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Impacts of Diabetic Neuropathy on the Human Neuromuscular System

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Graduate Program in Kinesiology

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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IMPACTS OF DIABETIC NEUROPATHY ON THE HUMAN NEUROMUSCULAR SYSTEM

(Thesis Format: Integrated Article)

By

Matti Douglas Allen

Graduate Program in Kinesiology

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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2014

THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES
ABSTRACT

Diabetes mellitus (DM) imparts vascular and metabolic stressors that cause damage and dysfunction to the human nervous system. The disorder associated with this dysfunction is termed diabetic polyneuropathy (DPN). Although DPN has been associated with muscle weakness and atrophy, the extent of its impacts on the neuromuscular system is not well understood. The five studies presented in my thesis investigated how DPN affects the neuromuscular system in humans, from the motor neuron to skeletal muscle contractile properties, using a combination of electromyography (EMG), dynamometry and magnetic resonance imaging (MRI) techniques.

The purpose of Studies 1 and 2 was to determine whether the neurogenic loss of motor units underlies the muscle weakness and atrophy associated with DPN, and to investigate how these changes may differ in an upper and lower limb muscle. I determined DPN patients feature reduced motor unit estimates (MUNEs) compared to controls, and progression of motor unit loss in DPN may follow a distal to proximal or nerve length-dependent pattern.

The purpose of Study 3 was to assess the stability of neuromuscular transmission in patients with DPN compared with healthy controls, using a novel set of electrodiagnostic parameters obtained via quantitative EMG. I determined DPN patients have less stable neuromuscular transmission, and they feature intermittent conduction failure at a relatively low contraction intensity.

The purpose of Study 4 was to investigate skeletal muscle contractile properties and morphology in DPN patients. I determined DPN patients possess slowed muscle, with greater proportional amounts of non-contractile intramuscular tissue compared to controls.

The purpose of Study 5 was to explore the fatigability of DPN patients during a sustained maximal voluntary contraction (MVC). I determined DPN patients have less
endurance than controls, and their increased fatigability may be associated with neuromuscular transmission failure.

Overall these foundational explorations greatly expand our knowledge of how DPN can impact the neuromuscular system in humans. Furthermore, the studies contained within my thesis may help direct further useful studies and strategies to understand, and direct clinical support in those with DPN.

KEYWORDS
Diabetic polyneuropathy (DPN); human; motor nervous system; muscle; weakness; motor unit; electromyography (EMG); fatigue; magnetic resonance imaging (MRI); dynamometry
CO-AUTHORSHIP STATEMENT

This thesis contains material from published manuscripts (Chapters 2-6). On all manuscripts, Matti D. Allen was the first author and Dr. Charles L. Rice, Dr. Kurt Kimpinski, and Dr. Timothy J. Doherty were co-authors. In Ho Choi was a co-author on Chapter 2. Maddison Hourigan was a co-author on Chapter 4. Brendan Major was a co-author on Chapter 5. All experimental data presented in this thesis were collected, analyzed, and interpreted by Matti D. Allen.
ACKNOWLEDGMENTS

Over the course of my PhD, and the broader decade I have enjoyed at the University of Western Ontario, I have found myself obliged to thank many individuals for their time, energy, expertise, patience, perspective and friendship. I do not have the space to thank everyone by name and specific contributions, lest this prologue’s length exceed the thesis itself. However, I will briefly give acknowledgment to some key figures who were integral in the development and completion of this thesis.

“Do not train a child to learn by force or harshness; but direct them to it by what amuses their minds, so that you may be better able to discover with accuracy the peculiar bent of the genius of each.” – Plato

Charles L. Rice – a patient teacher and exemplary mentor. I was a fortunate student to have CLR help guide me through this experience. Charles fostered an academic environment in which the ideal amount of supervision was provided, at times allowing me to make my own mistakes, whilst ensuring I learnt from them. Perhaps most importantly, Charles supported my research questions whole-heartedly, despite those questions often residing outside his usual realm of academic inquiry. I am very thankful for that extraordinary support. Throughout my PhD, Charles has given me every opportunity to be successful and I will be sure to apply my learnings from the Rice lab throughout the rest of my life.

“If you cannot see where you are going, ask someone who has been there before.” – Anonymous

Tim Doherty and Kurt Kimpinski – two mentors who played crucial roles in the development and success of my thesis projects, as well as my career choices moving forward. Tim afforded me my first opportunity to partake in academic research as my MSc advisor in 2007 and has supported my work as a researcher ever since. Kurt took on the lead clinical role during my PhD investigations, allowing me to recruit appropriate patient participants while serving as the conduit for all relevant clinical information. These gentlemen went out of their way to help me walk my desired path. The help will always be appreciated and never forgotten.
“It is easier to find men who will volunteer to die, than to find those who are willing to endure pain with patience.” – Julius Caesar

Over the past three years, many individuals, often strangers to me, have volunteered substantial amounts of time and effort as participants in my studies. They did this knowing no tangible reward was forthcoming; they did this knowing my assessments would involve some discomfort. I acknowledge those individuals, for their efforts were everything to this thesis.

“Talent wins games, but teamwork wins championships.” – Michael Jordan

“Rule Number 5.” – Chopper Read

I have been fortunate to share a laboratory environment with a fantastic, and eclectic, group of individuals. And in reality, my positive collaborative experiences extend well beyond the confines of the neuromuscular lab, across Kinesiology and UWO at large. I thank my colleagues for their willingness to teach and their willingness to lend a hand; for setting examples worth following; and for creating a working environment that was simultaneously fun and productive. When a strong work ethic and enthusiastic, collaborative setting prevail, success will surely follow.

“Walking with a friend in the dark is better than walking alone in the light.” – Helen Keller

“A happy family is but an earlier heaven.” – George Bernard Shaw

My friends are obliquely referred to in each of the previous sections of these acknowledgements, as they serve interchangeably as mentors, collaborators, volunteer participants and critics. I must also thank them for all of the laughs and stories we have shared and created. Specifically, I owe much to my girlfriend, Nicole Coverdale, who has been a veritable wellspring of positive energy and a resolute companion. Finally, I thank my family, especially Gerry and Malle Allen. Any success I have in life is a testament to their love and unwavering support.

Matti Douglas Allen
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List of abbreviations

AAR – amplitude to area ratio
ALS – amyotrophic lateral sclerosis
ANOVA – analysis of variance
BDNF – brain-derived neurotrophic factor
BMI – body mass index
CD – contraction duration
CMAP – compound muscle action potential
CSA – cross-sectional area
DE-STA – decomposition-enhanced spike triggered averaging
DM – diabetes mellitus
DN – diabetic neuropathy
DPN – diabetic polyneuropathy
DQEMG – decomposition-based quantitative electromyography
EDB – extensor digitorum brevis
EMG – electromyography
FDI – first dorsal interosseous
HRT – half relaxation time
ITT – interpolated twitch technique
IGF – insulin-like growth factor
LDL – low density lipoprotein
MNCS – motor nerve conduction study
MNCV – motor nerve conduction velocity
MU – motor unit
MUAP – motor unit action potential
MUAPT – motor unit action potential train
MUFR – motor unit firing rate
MUNIX – motor unit number index
MUP – motor unit potential
MUNE – motor unit number estimate
List of abbreviations (continued)

NF – near fibre
NT3 – neurotrophin 3
NT4 – neurotrophin 4
OxyLDL – oxidized low density lipoprotein
RMS – root-mean-square
SFEMG – single fibre electromyography
SMUP – surface motor unit potential
SNAP – sensory nerve action potential
SNCS – sensory nerve conduction study
TA – tibialis anterior
TPT – time to peak torque
Vpp – voltage peak to peak
Chapter 1

1 General Introduction

Voluntary movement is a fundamental attribute expressed by nearly all forms of animal life. In humans, similar to many animals, this voluntary movement is accomplished via activation and control of the neuromuscular system. Thus, given the understandable reverence humanity places on movement, it comes as no surprise that humans have been fascinated by the study of neuromuscular physiology for millennia. With utmost brevity, this fascination manifests within the ancient writings of Hippocrates and Galen; the musings of da Vinci and Vesalius during the European Renaissance; and through the seminal discoveries of Galvani\(^1\), Duchenne\(^2\), Adrian & Bronk\(^3,4\), Denny-Brown\(^5\), Sherrington\(^6\), Henneman\(^7\) and others during the 18\(^{th}\), 19\(^{th}\) and 20\(^{th}\) centuries. In spite of the immense growth of our understanding, complete discernment of many workings within neuromuscular physiology remain elusive. Particular among these mysteries are questions concerning the impacts of disease on muscle and nerve. Deeper understanding of the impacts of these diseases may eventually lead to improved diagnostic methods and enhanced treatment options, as well as models of nature’s experiments to help understand basic physiology. Hence it has been observed, “Physiology is the stepchild of medicine. That is why Cinderella often turns out the queen.” (Martin H. Fischer)

1.1 The Human Neuromuscular System and the Motor Unit

The primary function of the neuromuscular system is to provide humans with the means for movement and locomotion. In parsimonious terms, the system is comprised of (1) α motor neurons which innervate, and thus control, (2) skeletal muscle fibres. Motor neurons are responsible for relaying signals, in the form of action potentials, from the central nervous system (i.e. the brain and spinal cord) to skeletal muscle which initiates
contraction. Muscle fibres are then responsible for contracting via the actomyosin complex, thus generating the force necessary for movement.

Each individual α motor neuron and the muscle fibres that it innervates form the motor unit (Figure 1.1): the fundamental functional contractile unit in neuromuscular physiology. The size of motor units varies greatly from unit to unit, and muscle to muscle, from dozens of muscle fibres per unit (e.g. external rectus) to thousands of muscle fibres per unit (e.g. medial gastrocnemius). The motor unit composition of a muscle is thought generally to reflect that muscle’s function. For example muscles involved with fine, complex movements (i.e. hand muscles) are usually comprised of many, relatively smaller motor units. In contrast, muscles involved with gross limb movement (i.e. the knee extensors) usually consist of relatively fewer, larger motor units.

Figure 1.1 The motor unit.

Two individual motor units are depicted. The α-motor neuron cell bodies are located in the ventral horn of the spinal cord. Neuron axons exit the ventral horn into the periphery and synapse with their respective muscle fibres. Each motor unit is composed of an individual α-motor neuron and all of the muscles fibres it innervates.
Under most conditions, when contracting voluntarily, smaller motor units tend to be recruited prior to larger motor units, a concept coined the Henneman size principle[^7][^10][^11]. The general result of this principle is that relatively smaller, weaker, slower, fatigue resistant muscle fibres are recruited first before larger, stronger, faster, less fatigue-resistant muscle fibres. This forms one important aspect of the orderly recruitment of muscle and is occasionally referred to as spatial summation[^3][^4][^12][^13]. In contrast, temporal summation refers to the modulation of motor unit firing rate whereby muscle force output is changed by increasing or decreasing the frequency at which motor neurons signal muscle to contract[^3][^4]. These two processes work concurrently and in concert with one another to allow for a high degree of smooth and graded control in muscle force generation in conjunction with motor drive and coordination from spinal and supraspinal levels.

Of course, the neuromuscular system features far greater complexity than is outlined above and many questions remain unresolved. Investigating pathophysiological alterations to nerve and muscle due to aging or disease (e.g. diabetic neuropathy) can provide useful and unique avenues for further insight into basic neuromuscular physiology and the capacities and limitations of the system.

