain 60 rpm. Therefore, no incremental exercise tests were terminated solely due to a plateau in $\dot{V}O_2$.

The American College of Sports Medicine recommends specific criteria for determining whether maximal effort was reached during an incremental exercise test

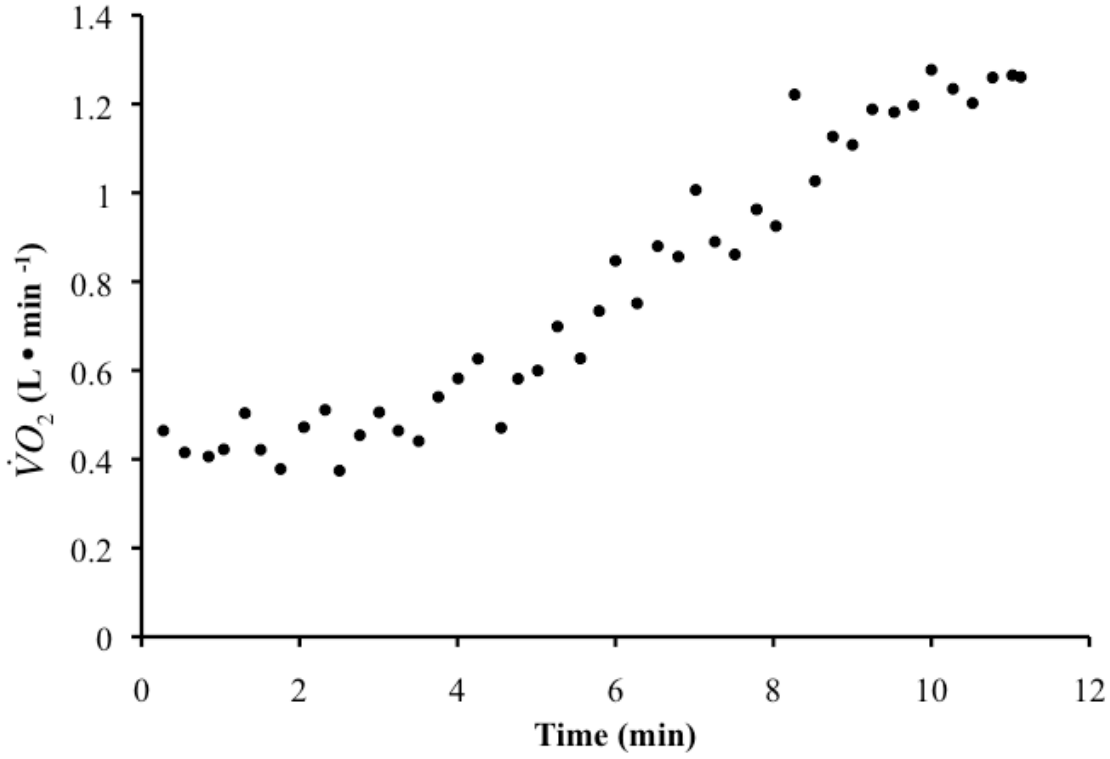

Figure 3-1. Example of a plateau in $\dot{V}O_2$.

Data shown are from a single subject during one of the incremental cycle ergometer tests to exhaustion. The subject cycled at 0 W for the first 3 min, after which, power output increased by 15 W each min until exhaustion. $\dot{V}O_2$ was averaged over 15 s periods throughout the test. $\dot{V}O_2$ increased by less than 100 mL· min^{-1} during the last stage (from 10 – 11 min) compared to the previous stage (from 9 – 10 min), indicating a plateau in $\dot{V}O_2$.

(Heyward, 2006). According to these guidelines, in this thesis, an exercise test was considered maximal if there was a plateau in $\dot{V}O_2$, or if 2 of the following 3 criteria were met:

[1] HR within 10 beats of age-predicted maximum HR (220 – age)

[2] RER > 1.10

[3] $[BLa^-] > 8.0$

3.3 Muscle Contractile Response

Before and immediately following each exercise test, right quadriceps muscle MVC and VAR were determined using a twitch interpolation technique (Merton, 1954; Belanger and McComas, 1981; Shield and Zhou, 2004). Subjects sat on the chair of a Biodex isokinetic dynamometer (Biodex System 3, New York, USA) with their right leg attached to the immobilized, rotating arm just above the lateral malleolus, and knee angle fixed at 90^0. The torso and hip were secured to prevent additional movement (Figure 3-2).

The femoral nerve was stimulated transcutaneously via 2 rectangular carbon rubber stimulation pads (2.5 cm x 5 cm) with conducting gel. One was placed anteriorly on the inguinal crease and the other posteriorly on the gluteal fold (de Ruiter *et al.*, 2004). Electrical stimulation was with a single, 300 V, 200 μs square-wave pulse (Digitimer DS7AH Stimulator, Digitimer Ltd., Welwyn Garden City, England). Prior to testing, supramaximal current was determined by progressively increasing the current until twitch amplitude no longer increased. At this current, it was assumed that all motor units beneath the stimulation pads were maximally activated (de Ruiter *et al.*, 2004). Subjects were instructed to relax prior to each stimulus to minimize conscious attempts to aid or impede the stimulated contraction. They completed 3 x 5 s MVCs with a 5 min rest between contractions. An electrical impulse was sent 2.5 s prior to each MVC, as soon as subjects reached maximal voluntary torque (i.e.: the torque output levelled off) and 2.5 s after each MVC for interpolated twitch analysis. Twitch amplitude prior to each MVC

Figure 3-2. Subject set-up for muscle contractile response tests.
Subjects were seated in the Biodex chair with their hip and torso strapped down to minimize movement during each MVC. The knee was aligned with the axis of rotation of the Biodex arm and the ankle pad was positioned just above the ankle. The Biodex arm was fixed in position such that the leg hung vertically and there was a 90^{0} knee angle.

(T_{pre}) was used to determine the contractile response to the supramaximal stimulus in order to monitor the development of peripheral fatigue. The interpolated twitch (IT) and the twitch following the MVC (T_{post}) were used to calculate VAR. All subjects were given the opportunity to practice an MVC without interpolated twitch analysis prior to the start of testing. All subjects also received visual feedback and verbal encouragement throughout each MVC attempt.

Following an additional 5 min rest, each subject performed a 10 s contraction at 50 % of their highest day 1, pre-exercise MVC aided by visual feedback. sEMG during the 50 % MVC contraction was compared to that during the reference MVC contraction in order to estimate relative motor unit activation. Root mean square (RMS) sEMG during each 50% MVC contraction was used as a secondary method to quantify peripheral fatigue within the quadriceps muscles. Fatigue would require increased motor unit recruitment and/or increased frequency of firing of individual motor units, which would result in an increase in the RMS sEMG signal amplitude (see section 3.5)

The Biodex isokinetic dynamometer was calibrated according to the manufacturer's instructions prior to each testing session. All wires and electrodes remained attached throughout each incremental exercise test, so that interpolated twitch analysis could be repeated as quickly as possible after exercise.

3.3.1 Interpolated Twitch Analysis and VAR Calculation

The formula for calculating VAR using interpolated twitch analysis is as follows:

$$VAR = (1 - IT/T_{post}) \times 100$$

Where IT is the increase in torque associated with the stimulus superimposed upon the MVC and T_{post} is the amplitude of an electrically induced twitch produced by the identical stimulus but in a relaxed and potentiated muscle following the MVC (Gandevia, 2001; Shield and Zhou, 2004). It is assumed that the same magnitude of potentiation exists at the point of superimposed stimulation as 5 s after the MVC, although this has not been directly tested (Gandevia, 2001). The proportion of motor units activated by the electrical stimulus at rest that are not voluntarily activated at their maximal frequency is reflected by the ratio of IT to T_{post} amplitude. Thus, $1 - IT/T_{post}$ represents the proportion

of the motor units that are maximally activated with voluntary effort. VAR is simply this ratio, expressed as a percentage.

3.3.2 Surface Electromyography (sEMG)

sEMG was recorded throughout the muscle contractile response tests. Prior to the 1st testing session, the subject was seated in a chair and asked to extend their right leg by contracting the quadriceps femoris muscles, such that the muscle bellies of the right vastus medialis (VM), rectus femoris (RF) and vastus lateralis (VL) muscles could be landmarked. A 4 cm x 8 cm area of skin on the center of each of the 3 muscle bellies as well as a 2 cm x 4 cm area over the head of the right fibula were shaved and cleansed with alcohol in order to remove any hair, dead skin cells and oil from the surface of the skin. This was done to ensure a good contact between skin and electrode as well as to minimize noise in the sEMG signal. Two sEMG electrodes (Softrace pediatric Ag-AgCl electrode, ConMed Corporation, Utica, NY) were affixed longitudinally to the shaved area on each muscle belly parallel to the assumed orientation of the muscle fibres, at an inter-electrode distance of 3 cm. A single electrode was placed over the head of the fibula to act as a ground. Electrode outlines were marked with a waterproof marker to ensure identical placement on the 2nd day. The electrodes had an adhesive backing, but they were covered with Cover-Roll stretch tape (Beiersdorf AG, Hamburg, Germany) to ensure that they did not move during the testing session.

3.4 Data Analysis

Both torque and sEMG data were collected and analyzed with WinDaq Pro+ data acquisition software (DataQ Instruments Inc., Akron, OH, USA) at a sampling frequency of 3000 Hz. Contractile response data were recorded as raw voltage from the Biodex dynamometer and converted to torque based on pre-testing calibration. Peak MVC torque was calculated as the average of the 500 data points (0.167 s) prior to the superimposed electrical stimulus. Only the strongest of the 3 MVC trials was analysed. RMS EMG was calculated during each MVC using the 500 data points immediately prior to the

interpolated twitch, after taking the apparent electromechanical delay (80 data points or 0.026 s) into account. RMS EMG was calculated during each 50 % MVC effort using the 1 s period during which measured torque was as close to target torque as possible. Again, the apparent electromechanical delay was taken into account.

In order to ensure that estimates of VAR were accurate, it was essential that the superimposed stimuli be delivered as close to each subject's actual peak MVC torque as possible. A comparison was made between the peak MVC used for analysis (using the average of the 500 data points prior to IT) and the single highest torque data point attained during each MVC, to ensure that this was the case.

Differences in age, weight, height and body mass index (BMI) between groups were analyzed using independent T-tests. Scores on the SF-36 questionnaire were analyzed by a 2-way, group by scale analysis of variance (ANOVA). Changes in time to exhaustion, metabolic variables as well as muscle contractile functions between groups and over time were determined by 2-way, group by time ANOVAs, with time being a repeated measure. If an interaction was observed, Tukey's HSD post hoc tests were used to identify simple main effects. If no interaction was observed, then main effects (group or time) were considered. T_{pre} and T_{post} amplitude were initially analyzed with a 3-way group by pre/post by time ANOVA with both pre/post and time being repeated measures. If an interaction was observed, then simple main effects were identified by post-hoc testing. If there was no interaction, then the respective 2-way ANOVAs were completed as above. Correlations between each SF-36 scale and the change in VAR from before to after exercise on the 2^{nd} day were calculated using Pearson's product moment correlation coefficient.

All data are expressed as mean ± SD and a p-value < 0.05 was considered statistically significant. All calculations were performed using Microsoft Excel 2003 (Microsoft, Redmond, Washington, USA) and the Statistical Package for Social Sciences (SPSS) for Windows version 15.0 (SPSS Inc., Chicago, Illinois, USA).

Chapter Four: Results

4.1 Subject Characteristics

There were no statistically significant differences with respect to age, weight, height or aerobic capacity (as determined during the 1st incremental exercise test) between CON and ME/CFS groups (Table 4-1). There were, however, significant differences between groups with respect to daily function, as assessed by the SF-36 health survey questionnaire. ME/CFS scored lower than CON on all 10 scales (Figure 4-1). All differences between groups were significant ($p<0.004$), except for the role emotional scale where a trend was evident ($p=0.056$). Furthermore, a comparison to the population norms provided by Ware (2000) revealed that ME/CFS subjects scored at least 1 SD below the norm on the physical function, role physical, body pain, general health, vitality, social function and combined physical components scales (Figure 4-1). In contrast, CON subjects were above average on all 10 scales.

There was a significant correlation between several of the SF-36 scales and change in VAR from before to after the 2nd incremental exercise test for all subjects (Table 4-2). Those subjects who scored higher on the SF-36 scales had less of a decrease in VAR. The strongest correlations were found with the vitality, role physical, general health, physical component, physical function, body pain and social function scales. There were no significant correlations found between SF-36 scales and any of the metabolic variables ($\dot{V}O_{2peak}$, P_{peak}, HR_{peak}, LT, $[BLa^{-}_{peak}]$ or RPE_{peak}) ($p>0.10$).

4.2 Incremental Exercise Tests

Of the 36 incremental exercise tests completed in this study (18 subjects, 2 tests per person), 32 were considered maximal according to the American College of Sports Medicine guidelines (Heyward, 2006) stated in section 3.2. One ME/CFS subject did not satisfy the criteria on either test ($\dot{V}O_{2peak}$ = 1.12 L·min-1 and 1.32 L·min-1 on the 1st and 2nd incremental tests, respectively) . Two control subjects did not meet the criteria for maximal effort on the 1st test, but did on the 2nd test. However, for 1 of these subjects,

Table 4-1. Subject characteristics.

	ME/CFS (n = 8)	CON (n = 8)	p-value
Age (yrs)	46.1 ± 7.9	45.6 ± 7.9	0.883
Weight (kg)	63.6 ± 13.5	67.3 ± 12.8	0.557
Height (cm)	168 ± 7	166 ± 5	0.464
Body Mass Index ($kg \cdot m^{-2}$)	22.6 ± 4.9	24.5 ± 4.3	0.398
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	23.8 ± 6.6	23.5 ± 2.8	0.908
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	1.45 ± 0.28	1.56 ± 0.22	0.389

Data are presented as mean ± SD.

$\dot{V}O_{2peak}$ is from the first incremental exercise test.