### 1.2 Type 2 Diabetes and Diabetic Neuropathy

Type 2 diabetes mellitus (DM) is a metabolic disorder marked by high blood sugar (hyperglycemia) associated with insulin resistance or a relative dearth of insulin[^14]. In comparison, type 1 DM is an autoimmune mediated disorder leading to a total loss of insulin production due to the destruction of insulin-producing beta cells within the pancreas[^14][^15]. Type 2 DM accounts for approximately 90% of all cases of DM, and is closely associated with obesity, sedentarianism, and poor dietary habits[^16]. Given its greater prevalence and burden upon society, the study of type 2 DM and its neuropathic complications will form the focus of this thesis. Thus, throughout this dissertation hereafter, “DM” shall refer to type 2 DM specifically, unless otherwise noted. Approximately 6% of the world’s adult population is living with DM[^16] and it is
recognized as a global health epidemic by the World Health Organization. Additionally, DM is closely associated with many complications including vascular disease, retinopathy, nephropathy, and of direct relevance to this dissertation, peripheral neuropathy.

Diabetic polyneuropathy (DPN) generally refers to nerve dysfunction as a complication of DM. It is estimated that 50% of patients with DM will develop DPN at some point in their lives. In its broadest context, DPN can refer to any of a group of neuropathic conditions with relatively heterogeneous patterns of neurological involvement. However, two major subgroups of DPN have been proposed: typical DPN and atypical DPN. Atypical DPNs are rarer and can feature acute, sub-acute or chronic onset of symptoms, with a monophasic or fluctuating course. Additionally, atypical DPNs often feature burning pain and hyperalgesia to touch, along with minimal sensory loss and motor dysfunction. In contrast, typical DPN is described as a chronic, symmetrical, length-dependent sensorimotor polyneuropathy. Typical DPN features sensory (i.e. numbness and paresthesia) and motor (i.e. atrophy and weakness) dysfunction progressing in a length-dependent manner. “Length-dependent” refers to the length of sensory and motor neuron axons, meaning symptoms (i.e. numbness and weakness) typically manifest first in the feet, progressively moving into the legs and hands; with continued advancement in a distal to proximal pattern. Epidemiological studies report typical DPN is the most common form of DPN, as its name indicates. Throughout this dissertation, the term DPN shall refer to the typical, sensorimotor form of the disease.

1.3 Pathophysiology of DPN

The precise mechanisms underlying the development of DPN are not entirely clear. However, DPN is likely caused by diabetes-related metabolic or vascular disturbances that are not mutually exclusive, and may be interrelated or synergistic. These mechanisms, briefly outlined below, lead to axonal loss via a dying-back, or retrograde degradation, as well as peripheral nerve demyelination.
Vascular disease is a known complication associated with DM\textsuperscript{22}. Indeed microangiopathy has been shown to develop early in DPN, and these abnormalities may directly relate to subsequent nerve dysfunction. More specifically, microangiopathy within the vasa nervorum (which provide the blood supply to peripheral nerves) may cause nerve dysfunction as a result of ischemic damage. Vasodilation is limited in DM due to disrupted nitric oxide signaling in blood vessels\textsuperscript{23}. Additionally, DM is associated with increases in blood viscosity\textsuperscript{24}, impaired oxygen release from blood to tissue\textsuperscript{25}, and dysfunctional structural alterations of red blood cells\textsuperscript{26}. Thus these combined changes may contribute to chronic hypoxia and ischemia resulting in nerve damage\textsuperscript{21}. However, whether microangiopathy and the resultant hypoxia are the primary causes of DPN is the subject of debate. Whereas some studies have found decreases in nerve blood flow associated with DPN, others have actually found increases\textsuperscript{27} or no changes in nerve blood flow\textsuperscript{28}, despite the clear development of impaired nerve function (see \textsuperscript{21} for review). The reasons for these disparate findings may be related to technical differences in the way blood flow was measured, as well as key differences in the experimental model or population studied\textsuperscript{21}.

The other postulated mechanisms underlying DPN are related to a variety of metabolic and cellular signaling abnormalities that are consequences of DM and chronic hyperglycemia (see Figure 1.2). Peripheral nerves are especially vulnerable to hyperglycemic-mediated damage due to their reliance on GLUT-1 and -3 glucose transporters\textsuperscript{29,30,31,32}. GLUT-1 and -3 transporters are insulin-independent and are dependent on extracellular blood glucose concentrations. Therefore, under diabetic conditions (i.e. hyperglycemia and insulin resistance) relatively large quantities of glucose will be transported into neural tissue. When intraneural glucose concentrations become too high, several pathways may become active leading to neural dysfunction or cell death. These cellular processes that may result in damage include: (1) increased reactive oxidative species (ROS) formation as a result of reverse mitochondrial electron flow due to excess NAD and FADH accumulation\textsuperscript{33}; (2) excess sorbitol production leading to cellular osmotic stress\textsuperscript{34}; (3) increased hexosamine pathway activation causing a chronic inflammatory response\textsuperscript{35}. Additionally, the hyperglycemia-related increase in advanced glycated end-products (AGEs), caused by the reaction of glucose with various
fats and proteins, may impose an inflammatory-mediated stressor on neurons\textsuperscript{36}. Nerve cell stress in DM may also occur due to the dyslipidemia, for example through oxidized low density lipoproteins (oxyLDLs) or toxic (i.e. high) concentrations of intracellular fats which may act through mitochondrial-induced cell death or inappropriate lysosome activation, respectively\textsuperscript{37,38}. Finally, impairments in nerve cell interaction with various neurotrophic and growth factors may play a role in the development of neuropathy and impaired regeneration. Deficiencies in insulin, insulin-like growth factor (IGF), brain derived growth factor (BDNF), and neurotrophin 3 and 4 (NT3, NT 4) signaling as a result of diabetes may play a role in the development and progression of DPN\textsuperscript{39}. The aforementioned postulated mechanisms underlying DPN form a limited list and brief description that suit the purposes of this dissertation; more comprehensive discussions of this subject matter can be found elsewhere\textsuperscript{31,40}. 

Figure 1.2 Metabolic mechanisms of nerve damage in diabetic neuropathy

Chronic hyperglycemia and hyperlipidemia in diabetes mellitus may activate multiple damaging mechanisms in neurons. Note: vascular causes potentially underlying diabetic neuropathy are not shown. Abbreviations: AGE, advanced glycation end product; GLUT, glucose transporter; LDL, low density lipoprotein; LDLR, LDL receptor; LOX1, oxidized LDL receptor 1; oxLDL, oxidized LDL; RAGE, receptor for advanced glycation end products. Adapted from: Vincent et al 2011
1.4 Clinical Manifestations of DPN

Confirmed clinical DPN is defined as: “an abnormal nerve conduction study and a symptom or symptoms or a sign or signs of sensorimotor polyneuropathy” that can be attributed to DM and no other cause of polyneuropathy\textsuperscript{19,21}. Some of these signs, symptoms and abnormal nerve conduction studies are described briefly below, and reviewed elsewhere\textsuperscript{21}.

Patients with DPN typically present with sensory abnormalities, including numbness, paresthesia, hyperalgesia, or allodynia\textsuperscript{19}. These symptoms usually first appear in the feet and may advance into the legs, hands and even more proximally over time and with increasing disease severity\textsuperscript{19,41}. The distribution of sensory symptoms is sometimes referred to as “stocking and glove”, which reflects the length-dependent progression of the disease. In clinic, these sensory abnormalities are often assessed through physical neurological examination, quantitative sensory testing (QST), and Semmes-Weinstein monofilaments\textsuperscript{19}.

Additionally DPN patients often present with dysfunction of the motor nervous, or neuromuscular system\textsuperscript{42,43}. The earliest of these signs is usually atrophy of the extensor digitorum brevis (EDB) muscle in the foot; which may be followed by wasting and weakness of the ankle dorsiflexors\textsuperscript{44,45}. This muscle weakness may lead to altered gait and foot posture, as well as other neuromuscular consequences outlined in greater detail in the next section of this dissertation\textsuperscript{44}. Finally, DPN patients may also display a loss of reflexes at the ankle or knee\textsuperscript{17}; foot ulceration\textsuperscript{46}; and dysfunction of the autonomic nervous system, including distal anhidrosis, constipation, impotence, orthostatic intolerance, among other symptoms\textsuperscript{47}.

Electrophysiological testing is an integral aspect of any clinical assessment of suspected DPN, and is considered to be the gold standard in DPN evaluation\textsuperscript{19,21,48}. These measures, namely nerve conduction studies, have been shown to be clinically reliable and sensitive to the neurological changes associated with DPN. The elicitation of a sural nerve sensory nerve action potential (SNAP) that has a reduced amplitude (<6 µV) or is absent when recording from the posterior aspect of the ankle has proven to be the earliest
electrophysiological sign of DPN\textsuperscript{51}. A prolonged peak sural nerve sensory nerve conduction latency (>4.9 ms) is another potential electrophysiological sign of DPN\textsuperscript{49}. In more severe cases of DPN, abnormalities in fibular nerve motor nerve conduction velocities (MNCV; <40 m/s) or reduced compound muscle action potential (CMAP) amplitudes from the anterolateral compartment of the leg (i.e. tibialis anterior; <4.0 mV) are signs of detectable motor involvement\textsuperscript{49}. In addition, other electromyographic-related measures have been used to evaluate nerve dysfunction in DPN such as motor unit number estimates (MUNE)\textsuperscript{50} and neuromuscular transmission stability (i.e. jitter)\textsuperscript{51} which will be discussed in further detail in this dissertation (Sections 1.5 and 1.9). Aside from their clinical relevance the aforementioned electrophysiological measures listed previously can provide detailed information regarding the status of the sensory and motor nervous systems in a minimally invasive manner which can prove invaluable in the context of scientific, physiological investigations.

1.5 Neuromuscular Consequences of DPN

Although early and mild cases of DPN predominantly feature abnormalities of the sensory nervous system, it is clear stressors associated with DM may target motor neurons\textsuperscript{42} and thus DPN can be associated with substantial dysfunction of the neuromuscular system (see \textsuperscript{43} for a brief review).

DPN-related neuromuscular dysfunction may manifest in altered motor neuron properties including reduced firing rates\textsuperscript{52}, increased firing rate variability\textsuperscript{52} and motor neuron excitability changes\textsuperscript{53}. Additionally, animal studies have shown DPN can result in changes at the muscle-level including disrupted t-tubules\textsuperscript{54}, dysfunctional ion pumps and channels\textsuperscript{54–57}, and degenerated mitochondria\textsuperscript{33,54}. However, perhaps the most obvious neuromuscular consequence of DPN is skeletal muscle atrophy and resultant weakness\textsuperscript{45,58,59}. Loss of isometric strength in the ankle dorsiflexors and plantar flexors, as well as knee extensors has been previously reported in DPN patients\textsuperscript{58,60,61}. A primary mechanism underlying this strength loss is muscle atrophy\textsuperscript{62}. For example, as mentioned in the previous section, atrophy of EDB is a sensitive and early indicator of DPN\textsuperscript{63}.
Additionally, magnetic resonance imaging (MRI) has been used to show substantial muscular atrophy in leg muscles of DPN patients, along with increased fatty infiltration of muscle\textsuperscript{64}. The same study also reported decreased: plantar flexion strength; isokinetic power; as well as decreased strength or power per unit of muscle volume (i.e. decreased muscle quality)\textsuperscript{64}. It remains unknown if the loss of power production is due to strength loss alone, or if a concomitant loss of muscle contractile velocity also occurs. The DPN-related decrease in muscle quality could be due to increases in antagonist co-activation, reductions in myocellular contractile protein expression, disruption in Ca\textsuperscript{2+} handling, or intermittent neuromuscular transmission failure.

It is not clear how DPN may affect neuromuscular fatigability. A previous study investigating type 1 DM found patients actually have greater relative muscular endurance versus healthy controls\textsuperscript{61}. However, the majority of patients included in that previous study did not feature substantial motor involvement in their neuropathy and no measures of voluntary activation were made. In contrast, studies reporting on clinical populations other than DPN that involve dysfunction of physiological systems in common with DM and DPN have found increases in fatiguability. For example, it has been shown previously that individuals with metabolic disease without neuropathy (e.g. type 1 DM)\textsuperscript{65}, vascular disease (e.g. chronic heart failure)\textsuperscript{66}, or nerve disease (e.g. amyotrophic lateral sclerosis [ALS])\textsuperscript{67}, fatigue more quickly than controls. Thus it remains to be determined how neuromuscular fatigue is impacted by DPN.

The neuromuscular consequences described previously can have major impacts, which are perhaps magnified by deficits in the sensory nervous system, in relation to functional status and quality of life. Weakness of the dorsi- and plantar flexors is associated with gait abnormalities and increases in fall risk\textsuperscript{44,68}. Gait abnormalities can lead to damaged, malfunctioning ankle joints, as well as the development of ulcers\textsuperscript{46,60}, which are closely associated with lower-limb amputation\textsuperscript{69}. Increased fall risk in DPN patients is an especially important concern as patients with DM are at greater risk of injurious falls (e.g. hip fractures) compared to healthy controls\textsuperscript{70}. Finally, these functional decrements may limit the patient’s ability to actively participate in exercise-based rehabilitation programs, which are an effective avenue to maintain glycemic control and
reduce DM-related disease severity\textsuperscript{71}. Since neuromuscular consequences of DPN can lead to such unfortunate declines in functional ability and quality of life, their study is important and may lead to enhanced strategies of compensation.

1.6 Motor unit number estimations (MUNE) in humans

In humans, the loss of motor units has been acknowledged as a key physiological process underlying various neuromuscular diseases\textsuperscript{72} as well as contributing to functional declines of the NM system associated with natural adult aging\textsuperscript{73}. Therefore developing methodologies for quantifying the number of functional motor units in a muscle in vivo has been of great interest in clinical neurophysiology. Standard electrophysiological measures such as motor nerve conduction studies (MNCS) and needle electromyography (EMG) serve as qualitative proxy measures of motor unit numbers; however they are not sensitive to mild degrees of motor unit loss, particularly following successful collateral reinnervation that may occur in some diseases and presumed to occur as part of adult aging\textsuperscript{72,74}.

The concept of the motor unit number estimate (MUNE) was developed to help address the limitations associated with standard clinical electrophysiological techniques in quantifying motor unit populations within a muscle. Due largely to improvements in computer processing capabilities, MUNE techniques have been greatly improved since their original inception in the 1970s\textsuperscript{72,74}. Although MUNE actually refers to several different specific electrophysiological methodologies, each MUNE technique is conceptually similar in that it is based upon the ratio between the maximal compound muscle action potential (CMAP) divided by the mean surface motor unit potential (SMUP). The CMAP is representative of the maximal electrophysiological size of the entire motor unit pool within a muscle, and the mean SMUP is representative of the size of the average single constitutive motor unit. The primary difference between each MUNE technique is how the mean SMUP is derived.

Since 1971 when MUNE was first reported\textsuperscript{75}, different techniques have been developed. Each methodology features various strengths and limitations, and at present
no single MUNE technique is accepted as the ‘gold standard’\textsuperscript{72,74}. Indeed, no gold standard exists even pertaining to the physical counting of motor units or motor axons due to technical limitations in histochemistry. Some common electrophysiological MUNE techniques include: (1) incremental stimulation, (2) multiple point stimulation, (3) statistical, (4) motor unit number index (MUNIX) and (5) spike triggered averaging (STA) with EMG decomposition. These techniques possess various inherent advantages and limitations which have been previously reviewed in the literature\textsuperscript{72,74} and, aside from the STA with EMG decomposition technique, will not be discussed within the confines of this thesis.

1.7 Spike triggered averaging (STA) and STA with decomposition-based quantitative electromyography (DQEMG) MUNE

Spike triggered averaging (STA) MUNE combines simultaneous collection of needle and surface EMG in a given muscle during voluntary contractions\textsuperscript{76}. During a low-level voluntary contraction, the EMG operator positions the needle electrode so that only the single motor unit of interest is being recorded. This needle-detected motor unit potential (MUP) is then used as a trigger to time lock the response from the same motor unit being recorded at the surface electrode. This process is repeated until sufficient (approximately 20) single surface motor unit potentials (SMUP) have been recorded to provide a representative mean\textsuperscript{76}. The mean SMUP is then divided into the maximal CMAP to provide a MUNE, similar to other techniques. This technique has been improved upon with the inclusion of EMG decomposition software which allows for decomposition-enhanced STA (DE-STA). DE-STA MUNE is based upon the same premise as traditional STA MUNE, however it is much more efficient and allows the investigator to sample from a wider range of the motor unit recruitment profile of a muscle\textsuperscript{77–79}. This means DE-STA can record motor units that are recruited at higher intensities compared to traditional STA, which is generally limited to recording low-threshold motor units. Thus DE-STA may provide a more representative sample of motor unit size compared to STA without decomposition. DE-STA involves the application of decomposition algorithms to the EMG interference pattern, allowing the software to
decompose the signal into its individual, constituent MUP trains (MUPTs). This allows a greater number of individual motor units to be sampled simultaneously, during higher intensity contractions. Another advantage of this MUNE technique is that it allows for the collection of needle EMG data that may provide other useful information regarding the physiological status of the neuromuscular system including: motor unit firing rates, neuromuscular transmission stability (i.e. jitter and jiggle), and transmission failure (via % blocking). A common software used to perform DE-STA is decomposition based quantitative EMG (DQEMG), which will be described in greater detail in a subsequent section (Section 1.9).

1.8 Clinical usage of MUNE

The MUNE methods previously outlined have been applied in various clinical models of human neurological or neuromuscular disease. ALS has been a frequent target for the application of MUNEs given this disease involves directly large and rapid loss of motor neurons. In the United States, the Food and Drug Administration (FDA) has not yet accepted MUNE as a primary endpoint measure in ALS clinical trials because the clinical meaning of MUNE has not been sufficiently established. However, MUNE results in ALS clinical trials have been corroborated by other standard clinical measures. Moreover, in ALS, MUNE has proven to be more sensitive than strength testing in predicting the onset of ALS-associated symptoms. Beyond ALS, MUNE has been used to detect motor unit losses in spinal muscle atrophy, Charcot Marie Tooth neuropathy, stroke-related hemiparetic muscle and, most relevant to this dissertation, diabetic polyneuropathy. Additionally, MUNE techniques have been used multiple times in the literature to examine how the neuromuscular system changes with adult aging.

The lone previous study using MUNE more than 35 years ago in human diabetic neuropathy found significantly fewer motor units in DPN patients compared with age-matched controls in the extensor digitorum brevis (EDB) muscle. EDB was likely selected for study due to its known involvement in DPN, especially given the length-
dependent progression of the disease. MUNE results in DPN patients were positively correlated with a decrease in motor nerve conduction velocities, a standard measure of neuromuscular dysfunction associated with DPN. Additionally, Hansen and Ballantyne reported MUNEs were negatively related to: age, duration of diabetes and MUP size. This study helped establish the potential clinical utility of MUNE in examining patients with DPN, and was the first to electrophysiologically show DPN was related to a loss of motor units. However, studying EDB in the context of MUNEs and DPN has important limitations. The functional status (e.g. strength) of EDB is difficult to assess; moreover EDB has relatively limited functional importance in day-to-day activities of living compared to other limb muscles. Additionally, when examining EDB it may be difficult to definitely attribute motor unit loss to the neuronal impacts of diabetes per se, or mechanical damage induced via altered gait and sensory and proprioception dysfunctions. Furthermore, in DPN, it remains unclear how data acquired via MUNE may integrate with other complementary measures (i.e. muscle strength, muscle morphology) in providing comprehensive information regarding the impacts of DPN on the neuromuscular system in humans.

1.9 Decomposition-based quantitative electromyography (DQEMG)

Quantitative assessment of motor unit action potentials (MUAPs) from individual motor units can provide useful and interesting information for the physiological appraisal of the neuromuscular system. One method that is used to accomplish this assessment is through using the computer software decomposition-based quantitative EMG (DQEMG). DQEMG software combines intramuscular EMG and surface EMG with a series of algorithms that can decompose a complex EMG pattern into its individual, constituent motor unit action potential trains (MUAPTs). Subsequently, DQEMG software provides more detailed information for each MUAPT including: firing rate, firing rate variability, size, shape, and stability. Additionally, DQEMG can be used to derive a MUNE via the DE-STA method, as described previously (Section 1.7). Further details regarding DQEMG are subsequently presented.
DQEMG typically involves the collection of both micro and macro signals\(^92\). Micro signals are derived via intramuscular, needle EMG electrodes which provide selective, detailed spatial and temporal information regarding individual motor units. Macro signals are usually collected via surface electrodes which provide information regarding overall size and spatial distribution of single motor units. Needle EMG data can be acquired via concentric, monopolar or single fibre needle electrodes during sustained, isometric voluntary contractions, typically of 30 seconds in duration. For the purposes of this brief description, discussion will be limited to data acquired via concentric needle electrode, although the general principles for each needle-type are the same.

The aforementioned micro signal, collected during low to moderate level muscle contractions, originally consists of a complex EMG interference pattern from multiple motor units. This EMG pattern is decomposed into its constituent individual MUAPTs using a series of algorithms which classify and cluster individual MUAPs based on MUAP shape, firing rate, and firing rate variability. The macro (surface EMG) signal is analyzed in temporal alignment with the decomposed micro signal. This is performed by using each individual MUAP as a trigger which locks to a 100 ms epoch of the macro signal. This method allows for the derivation of surface motor unit potentials (SMUPs), which correspond to the relative size of individual motor units within a muscle, and are used to calculate a MUNE as described previously (Section 1.6). MUNEs calculated using DQEMG software have been reported with high degrees of intra- and inter-rater reliability\(^79,94\). It is noteworthy that contraction intensity has been shown to have a marked effect on DQEMG-derived MUNEs\(^93,95\). Studies performed in the first dorsal interosseous (FDI)\(^93\) and tibialis anterior (TA)\(^73\) have found with increasing contraction intensities, from 10%-50% MVC, mean SMUPs increase resulting in decreased MUNEs. McNeil et al (2005) concluded, in the TA, a contraction intensity of 25% MVC includes recruitment of the majority of low- and high-threshold motor units, and thus provides the most representative MUNE compared to other contraction intensities\(^73\).
1.10 Analysis of individual motor unit action potential trains (MUAPTs)

In addition to providing CMAPs, SMUPs and MUNEs, DQEMG can provide detailed information regarding the temporal, spatial and morphological characteristics of individual needle-detected MUPs\textsuperscript{78,92}. Some of these parameters include: MU firing rates, MU firing rate variability, MUP size (amplitude, area), MUP shape and complexity (turns, phases), MUP density (fibre count) and MUP stability (jitter, jiggle, % blocking). Increases in MUP size and complexity are electrophysiological signs of enlarged, collaterally reinnervated motor units, which are associated with various disease processes\textsuperscript{96} as well as adult aging\textsuperscript{76,97,98}. These parameters are commonly included in a clinical, intramuscular needle EMG assessment. With major involvement of the neuromuscular system in DPN, abnormal findings indicative of skeletal muscle denervation have been reported\textsuperscript{50,51,99}.