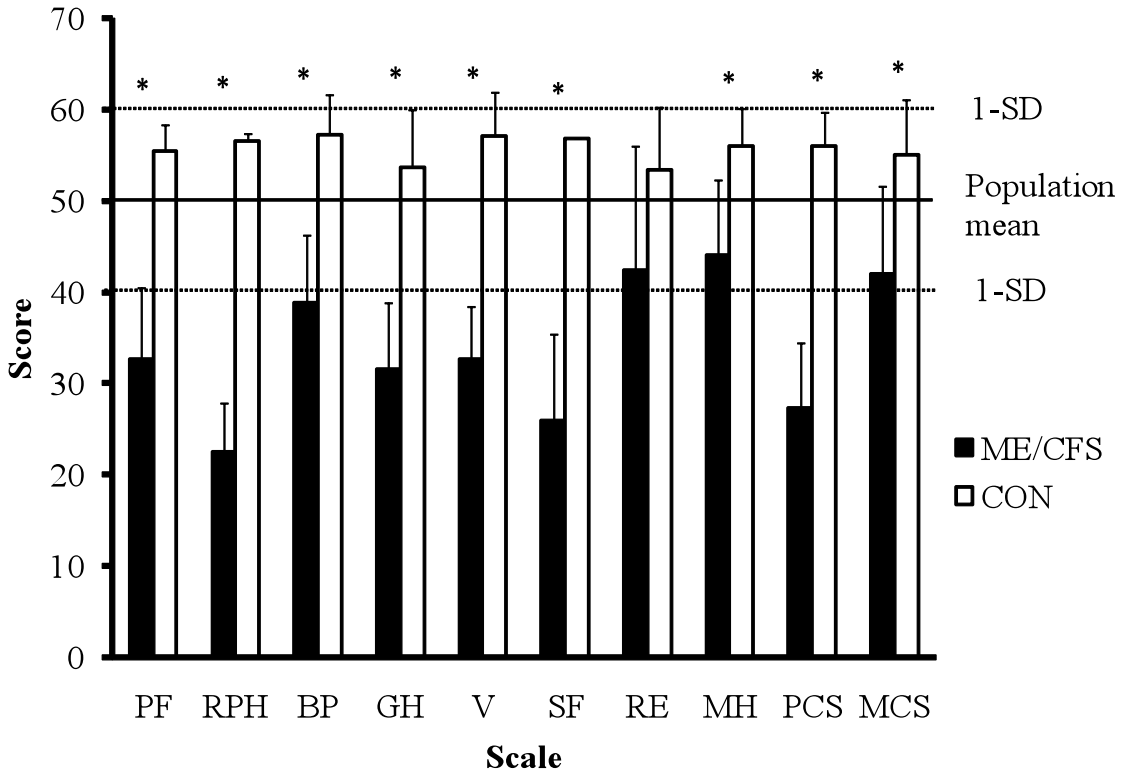

Figure 4-1. Scaled SF-36 health survey questionnaire scores for ME/CFS and CON.

Normal population mean and SD are indicated with solid (—) and dotted (---) lines, respectively. PF = physical function, RPH = role physical, BP = body pain, GH = general health, V = vitality, SF = social function, RE = role emotional, MH = mental health, PCS = physical components scale, MCS = mental components scale. Error bars indicate 1-SD from the mean. * Significant difference between ME/CFS and CON, $p<0.05$.
(n = 9 ME/CFS, 9 CON)

Table 4-2. Correlation between scores on the SF-36 questionnaire and change in VAR from pre- to post- exercise on the 2nd day

SF-36 Scale	R value
Physical Function	0.70*
Role Physical	0.80 *
Body Pain	0.68*
General Health	0.74 *
Vitality	0.87 *
Social Function	0.62*
Role Emotional	0.09
Mental Health	0.49
Physical Components Scale	0.85 *
Mental Components Scale	0.36

Values shown are Pearson product moment correlation coefficients

* Significant correlation, $p<0.05$

(n = 8 ME/CFS; 7 CON)

$\dot{V}O_{2peak}$ was less than 100 mL·min^{-1} lower on the 1st test than on the 2nd test, which satisfies the criteria for a plateau in $\dot{V}O_2$. Therefore, this test was considered to have required maximal effort. $\dot{V}O_{2peak}$ for the control subject who did not satisfy the criteria for maximal effort on the 1st test was 1.22 L·min^{-1} and 1.68 L·min^{-1} for the 1st and 2nd tests, respectively. Statistical analysis of all of the metabolic variables was completed with and without the inclusion of the 3 submaximal tests. The results were the same, however, it was chosen to present only the data from the 8 ME/CFS subjects and 8 CON subjects in which maximal effort was reached during both incremental exercise tests.

There was no difference in $\dot{V}O_{2peak}$, P_{peak}, HR_{peak}, RER_{peak} or LT between groups or from test 1 to test 2 ($p>0.10$) (Table 4-3). Despite these similarities, time to exhaustion was significantly shorter for the ME/CFS subjects compared to the control subjects ($p=0.034$). There was no difference in [BLa^-] from test 1 to test 2 in either group, and although [BLa^-] at LT was the same in both groups, the difference in [BLa^-_{peak}] between groups approached significance ($p=0.08$) (Table 4-3). RPE_{peak} was lower in ME/CFS than CON during both exercise tests, although no differences were seen at LT on either day (Table 4-3). However, when RPE was expressed relative to HR both at LT and at maximal effort, the HR:RPE ratios were not significantly different between groups or from test 1 to test 2 ($p>0.10$) (Table 4-3).

Although there was no significant difference in $\dot{V}O_{2peak}$ from test 1 to test 2, there was some variability in $\dot{V}O_{2peak}$ from day to day. For the most part, this reflected small changes in the time to exhaustion, and not changes in efficiency. Figure 4-2 shows the $\dot{V}O_2$ over time for the subject who had the greatest change in time to exhaustion from test 1 to test 2. This happened to be the subject who did not satisfy maximal effort criteria on the 1st test, but did on the 2nd test. This subject lasted nearly 2 min longer on the 2nd day. $\dot{V}O_2$ increased similarly in both tests, and it appears as though the subject was simply able to push themselves to exercise longer on the 2nd day and therefore reach a higher $\dot{V}O_{2peak}$.

Table 4-3. Metabolic response to 2 incremental cycle ergometer tests to exhaustion completed 24 hrs apart in healthy women and those with ME/CFS.

	ME/CFS (n = 8)		CON (n = 8)	
	Test 1	Test 2	Test 1	Test 2
LT				
LT (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$)	17.7 ± 4.6	16.7 ± 2.9	17.2 ± 4.0	17.8 ± 4.5
LT (% $\dot{V}O_{2peak}$)	72.0 ± 9.5	72.0 ± 9.0	72.8 ± 12.2	72.0 ± 8.3
P at LT (W)	75 ± 32	64 ± 41	87 ± 18	90 ± 15
HR at LT (bpm)	139 ± 9	140 ± 15	138 ± 15	141 ± 17
[BLa^-] at LT (mM)	3.6 ± 0.4	3.1 ± 0.8	4.0 ± 1.1	3.8 ± 0.8
RPE at LT	14 ± 3	14 ± 2	14 ± 2	15 ± 1
HR:RPE (bpm)	10.9 ± 3.1	10.2 ±1.3	9.5 ± 0.5	9.3 ± 0.9
End exercise				
Time to exhaustion (s)	612± 132*	630±105*	698 ± 75	697 ± 53
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	23.8 ± 6.6	23.3 ± 6.0	23.5 ± 2.8	24.5 ± 3.9
P_{peak} (W)	105 ± 29	108 ± 29	125 ± 20	125 ± 15
HR_{peak} (bpm)	160 ± 17	165 ± 18	172 ± 7	169 ± 12
$[BLa^-]_{peak}$ (mM)	6.1 ± 2.4	5.3 ± 2.4	7.4 ± 1.4	7.3 ± 2.2
RPE_{peak}	17 ± 2*	17 ± 2*	19 ± 1	19 ± 1
HR:RPE (bpm)	9.4 ± 1.5	9.5 ± 1.0	9.3 ± 0.6	8.9 ± 0.8
RER_{peak}	1.24± 0.16	1.27±0.14	1.27 ± 0.11	1.28±0.09

Data are expressed as mean ± SD. CON = control, LT = lactate threshold, P = power output, HR = heart rate, [BLa-] = blood lactate concentration, RPE = rating of perceived exertion, O_{2peak} = O_2 consumption. There was no group interaction and there were no differences in any variable from test 1 to 2. * Group main effect, $p<0.05$

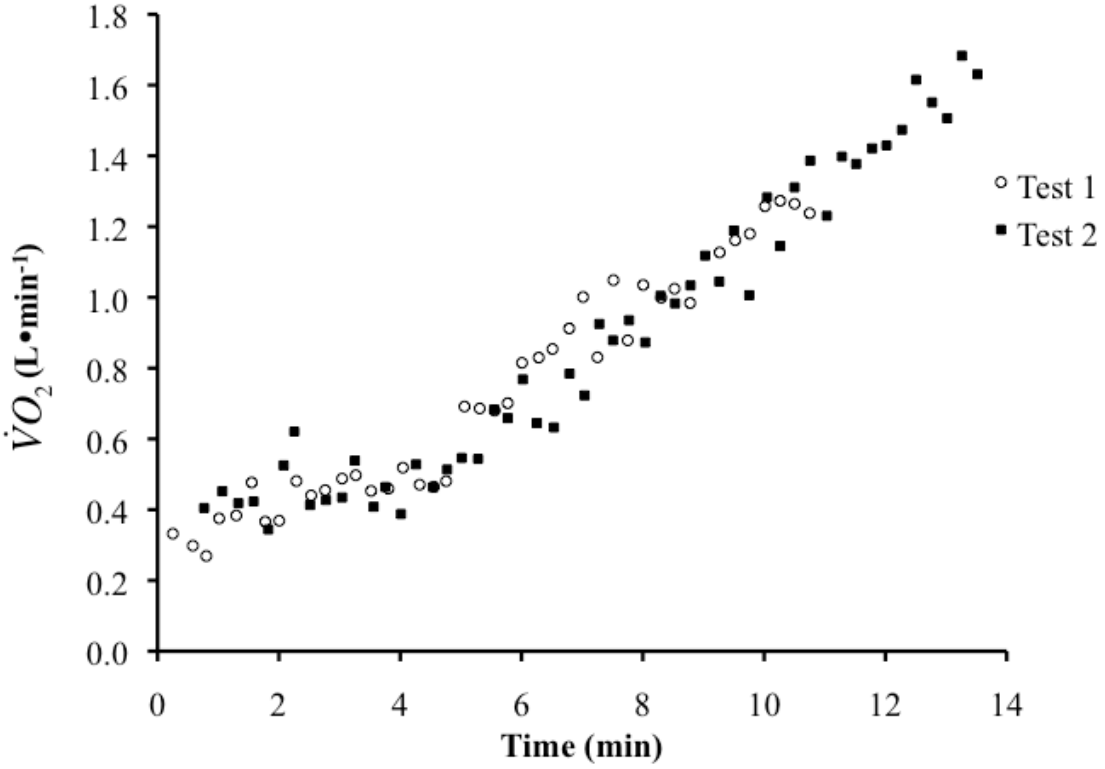

Figure 4-2. Example of variability in $\dot{V}O_2$ from test 1 to test 2.

Data shown are from a single subject during 2 incremental cycle ergometer tests to exhaustion completed 24 hrs apart. The subject cycled at 0 W for the first 3 min, after which, power output increased by 15 W each min until exhaustion. $\dot{V}O_2$ was averaged over 15 s periods throughout the test. Note that test 1 did not reach the criteria for a maximal effort, while test 2 did. No $\dot{V}O_2$ plateau was reached, however, secondary criteria for maximal effort (see section 3.2) were met.

4.3 Muscle Contractile Response

It took 95 ± 30 s and 83 ± 30 s (range: 45-150 s, as long as no technical difficulties were encountered) from the end of exercise until the first post-exercise electrical stimulus was delivered on days 1 and 2, respectively. Individual data are shown in Appendix E. One ME/CFS subject was unable to tolerate supramaximal electrical stimuli on the 2nd day, and technical difficulties prevented interpolated twitch analysis for 2 CON subjects on the 2nd day as well. No VAR was calculated nor were twitches analysed under these circumstances. For 1 of these subjects, no MVC was recorded on the 2nd day either. Data from these subjects on day 1 were included in figures showing individual data points, however, these subjects were excluded from the ANOVA and correlation analyses since they do not represent complete data sets. Therefore, MVC analysis was completed for 9 ME/CFS subjects and 8 CON subjects, while VAR analysis was completed for 8 ME/CFS subjects and 7 CON subjects.

Figures 4-3 and 4-4 show sample torque tracings of MVCs with electrical stimulation before, during and after. Figure 4-3 is an example of a subject with a VAR ~ 100 % while Figure 4-4 is an example of a subject with a VAR ~ 80 %. Note the difference in IT amplitude between figures.

Peak MVC torque measured before and after exercise on both days is shown in Figure 4-5. Baseline MVC was not different between groups (ME/CFS 85.1±24.1 N·m; CON 90.5±19.4 N·m), showing that all subjects were equally as strong. In general, MVC decreased after exercise and then recovered from day 1 to day 2. There was a significant group by time interaction with respect to MVC magnitude ($p=0.040$), indicating a larger decrement in MVC in ME/CFS subjects compared to CON subjects after exercise on day 2. While there was no statistically significant difference between groups in the decrease in MVC after exercise on day 1 (ME/CFS -11.4 ± 12.3 %; CON -17.4 ± 6.2 %; $p=0.298$), ME/CFS subjects showed a 2-fold greater decrease in MVC on day 2 following exercise (-20.0 ± 17.1 %) compared to CON subjects (-10.8 ± 8.7 %) ($p=0.023$). Despite the changes in MVC torque, RMS EMG for each of the 3 muscles (VM, RF and VL) measured at peak MVC prior to IT was the same in both groups and for all 4 MVCs (Table 4-4).

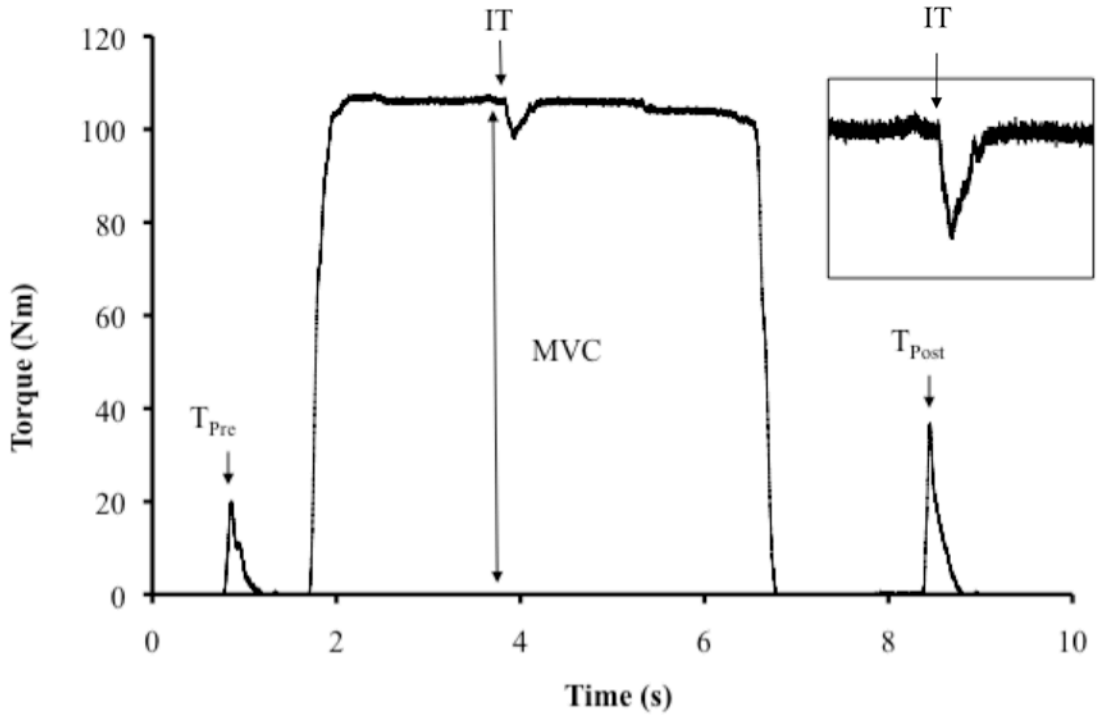

Figure 4-3. Sample MVC torque tracing for a subject with VAR ~ 100%.