MUP stability refers to the integrity of neuromuscular transmission, which can be examined in detail through the assessment of the degree of variability in the shape of consecutively detected MUPs\textsuperscript{100}. The two key properties related to MUP shape variability are termed jitter and jiggle\textsuperscript{100,101}. Jitter refers to the variability of the time intervals between pairs of individual muscle fibre potentials from a single MUP, and jiggle refers to the variability in overall MUP shape from one MUP discharge to the next (see Figure 4.1). Increases in both jitter and jiggle have been reported under conditions of neuromuscular transmission disturbance and can reflect early axonal denervation\textsuperscript{51,102,103}. Specific to DPN, a previous study using single fibre electromyography (SFEMG) found increased jitter in the tibialis anterior (TA) of patients with DPN, however jiggle was not reported\textsuperscript{51}. Additionally, increased fibre density (fibre count) in patients with DPN compared to controls was observed, which is indicative of reinnervation similar to the interpretation of enlarged, more complicated MUPs\textsuperscript{51}.

A limitation associated with traditional MUP morphological parameters (e.g. size, complexity) as well as traditional jitter and jiggle, is they can be difficult to assess at voluntary contraction levels greater than very light (approximately 5-10\% of MVC). This is due to increases in EMG signal intensity and complexity that may lead to electrophysiological contamination of single MUPT analysis. Thus most clinical needle
EMG studies are performed at minimal contraction intensities, often using SFEMG. This constrains most clinical needle EMG assessments to relatively few and exclusively low threshold motor units, limiting the scope of data obtained. One potential solution to this issue involves the development of near fibre (NF) MUP parameters that are obtained via a high-pass filtered MUP template, which therefore focuses on the electrophysiological contributions of muscle fibres that are nearest (within ~350 µm) to the needle electrode centre of detection. Filtering the EMG signal in this way reduces contributions from electrophysiological signals located greater than ~350 µm away from the needle detection surface, and thus this analysis may prove more robust than traditional MUP analysis, especially during MUNE protocols which require sustained, moderate level (25% MVC) contractions. Some NF parameters of interest include: NF count (a measure of fibre density), NF jiggle (which can be used to assess the neuromuscular transmission variability of the near fibers), NF dispersion (which measures the temporal dispersion of the near fiber contributions) and maximum NF interval (which is the maximum time interval between temporally adjacent near fiber contributions to a NF MUP). At present, no study has reported these measures in patients with neuropathy therefore their utility remains poorly understood.

Thus, DQEMG provides a unique and convenient platform to combine MUNE and needle-detected MUP analysis measures to gain a more detailed understanding of pathological alterations to the neuromuscular system imposed by DPN. Further study using DQEMG software is warranted to determine how changes in neuromuscular transmission stability and neuromuscular remodeling may relate to one another, as well as other aspects of functional capacity and disease severity in patients with DPN.
1.11 Purposes and Hypotheses

Broadly stated, the primary purpose of the studies described herein (Chapters 2, 3, 4, 5, & 6) was to investigate the physiological impacts of DPN on the neuromuscular system in humans. More specific objectives and hypotheses are as follows:

1. Purpose: To determine if the loss of strength reported in patients with DPN is related to a loss of motor units, as quantified using DQEMG (Chapter 2).
   a) It was hypothesized that DPN patients would have fewer motor units compared to controls, and MUNE would be positively associated with strength.

2. Purpose: To determine if a length-dependency exists regarding the DPN-related loss of motor units in humans (Chapter 3).
   a) It was hypothesized that MUNE of the TA would be more greatly affected by DPN than FDI.

3. Purpose: To determine how DPN affects the stability of neuromuscular transmission using the electrophysiological parameters jiggle, jitter and percent blocking (Chapter 4).
   a) It was hypothesized that patients with DPN would feature a greater degree of neuromuscular transmission instability (i.e. greater jiggle, jitter and % blocking) compared to healthy controls.

4. Purpose: To assess the effect of DPN on dorsiflexor muscle contractile properties, including contractile speed and muscle morphology, in association with DPN-related denervation (Chapter 5)
   a) It was hypothesized DPN patients would have weaker, slower muscles with greater proportions of non-contractile intramuscular tissue that would be negatively correlated with MUNE.
5. Purpose: To examine the neuromuscular fatigability of patients with DPN using sustained isometric contractions and identify possible mechanisms underlying differences from controls (Chapter 6).

   a) It was hypothesized DPN patients would be relatively more resistant to fatigue due to slowed muscle properties and the less absolute strength.
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Chapter 2

2 Motor unit loss and weakness in association with diabetic neuropathy in humans

2.1 Introduction

The most common form of diabetic neuropathy is a length dependent symmetrical neuropathy with predominant involvement of sensory fibres. In more severe forms there is progressive involvement of motor fibres. Animal models show that relative to the sensory nervous system, motor axons are more resistant to the effects of diabetes mellitus (DM). Nonetheless these models indicate motor axons are targeted by DM and undergo degeneration. In humans, motor axon or motor unit (MU) loss as a complication of DM has been quantified in the extensor digitorum brevis (EDB). However, in intrinsic foot muscles such as the EDB it may be difficult to differentiate between axonal loss caused by physical trauma versus damage caused by biochemical stressors associated with diabetes. Furthermore, investigating MU loss in more functional muscles (e.g. tibialis anterior) allows for the assessment of other relevant clinical and physiological properties such as strength and fatiguability. Thus the purpose of this study was to estimate the number of functioning MUs in the tibialis anterior, using an electrophysiological method, in a group of patient with DM in comparison to age and sex matched controls.

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1 A version of this chapter has been published. Used with permission from John Wiley and Sons, Inc.

2.2 Materials and Methods

Participants

Six patients (4 men and 2 women; age, 63 ± 16.1 years) were recruited for this study. All patients met criteria for the diagnosis of DM and had clinical and electrophysiological features of confirmed diabetic polyneuropathy (DPN)\(^2\). Additionally, all patients had a thorough history and clinical examination to exclude other causes of nerve injury (i.e. compressive mononeuropathies or L5-S1 radiculopathies). Six healthy, age and sex-matched controls (4 men and 2 women; age, 62 ± 16.3 years) were recruited from the community. The study was approved by the local university’s research ethics board. Informed, oral and written consent was obtained from all participants prior to testing.

Motor Unit Number Estimates (MUNE) and Dorsiflexor Strength

To assess dorsiflexion strength, participants were seated in a custom isometric dynamometer. Surface electromyography (EMG) was collected from the tibialis anterior using self-adhering Ag-AgCl electrodes. Intramuscular EMG signals were recorded via a disposable concentric needle electrode inserted into the TA, 5-10 mm proximal to the active surface electrode.

All EMG data were acquired using customized software on a Neuroscan Comperio system (Neurosoft, El Paso, TX). The algorithms used for decomposition-based quantitative EMG (DQEMG) and decomposition-enhanced spike-triggered averaging (DE-STA) have been described in detail elsewhere\(^6,7\). In brief, the surface and intramuscular EMG signals were bandpass filtered at 5 Hz to 1 kHz and 10 Hz to 10 kHz, respectively. Surface electrodes (Marquette Medical Systems, Jupiter, FL) were applied to the TA muscle using standard methods and the maximum CMAP for the TA muscle was obtained by supra-maximal stimulation of the fibular nerve, just distal to the fibular head, with a hand held stimulating electrode. Next, three maximal voluntary dorsiflexion contractions (MVCs) were performed. Subsequently, participants matched a target line of 25% MVC for all isometric contractions because this contraction intensity has been
shown to be the most effective intensity for obtaining a representative motor unit number estimation (MUNE) in the tibialis anterior (TA)\(^8\). Surface and concentric needle (Model N53153; Teca, Hawthorne, NY) EMG were collected simultaneously while participants sustained a steady target torque for each contraction for \(\sim 30\) s. These contractions were repeated until at least 20 suitable trains of motor unit potentials (MUPs) and their respective surface-motor unit potentials (S-MUPs) were collected.

Decomposed EMG signals were reviewed off-line to determine the acceptability of the needle-detected MUP trains and their corresponding S-MUPs. A computer algorithm aligned the negative onset markers for all accepted S-MUPs and created a mean S-MUP template based upon their data-point by data-point average. A MUNE was derived by dividing the negative-peak amplitude of the CMAP by the negative peak amplitude of the mean S-MUP\(^9\).

Torque signals were collected and sampled online at 500 Hz using Spike2 software (Cambridge Electronic Design, Cambridge, UK) and analyzed off-line to determine voluntary isometric torques (strength).

**Statistics**

Group CMAP, MUNE and isometric dorsiflexion strength data were compared using a one way ANOVA. Group mean S-MUP data were not normally distributed, thus these data were compared using a Kruskal-Wallis one way analysis of variance by ranks. All data are presented as group means ± standard deviations. Significance was set at \(p \leq 0.05\).

**2.3 Results**

All results all presented in Table 2.1. Control (n=6) and DPN (n=6) groups were age and sex matched (\(p>0.05\)). DPN patients were found to be \(\sim 60\%\) weaker in isometric dorsiflexion strength (Nm) versus controls (\(p<0.05\)). DPN patients possessed significantly reduced (\(\sim 40\%)\) CMAP negative peak amplitudes (mV) and enlarged S-MUP (\(\sim 50\%)\) negative peak amplitudes (µV) (\(p<0.05\)). These changes resulted in \(\sim 60\%\)
fewer MUs based on MUNE in DPN patients when compared to controls (p<0.05). DPN patients had mean motor unit firing rates of 10.2 ± 1.5 pulses per second; controls had mean motor unit firing rates of 12.1 ± 0.9 (p<0.05). During the 25% MVC contractions DPN patients produced 29.0 ± 3.6% of maximal RMS EMG; controls produced 24.5 ± 6.4% of maximal RMS EMG (p>0.05).
<table>
<thead>
<tr>
<th>Measure</th>
<th>Control (6)</th>
<th>DPN Patient (6)</th>
<th>% Difference</th>
</tr>
</thead>
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<tr>
<td>Age</td>
<td>62 ± 16.3</td>
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<tr>
<td>Dorsiflexion Strength (Nm)</td>
<td>39.0 ± 10.6</td>
<td>15.9 ± 4.0*</td>
<td>59.3%</td>
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<tr>
<td>TA CMAP NP Amplitude (mV)</td>
<td>6.2 ± 1.3</td>
<td>3.9 ± 0.9*</td>
<td>38.1%</td>
</tr>
<tr>
<td>TA S-MUP NP Amplitude (µV)</td>
<td>57 ± 19</td>
<td>109 ± 47*</td>
<td>47.4%</td>
</tr>
<tr>
<td>TA MUNE (#)</td>
<td>111 ± 22</td>
<td>45 ± 27*</td>
<td>59.1%</td>
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**Nerve Conduction Studies**

<table>
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<th>Control (6)</th>
<th>DPN Patient (6)</th>
<th>% Difference</th>
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</thead>
<tbody>
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<tr>
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<tr>
<td>Tibial Nerve CV (m/s)</td>
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<td>33.9 ± 2.9</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2.1** Strength and motor unit number estimates of the tibialis anterior in controls and patients with diabetic polyneuropathy

Nerve conduction study data presented in the patient group are outside of normal ranges. DPN = diabetic polyneuropathy; Nm = Newton\*metre; TA = tibialis anterior; CMAP = compound muscle action potential; NP = negative peak; mS-MUP = mean surface motor unit potential; MUNE = motor unit number estimate; SNAP = sensory nerve action potential; CV = conduction velocity. * denotes significant difference between groups, p<0.05; # sural nerve responses were absent in all patients except one.
2.4 Discussion

DM imposes unique, chronic forms of damage and degeneration to the peripheral nervous system. Whilst damage to the sensory nervous system is often observed clinically, much less is known about the long-term effect of DM on the motor nervous system. Our results provide new evidence in humans of MU loss with DM, in association with significant decreases in strength, using DQEMG-derived MUNE.

Using various electrophysiological MUNE techniques, MU loss has been previously documented in other diseases affecting the nervous system and in DM in an intrinsic foot muscle. MU loss and associated muscular weakness as a result of DM has not been directly quantified in the TA in humans despite the inclusion of related clinical signs such as atrophy, weakness, and reduced CMAPs in the description of patients with DPN. Our findings indicate the weakness, muscular atrophy, and increased fatigue reported in DPN patients may be partially accounted for by a loss of MUs. However, MUNE does not allow for the differentiation between distal axonal retraction versus motor neuron death.

In rodent models of chronic experimental diabetes, motor neurons undergo distal, axonal dropout, whereby motor neurons gradually withdraw their terminals from distal innervation of their associated muscle. Additionally it has been suggested that smaller MUs are preferentially targeted or there is a general enlargement of surviving MUs through compensatory sprouting. Our results of pathologically enlarged S-MUPs within the patient group also indicate an ongoing process of denervation and collateral reinnervation in DM patients. However, the observation of reduced strength in the DM group indicate the process of denervation is outpacing collateral reinnervation, leading not only to a reduction in MUs but also to probable atrophy of muscle tissue and concomitant loss of strength.

The TA was selected for investigation in the present study due to the distal to centripetal progression of diabetic neuropathy and its functional relevance in mobility, balance and association with fall-risk. Given the pattern of the development of weakness in patients with diabetic neuropathy it seems likely that MUs would be
preserved in more proximal limb muscles, such as the quadriceps or biceps brachii, although this remains to be determined\textsuperscript{12,14}.

The results from the present study indicate DQEMG-derived MUNE may have some clinical utility in predicting functional impairment, tracking DM progression and disease management. This may be particularly useful in individuals whose diabetic neuropathy features a detectable motor component (i.e. muscle wasting, reduced CMAP, weakness) using standard clinical measures (i.e. manual muscle testing, motor nerve conduction studies). Additionally, it remains to be determined how MU loss in DM patients relates to other relevant clinical and functional measures.
References


Chapter 3

3 Length dependent loss of motor axons and altered motor unit properties in human diabetic polyneuropathy

3.1 Introduction

Diabetic polyneuropathy (DPN) is a generalized disorder of the peripheral nervous system caused by metabolic and vascular stressors associated with diabetes mellitus (DM). The pathophysiology underlying this nervous system dysfunction may be due to a combination of various metabolic and vascular factors, and is correlated with disease duration and quality of serum glucose control (e.g. as indicated by HbA1c levels). In the early stages of DPN, patients often present with predominant involvement of the sensory nervous system and no clinical impairment in motor function is detectable. Regardless of the underlying causes, DPN commonly develops as a length-dependent, or progressive centripetal degeneration of peripheral nerve axons. This manner of disease progression typically leads to development of length dependent neurological complications first in the feet, followed by the legs and hands.

Although motor neurons may be more resistant than sensory axons to the dysfunction and degeneration associated with diabetes mellitus, studies in animal and human models nonetheless have shown that the motor system is affected. Indeed, whereas sensory dysfunction can lead to injury resulting from lack of sensation, ulceration and amputation; dysfunction of the motor nervous system is associated with muscle atrophy and weakness. Muscle weakness may therefore contribute to the higher risk of developing physical disability in patients with diabetes.

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Muscular atrophy and weakness associated with DM may be partially a result of motor unit or motor axon loss. Electrophysiologically-derived motor unit number estimates (MUNEs) in human patients with DPN have been shown to be significantly reduced in one distal muscle of the lower limb, specifically an intrinsic foot muscle, the extensor digitorum brevis (EDB)\(^{10}\) and, in preliminary findings, in the tibialis anterior (TA)\(^{13}\). In EDB, these pathological changes in motor unit number and properties were correlated with age and disease duration\(^{10}\). Motor unit loss has been reported concurrent with significant muscular weakness when compared with age and sex-matched human controls\(^{13}\). In addition to reduced MUNEs, changes in motor neuron properties have been reported in patients with DPN. Abnormal single-fiber electromyography recordings (increased jitter) have been found in DPN patients, suggestive of reinnervation of the motor system, which correlated with glycemic control\(^{3}\). Also, previous studies indicate patients with DM show lower mean motor unit discharge rates versus controls and greater variability in discharge rates of action potential trains during sustained low level contractions\(^{17}\). These findings may be related to changes in recruitment strategy due to neuromuscular remodeling and altered excitability of motor units\(^{18}\). However it is not known how these results may compare to potential changes in motor unit numbers of more proximal or upper limb muscles, or how they relate more broadly to indicators of disease severity, such as glycemic control.

Decomposition-based quantitative electromyography (DQEMG) is an electrophysiological technique based on surface and intramuscular EMG, in conjunction with dedicated computer algorithms used to decompose complex EMG signals\(^{19,20}\). This technique can be used to provide reliable MUNEs, as well as other valuable information concerning motor unit properties in humans, including motor unit size, complexity, stability, recruitment order, and rate coding strategies\(^{21-23}\). Although this useful technique has been applied to study various clinical and special populations, including amyotrophic lateral sclerosis\(^{21}\), compressive neuropathies\(^{24}\), knee osteoarthritis\(^{23}\), and adult aging\(^{25}\), it has not been fully employed in the investigation of diabetic neuropathy (Chapter 2).

The purpose of the present study was to estimate the number of motor units and assess their electrophysiological properties in an upper (first dorsal interosseus [FDI])
and lower limb (TA) muscle of patients with DPN using DQEMG. We sought to compare how motor unit numbers and their properties were affected by DPN and whether changes in these properties were related. Additionally, the associations between these different neuromuscular properties with functional parameters (e.g. strength) and measures of serum glucose control (e.g. HbA1c levels) were investigated across muscles. We hypothesized DPN patients would feature significantly reduced MUNEs, and enlarged motor units in both TA and FDI when compared with age matched controls. Additionally, we hypothesized these differences would be more pronounced in the TA versus the FDI due to the centripetal nature of DPN disease progression. Finally, we hypothesized negative relationships would exist between MUNEs and parameters of disease severity.

3.2 Methods

3.2.1 Participants

Twelve patients (7 men, 5 women; ages 32-78 years) with DPN were recruited. They met the criteria for diagnosis of type 2 non-insulin dependent DM with clinical and electrophysiological features of confirmed DPN. Additionally, they had a thorough history, and clinical and electrophysiological examination by an experienced neurologist with specialized training in neuromuscular disease to exclude other causes of nerve injury (i.e., other polyneuropathies, compressive mononeuropathies, radiculopathies). Patients with any neurological, metabolic or vascular diseases other than related to DM or DPN were excluded. Twelve healthy, age and sex-matched controls (7 men, 5 women; ages 29-77 years) were recruited from the community. Control participants were screened by physicians (neurologists) to ensure they met inclusion criteria. The study was approved by the local university research ethics board. Informed oral and written consent was obtained from all participants prior to testing.

3.2.2 DQEMG and Strength Assessment of Tibialis Anterior

Participants were seated in a custom isometric dynamometer designed to measure plantar and dorsiflexion at the ankle joint. In the dynamometer, the right ankle was
positioned at 30° of plantar flexion, with both knee and hip angles maintained at 90°. A C-shaped brace was secured directly on the distal aspect of the right thigh to minimize movement at the hip joint during contractions. Inelastic Velcro straps were placed over the dorsum of the foot to secure the foot to the dynamometer.

All testing was performed on the right (dominant) leg. Participants performed three dorsiflexion maximal voluntary contractions (MVCs), with at least 3 min of rest between attempts. Each MVC was held for approximately 3 seconds. Participants were provided with real-time visual feedback of their torque and verbally encouraged. Voluntary activation during the 2nd and 3rd MVC attempts was assessed using the interpolated twitch technique. This technique involves supramaximal percutaneous electrical stimulation of the fibular nerve just distal to the fibular head. The amplitude of the interpolated torque electrically evoked from a single 100μs stimulus during the plateau of the MVC was compared with a single (100μs) resting twitch evoked ~1s following the MVC. Voluntary activation was calculated as a percent using the following equation: \([1 - (\text{interpolated twitch} / \text{resting twitch})] \times 100\). The peak torque of the three MVC attempts was taken as the maximal torque for the participant. All torque signals were collected and sampled online at 500 Hz using Spike2 software (Version 7.11; Cambridge Electronic Design Ltd., Cambridge, United Kingdom) and analyzed off-line to determine voluntary isometric torques (strength).

Surface electromyography (EMG) was collected from the TA using self-adhering Ag-AgCl electrodes (1 cm x 3 cm). The active electrode was placed over the TA motor point, approximately 7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia. This placement was adjusted to maximize the TA compound muscle action potential (CMAP) amplitude as necessary. The reference electrode was placed over the distal tendon of the TA. A ground electrode was placed over the patella.

Decomposition-based quantitative electromyography (DQEMG) data were acquired using decomposition enhanced spike triggered averaging (DE-STA) software, described in detail elsewhere. Intramuscular EMG signals were recorded via a disposable concentric needle electrode (Model N53153; Teca Corp., Hawthorne, NY).
inserted into the TA, 5-10 mm distal to the active surface electrode. The surface and intramuscular EMG signals were bandpass filtered at 5 Hz to 1 kHz and 10 Hz to 10 kHz, respectively. Surface EMG was sampled at 3 kHz; intramuscular EMG was sampled at 30 kHz. To evoke the maximum CMAP a bar electrode held distal to the fibular head delivered supramaximal electrical stimuli to the common fibular nerve. Subsequently, participants matched a target line of 25% MVC, visible on a computer monitor, for all isometric dorsiflexion contractions during which the intramuscular needle electrode was inserted and manipulated in the muscle. This contraction intensity has been shown to be the most effective intensity for obtaining a representative motor unit number estimation (MUNE) in the tibialis anterior (TA)\textsuperscript{22}. Surface and intramuscular EMG were collected while participants sustained a steady target torque for each contraction held for ~30 s. Between contractions, the concentric needle electrode was repositioned in order to obtain to sample from different motor units. These procedures were repeated until at least 20 suitable trains of motor unit potentials (MUPs) and their respective surface-motor unit potentials (S-MUPs) were collected.

Decomposed EMG signals were reviewed off-line to determine the acceptability of the needle-detected MUP trains and their corresponding S-MUPs. A computer algorithm aligned the negative onset markers for all accepted S-MUPs and created a mean S-MUP template based upon their data-point by data-point average. All MUP and S-MUP markers were subsequently reviewed visually by the operator. A MUNE was derived by dividing the negative-peak amplitude of the CMAP by the negative peak amplitude of the mean S-MUP\textsuperscript{29}.

In addition to providing MUNEs, the DQEMG software measures firing rates (Hz) of individual motor unit action potential trains (MUAPTs), and provides overall mean firing rates per participant at a relative target level of contraction. Finally, the DQEMG system allows for the measurement of properties of needle detected-MUPs, including MUP amplitude, area, duration, and area to amplitude ratio (AAR). The calculation of the MU size index (SI [SI = 2 x log(amp) + area/amp]), a stable measure useful in the detection of neurogenic processes, was also performed\textsuperscript{30}. All markers were automatically placed by the DQEMG software and were subsequently inspected manually
and adjusted where appropriate. The DQEMG technique described has been shown to possess similar test-retest values within individuals\textsuperscript{31}, and high degrees of intra- and inter-rater reliability in control and clinical populations\textsuperscript{32,33}.