Inset shows a magnification of IT. Note that IT amplitude is negligible. Also note that T_{post} is larger than T_{pre}. T_{pre} = pre-MVC stimulated twitch, MVC = maximal voluntary contraction amplitude, IT = superimposed electrical stimulus which may/may not result in an increase in torque, T_{post} = post-MVC stimulated twitch.

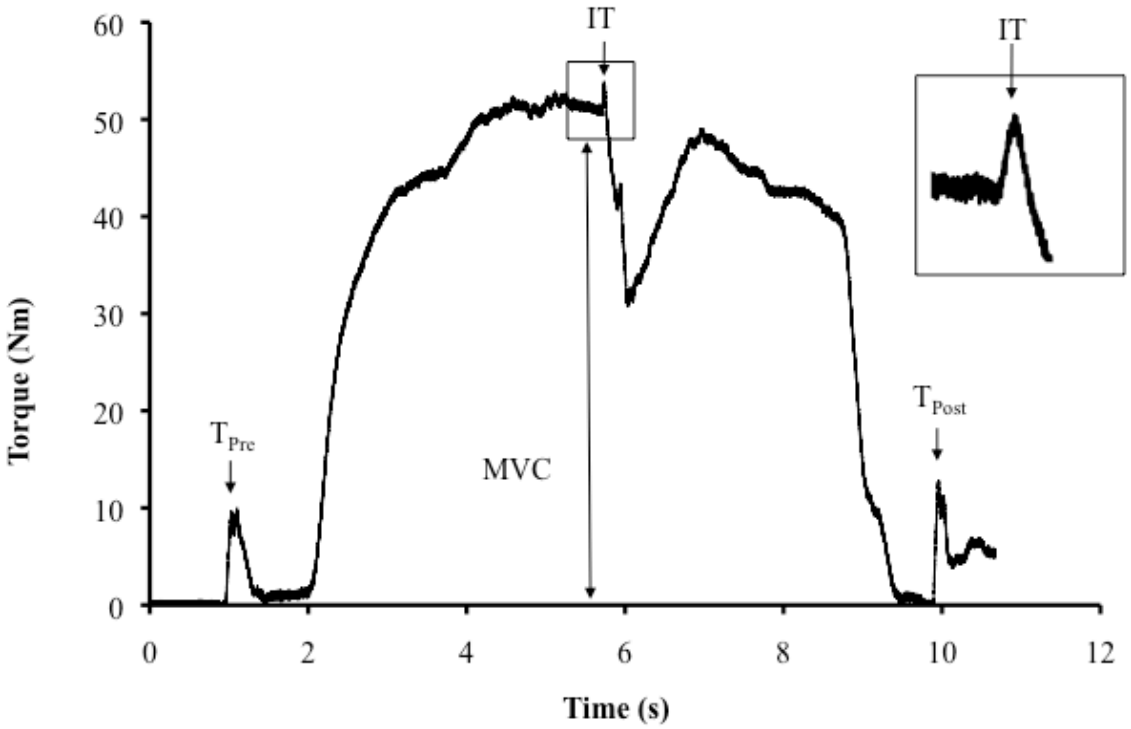

Figure 4-4. Sample MVC torque tracing for a subject with VAR ~ 80 %.

Inset shows a magnification of IT. T_{pre} = pre-MVC stimulated twitch, MVC = maximal voluntary contraction amplitude, IT = superimposed electrical stimulus which may/may not result in an increase in torque, T_{post} = post-MVC stimulated twitch.
For viewing purposes, this figure was digitally filtered using the following equation:
Y= [(Y-2) + (Y-1)*3 + Y*5 + (Y+1)*3 + (Y+2)]/13

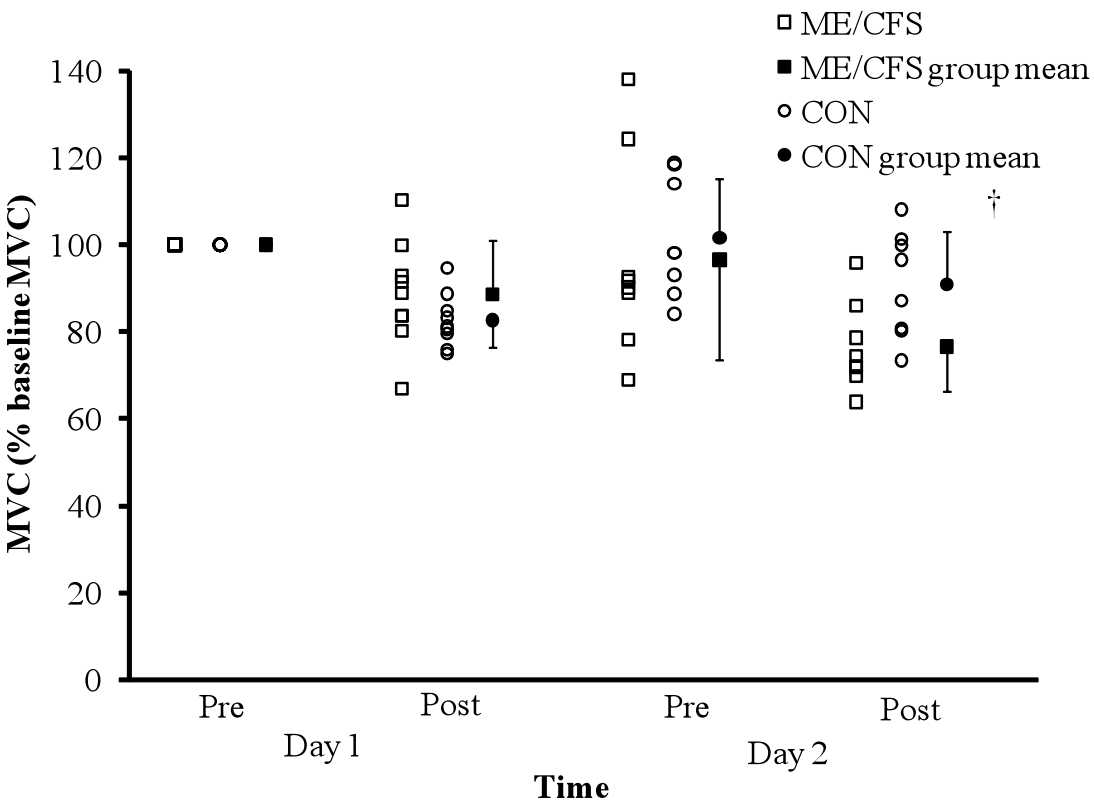

Figure 4-5. MVC pre- and post- exercise on day 1 and day 2.

Individual subjects are indicated by open symbols (ME/CFS □, CON ○), and group means by closed symbols (■, ●). Error bars indicate 1-SD from the mean. Note that the group mean symbols are overlapped on day 1 prior to exercise. There was a significant group by time interaction ($p=0.040$). § Significant difference between ME/CFS and CON, $p<0.05$.

(n = 9 ME/CFS; 8 CON)

Table 4-4. RMS EMG for vastus medialis, rectus femoris and vastus lateralis muscles during MVC.

		RMS EMG (V)					
		ME/CFS			CON		
		VM	RF	VL	VM	RF	VL
Day 1	Pre ex	0.21 ± 0.11	0.37 ± 0.26	0.29 ± 0.19	0.26 ± 0.15	0.39 ± 0.28	0.26 ± 0.13
	Post ex	0.24 ± 0.15	0.44 ± 0.30	0.43 ± 0.32	0.24 ± 0.12	0.43 ± 0.34	0.27 ± 0.27
Day 2	Pre ex	0.25 ± 0.15	0.38 ± 0.20	0.36 ± 0.19	0.27 ± 0.20	0.39 ± 0.20	0.28 ± 0.14
	Post ex	0.17 ± 0.11	0.40 ± 0.24	0.29 ± 0.16	0.24 ± 0.16	0.39 ± 0.19	0.31 ± 0.17

Data are shown as mean ± SD. RMS EMG was calculated both before and after each incremental exercise test using the 500 data points prior to IT stimulation during MVC. There were no statistically significant differences in RMS EMG for any of the 3 muscles. VM = vastus medialis, RF = rectus femoris, VL = vastus lateralis, pre ex = pre incremental exercise test, post ex = post incremental exercise test.
(n = 9 ME/CFS; 8 CON)

As can be seen in Figure 4-6, initial VAR was not significantly different between groups (p=0.19) and changed similarly to MVC with respect to time. There was no group by time interaction (p=0.095), although there was a trend towards a greater decrease in ME/CFS subjects after exercise. VAR decreased by 18.6 ± 15.5 % after exercise on day 2 in ME/CFS compared to 1.5 ± 6.2 % in CON. Overall, there was a group main effect such that VAR was lower in ME/CFS (81.9 ±1 4.1 %) than CON subjects (93.2 ± 13.8 %) (p=0.012). There was also a time main effect such that VAR was lower after each incremental exercise test (p=0.033).

To have confidence in the reported VAR values, it was important to determine whether or not the superimposed twitch was delivered close to each subject's true MVC. The MVC torque used to determine VAR (average of the 500 data points prior to the electrical stimulus) was compared to the single highest torque value attained during each MVC for each subject. The mean difference between peak MVC used for analysis and the highest torque value was 5.6, 4.8, 5.3 and 3.8 % for the MVC pre and post exercise on day 1 and day 2 respectively. Individual data are shown in Appendix F.

Figure 4-7 illustrates the amplitudes of both T_{pre} and T_{post} before and after exercise on both days. There was no significant 3-way interaction between group, time and whether the twitch was elicited before or after the MVC (p=0.217). There was no 2-way interaction between group and time (p=0.190) or time and pre/post (p=0.100) but there was a significant interaction between group and pre/post (p=0.03) such that there was a greater increase in T_{post} compared to T_{pre} in the control subjects. There was no group main effect in any analysis, but there was a time main effect indicating that T_{post} was greater than T_{pre} in all subjects at all time points (p<0.001) and that all twitch amplitudes were smaller after exercise than before exercise on both days (p=0.010).

There were no group by time interactions or group main effects with respect to RMS EMG data for VM (Figure 4-8), RF (Figure 4-9) and VL (Figure 4-10) during the 50 % MVC contractions. There was, however, a main effect of time for both the RF (p=0.001) and VL (p=0.006) muscles. In both cases, RMS EMG was significantly higher in all subjects after the incremental exercise test on both days, while RMS EMG was the

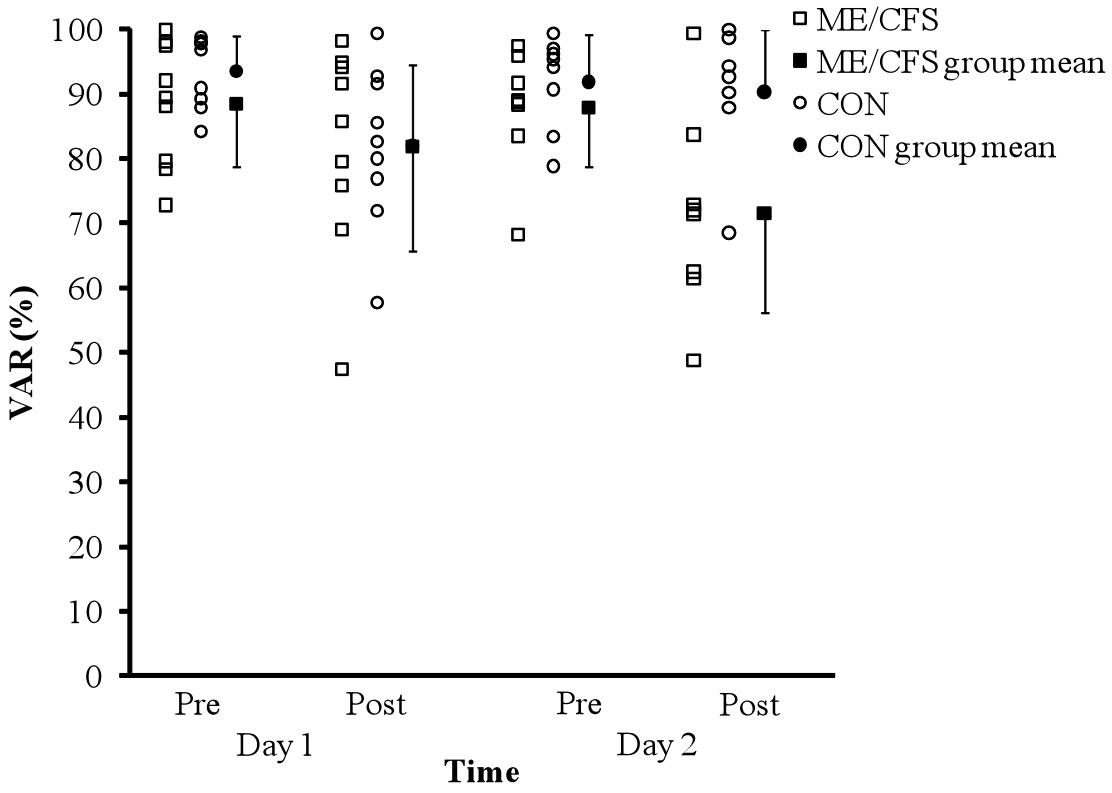

Figure 4-6. VAR pre- and post- exercise on day 1 and day 2.