3.2.3 DQEMG of First Dorsal Interosseus

For the FDI, participants were seated in a straight-back chair with their feet on the floor. The participant’s forearm and hand were secured in a custom-built apparatus designed to isolate the second (index) digit during isometric abduction contractions\textsuperscript{34}. This apparatus was designed to minimize any movement in the wrist and hand, while allowing for isolated index finger abduction (FDI contraction) through the immobilization of the wrist, thumb and digits three to five with Velcro straps. Torque data was not recorded for the FDI.

Surface EMG was collected using self-adhering Ag-AgCl electrodes (1 cm by 2.5 cm); the active electrode was placed over the motor point of the FDI muscle and the reference electrode was placed over the second metacarpal phalangeal joint. A ground electrode was applied to the medial aspect of the wrist, distal to the stimulation site. To obtain a maximal FDI CMAP supramaximal stimulation was applied to the ulnar nerve at the wrist (~7 cm proximal to the active electrode).

Participants performed three index finger abduction maximal voluntary contractions (MVCs), with at least 3 min of rest between attempts. Each MVC was held for approximately 3 seconds during which the participant was verbally encouraged. Visual and auditory feedback were provided to the participant via surface EMG activity, and maximal RMS EMG activity was recorded during these MVCs. The intensity of subsequent contractions was based on a percentage (25%) of relative maximal RMS activity (% MVC-RMS)\textsuperscript{28,29}.

DQEMG data were acquired from the FDI using similar equipment, filter and amplification parameters to the protocol in the TA (described above). The concentric needle electrode was inserted into the FDI 2-5 mm distal or proximal to the active electrode. Participants performed ~30 s FDI contractions, while matching a target line of 25% MVC-RMS, during which surface and intramuscular EMG were collected. These
procedures were repeated until at least 20 suitable trains of MUPs and their respective S-MUPs were collected. Decomposed EMG signals of the FDI were analyzed using the same methodology used in the TA (described above).

3.2.4 Statistics

Mean values ± standard deviations are presented in the text. Normally distributed data were analyzed using a one-way ANOVA; the level of significance was set at $P \leq 0.05$. When a significant main effect was detected, Tukey’s post-hoc test was performed. Non-normally distributed data were analyzed using a Kruskal-Wallis one-way ANOVA on ranks, with Dunn’s Method used as the post-hoc test. Effect sizes for comparisons between groups are presented as Cohen’s d. Relationships between variables were testing using Pearson’s Product Moment Correlation. Data analyses were performed using SigmaPlot software (Version 12.2; Systat Software, Chicago, Illinois, US).

3.3 Results

3.3.1 Participant Characteristics

Participant characteristics are presented in Table 3.1. No significant differences were detected for age or height ($p>0.05$) between groups. DPN patients had significantly greater body mass ($p<0.05$) and BMI ($p<0.05$) than controls. Maximum ankle dorsiflexion strength was 40% lower in the DPN group versus controls ($p<0.05$), with no group differences for voluntary activation ($p>0.05$). Data characterizing the clinical history and status of the DPN patient group are presented in Table 3.2. In both groups, mean TA and FDI CMAP amplitudes were above our clinic’s lower limit of normal, which are 4.0 mV and 10 mV, respectively.
# Table 3.1 Participant Characteristics

MVC – maximal voluntary contraction. * Denotes significant difference between groups (p<0.05).

<table>
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<th>Control (n = 12)</th>
<th>DPN Patient (n = 12)</th>
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<td><strong>Anthropometric Parameters</strong></td>
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<td></td>
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<td>Male/Female</td>
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<td>7/5</td>
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<tr>
<td>Age (years)</td>
<td>63.8 ± 15.5</td>
<td>65.8 ± 15.4</td>
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<tr>
<td>Height (m)</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
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<td>Weight (kg)</td>
<td>73.8 ± 6.1</td>
<td>83.1 ± 7.4*</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.4 ± 3.1</td>
<td>28.9 ± 3.7*</td>
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<tr>
<td><strong>Dorsiflexion Properties</strong></td>
<td></td>
<td></td>
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<tr>
<td>Dorsiflexion MVC (Nm)</td>
<td>34.1 ± 9.5</td>
<td>22.3 ± 7.2*</td>
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<tr>
<td>Voluntary Activation (%)</td>
<td>98.3 ± 1.9</td>
<td>97.6 ± 2.1</td>
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Table 3.2 Clinical characteristics of DPN patient group

SNAP – sensory nerve action potential; TA – tibialis anterior; NP - negative peak; CMAP – compound muscle action potential; CV – conduction velocity; FDI – first dorsal interosseous. * SNAP responses were absent in 10 out of 12 patients.

3.3.2 Decomposition-based Quantitative Electromyography Measures

Results from DQEMG of the TA and FDI are presented in Tables 3.3 & 3.4. In both muscles, DPN patients featured ~25% greater duration for mean needle-detected motor unit potentials (NMUPs) than controls (p<0.05), however, mean NMUP amplitude (peak-to-peak amplitude) and area to amplitude ratios (AAR) were not different between
groups (p>0.05). In examining NMUP complexity, groups did not differ in the number of phases (p>0.05), although the DPN patient group featured ~25% more turns than controls in both muscles (p<0.05). In the TA and FDI, the DPN patient group had lower mean firing rates during the sustained, submaximal contractions compared to controls (p<0.05). Motor unit size index and % RMS EMG were not different between groups in either muscle (p>0.05).

When examining the TA, DPN patients were found to have ~40% larger mean surface motor unit potentials (S-MUP) than controls (p<0.05). Maximal compound muscle action potentials (CMAP) (negative peak-amplitude) were significantly smaller (~30%) in DPN patients compared to controls (p<0.05). Additionally, the DPN patient group featured a ~45% lower MUNE compared to the control group (p<0.05) (Figure 3.1).

In the FDI, SMUP amplitudes were not different between groups (p>0.05). Maximal CMAP (negative peak-amplitude) were significantly smaller (~20%) in DPN patients compared to controls (p<0.05). Finally, the DPN patient group featured a ~30% lower MUNE compared to the control group (p<0.05) (Figure 3.1). In both TA and FDI, individual MUNE data for DPN patients and controls are illustrated in Figure 3.2.
<table>
<thead>
<tr>
<th>N-MUP Parameters</th>
<th>Control</th>
<th>DPN Patient</th>
<th>Effect Size</th>
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<tr>
<td>Peak-to-peak voltage (µV)</td>
<td>803.0 ± 145.0</td>
<td>1056.0 ± 468.0</td>
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<td>Duration (ms)</td>
<td>8.4 ± 1.54</td>
<td>10.8 ± 1.6*</td>
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<td>AAR (ms)</td>
<td>1.6 ± 0.3</td>
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<td>Phases (#)</td>
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<td>Turns (#)</td>
<td>3.3 ± 0.6</td>
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<tr>
<td>Negative peak amplitude (µV)</td>
<td>77.0 ± 20.0</td>
<td>116.0 ± 61.0*</td>
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<tr>
<td>Mean Firing Rate (Hz)</td>
<td>12.2 ± 1.7</td>
<td>10.1 ± 1.2*</td>
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<tr>
<td>% RMS EMG</td>
<td>20.7 ± 6.1</td>
<td>23.3 ± 4.3</td>
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<td>Motor Unit Size Index</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.6</td>
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<th>MUNE</th>
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<tr>
<td>CMAP (mV)</td>
<td>6.8 ± 1.1</td>
<td>5.0 ± 1.7*</td>
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<tr>
<td>SMUP (µV)</td>
<td>77.0 ± 20.0</td>
<td>116.0 ± 61.0*</td>
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<td>MUNE (#)</td>
<td>99.0 ± 19.0</td>
<td>54.0 ± 25.0*</td>
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**Table 3.3** Decomposition-based quantitative electromyography of tibialis anterior

N-MUP – needle detected motor unit potential; AAR – area to amplitude ratio; RMS EMG – root mean squared electromyography; CMAP – compound muscle action potential; SMUP – surface detected motor unit potential; MUNE – motor unit number estimation. Effect size presented as Cohen’s D. CMAP and SMUP data are presented as negative peak amplitudes. * Denotes significant difference between groups (p<0.05).
### N-MUP Parameters

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<tr>
<td>Peak-to-peak voltage (µV)</td>
<td>839.0 ± 124.0</td>
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<td>Duration (ms)</td>
<td>8.0 ± 0.74</td>
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<td>AAR (ms)</td>
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<td>Turns (#)</td>
<td>2.6 ± 0.2</td>
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### S-MUP Parameters

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<td>Negative peak amplitude (µV)</td>
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### Other

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<tr>
<td>% RMS EMG</td>
<td>20.7 ± 1.6</td>
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<tr>
<td>Motor Unit Size Index</td>
<td>0.9 ± 0.3</td>
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### MUNE

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<td>12.7 ± 1.6</td>
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<td>MUNE</td>
<td>107.0 ± 33.0</td>
<td>73.0 ± 18.0*</td>
<td>0.96</td>
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**Table 3.4** Decomposition-based quantitative electromyography of first dorsal interosseous

N-MUP – needle detected motor unit potential; AAR – area to amplitude ratio; RMS EMG – root mean squared electromyography; CMAP – compound muscle action potential; SMUP – surface detected motor unit potential; MUNE – motor unit number estimation. Effect size presented as Cohen’s D. CMAP and SMUP data are presented as negative peak amplitudes. * Denotes significant difference between groups (p<0.05).
Figure 3.1 Comparison of relative differences between DPN patients and controls in key electrophysiological variables between tibialis anterior (TA) and first dorsal interosseous (FDI). All relative differences between DPN patients and controls were significant, excluding FDI mSMUP.
**Figure 3.2** Motor unit number estimates in diabetic neuropathy

Motor unit number estimates in the tibialis anterior (panel A) and first dorsal interosseous (panel B) in DPN patients and age-matched controls. Group means are represented by enlarged symbols and feature standard deviation error bars.
Figure 3.3 Relationship between muscle action potential size and motor unit number estimates

Relationship between compound muscle action potential (CMAP) amplitude and motor unit number estimates (MUNEs) in the tibialis anterior of DPN patients (black squares) and controls (grey circles). Dotted line represents lower limit of normal (4.0 mV) for CMAP amplitude. Group means are represented by enlarged symbols and feature standard deviation error bars.
3.3.3 Tibialis Anterior versus First Dorsal Interosseus Motor Unit Properties

In patients with DPN, the TA has undergone greater motor unit loss in comparison to the FDI (see effect sizes in Tables 3.3, 3.4). Patients with DPN featured a ~45% lower MUNE in the TA, versus a ~30% lower MUNE in FDI. CMAPs were ~30% smaller in the TA versus ~20% smaller in FDI. Furthermore, in the TA, DPN patients were found to have mean SMUPs enlarged by ~40% whereas in FDI there were no differences.

3.3.4 Disease Severity, Age, Strength and Motor Unit Number Estimates

Within the DPN patient group, in either muscle, no significant relationships were found between MUNEs and: HbA1c levels, blood lipid profiles, duration of DM, duration of DPN, or age (p>0.05). TA MUNE and dorsiflexion strength were found to be positively related to one another (r = 0.79; r² = 0.62; p<0.05) within the DPN group.