Individual subjects are indicated by open symbols (ME/CFS □, CON ○), and group means by closed symbols (■, ●). Error bars indicate 1-SD from the mean. The group mean data points post exercise on day 1 are overlapped. There was a significant group main effect ($p=0.012$), with ME/CFS having a lower VAR than CON.
(n = 8 ME/CFS; 7 CON)

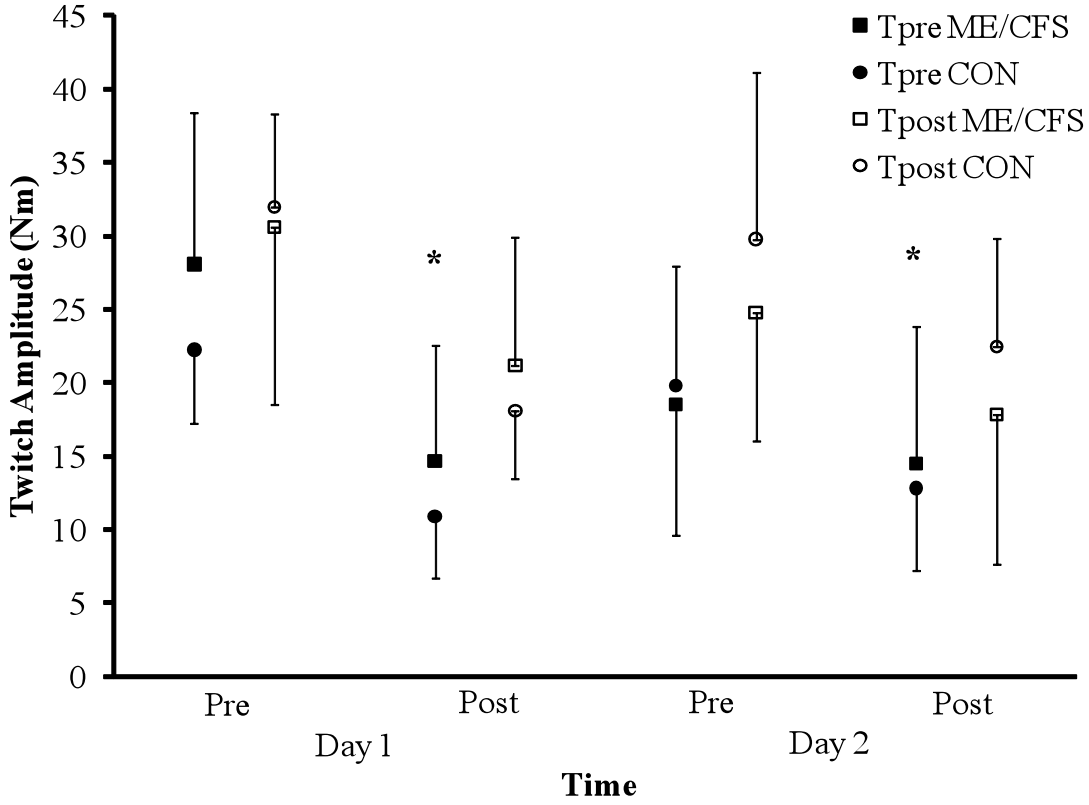

Figure 4-7. Amplitude of electrically evoked twitches pre- and post- exercise on day 1 and day 2.

Twitches evoked from rest prior to each MVC (T_{pre}) are shown by closed symbols (ME/CFS ■; CON ●) while twitches evoked from rest following each MVC (T_{post}) are shown by open symbols (ME/CFS □; CON ○). Electrical stimulation was delivered before and after 2 incremental bicycle tests to exhaustion completed 24 hrs apart. There were no statistically significant group by time interactions. There was no group main effect. There was a time main effect; * significant difference from T_{pre} prior to exercise on day 1, $p<0.05$

(n = 8 ME/CFS; 7 CON)

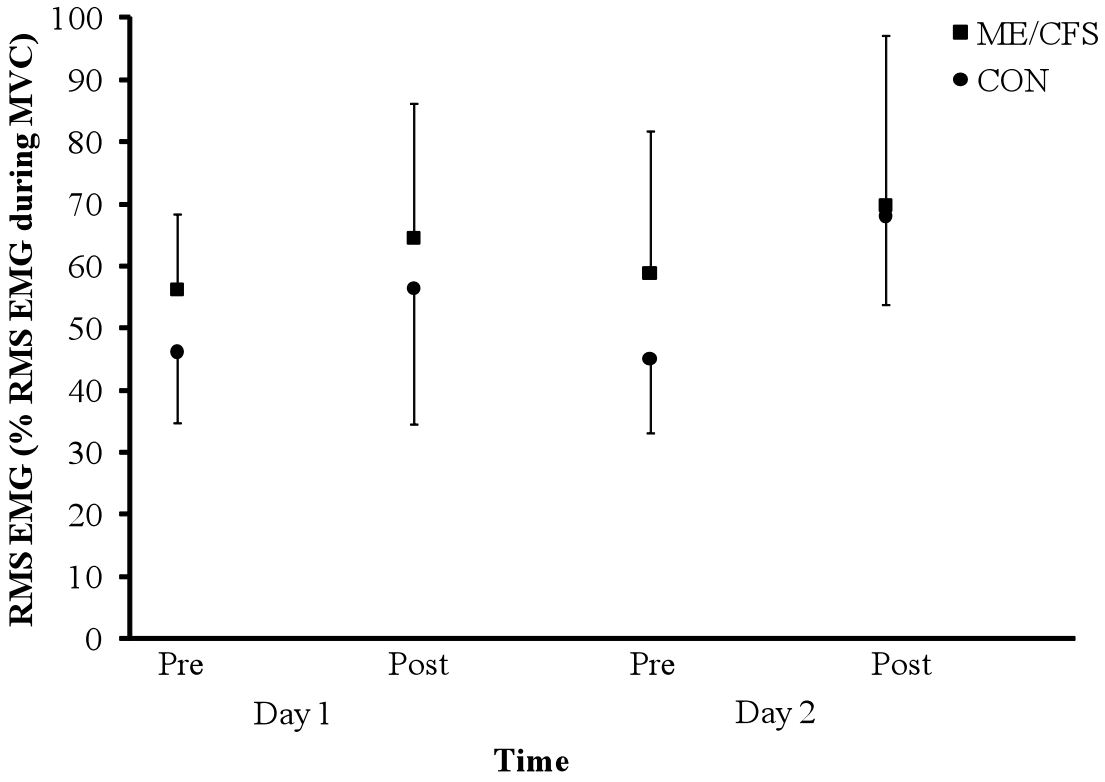

Figure 4-8. RMS EMG for vastus medialis during 50% MVC contractions pre- and post- exercise on day 1 and day 2.

50% MVC contractions were repeated before and after 2 incremental bicycle exercise tests to exhaustion completed 24 hrs apart. Mean (ME/CFS ■; CON ●) RMS EMG is expressed as a % of the RMS EMG recorded during the pre-exercise MVC on day 1. Error bars represent 1 SD from the mean. There were no significant differences between groups or over times.

(n = 9 ME/CFS; 9 CON)

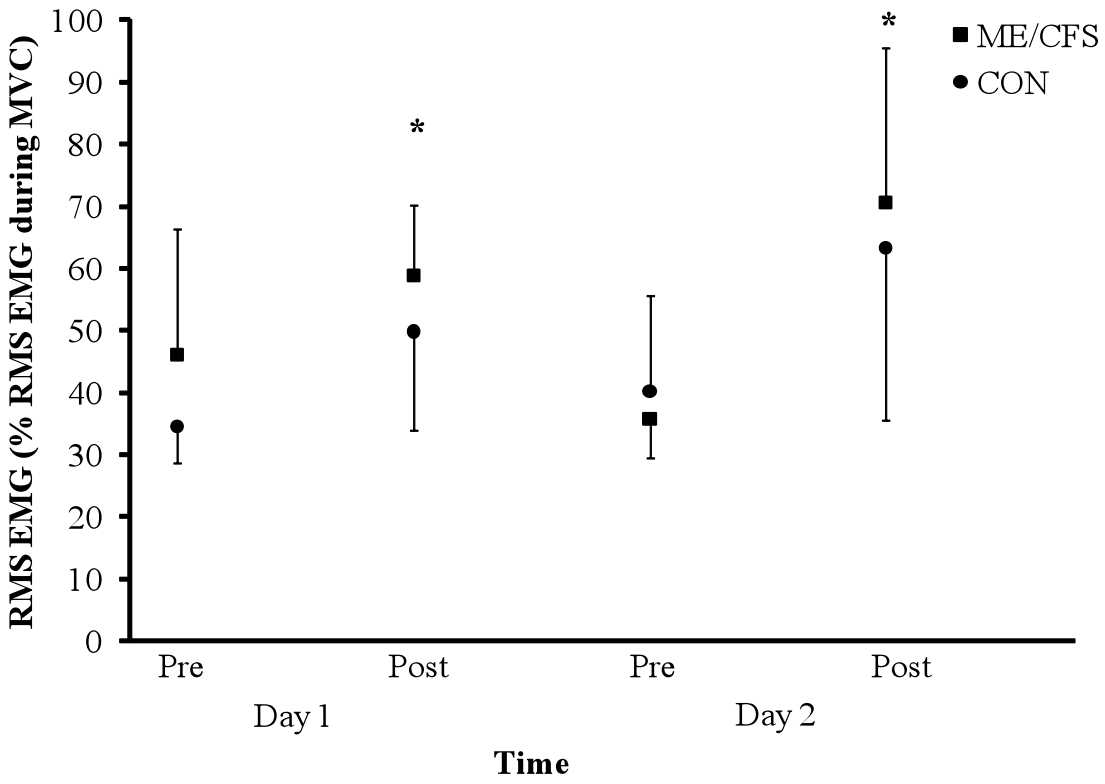

Figure 4-9. RMS EMG for rectus femoris during 50% MVC contractions pre- and post- exercise on day 1 and day 2.

50% MVC contractions were repeated before and after 2 incremental bicycle exercise tests to exhaustion completed 24 hrs apart. Mean (ME/CFS ■; CON ●) RMS EMG is expressed as a % of the RMS EMG recorded during the pre-exercise MVC on day 1. Error bars represent 1 SD from the mean. There was no group by time interaction. There was no group main effect, but there was a time main effect ($p=0.001$). * Significantly different from RMS EMG prior to exercise on day 1, $p<0.05$
(n = 9 ME/CFS; 9 CON)

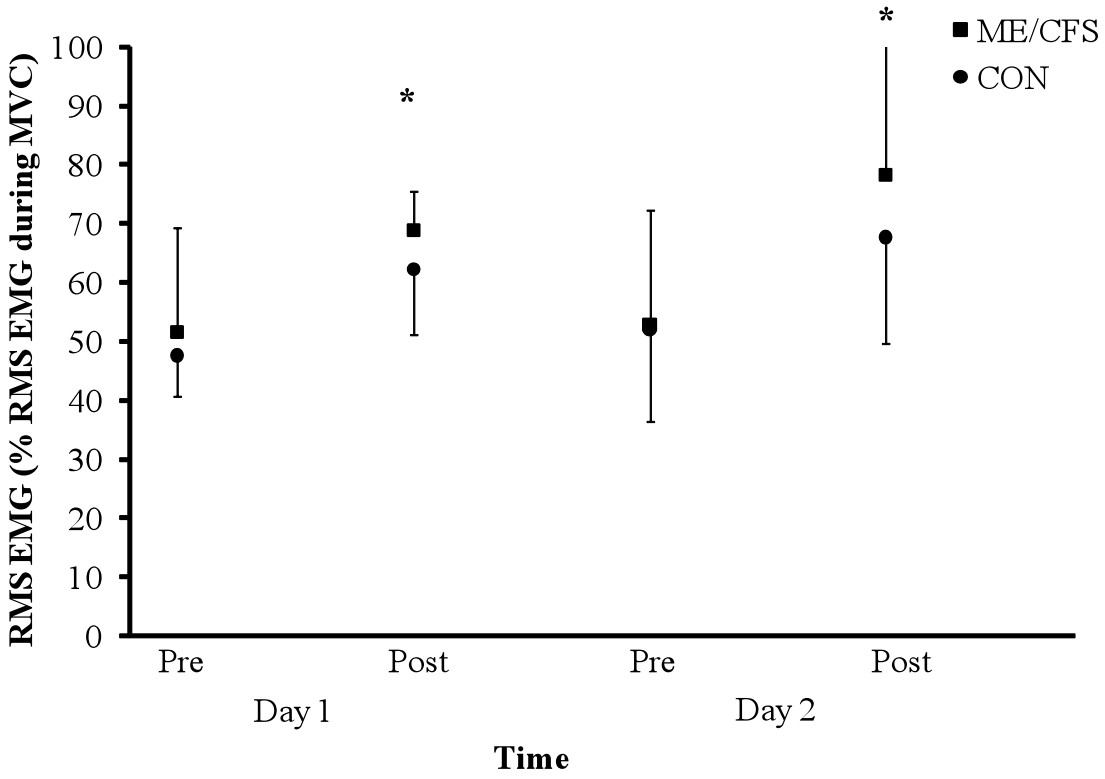

Figure 4-10. RMS EMG for vastus lateralis during 50% MVC contractions pre- and post- exercise on day 1 and day 2.

50% MVC contractions were repeated before and after 2 incremental bicycle exercise tests to exhaustion completed 24 hrs apart. Mean (ME/CFS ■; CON ●) RMS EMG is expressed as a % of the RMS EMG recorded during the pre-exercise MVC on day 1. Error bars represent 1 SD from the mean. There was no group by time interaction. There was no group main effect, but there was a time main effect ($p=0.006$). * Significantly different from RMS EMG prior to exercise on day 1, $p<0.05$
(n = 9 ME/CFS; 9 CON)

same prior to exercise on both days. Unlike RF and VL there was no significant main effect of time in the RMS EMG for VM ($p=0.558$).

Chapter Five: Discussion

This study monitored the development of both central and peripheral fatigue following repeated incremental exercise tests in women with ME/CFS and sedentary but otherwise healthy control subjects. All subjects reached the same $\dot{V}O_{2peak}$ on both incremental exercise tests to exhaustion, and there was no difference in $\dot{V}O_{2peak}$ between groups. VAR was lower in the ME/CFS group than in the control group, providing evidence to suggest that there is greater CAF in ME/CFS subjects. Somewhat surprisingly, there was no evidence to suggest that the larger drop in MVC after the 2nd exercise test was due to greater central or more persistent peripheral fatigue in the ME/CFS group. Unlike the MVC measurements, there was no significant group by time interaction with respect to VAR, which suggests that central fatigue was not different between groups. This is supported by the RMS EMG data from the MVCs, which shows that central motor drive was consistent across all trials. If there had been increased central fatigue in the ME/CFS group, we would have expected to see a significantly larger drop in VAR in the ME/CFS group accompanied by a drop in RMS EMG during the MVC. It should be noted however, that although the group by time interaction for VAR was not significant (p=0.095), with the exception of 1 CON and 1 ME/CFS subject, VAR on day 2 after exercise was lower in all ME/CFS subjects than all CON subjects. The fact that there were no differences in T_{pre} amplitude or RMS EMG at 50% MVC torque between groups at any time point suggests that both the magnitude of peripheral fatigue caused by the incremental exercise tests and the extent of recovery from day 1 to day 2 were similar in both ME/CFS and control subjects.

Indeed, the larger drop in MVC in the ME/CFS group despite the finding that the indices of both central and peripheral fatigue measured in this thesis remained similar between groups, can be explained by the presence of LFF. Both the decrease in T_{pre} amplitude and the increase in RMS EMG amplitude at 50% MVC torque are due to the rightward shift in the force-frequency relationship associated with LFF. This shift also explains how VAR can appear to decrease while central motor drive remains consistent, as suggested by the consistent RMS EMG amplitude observed during each MVC. Finally,

the greater level of CAF in the ME/CFS subjects explains why, in the presence of the same magnitude of LFF as the control subjects, MVC dropped more in the ME/CFS subjects after the 2nd exercise test. This will be discussed in more detail in section 5.3. Although none of the changes in muscle contractile function in the ME/CFS group resulted in an inability to reach the same $\dot{V}O_{2peak}$ on the 2nd incremental exercise test, central fatigue, CAF and LFF may still contribute to the symptoms of post-exertional malaise.

5.1 Subject Characteristics

Mean $\dot{V}O_{2peak}$ for the subjects in this study was 23 $mL \cdot kg^{-1}min^{-1}$, while the normal $\dot{V}O_{2peak}$ for women aged 40-50 is 28-33 $mL \cdot kg^{-1} \cdot min^{-1}$ (Heyward, 2006). Previous studies measuring $\dot{V}O_{2peak}$ in ME/CFS subjects using a variety of incremental protocols have reported values that are slightly higher than those presented in this thesis (Ottenweller *et al.*, 2001; Wallman *et al.*, 2004; VanNess *et al.*, 2007), confirming that all subjects participating in this study were very unfit. Since cardiovascular fitness was the same in both groups, it can be concluded that neither the observed change in MVC nor the difference in VAR between groups was due to a difference in fitness levels.

Significantly lower scores on the SF-36 health survey questionnaire suggest that there is considerable quality of life impairment associated with ME/CFS. Other studies using the SF-36 questionnaire with both ME/CFS patients and control subjects have reported similar scores in all of the subscales (Nijs *et al.*, 2010; VanNess *et al.*, 2010). The stringent inclusion criteria used for the control group in the present study, specifically that they could not meet more than 1 Fukuda ME/CFS criterion on the CDC CFS questionnaire, likely explains why the control subjects scored above average on all scales.

The correlation between changes in VAR from before to after exercise on day 2 with scores on the SF-36 questionnaire suggested that individuals who scored lower on certain SF-36 subscales experienced a greater drop in VAR. The SF-36 questionnaire is thought to be a valid, reliable and sensitive measure of the symptoms of post-exertional

malaise. It has also been used to monitor the development of post-exertional malaise in individuals with ME/CFS following different exercise protocols (Nijs *et al.*, 2008a; Nijs *et al.*, 2008b; Nijs *et al.*, 2010; VanNess *et al.*, 2010). Since VAR is an index of central fatigue, and it is related to CAF, the correlation between change in VAR on the 2nd day and SF-36 scores suggests that central fatigue and CAF may be related to the symptoms of post-exertional malaise. Specifically, greater central fatigue and CAF (as indicated by a larger decrease in VAR), may be associated with lower scores on the SF-36 questionnaire, and therefore with the symptoms of post-exertional malaise.

While this questionnaire is clearly able to differentiate between healthy individuals and those with ME/CFS, it is unlikely to be able to distinguish between ME/CFS and related disorders such as fibromyalgia and major depression. This must be considered when using the SF-36 questionnaire as part of the diagnostic procedure for ME/CFS. Previously, the physical function scale was used on its own to monitor changes in symptoms and to evaluate various ME/CFS therapies (White *et al.*, 2007). Assuming that the change in VAR on the 2nd day is characteristic of individuals with ME/CFS, the relationship between it and several of the SF-36 sub scales suggests that the vitality, role physical, general health, physical components, body pain and social function scales might be useful, in addition to the physical function scale, for identifying the symptoms of post-exertional malaise.

Despite differences in SF-36 scores between groups, there was no difference in exercise performance. This suggests not only that an ability to repeat maximal effort exercise on consecutive days is not necessarily indicative of a high quality of life, but also that repeated incremental exercise tests are not necessarily sensitive to the factors influencing quality of life in ME/CFS patients. Together, these findings suggest a limited usefulness for repeated incremental exercise tests in the diagnosis of ME/CFS.

5.2 Incremental Exercise Tests

Dual exercise test protocols similar to the one used in the present study have been completed with individuals suffering from a variety of chronic diseases including pulmonary hypertension (Hansen *et al.*, 2004), renal disease (Koufaki *et al.*, 2001),

valvular heart disease (Lehmann and Kölling, 1996), restrictive lung disease (Marciniuk *et al.*, 1993) and cystic fibrosis (McKone *et al.*, 1999). In all cases, $\dot{V}O_{2peak}$ was found to be a reliable measure that varied only slightly from day-to-day.

In this thesis, consistent values for $\dot{V}O_{2peak}$, HR_{peak} and LT were obtained for each individual from test 1 to test 2, showing that these values were reproducible within the tested populations. The coefficients of variation for $\dot{V}O_{2peak}$, HR_{peak} and LT were 5.5 %, 2.8 % and 8.4 % in ME/CFS and 4.6 %, 3.4 % and 6.0 % in CON. This variation was mostly related to small changes in time to exhaustion from test 1 to test 2, rather than changes in efficiency. It remains to be seen whether the greater variability in $\dot{V}O_{2peak}$ and LT in ME/CFS subjects is related to the disease or not.

It was quite surprising to find that despite the ME/CFS subjects stopping sooner than the control subjects, both groups reached the same $\dot{V}O_{2peak}$ on both tests. Previous studies assessing $\dot{V}O_{2peak}$ in ME/CFS subjects and controls either reported similar values between groups and no differences in time to exhaustion (Ottenweller *et al.*, 2001; VanNess *et al.*, 2007) or lower values in ME/CFS along with shorter time to exhaustion (Sisto *et al.*, 1996). A study by De Becker *et al.* (2000) reported lower $\dot{V}O_{2peak}$ in ME/CFS subjects, yet their times to exhaustion are irrelevant because control subjects and ME/CFS subjects completed tests at different power outputs. The shorter time to exhaustion presented in this thesis combined with the fact that all subjects reached the same level of peripheral fatigue may indicate that ME/CFS subjects fatigue at a faster rate than control subjects. Since indices of skeletal muscle fatigue (see the following section) were only measured at the end of each exercise test, this hypothesis cannot be proven nor disproven without further research. No previous studies have investigated the rate of development of skeletal muscle fatigue during an incremental exercise test in ME/CFS patients and few, if any, have looked at this in control subjects either.

This thesis is not the first study to have reported similar $\dot{V}O_{2peak}$ in ME/CFS subjects and sedentary but otherwise healthy controls. In 2001, Ottenweller and colleagues measured the response to an incremental treadmill walking protocol to exhaustion in individuals with ME/CFS and sedentary controls. Mean $\dot{V}O_{2peak}$ in the

control group was 30.4±1.2 mL·kg^{-1}·min^{-1} vs. 28.2±1.7 mL·kg^{-1}·min^{-1} in ME/CFS subjects. Studies that have reported lower $\dot{V}O_{2peak}$ in ME/CFS subjects compared to control subjects likely used less stringent criteria to determine maximal effort (i.e.: RER > 1.0 and HR within 15 % of age-predicted maximum vs. RER > 1.1 and HR within 10 beats of age predicted maximum) (Riley *et al.*, 1990; De Becker *et al.*, 2000) or incremental protocols that were inappropriate (i.e.: increments in power output between stages were too large) (Sisto *et al.*, 1996) for such a sedentary population.

VanNess *et al.* (2007) completed a repeated incremental cycle ergometer exercise protocol nearly identical to the one presented in this thesis, in ME/CFS subjects and sedentary control subjects. They found that $\dot{V}O_{2peak}$ was similar between groups during their 1st test (ME/CFS 26.2±4.9 mL·kg^{-1}·min^{-1} vs. CON 28.4±7.2 mL·kg^{-1}·min^{-1}), yet $\dot{V}O_{2peak}$ was significantly lower in the ME/CFS group during the 2nd test (ME/CFS 20.5±1.8 mL·kg^{-1}·min^{-1} vs. CON 28.9±8.0 mL·kg^{-1}·min^{-1}). Since baseline $\dot{V}O_{2peak}$ was lower in this thesis than in the study by VanNess *et al*., the contrasting findings regarding the ability of ME/CFS subjects to reach the same $\dot{V}O_{2peak}$ on the 2nd incremental exercise test cannot be explained by poorer aerobic fitness. Although VanNess and colleagues used a ramp incremental protocol while this thesis used a step incremental protocol, it is unlikely that this contributed to the different results. A separate comparison of ramp vs. step incremental protocols in subjects with ME/CFS found no difference in $\dot{V}O_{2peak}$ between the protocols (Nijs *et al.*, 2007). The most likely reason for the discrepancy between the 2 studies is that only a subset of individuals with ME/CFS actually expresses an inability to repeat performance on dual incremental exercise tests. Out of 100s of dual incremental exercise tests, only about half of the ME/CFS subjects have demonstrated an inability to reach the same $\dot{V}O_{2peak}$ on the 2nd incremental exercise test (J.M. VanNess and S.R. Stevens, personal communication, June 25, 2010). Furthermore, the lack of differences in $\dot{V}O_{2peak}$, P_{peak}, HR_{peak} or LT between groups in this thesis suggests that neither these measures nor dual graded exercise tests are appropriate diagnostic tools for ME/CFS.

Although RPE was not different between groups at submaximal intensities, it was significantly lower in the ME/CFS subjects at the end of exercise. This is in contrast to many previous studies where abnormally high RPE was reported in ME/CFS (Riley *et al.*, 1990; Gibson *et al.*, 1993; Fulcher and White, 2000; Georgiades *et al.*, 2003; Wallman *et al.*, 2004; Neary *et al.,* 2008; Light *et al.*, 2009). Although several studies have shown no differences in RPE in ME/CFS subjects compared to control subjects (Lloyd *et al.*, 1991; Sisto *et al.*, 1996; Cook *et al.*, 2003), none to date have reported lower RPE for those with ME/CFS. It is unlikely that the lower RPE_{peak} in this thesis was due to a lack of effort on the part of the ME/CFS subjects, as 8 out of 9 satisfied the criteria for maximal effort on both incremental tests. However, it is possible that had the ME/CFS subjects been able to exercise as long as the control subjects, they may have reached the same level of perceived exertion as the control group. Another theory is that this finding may indicate the extent of functional decline in the ME/CFS subjects. If they were aware that they used to be able to exercise much harder prior to becoming sick, then they may have perceived their current effort as not as high.

The significantly lower RPE_{peak} as well as the trend towards lower $[BLa^{-}_{peak}]$ in ME/CFS despite normal levels at LT are also not due to differences in exercise intensity. All subjects completed the same incremental exercise protocol, and since both LT and P_{peak} were similar between groups, the relative intensity of each exercise test was similar. However, the shorter absolute exercise duration in ME/CFS subjects compared to CON could explain the trend towards lower $[BLa^{-}_{peak}]$.

The lower RPE_{peak} and the trend towards lower $[BLa^{-}_{peak}]$ in ME/CFS, despite similar levels at LT, could be explained by impaired HPA axis and sympathetic nervous system function. Cortisol and epinephrine are 2 key compounds released by these systems and low levels have been associated with fatigue (Brosnan and Gowing, 1996; Streeten and Anderson, 1998). Many studies have reported decreased cortisol levels in ME/CFS (Cleare *et al.*, 1995; Cleare, 2001; Jerjes *et al.*, 2005; Jerjes *et al.*, 2006) and 1 study has shown a decreased epinephrine response to maximal effort exercise in ME/CFS (Ottenweller *et al.*, 2001). However, other studies have found no differences in cortisol levels between ME/CFS subjects and control subjects (Cleare, 2003; Di Giorgio *et al.*,

2005). Thus, there is not irrefutable evidence that HPA axis and sympathetic function are impaired in all ME/CFS patients. The variable findings may be due to the different sampling protocols that were used, as well as the unique circumstances under which each study monitored hormone response. Furthermore, several studies collected single hormone samples, despite the fact that cortisol is released on a diurnal pattern. Generalization of the results from a single sample to overall cortisol release may be misleading if the diurnal pattern is shifted, as has been suggested in ME/CFS (Di Giorgio *et al.*, 2005).

Normally, LT is associated with a spike in epinephrine release which stimulates both glycogenolysis and glycolysis (Mazzeo and Marshall, 1989). This increases the availability and metabolism of glucose, as well as the production of lactic acid, in order to provide energy for higher intensity exercise. Thus, a smaller-than-expected epinephrine response at and above LT in ME/CFS, due to impaired HPA axis and sympathetic nervous system function, could result in impaired glycogenolysis and lower [BLa^-_{peak}]. This could explain the findings of Ottenweller et al., described above (2001). Additionally, plasma epinephrine concentration has been linked to RPE such that higher concentrations of epinephrine in the plasma were associated with higher RPE (Skrinar *et al.*, 1983). Therefore, reduced epinephrine release in the ME/CFS groups could also explain the lower RPE_{peak} reported in this group.

Epinephrine upregulates glycogenolysis and glycolysis by binding to adrenergic receptors on the cell membrane and activating second messengers within the cell (i.e.: cyclic AMP). These second messengers then activate cellular kinases which phosphorylate various enzymes to increase their activities and stimulate glycogenolysis and glycolysis. A recent study by Light *et al.* (2009) reported lower levels of adrenergic receptor mRNA both before and after 25 min of submaximal bicycle exercise in ME/CFS subjects compared to control subjects. This might suggest that there are fewer adrenergic receptors in ME/CFS patients. Therefore, the lower RPE_{peak} and the trend towards decreased [BLa^-_{peak}] may actually be the result of a decrease in the number of adrenergic receptors in ME/CFS subjects rather than decreased epinephrine release. Indeed, a decrease in the number of adrenergic receptors would decrease the efficiency of the

sympathetic nervous system, and impair its ability to respond to stimuli such as high intensity exercise.

In addition to comparing raw RPE values associated with specific workloads, it has been proposed to investigate the HR:RPE ratio (Neary *et al.,* 2008). By expressing RPE relative to HR, it is possible to assess whether perceived exertion is altered for a given physiological intensity (as indicated by HR). Despite the fact that RPE was significantly lower in the ME/CFS group at maximal exercise on both days, while there was no difference in HR_{max} between groups, there was no significant difference in the HR:RPE ratio at the end of exercise between groups or from test 1 to test 2. The most likely explanation for this finding is that the large inter-individual variability in HR_{peak} resulted in insufficient power to detect a statistically significant difference given the sample size in this thesis. In general, HR_{peak} appeared to be lower in the ME/CFS subjects compared to control subjects. This, combined with the lower RPE_{peak} would explain why there was no significant difference between groups with respect to the HR:RPE ratio. Since neither HR nor RPE were different between groups at LT, it follows that the HR:RPE ratio was the same in both groups.