3.4 Discussion

This study has demonstrated that patients with DPN have fewer motor axons, which may be related to a loss of MUs, in both distal lower (TA) and upper limb (FDI) muscles compared to healthy, age-matched controls. Neuromuscular properties of the TA were more severely affected by DPN than FDI in terms of degree of motor unit loss and motor unit remodeling. The number of motor units in the TA was found to be significantly positively related to dorsiflexion strength in the DPN patient group. Using DQEMG, patients with DPN were found to have more complex motor units and lower mean motor unit firing rates compared to controls at similar levels of contractile intensity. Finally, in the DPN group, no significant relationships were found between motor unit loss and disease severity, disease duration or age in either muscle.
3.4.1 DPN-Related Motor Axon Loss and DQEMG-Derived MUNE

In the present study, the observed motor axon loss associated with DPN in the TA and FDI is consistent with findings previously reported in extensor digitorum brevis\textsuperscript{10}, and preliminary findings reported in the TA of a small group of DPN patients\textsuperscript{13}. The reduced MUNEs in DPN patients are likely primarily due to distal axonal retraction of motor neurons from their associated muscle fibres and concurrent inadequacy of compensatory collateral reinnervation, rather than cell death of motor neurons at the perikaryal level\textsuperscript{7}. Using a rodent model of experimental diabetes, it has been found that although motor neurons are resistant to immutable loss, indeed, they gradually withdraw their terminals from distal innervation of skeletal muscle\textsuperscript{7}. A key difference between the present study and the report by Hansen and Ballantyne\textsuperscript{10} is that they used a modified version of the incremental MUNE technique, whereas we have employed DE-STA. The results from the incremental technique can be affected by several important factors including the effects of ‘alternation’; the instability caused by neuropathy; and the assumption that MUs electrically stimulated at low intensities are representative of the entire MU pool\textsuperscript{36}, which may overestimate the total number of functioning motor units. DQEMG-derived MUNE accounts for these limitations through its use of voluntary contractions and algorithms designed to compensate for neuromuscular instability in detecting single MUPs\textsuperscript{19}. Additionally, at 25% of maximal contractile intensity, the majority of MUs are recruited in both FDI and TA\textsuperscript{37,38}. Also, the MUNE technique in the present study has the added benefit of providing data pertaining to needle detected-MUPs\textsuperscript{19}. Although all subjects tolerated the needle component of the assessment very well, the reliance on needle-electrode insertion with this technique may be uncomfortable for some DPN patients, especially those with symptoms of painful diabetic neuropathy. It is also important to note this MUNE protocol has been shown to have high degrees of intra- and inter-rater reliability\textsuperscript{32,33}, and produces similar test-retest values within subjects, thus supporting its potential utility in following patients longitudinally\textsuperscript{31,39}. Finally, it should be noted that DQEMG-derived MUNE is especially useful for studying more proximal muscles (e.g. TA, soleus, trapezius) versus some other MUNE techniques\textsuperscript{13,33,40}. 
In addition to technical differences noted above, there are functional differences between the TA and EDB that may limit a direct comparison. In older adults, it is not uncommon to observe reduced CMAP amplitudes in fibular motor nerve conduction when recording from EDB, but not from the TA of the same individuals. As an intrinsic muscle on the dorsum of the foot, the EDB may be more susceptible to lifelong mechanical damage regardless of DM, but unlike the TA, relevant functional impairments of the EDB may not affect important lower limb functions related to locomotion, postural stability and the risk of falls.

3.4.2 Length-Dependent Motor Axon Loss in DPN

The degree of motor unit loss in EDB, TA and FDI is variable and the observed pattern appears to reflect the length-dependent nature of DPN disease progression. In EDB, a more distally located muscle than FDI or TA, a previous study reported ~63% fewer motor units in DPN patients compared to controls. In comparison, the present study found reduced MUNEs in DPN patients of ~50% and ~30% in the TA and FDI respectively (Figure 3.1). In this context, interpretation of our results with the prior study must be done with caution because the DPN patients studied by Hansen and Ballantyne featured a shorter mean duration of DM compared to the present study (11.7 vs 16.1 years). Assuming that for any patient a greater loss of motor units occurs with longer disease duration, it seems plausible the greater relative loss of motor units in EDB is a reflection of the pattern (peripheral to central) of disease progression rather than an artifact of inherent differences between DPN patient populations, or due to different MUNE techniques as mentioned above. Furthermore, greater differences in other electrophysiological parameters, including maximal CMAP amplitudes and mean SMUP amplitudes, in the TA versus the FDI fits the general pattern of DPN progression.

These findings highlight that in human DPN, reductions in axonal loss and presumably motor unit numbers follow the same length-dependent pattern of progression as other clinical signs and symptoms (i.e. sensory abnormalities, weakness, muscle atrophy, autonomic dysfunction). Furthermore, the significant and strong positive relationship between TA MUNE and dorsiflexion strength ($r = 0.79$) may indicate that a loss of MUs is an important factor in dorsiflexor weakness. In human adult aging, loss of
motor neurons has been purported as an important but often overlooked factor related to muscle atrophy and associated loss of strength (i.e. sarcopenia).\textsuperscript{43,44} Since innervation is necessary for the maintenance of muscle mass, and thus strength, it seems likely the motor unit loss reported in DPN patients in the present study is a key factor leading to the concomitant loss of strength. Indeed, previous studies have noted a positive relationship between MUNE and strength in clinical and special populations including post-polio syndrome,\textsuperscript{45} amyotrophic lateral sclerosis,\textsuperscript{46} Charcot-Marie-Tooth disease\textsuperscript{47} and adult aging.\textsuperscript{25} Additionally, in a rodent model of DPN, motor unit loss has been documented in the early stages of DPN prior to the manifestation of standard electrophysiological, behavioral or clinical signs including strength loss.\textsuperscript{12} As weakness of the TA is associated with increased postural instability and greater likelihood of injurious falls\textsuperscript{41}, early detection of motor unit loss in DPN patients could prove useful in the prescription and adoption of preventative or compensatory interventions (e.g. aggressive glycemic control, strength training, pharmacological interventions).

In addition to motor axon loss, the present study provides indirect evidence of neuromuscular remodeling and collateral reinnervation via significantly greater needle-detected MUP durations; greater MUP complexity (i.e. turns), and greater mean SMUP amplitudes in the TA of DPN patients compared to controls\textsuperscript{25} (Tables 3.3 and 3.4). These findings in the TA of DPN patients may reflect the ongoing process of distal, axonal loss of \(\alpha\)-motor neurons resulting in denervation of their muscles fibres, and compensatory collateral reinnervation of those orphaned muscle fibres by neighboring \(\alpha\)-motor neurons that remain intact and functional. This process has been documented in animal models of DM following nerve crush injury\textsuperscript{48}, and leads to the development of larger, more complex individual motor units, a result that is detectable via MUPs with more turns and larger average SMUPs. In early DPN, this process may preserve muscle fibres and maximal CMAP amplitudes\textsuperscript{48}. This result stands in contrast to our findings in the FDI in which no differences were found in mean SMUP size between DPN patients and controls. This may be due to FDI undergoing a lesser degree of neuromuscular remodeling and collateral reinnervation as TA in the DPN patient group as indicated by less substantial losses in MU numbers.
3.4.3 DQEMG Compared to Standard Electrodiagnostics

The mean CMAPs in the TA (5.0 mV) and FDI (10.3 mV) of the DPN patient groups were greater than the lower limits of normal used in our EMG clinic (4.0 mV and 10 mV, respectively)\textsuperscript{35}. Based on this electrodiagnostic criterion alone, as a group, the DPN patients would not be considered to have undergone significant motor axonal loss (Figure 3.3). At this time, no standardized lower limits of normal exist for TA and FDI MUNEs. However, DPN patients featured substantially fewer MUs versus controls, and these differences were greater than the differences reported in CMAP amplitudes, particularly in the TA (ΔTA: MUNE ~45%, CMAP ~30%; ΔFDI MUNE ~30%, CMAP ΔFDI ~20%). Given MUNE takes into account the process of neuromuscular remodeling and collateral reinnervation via the mean SMUP, MUNE may provide a more sensitive measure of motor unit loss in comparison to nerve conduction studies alone. In addition, standard needle EMG studies would also be insensitive to this extent of axonal loss. Whereas clear differences were found in MUNEs between groups, differences in some needle detected MUP parameters, such as AAR, size index and number of phases, were not apparent (Tables 3.3 and 3.4). Thus, our results support the potential inclusion of MUNE as a useful tool in identifying patients in the early stages of motor-involved DPN, allowing for earlier detection and intervention.

In addition to motor axon loss and neuromuscular remodelling, our findings illustrate a decrease (~15%) in motor unit firing rates in DPN patients compared to controls at similar contractile intensities (approximately 25%MVC). This result corroborates a similar finding in the vastus lateralis of type 2 DM patients during low intensity, ramped knee extension contractions\textsuperscript{17}. They attributed this finding of neurophysiological adaptation to a slowing of inherent muscle contractile properties which has been reported in diabetic rodent skeletal muscle\textsuperscript{49}. Additionally, the decreased firing rates observed in DPN patients may reflect changes in intrinsic properties of α-motor neurons related to excitability. In humans, excitability increases in motor neurons as a result of Na+/K+ pump dysfunction were reported to be associated with DPN\textsuperscript{50}. This could result in changes in recruitment patterns, specifically a greater reliance on recruitment of additional motor units, rather than firing rate increases, as a means of
grading force output. Furthermore, given the enlargement of motor units found in the TA of DPN patients, presumably fewer motor units would be necessary at lower relative firing rates to produce a similar relative level of force output. Our results support this notion given the lower recruitment and firing rate indices (Tables 3.3 and 3.4) found in both TA and FDI of DPN patients compared to controls.

3.4.4 Motor Axon Loss and Disease Severity

We did not find any significant relationships between MUNEs and disease duration or disease severity of DM (e.g. HbA1c levels). This is most likely due to the modest sample size of the present study and a result of the complex interaction of the various factors that could drive axonal and MU losses observed in this sample of patients. In insulin-dependent DM, HbA1c levels have been identified as one of the strongest predictors of the development and severity of other signs and symptoms (e.g. numbness, electrophysiological measures) associated with DPN, and therefore the lack of relationship between MUNE and HbA1c levels is somewhat surprising. Previous reports featuring substantially larger sample sizes have found significant relationships between motor dysfunction and neuromuscular remodeling with glycemic control. Specifically, Bril and colleagues found a positive relationship between increased single-fibre EMG jitter, a marker of neuromuscular remodeling, and glycemic control. Additionally, significant negative relationships between DM disease duration and MUNE, as well as age and MUNE has been reported; however they did not examine the role of disease severity, as marked by hyperglycemia or dyslipidemia, in motor unit loss. Furthermore, it is important to note HbA1c values recorded at the time of inclusion in this study only reflect glycemic control for approximately the previous six months. Thus the patient’s history beyond this six month window is unaccounted for which could pervasively influence neuromuscular integrity. Additionally, some recent reports suggest that glycemic control in type 2 diabetics may be less important than vascular factors in relation to the development and severity of DPN. Conceivably, the duration of diabetes, the severity of diabetes, genetic predisposition, age and physical activity patterns could all affect MUNE in DPN patients.
3.4.5 Conclusions

Regardless of these potential relationships, our study demonstrates significant reduction of MUNE in the TA and FDI in association with DPN. The magnitude of motor unit loss appears to be muscle dependent, and follows a length-dependent pattern. Needle-detected motor unit properties are also altered in DPN patients, specifically mean firing rates and MUP turns. Finally, our study may lend support to the use of DQEMG as a potential measure that is sensitive to changes not detected by standard nerve conduction studies associated with DPN. The DQEMG-derived MUNE technique described in the present study is particularly useful in studying more proximal muscles in comparison to other MUNE methods. The use of DQEMG could be incorporated into larger studies to help identify DPN patients in the early stages of neuromuscular degeneration allowing for earlier, and perhaps more effective intervention.
References


Chapter 4

4 Increased neuromuscular transmission instability and motor unit remodeling with diabetic neuropathy as assessed using novel near fibre motor unit potential parameters

4.1 Introduction

Diabetic polyneuropathy (DPN) is a progressive neuropathic disorder characterized by distal axonal loss\(^1\) and impaired regeneration\(^2\). Early signs and symptoms may be predominantly sensory in nature\(^3\), however there is known involvement of the motor system, including loss of motor axons or motor units\(^4\)–\(^7\) which has been associated with muscle weakness\(^6\). Electrophysiologically, the demyelinating component of this neuropathy can be evaluated through nerve conduction studies (NCS), however NCS provide limited information regarding axonal properties and no information with regard to collateral reinnervation (axonal regeneration).