Although the magnitude of the HR:RPE ratios reported in this thesis for the ME/CFS group are similar to those reported by Neary *et al.* (2008), those reported for the control group are lower in this thesis. The discrepancy in these results can possibly be attributed to differences in fitness between the control groups used in the 2 studies. The $\dot{V}O_{2peak}$ in the control group from the study by Neary and colleagues (33.0 ± 3 $mL \cdot kg^{-1} \cdot min^{-1}$) was significantly higher than both their ME/CFS group (23.8 ± 10 $mL \cdot kg^{-1} \cdot min^{-1}$) as well as the control group used in this thesis (23.5 ± 2.8 $mL \cdot kg^{-1} \cdot min^{-1}$). The superior fitness of the control group used by Neary *et al.* may have influenced their perceived exertion such that it was lower for any given heart rate when compared to less fit individuals. This would explain why the HR:RPE ratio in those control subjects was significantly higher than both that of the ME/CFS subjects in either study and the control subjects in this thesis.

Exercise protocols of varying intensities and durations have been suggested as part of the treatment for ME/CFS, since regular exercise has been effective in improving

quality of life in both healthy and other chronically ill populations (Pedersen and Saltin, 2006). In a systematic review published in 2006 by Chambers and colleagues, the majority of RCTs investigating the effects of exercise therapy in ME/CFS showed an improvement in both physical and psychological symptoms. However, most studies also reported exacerbations of symptoms in some subjects. The effect that regular exercise therapy programs have on the development of central fatigue, CAF and LFF in ME/CFS remains unknown. Perhaps those individuals who experienced exacerbations in symptoms responded to the exercise program with greater than normal central fatigue and prolonged CAF as well as LFF.

5.3 Muscle Contractile Response

There was no evidence that individuals with ME/CFS were weaker than healthy but otherwise sedentary individuals, as there was no difference in MVC between groups at baseline. Similar findings were reported by Gibson and associates in 1993, who measured quadriceps femoris MVC prior to and 5 minutes after an incremental bicycle test to exhaustion in individuals with ME/CFS and sedentary controls. They found no differences in MVC between groups either before or after exercise. Other studies by Samii *et al*. (1996), Sisto *et al.* (1996) and Fulcher and White (2000) also found that ME/CFS subjects were as strong as healthy control subjects.

The comparison of the highest torque (single data point) obtained by each subject during each MVC contraction to the MVC calculated as the average of the 500 data points prior to IT revealed that there was a 3 – 5 % difference between these values (Appendix F). This difference can be attributed, in part, to the noise in the signal obtained from the Biodex dynamometer. The noise appears to be ~ 3 - 4 N·m in most torque tracings, which is ~ 2 % for a 150 N·m MVC and ~ 6 % for a 50 N·m MVC. The MVCs in this thesis ranged from 46 – 139 N·m. The largest difference between the single highest torque data point and the calculated MVC was 11 N·m and only 8 times was the difference larger than 7 N·m .While in many instances, the highest torque data point actually occurred within the 500 data points used to calculate MVC, there were others

where it did not. Out of the 70 MVC contractions analysed (18 subjects * 4 MVCs each = 72, less the 2 MVCs not obtained from 1 CON subject on day 2 due to technical difficulties), only 10 times did the highest torque data point fall outside the 500 data points used to calculate MVC.

Mean VAR was lower in ME/CFS than CON which, on its own, indicates the presence of persistent CAF. This finding supports previous studies that have reported reduced central muscle activation (Kent-Braun *et al.*, 1993; Schillings *et al.*, 2004), M-wave amplitude (Jammes *et al.*, 2005) and MEP amplitude (Samii *et al.*, 1996) in ME/CFS. Although Lloyd et al. (1991) found no evidence of impaired central activation in ME/CFS, they had an inclusion criteria of VAR > 95 %, which might explain the different result. It is also possible that they did not collect data at a high enough frequency (only 50Hz) to be able to detect small changes in IT amplitude.

Despite the 17 % greater drop in VAR after exercise on the 2nd day in the ME/CFS subjects, there was only a trend towards a statistically significant group by time interaction. Due to the large inter-individual variability in VAR, it is possible that if we had recruited more subjects for this study, we may have had the power to detect a statistically significant interaction. This is a potentially valuable result with respect to improving the understanding of skeletal muscle fatigue in ME/CFS, and it deserves further investigation.

If central fatigue was increased in ME/CFS subjects after the 2nd exercise test, as could be inferred by the trend towards a significant group by time interaction with respect to VAR (see Figure 4-6), it would be expected that RMS EMG during the MVC measured after the 2nd exercise test would be lower than at baseline. However, this was not the case, as RMS EMG during the MVC did not change over time in either group (see Table 4-4). Although the presence of LFF cannot be confirmed, as a force-frequency relationship was not measured in this thesis, LFF can account for both the observed decrease in MVC and the trend towards decreased VAR in ME/CFS subjects compared to control subjects despite a constant central motor drive.

Figure 5-1 shows a typical force-frequency curve in a rested muscle (curve A) as well as the shift to the right associated with LFF in that muscle (curve B). The rightward

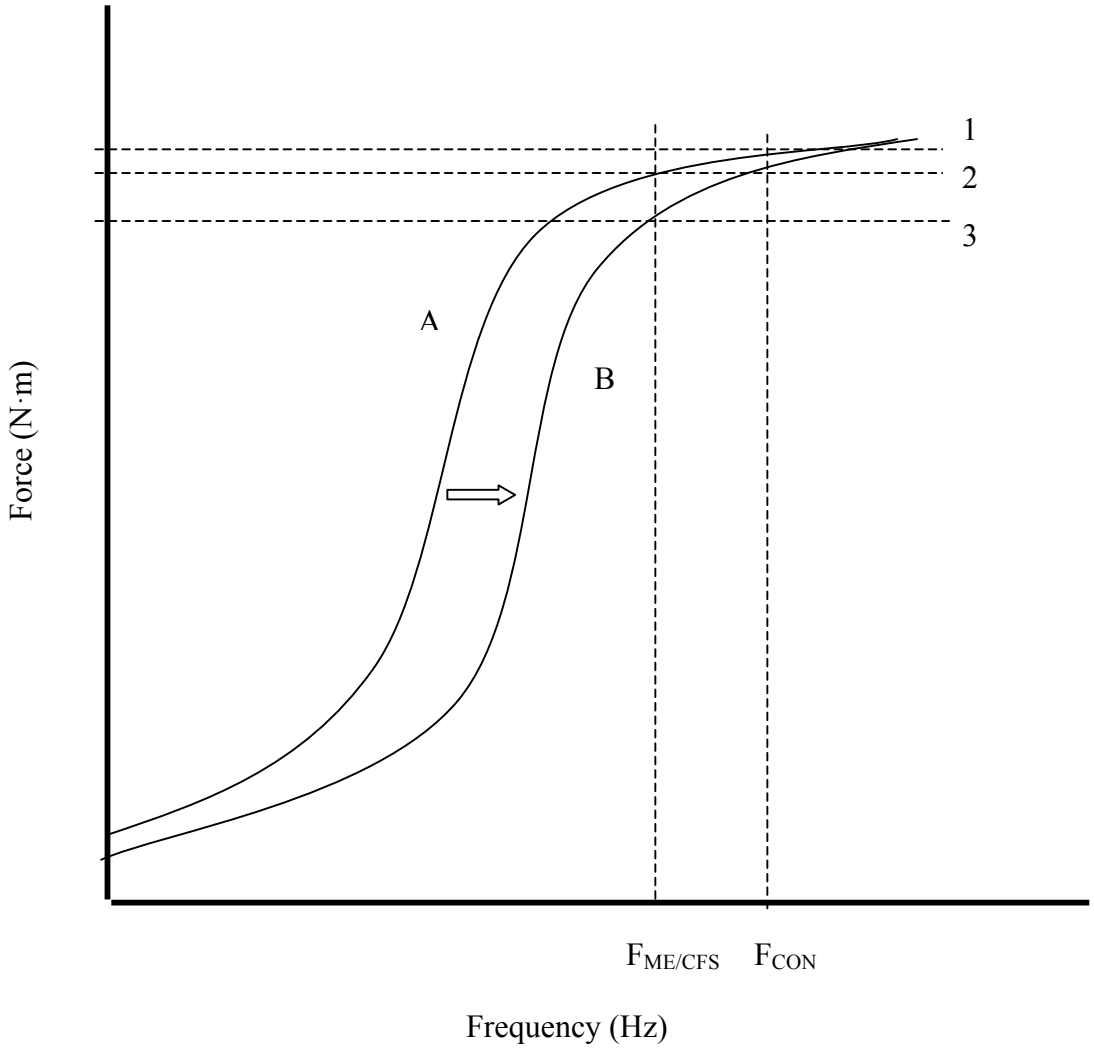

Figure 5-1. Effect of LFF on MVC and VAR measurements.

Curve A represents the force-frequency curve at rest. Curve B represents the rightward shift that occurs with LFF. $F_{ME/CFS}$ represents the maximal voluntary rate of motor unit recruitment in the ME/CFS subjects. F_{CON} represents the maximal voluntary rate of motor unit recruitment in the control subjects. $F_{CON} > F_{ME/CFS}$ because VAR was higher in the control subjects. Lines 1, 2, and 3 indicate MVC associated with a specific frequency of motor unit recruitment and the presence (or absence) of LFF. See text for detailed description.

shift reflects the fact that in order to generate the same force in a muscle with LFF as in the rested state, a higher frequency of motor unit activation is required. LFF results in a decrease in twitch amplitude, as is shown in Figure 5-1 by the fact that curve B crosses the y-axis below curve A. This explains why both T_{pre} and T_{post} amplitude were significantly lower after each incremental exercise test compared to before the test.

It can also be seen from Figure 5-1 that a higher frequency of motor unit activation is required to generate 50 % MVC torque in the presence of LFF. This increased frequency of motor unit recruitment explains the observed increase in RMS EMG for both the RF and VL in both groups. The most likely explanation for why no increase was observed in the RMS EMG from the VM is simply that recruitment of these motor units was not adjusted to compensate for the presence of fatigue.

Finally, Figure 5-1 can also be used to illustrate how LFF explains both the large decrease in MVC after the 2^{nd} exercise test in the ME/CFS group as well as how VAR tended to decrease in the ME/CFS group despite the fact that RMS EMG during each MVC did not change. As mentioned above, the lower overall VAR in the ME/CFS subjects vs. the control subjects suggests that there was greater CAF in the ME/CFS group. From this, it can be inferred that the frequency of motor unit activation during the MVC was lower in the ME/CFS group than in the control group. Therefore, the ME/CFS subjects may have been activating their muscles at frequency $F_{ME/CFS}$ while the control subjects were at F_{CON}. This placed the ME/CFS subjects at the intersection of curve A and line 2, while the control subjects were at the intersection of curve A and line 1. Since both groups were close to the plateau portion of the force-frequency curve, there was very little difference in MVC amplitude in the rested muscle (prior to exercise) between groups.

The incremental exercise test caused the development of LFF in all subjects, such that the force frequency curve shifted to the right and became curve B. On curve B, it is still possible for the muscle to generate true maximal power, albeit at a higher frequency of motor unit firing than in the non-fatigued state. Given that the RMS EMG amplitude at MVC was consistent in both groups from before to after exercise, it can be assumed that both groups were able to reach the same frequency of motor unit recruitment after the

incremental test as before. Therefore, the ME/CFS subjects were still at $F_{ME/CFS}$ while the control subjects were still at F_{CON}. The MVC generated by the ME/CFS subjects corresponded to the intersection of curve B and line 3, while MVC in the control subjects corresponded to the intersection of curve B and line 2. As is evident from Figure 5-1, this resulted in a relatively larger decrease in MVC in the ME/CFS subjects than the control subjects, because they were closer to the steep portion of the force-frequency curve. In order to generate the same MVC in the fatigued state as in the non-fatigued state, the frequency of motor unit firing needed to increase. Since the ME/CFS subjects were voluntarily generating a much smaller proportion of true muscle maximum force after exercise compared to the control subjects, VAR also trended towards a decrease. This is how VAR appeared to decrease, despite the fact that RMS EMG at MVC stayed constant.

Several authors have acknowledged the existence of LFF and that it can persist for days following a fatiguing effort (Fitts, 1994; Jones, 1996). LFF is thought to be caused by a disruption in excitation-contraction coupling associated with decreased Ca^{2+} release from the SR. In order to confirm the presence of LFF in ME/CFS subjects following a bout of exercise, a force-frequency curve for the muscle(s) of interest would need to be determined both prior to and following the exercise. Additionally, muscle biopsies taken immediately following electrical stimulation of the muscle could provide information regarding Ca^{2+} release from the SR in response to a specific level of stimulation. If Ca^{2+} release as a result of electrical stimulation was lower after exercise than before, it would support the hypothesis that LFF is present. To date, no researchers have obtained muscle biopsies from ME/CFS subjects. Due to the invasive and potentially painful nature of muscle biopsies, it may be difficult to obtain them from a group of ME/CFS subjects.

Baseline VAR was lower in both groups than had been previously reported for the quadriceps femoris muscle group (Kent-Braun *et al.*, 1993; Kent-Braun, 1999). The lower VAR in ME/CFS subjects is likely due to differences in the formula used to calculate VAR between studies. Kent-Braun *et al.* (1993) measured central activation as the ratio between MVC and (MVC+ superimposed tetanus). The lower VAR in the control subjects may be attributed to the fact that they were quite sedentary and unfit.

Regular physical activity not only trains the cardiovascular system, but it also improves neuromuscular coordination to optimize muscular contractions (Enoka, 1997).

The finding that T_{post} was consistently higher than T_{pre} at each time point ($p<0.001$) is evidence that muscular contraction causes not only fatigue, but also potentiation, and that the amplitude of successive contractions is dependent upon the net of fatigue and potentiation present in the muscle (Rassier and MacIntosh, 2000). This also suggests that within 2 -3 s of a 5 s MVC, there is more potentiation than fatigue present in the muscle. Similar findings have been reported following brief tetanic contractions in rat gastrocnemius muscles (MacIntosh and Rassier, 2002), as well as following 10 s quadriceps MVCs in humans (Hamada *et al.*, 2000).

The increase in twitch amplitude from T_{pre} to T_{post} was less in the ME/CFS subjects than in the control subjects (Figure 4-6), suggesting that either more fatigue or less potentiation was present (or some combination of both) in the ME/CFS subjects. Since none of the indices measuring fatigue were different between groups, it is hypothesized that less potentiation was present. This is supported by the finding that VAR was lower in ME/CFS compared to control subjects. Firstly, lower VAR would, in theory, lead to smaller increases in intracellular [Ca^{2+}] during each MVC. Considering that potentiation is thought to occur via phosphorylation of the myosin light chain by myosin light chain kinase, and that myosin light chain kinase is activated by Ca^{2+} in the cytosol, it follows that lower levels of voluntary muscle activation would lead to less potentiation (Rassier and MacIntosh, 2000; MacIntosh and Rassier, 2002; MacIntosh *et al.*, 2006). Secondly, according to the size principal of motor unit recruitment, the last motor units recruited during a maximal voluntary contraction are typically the largest, fast-twitch motor units (MacIntosh *et al.*, 2006). It has also been suggested that fast-twitch muscles are capable of exhibiting greater potentiation than slow-twitch muscles. Therefore, it is possible that the ME/CFS subjects voluntarily recruited fewer fast-twitch motor units than the control subjects, which could also lead to less potentiation. Although this possible difference in potentiation between groups is an interesting finding from this study, further analysis was not performed because this was not an a priori stated purpose of this thesis.

Chapter Six: Conclusions

6.1 Summary

It was found that women with ME/CFS were able to reach the same $\dot{V}O_{2peak}$ as sedentary but otherwise healthy women not only on the 1st incremental exercise test, but on the 2nd test as well. This finding is different than what was reported by VanNess and colleagues (2007) and serves to highlight the fact that ME/CFS seems to have a variable impact on several physiological systems. Indeed, it appears that only a subset of individuals with ME/CFS cannot reach the same $\dot{V}O_{2peak}$ on 2 incremental cycle ergometer exercise tests to exhaustion repeated 24 hrs apart. Despite the fact that the ME/CFS group was able to reach the same $\dot{V}O_{2peak}$ on both exercise tests, there was evidence of altered muscle contractile function that deserves further research.

First of all, there was evidence that women with ME/CFS have greater CAF than control subjects with respect to activating the quadriceps femoris muscles. While this did not preclude the ME/CFS subjects from exercising to maximal effort or reaching the same $\dot{V}O_{2peak}$ as sedentary but otherwise healthy control subjects, it may contribute to the symptoms of post-exertional malaise. Secondly, although the group by time interaction with respect to VAR did not reach statistical significance (p=0095), it was noted that with the exception of 1 ME/CFS subject and 1 CON subject, VAR was lower in all ME/CFS subjects than control subjects after the 2nd exercise test. Due to the large inter-individual variation in VAR, it is possible that a larger sample size might have allowed us to detect a statistically significant interaction. This is a potentially valuable finding as it relates to the development of central fatigue in ME/CFS subjects. Furthermore, the correlation between several SF-36 questionnaire sub scales and change in VAR on the 2nd day suggests that changes in central motor unit activation may play a role in the symptoms of post-exertional malaise. As was previously described, the SF-36 questionnaire has been commonly used to monitor the symptoms of post-exertional malaise, while VAR is an indicator of central fatigue.

Finally, based on the indices measured in this thesis, there was no evidence to suggest that repeated bouts of maximal effort exercise caused a greater central fatigue or

a more persistent peripheral fatigue response in the ME/CFS subjects. Despite this finding, MVC decreased more in the ME/CFS group than in the control group. It is hypothesized that LFF caused by the incremental cycle ergometer tests to exhaustion in both groups, combined with the fact that CAF was greater in the ME/CFS subjects, was responsible for this drop in MVC on the 2nd day.

6.2 Limitations

There are several limitations to this study that should be acknowledged. First of all, given the nature of ME/CFS and the debilitating effects that high intensity exercise can have on these individuals, those with very severe forms of ME/CFS were excluded from this study. Therefore, the results presented in this thesis, while applicable to those who participated, may not be appropriately extrapolated to all individuals suffering from ME/CFS.

Secondly, there is some evidence to suggest that interpolated twitch analysis is not able to accurately detect changes in voluntary muscle activation (Shield and Zhou, 2004; Taylor *et al.*, 2008; De Haan *et al.*, 2009). First of all, the equipment used to measure torque must be sensitive to the very small changes in torque associated with an interpolated twitch. Although it has been proposed that using doublet (or larger) stimuli instead of twitches may alleviate this problem, Behm *et al.* (1996) showed that estimates of VAR were the same whether singlet, doublet or quintuplet superimposed stimuli were used. Furthermore, it has also been suggested that larger superimposed stimuli introduce more noise into the recording, which can confound the results (Gandevia, 2001). The fact that larger stimuli are more painful than twitches, combined with the limited evidence to suggest that they are actually better than twitches for detecting changes in VAR, are the reasons why interpolated twitches were used in this thesis.

Another issue with interpolated twitch analysis is that transcutaneous nerve stimulation can activate both agonist and antagonist muscle groups, resulting in a smaller IT and thus an overestimation of VAR. Careful placement of the stimulation pads has been suggested in order to minimize this effect (Taylor *et al.*, 2008). Finally, De Haan and colleagues argue that the increase in torque with a superimposed twitch is not linear

all the way from submaximal to maximal voluntary torque, leading to an overestimation of VAR (De Haan *et al.*, 2009). Although this is an acknowledged shortcoming of interpolated twitch analysis, this method has been shown to be effective in measuring changes in voluntary muscle activation with fatigue and between different subject groups (Kent-Braun *et al.*, 1993; Gandevia, 2001; Schillings *et al.*, 2004; Taylor *et al.*, 2008).

Thirdly, while sEMG is a non-invasive method of measuring relative motor unit activation at different torque outputs, it relies on several assumptions that may not always be true. First of all, the signal detected by the sEMG electrodes is distorted by superficial fascia, fat, skin and hair between the electrodes and the motor units. The greater the distance between the signal and the electrode, the greater the distortion. While shaving the skin underneath the electrodes and cleansing it with alcohol minimizes the distortion caused by hair and dead skin cells, it does not eliminate it entirely. Next, during an isometric contraction there is still movement of the muscle fibres relative to the surface of the skin. This automatically changes the position of the electrical signal relative to the sEMG electrodes, introducing error into the measurement. Furthermore, proper placement of the electrodes is important to minimize the chances of mistakenly detecting electrical signals from muscles co-contracting with the one in question (cross talk). In order to get the best signal, sEMG electrodes must be positioned parallel to muscle fibre direction and not over the NMJ. Unfortunately, it is not possible to visualize either of these with the naked eye. Thus, to minimize all of these sources of error in this thesis, electrode placement was checked prior to testing to ensure that both the signal amplitude and the signal-to-noise ratio were fairly large. If this was not the case, electrode position was adjusted to obtain a better signal. Finally, the position of the electrodes was clearly marked to ensure identical placement from day 1 to day 2. Despite the shortcomings of sEMG, it was chosen instead of needle EMG because of its non-invasive nature.

Fourth, despite efforts to initiate the interpolated twitch analysis immediately following each incremental exercise test, this was not the case. It took from 45–150 s (as long as no technical difficulties were encountered) to move each subject from the cycle ergometer to the dynamometer chair, strap them in, and deliver the 1st stimulus. In this time period, some initial recovery will have occurred which could have confounded the

results. It was assumed that minimal recovery occurred during this time frame, as the subjects were passively seated and not allowed to perform any form of active recovery. As shown in Appendix E, the time to 1st stimulation was similar in most subjects. Furthermore, in those subjects where there was a large variation in the time to 1st stimulation from day 1 to day 2, there did not appear to be a consistent effect on either T_{pre} or MVC amplitude. Therefore, it is unlikely that the time to 1st stimulation would have had a systematic effect on the results of this thesis. Instead, it must be acknowledged that if we had been able to measure both central and peripheral fatigue even sooner after each incremental bicycle test, we may have been able to measure greater changes in these variables.

6.3 Further Directions

In both healthy individuals and those with common, chronic medical conditions such as diabetes, heart disease and arthritis, regular exercise programs can improve both health and quality of life (Pedersen and Saltin, 2006). Although, there are several studies reporting symptomatic benefits from graded exercise therapy in individuals with ME/CFS, a large number of patients participating in these exercise programs experience post-exertional malaise (Chambers *et al.*, 2006). Future research should focus on determining what kinds of exercise protocols (if any) can be introduced into this population without worsening CAF, or causing central fatigue, LFF and post-exertional malaise, while still providing the benefits of increased physical activity. It may very well be that individual monitoring and training prescription is required in order to ensure that exercise is beneficial for individuals suffering from ME/CFS.

Secondly, additional research investigating the contribution of LFF to post-exertional malaise in ME/CFS is warranted. This might include measuring force-frequency curves in ME/CFS subjects using electrical stimulation, as well muscle biopsies to measure Ca^{2+} release in response to electrical stimuli in both a rested and fatigued state. Studies investigating the rate of development of LFF (as well as central and peripheral fatigue) may also help to elucidate the effects that changes in muscle contractile function have on exercise performance and post-exertional malaise. Finally,

given the possible connection between VAR (and therefore central fatigue) and the symptoms of post-exertional malaise as indicated by the SF-36 questionnaire, further measurements of VAR before and after exercise in a larger sample of ME/CFS and healthy subjects is warranted.

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APPENDIX A: Subject Consent Form

Title: Repeated Exercise Capacity in women with ME/CFS
Sponsor: ME Research UK
Investigator(s): Elana Taub, Dr. Eleanor Stein and Dr. Brian MacIntosh

This consent form, which is part of the process of informed consent, gives you the basic idea of what the research is looking at and what you will be doing during your participation. In case you would like more detailed information about something mentioned here, or information not included here, please feel free to ask. Please read this carefully and try to understand any accompanying information.

BACKGROUND
As you are aware, diagnosing chronic fatigue syndrome is a difficult task, and very little is known about the physical side of the fatigue you experience. The purpose of this study is to evaluate potential tools or tests for the identification of chronic fatigue syndrome, or variations within this condition.

WHAT WOULD I HAVE TO DO?
You will fill out three questionnaires as part of the screening test to determine your qualifications for this study. In addition, you will be asked to fill out a Physical Activity Readiness Questionnaire (PAR-Q). There will be two experimental exercise tests, and each is to be completed twice with 24 hrs between visits: 1), cycling exercise to the limit of your tolerance and 2) testing your quadriceps muscle.

1) Incremental exercise test: You will ride a cycle ergometer, beginning at an easy intensity, and the intensity of exercise will increase gradually until you feel that you cannot continue the exercise. During this time, you will be breathing through a mouthpiece that collects and analyzes the air you breathe out. Heart rate will also be measured during this exercise. We will do finger pricks to collect blood for the measurement of lactate.
2) Quadriceps test: Five sets of electrodes will be placed on your thigh: two to stimulate the quadriceps muscle and the other three to measure the electrical response of that muscle. The skin will be shaved and wiped with an alcohol swab to remove the dead layer of skin before taping the electrodes in place. You will sit in a specialized chair attached to a leg extension machine. Your torso and leg will be anchored to the apparatus to prevent extra movement and measure the contractile response. The stimulation amperage will be gradually increased to get the highest possible contraction. Tell us if this hurts. You will be asked to give three maximal effort contractions, each lasting five seconds. Just prior to, during and immediately following each maximal contraction we will electrically stimulate your quadriceps to contract. After a short rest you will be asked to do a submaximal contraction (50% of maximum) for 10s. The electrical stimulation during the maximal contraction allows us to quantify muscle fatigue during this exercise.

Prior to arriving at the laboratory for testing, please adhere to the following guidelines:

- Refrain from eating or drinking for two hours prior to testing
- Refrain from consuming caffeine, alcohol or participating in heavy exercise for six hours prior to testing
- Be consistent in your morning routine between the two testing days
- Wear loose-fitting shorts and a comfortable T-shirt to allow placement of electrodes on your thigh and a blood pressure cuff on your arm

WHAT ARE THE RISKS?

The electrically stimulated muscle twitches may cause unusual feeling and discomfort. You can stop the test at any stage you wish. At the end of the incremental exercise test and muscle test, you may have minor leg muscle soreness, weakness and an uncomfortable feeling. There may be some bruising and tenderness in your finger tips from taking the blood samples.

WILL I BENEFIT IF I TAKE PART?

If you agree to participate in this study there is not expected to be a direct medical benefit to you. Your chronic fatigue may be improved during the study but there is no guarantee that this research will help you. The information we get from this study may help us to provide better treatments in the future for patients with chronic fatigue syndrome.

DO I HAVE TO PARTICIPATE?

Your participation in this research project is entirely voluntary.

WILL I BE PAID FOR PARTICIPATING, OR DO I HAVE TO PAY FOR ANYTHING?

You will not be paid for your participation in this research project. If you have to pay for parking, we will reimburse you for that expense.

IF I SUFFER A RESEARCH-RELATED INJURY, WILL I BE COMPENSATED?

In the event that you suffer injury as a result of participating in this research, no compensation will be provided to you by the University of Calgary, the Calgary Health Region or the Researchers. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages.

WILL MY RECORDS BE KEPT PRIVATE?

Information obtained during this research project is confidential. Nobody except the researchers will have access to your personal information. This information, however, may be used for statistical analysis or scientific purposes with your right to privacy retained.

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. If you have further questions concerning matters related to this research, please contact:

Elana Taub: @ (403) 220-7119 or etaub@kin.ucalgary.ca
Brian MacIntosh @ (403) 220-3421 or brian@kin.ucalgary.ca

If you have any questions concerning your rights as a possible participant in this research, please contact The Ethics Resource Officer, Internal Awards, Research Services, University of Calgary, at 220-3782.

Participant's Signature	Date
Investigator and/or Delegate's Signature	Date
Witness' Signature	Date

The University of Calgary Conjoint Health Research Ethics Board has approved this research study.

A copy of this consent form has been given to you to keep for your records and reference.

APPENDIX B: SF-36 Health Survey Questionnaire

SF-36 QUESTIONNAIRE

Name:_____ **Ref. Dr:**_____ **Date:** _____

ID#: _____ **Age:** _____ **Gender: M / F**

Please answer the 36 questions of the **Health Survey** completely, honestly, and without interruptions.

GENERAL HEALTH:
In general, would you say your health is:
◯ Excellent ◯ Very Good ◯ Good ◯ Fair ◯ Poor

Compared to one year ago, how would you rate your health in general now?
◯ Much better now than one year ago
◯ Somewhat better now than one year ago
◯ About the same
◯ Somewhat worse now than one year ago
◯ Much worse than one year ago

LIMITATIONS OF ACTIVITIES:
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
◯ Yes, Limited a lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Lifting or carrying groceries
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing several flights of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing one flight of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Bending, kneeling, or stooping
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking more than a mile
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking several blocks
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking one block
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Bathing or dressing yourself
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

PHYSICAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

Cut down the amount of time you spent on work or other activities
Yes No

Accomplished less than you would like
Yes No

Were limited in the kind of work or other activities
Yes No

Had difficulty performing the work or other activities (for example, it took extra effort)
Yes No

EMOTIONAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

Cut down the amount of time you spent on work or other activities
Yes No

Accomplished less than you would like
Yes No

Didn't do work or other activities as carefully as usual
Yes No

SOCIAL ACTIVITIES:
Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all Slightly Moderately Severe Very Severe

PAIN:
How much bodily pain have you had during the past 4 weeks?

None Very Mild Mild Moderate Severe Very Severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all A little bit Moderately Quite a bit Extremely

ENERGY AND EMOTIONS:
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

Did you feel full of pep?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you been a very nervous person?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt so down in the dumps that nothing could cheer you up?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt calm and peaceful?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Did you have a lot of energy?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt downhearted and blue?

- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Did you feel worn out?

- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Have you been a happy person?

- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Did you feel tired?

- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

SOCIAL ACTIVITIES:
During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little bit of the time
- None of the Time

GENERAL HEALTH:
How true or false is each of the following statements for you?

I seem to get sick a little easier than other people
Definitely true | Mostly true | Don't know | Mostly false | Definitely false

I am as healthy as anybody I know
Definitely true | Mostly true | Don't know | Mostly false | Definitely false

I expect my health to get worse
Definitely true | Mostly true | Don't know | Mostly false | Definitely false

My health is excellent
Definitely true | Mostly true | Don't know | Mostly false | Definitely false

APPENDIX C: CDC CFS Questionnaire

CDC Symptom Inventory

The next few questions are about physical symptoms that you may have experienced during the past six months.

Sore Throat

C.1 During the past six months, have you had a sore throat?

- ❑1 Yes
- ❑2 No → **(Skip to C.1f)**

C.1a During the past six months, how often have you had a sore throat?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.1b During the past six months, how bad was your sore throat?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.1c Prior to this past six months, for how long had you had a sore throat?

- ❑1 Less than 6 months → **(Skip to C.1e)**
- ❑2 6 – 12 months → **(Skip to C.1e)**
- ❑3 More than 12 months

C.1d For how many years have you had a sore throat?

_____ Record Number of Years

C.1e Do you consider your sore throat to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.1f Has a sore throat been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Tender Lymph Nodes and Swollen Glands

C.2 During the past six months, have you had tender lymph nodes or swollen glands in your neck or armpits?

- ❑1 Yes
- ❑2 No → **(Skip to C.2f)**

C.2a During the past six months, how often have you had tender lymph nodes or swollen glands?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.2b During the past six months, how tender were your lymph nodes or how swollen were your glands?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.2c Prior to this past six months, how long had you had tender lymph nodes or swollen glands?

- ❑1 Less than 6 months → **(Skip to C.2e)**
- ❑2 6 – 12 months → **(Skip to C.2e)**
- ❑3 More than 12 months

→ **C.2d For how many years have you had tender lymph nodes or swollen glands?**

_____ Record Number of Years

C.2e Do you consider your tender lymph nodes or swollen glands to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.2f Have tender lymph nodes or swollen glands been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Fatigue After Exertion

C.3 During the past six months, have you been unusually fatigued or unwell for at least one day after exerting yourself in any way?

- ❑1 Yes
- ❑2 No → **(Skip to C.3f)**

C.3a During the past six months, how often have you had unusual fatigue after exertion?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.3b During the past six months, how bad was your unusual fatigue after exertion?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.3c Prior to this past six months, for how long had you had unusual fatigue after exertion?

- ❑1 Less than 6 months → **(Skip to C.3e)**
- ❑2 6 – 12 months → **(Skip to C.3e)**
- ❑3 More than 12 months

C.3d For how many years have you had unusual fatigue after exertion?

_____ Record Number of Years

C.3e Do you consider your unusual fatigue after exertion to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.3f Has unusual fatigue after exertion been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Muscle Aches and Pains

C.4 During the past six months, have you had muscle aches or muscle pain?

- ❑1 Yes
- ❑2 No → **(Skip to C.4f)**

C.4a During the past six months, how often have you had muscle aches or muscle pains?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.4b During the past six months, how bad were your muscle aches or muscle pains?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.4c Prior to this past six months, for how long have you had muscle aches or muscle pains?

- ❑1 Less than 6 months → **(Skip to C.4e)**
- ❑2 6 – 12 months → **(Skip to C.4e)**
- ❑3 More than 12 months

C.4d For how many years have you had muscle aches or muscle pains?

_____ Record Number of Years

C.4e Do you consider your muscle aches or muscle pains to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.4f Have muscle aches or muscle pains been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Joint Pain

C.5 During the past six months, have you had pain in several joints?

- ❑1 Yes
- ❑2 No → **(Skip to C.5f)**

C.5a During the past six months, how often have you had joint pain?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.5b During the past six months, how bad was the joint pain?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.5c Prior to this past six months, for how long had you had joint pain?

- ❑1 Less than 6 months → **(Skip to C.5e)**
- ❑2 6 – 12 months → **(Skip to C.5e)**
- ❑3 More than 12 months

→ **C.5d For how many years have you had joint pain?**

_____ Record Number of Years

C.5e Do you consider your joint pain to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.5f Has joint pain been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Unrefreshing Sleep

C.6 During the past six months, has unrefreshing sleep been a problem for you?

- ❑1 Yes
- ❑2 No → **(Skip to C.6f)**

C.6a During the past six months, how often have you had unrefreshing sleep?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.6b During the past six months, how much of a problem was unrefreshing sleep?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.6c Prior to this past six months, for how long had you had unrefreshing sleep?

- ❑1 Less than 6 months → **(Skip to C.6e)**
- ❑2 6 – 12 months → **(Skip to C.6e)**
- ❑3 More than 12 months

→ **C.6d For how many years have you had unrefreshing sleep?**

_____ Record Number of Years

C.6e Do you consider unrefreshing sleep to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.6f Has unrefreshing sleep been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Headaches

C.7 During the past six months, have you had headaches?

- ❑1 Yes
- ❑2 No → **(Skip to C.7f)**

C.7a During the past six months, how often have you had headaches?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.7b During the past six months, how bad were your headaches?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.7c Prior to this past six months, for how long had you had headaches?

- ❑1 Less than 6 months → **(Skip to C.7e)**
- ❑2 6 – 12 months → **(Skip to C.7e)**
- ❑3 More than 12 months

→ **C.7d For how many years have you headaches?**

_____ Record Number of Years

C.7e Do you consider your headaches to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.7f Have headaches been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

Memory Problems and/or Concentration Problems

C.8 During the past six months, have you had forgetfulness or memory problems, or difficulty with thinking or concentrating that caused you to substantially cut back on your activities?

- ❑1 Yes
- ❑2 No → **(Skip to C.8f)**

C.8a During the past six months, how often have you had forgetfulness or memory problems, or difficulty with thinking or concentrating?

- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 A good bit of the time
- ❑4 Most of the time
- ❑5 All of the time

C.8b During the past six months, how bad were your forgetfulness or memory problems, or difficulty with thinking or concentrating?

- ❑1 Very mild
- ❑2 Mild
- ❑3 Moderate
- ❑4 Severe
- ❑5 Very severe

C.8c Prior to this past six months, for how long had you forgetfulness or memory problems, or difficulty with thinking or concentrating?

- ❑1 Less than 6 months → **(Skip to C.8e)**
- ❑2 6 – 12 months → **(Skip to C.8e)**
- ❑3 More than 12 months

C.8d For how many years have you had forgetfulness or memory problems, or difficulty with thinking or concentrating?

_____ Record Number of Years

C.8e Do you consider your forgetfulness, memory, thinking or concentration problems to currently be part of your ill-health?

- ❑1 Yes
- ❑2 No

C.8f Have forgetfulness, memory, thinking or concentration problems been a part of your ill-health in the past?

- ❑1 Yes
- ❑2 No

C.9. Which of the following symptoms has bothered you the most during the past six months?

Please check **one** box that describes that symptom that bothered you **most** during the past six months.

- ❑1 Sore throat
- ❑2 Tender lymph nodes or swollen glands in your neck or armpits
- ❑3 Unusual fatigue for at least one day after exertion
- ❑4 Muscle aches or pains
- ❑5 Joint pain
- ❑6 Unrefreshing sleep
- ❑7 Headaches
- ❑8 Memory or concentration problems

APPENDIX D: Adapted Karnofsky Scale / Bell Ability Scale

_____ **100**. No symptoms at rest or with exercise; normal overall activity; able to work or do house/home work full time without difficulty.

_____ **90**. No symptoms at rest; mild symptoms with vigorous activity; normal overall activity level; able to work full time without difficulty.

_____ **80**. Mild symptoms at rest; symptoms worsened by exertion; minimal activity restriction for activities requiring exertion; able to work full time with difficulty in jobs requiring prolonged standing or exertion.

_____ **70.** Mild symptoms at rest; some daily activity limitation noted; overall functioning close to 90% of expected except for activities requiring exertion; able to work full time.

_____ **60**. Mild to moderate symptoms at rest; daily activity limitation clearly noted; overall functioning 70% to 90%; able to work full time in light activity if hours flexible.

_____ **50**. Moderate symptoms at rest; moderate to severe symptoms with exercise or activity; overall activity level reduced to 70% of expected; unable to perform strenuous activities but able to perform light duties or desk work 4 to 5 hours a day, but requires rest periods.

_____ **40**. Moderate symptoms at rest; overall activity 50% to 70% of previous normal; able to go out of the house for short excursions; unable to perform strenuous activities; able to work sitting down at home 3 to 4 hours per day, but requires rest periods.

_____ **30**. Moderate to severe symptoms at rest; severe symptoms with exercise; overall activity reduced to
50% of expected; usually confined to house; able to perform light activity (desk work) 2 to 3 hours pe day
but requires rest periods.

_____ **20**. Moderate to severe symptoms at rest; unable to perform strenuous activity; overall activity 30-50% of expected; able to leave house only rarely; confined to bed or couch most of day; unable to concentrate more than 1 hour per day.

_____ **10**. Severe symptoms at rest; bedridden the majority of the time; rare travel outside the house; marked cognitive symptoms preventing concentration.

_____ **0**. Severe symptoms on a continuous basis; bedriddren; unable to care for self.

APPENDIX E: Individual data for the time to the 1st electrical stimulation after each incremental exercise test

Subject		Day 1			Day 2		
		Time to 1st stim (s)	T_{pre} (% baseline T_{pre})	MVC (% baseline MVC)	Time to 1st stim (s)	T_{pre} (% baseline T_{pre})	MVC (% baseline MVC)
ME/CFS	1	150	191	100	120	181	72
	2	180*	49	110	45	41	74
	3	90	78	84	180*	86	70
	4	120	72	67	60	55	64
	5	60	58	89	60	65	86
	6	60	51	93	90	65	85
	7	120	88	91	120	65	96
	8	150	51	84	150	45	79
	9	60	18	80	60	13	72
CON	1	90	43	83	75		81
	2	120	37	76	90	47	73
	3	70	60	89	60	37	80
	4	90	50	95	270*	56	100
	5	120	73	75	120	115	87
	6	75	40	85	75	32	108
	7	90	57	81	60	65	101
	8	60	43	80	60		
	9	90	31	81	90	43	97
MEAN		95			83		
SD		30			30		

Baseline T_{pre} and MVC were the T_{pre} and MVC amplitudes recorded on the 1st day prior to the incremental exercise test.

* Indicates tests where technical difficulties (ie: computer froze) extended the time until the 1st electrical stimulation was delivered following the incremental exercise test.

APPENDIX F: Individual comparisons of MVC value used to determine VAR (avg MVC) and highest torque value attained during each MVC

Subject		Day 1					
		Pre Exercise			Post Exercise		
		Avg MVC (N·m)	Peak MVC (N·m)	Diff (%)	Avg MVC (N·m)	Peak MVC (N·m)	Diff (%)
ME/CFS	1	116.4	126.7	8.1	116.3	119.6	2.7
	2	46.4	54.9	15.4	51.2	53.5	4.5
	3	75.9	84.1	9.8	63.5	67.9	6.5
	4	104.0	106.5	2.3	69.6	79.0	12.0
	5	103.2	108.5	4.9	91.7	98.0	6.5
	6	86.8	95.3	8.9	80.5	82.8	2.7
	7	59.8	65.7	9.0	54.7	56.4	3.0
	8	67.2	69.6	3.4	56.3	60.6	7.1
	9	106.4	107.8	1.3	85.2	86.3	1.2
CON	1	89.6	92.3	2.9	74.6	75.6	1.3
	2	116.3	119.6	2.7	88.2	89.0	0.9
	3	87.2	89.2	2.3	77.3	88.1	12.2
	4	58.6	60.8	3.6	55.4	57.8	4.0
	5	106.1	109.5	3.1	79.5	82.1	3.1
	6	78.7	83.4	5.7	66.8	72.1	7.4
	7	117.0	126.7	7.6	95.0	99.0	4.0
	8	82.6	83.9	1.6	65.8	67.6	2.7
	9	78.0	85.4	8.7	62.9	65.6	4.1
MEAN				5.6			4.8
SD				3.8			3.3

Avg VAR = average of the 500 data points prior to IT

Continued on next page.

Subject		Day 2					
		Pre Exercise			Post Exercise		
		Avg MVC (N·m)	Peak MVC (N·m)	Diff (%)	Avg MVC (N·m)	Peak MVC (N·m)	Diff (%)
ME/CFS	1	91.0	98.5	7.6	83.6	86.8	3.7
	2	57.7	59.1	2.4	34.5	36.7	5.8
	3	67.4	72.3	6.8	53.0	56.1	5.5
	4	71.6	75.3	4.9	66.5	70.6	5.8
	5	95.4	98.8	3.4	88.6	91.2	2.8
	6	73.0	84.5	13.6	73.5	79.0	7.0
	7	82.5	85.1	3.0	57.4	58.9	2.7
	8	60.6	65.4	7.2	52.9	55.2	4.2
	9	97.7	99.8	2.2	76.7	77.9	1.5
CON	1	87.9	92.3	4.8	72.4	75.3	3.9
	2	108.3	111.8	3.1	85.4	88.8	3.9
	3	77.4	80.9	4.3	69.9	74.7	6.5
	4	69.4	73.8	6.0	58.5	59.8	2.1
	5	89.2	96.6	7.6	92.4	98.3	6.0
	6	89.8	93.6	4.0	85.1	87.2	2.4
	7	139.1	144.7	3.9	118.6	118.6	0.0
	8						
	9	76.5	80.4	4.8	75.3	75.7	0.5
MEAN				5.3			3.8
SD				2.8			2.1

Technical difficulties resulted in no superimposed stimuli for control subject 8 on day 2 hence no MVC or VAR analysis was performed.