Standard concentric needle electromyography (NEMG) can provide more detailed information regarding the denervation-reinnervation process underlying DPN\(^8\). This is accomplished via the detection of spontaneous activity, polyphasic motor unit potentials (MUPs), and also the quantification of MUP amplitude, duration, and number of turns, as well as MU recruitment pattern\(^8\). Furthermore, the integrity of neuromuscular transmission can be examined in greater detail through the assessment of the degree of variability in the shape of consecutively detected MUPs\(^9\). Two key properties related to MUP shape variability are termed jitter and jiggle\(^9,10\). Jitter refers to the variability of the time intervals between pairs of individual muscle fibre potentials from a single MUP, and jiggle refers to the variability in overall MUP shape from one MUP discharge to the next.

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Increases in both jitter and jiggle have been reported under conditions of neuromuscular transmission disturbance and can reflect early axonal denervation\textsuperscript{5,11,12}. Additionally, increases in MUP instability (and thus jitter and jiggle) can be caused by variability in muscle fibre action potential velocity, a finding present in various myopathies\textsuperscript{13–15}. Specific to DPN, a previous study using single fibre electromyography (SFEMG) found increased jitter in the tibialis anterior (TA) of patients with DPN, however jiggle was not reported\textsuperscript{5}. Additionally, increased fibre density in patients with DPN compared to controls was observed, which is indicative of reinnervation presumably to compensate for prior denervation (motor unit remodeling)\textsuperscript{5}. It is not known how jitter and jiggle may relate to functional parameters such as strength or fatigue, however they may share a negative association as the disturbances that lead to increased neuromuscular instability may also be a factor related to a decrease in strength.

Novel parameters of near fiber (NF) MUPs have been developed. A NF MUP is calculated by high-pass filtering a MUP template waveform, and therefore is primarily comprised of contributions from fibers that are close to the needle detection surface\textsuperscript{16,17}. NF MUP parameters include: NF count (which, as described in Stashuk 1999\textsuperscript{17}, is a measure of fiber density), NF jiggle (which can be used to assess the neuromuscular transmission variability of the near fibers), NF dispersion (which measures the temporal dispersion of the near fiber contributions) and maximum NF interval (which is the maximum time interval between consecutive near fiber contributions to a NF MUP). NF MUP parameters theoretically can provide similar types of information as traditional MUP analysis (i.e. metrics of motor unit size (MUP amplitude, duration or area), fiber contribution dispersion (MUP # of phases or turns), and neuromuscular transmission instability (MUP jiggle)). However, because NF MUP parameters are dependent on the configuration and activity of the subset of motor unit fibers that are close (within ~350 µm) to the needle detection surface\textsuperscript{17–20} they can potentially provide more robust and detailed information concerning neuromuscular transmission variability and motor unit remodeling in comparison to conventional MUP parameters. This is particularly relevant when the MUPs and NF MUPs analyzed were extracted from a moderately complex interference pattern using electromyographic (EMG) signal decomposition methods (see Methods 4.2.3). At present, no study has reported these NF MUP parameter values for
patients with neuropathy, although it is expected that the general characteristics associated with neuropathic MUPs (increased size, complexity, and instability) will also be evident in neuropathic NF MUPs.

All of the aforementioned MUP and NF MUP parameters are obtained using a concentric needle-electrode and decomposition-based quantitative electromyography (DQEMG) software\textsuperscript{16,17}. DQEMG also provides data pertaining to motor unit firing rates and firing rate variability, as well as relative motor unit size, via surface motor unit potentials (SMUPs)\textsuperscript{7,21,22}. In addition, if a maximal compound muscle action potential (CMAP) waveform is appropriately recorded, DQEMG can be used to calculate a motor unit number estimate (MUNE), which can provide further insight regarding the denervation of a muscle\textsuperscript{7,21,22}.

The present study was designed to assess the effects of DPN on standard MUP and novel NF MUP parameters including NF jiggle, NF dispersion and maximum NF interval, compared to age and sex-matched controls. We compared results from the novel NF MUP parameters to standard MUP parameters including number of turns, area and traditional jiggle. Additionally we related measures of neuromuscular transmission variability and motor unit remodeling to dorsiflexion strength. Finally, we examined how changes in individual motor unit size, due to the DPN-associated denervation-reinnervation process, are related to NF MUP stability during sustained, low-level isometric contractions in patients with DPN. We hypothesized the following: (i) DPN patients would possess motor units with greater mean NF fiber count, NF jiggle, NF dispersion, and maximal NF interval; (ii) NF fiber count, NF jiggle and NF dispersion would be negatively related to dorsiflexion strength; and (iii) positive relationships would exist between standard parameters of MUP size and NF fibre count, NF jiggle and NF dispersion.
4.2 Methods

4.2.1 Participants

Twelve patients (7 men, 5 women; ages 32-78 years) with DPN were recruited. They met the criteria for diagnosis of type 2 (non-insulin dependent) diabetes mellitus (DM) with clinical and electrophysiological characteristics of confirmed DPN\textsuperscript{23}. Additionally, patients received a thorough consultation and electrophysiological examination by an experienced neurologist with specialized training in neuromuscular disease to exclude other causes of nerve injury (i.e., other polyneuropathies, compressive mononeuropathies or radiculopathies). Patients with any neurological, metabolic or vascular diseases other than related to DM or DPN were excluded. Twelve healthy, age and sex-matched controls (7 men, 5 women; ages 29-77 years) were recruited from the community. Control participants were screened by physicians (neurologists) to ensure they met inclusion criteria. The study was approved by the local university research ethics board. Informed oral and written consent was obtained from all participants prior to testing.

4.2.2 Tibialis Anterior DQEMG Data Acquisition

For the present investigation, the TA was selected due to its known involvement in DPN\textsuperscript{6,7} and its high-degree of accessibility for needle EMG study. Participants were seated in a custom isometric dynamometer designed to measure plantar and dorsiflexion at the ankle joint\textsuperscript{24}. In the dynamometer, the right ankle was positioned at 30° of plantar flexion, with both knee and hip angles maintained at 90°. A C-shaped brace was secured directly on the distal aspect of the right thigh to minimize movement at the hip joint during contractions. Inelastic Velcro straps were placed over the dorsum of the foot to secure the foot to the dynamometer\textsuperscript{25}.

All testing was performed on the right (dominant) leg. Participants performed three dorsiflexion maximal voluntary contractions (MVCs), with at least 3 min of rest between attempts. Each MVC was held for approximately 3 seconds. Participants were provided with real time visual feedback of their torque and verbally encouraged. Voluntary activation during the 2\textsuperscript{nd} and 3\textsuperscript{rd} MVC attempts was assessed using the
interpolated twitch technique (ITT)\textsuperscript{26}. This technique involves supramaximal percutaneous electrical stimulation of the fibular nerve just distal to the fibular head. The amplitude of the interpolated torque electrically evoked from a single 100 µs stimulus during the plateau of the MVC was compared with a single (100µs) resting twitch evoked ~1s following the MVC. Voluntary activation was calculated as a percent using the following equation: \([1 - (\text{interpolated twitch} / \text{resting twitch})] \times 100\). The ITT allows the operator to ensure the participant is providing a maximal voluntary effort during their MVC. The peak torque of the three MVC attempts was taken as the maximal torque for the participant. All torque signals were collected and sampled online at 500 Hz using Spike2 software (Version 7.11; Cambridge Electronic Design Ltd., Cambridge, United Kingdom) and analyzed off-line to determine voluntary isometric torques (strength).

Surface EMG signals were recorded from the TA using self-adhering Ag-AgCl electrodes (1 cm x 3 cm). The active electrode was placed over the TA motor point, approximately 7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia. This placement was adjusted as needed to maximize the TA CMAP amplitude. The reference electrode was placed over the distal tendon of the TA. A ground electrode was placed over the patella.

DQEMG EMG data were acquired using the protocol described in detail elsewhere\textsuperscript{16,27}. Intramuscular EMG signals were recorded via a disposable concentric needle electrode (Model N53153; Teca Corp., Hawthorne, NY) inserted into the TA, 5-10 mm distal to the active surface electrode. The surface and intramuscular EMG signals were bandpass filtered at 5 Hz to 1 kHz and 10 Hz to 10 kHz, respectively. Surface EMG signals were sampled at 3 kHz; intramuscular EMG signals were sampled at 30 kHz. To evoke the maximum CMAP a bar electrode held distal to the fibular head delivered supramaximal electrical stimuli to the common fibular nerve. Subsequently, participants matched a target line of 25% MVC, visible on a computer monitor, for all isometric dorsiflexion contractions. Prior to engaging in these contractions, the intramuscular needle electrode was inserted and manipulated in the muscle. This contraction intensity has been shown to be the most effective intensity for obtaining a representative MUNE in the tibialis anterior (TA)\textsuperscript{25}. Surface and intramuscular EMG signals were recorded during
a sustained 25% MVC contraction, which was held for ~30 seconds. Between contractions, the concentric needle electrode was repositioned in order to sample different motor units. These procedures were repeated until at least 20 suitable MUP trains and their respective surface-motor unit potentials (S-MUPs) were acquired.

4.2.3 Tibialis Anterior Decomposition-based Quantitative Electromyography Analysis

Decomposed intramuscular EMG signals were reviewed off-line to determine the acceptability of the extracted MUP trains and their corresponding S-MUPs. MUP trains were inspected visually to ensure that their MUP occurrence patterns were consistent with the expected activity of a single motor unit (consistent firing pattern and interdischarge coefficient of variation of < 0.3). Invalid MUP trains and their associated S-MUPs were excluded from further analyses. The DQEMG algorithms estimate a MUP and S-MUP template waveform. For these waveforms DQEMG automatically places markers related to onset, end, negative peak and positive peak positions. All MUP and S-MUP markers were subsequently reviewed visually by the operator. A MUNE was derived by dividing the negative-peak amplitude of the maximal CMAP by the negative peak amplitude of the mean S-MUP\textsuperscript{21,22}. These MUNE results have been previously reported\textsuperscript{7}.

The DQEMG algorithms also provide measures of the overall needle EMG signal intensity (measured in pulses per second), mean firing rates (Hz) of individual motor units based on their extracted MUP trains, and an overall mean firing rate per participant at a relative target level of contraction (25% of MVC). Additionally, the DQEMG system automatically calculates standard parameters of the MUP template, including peak-to-peak amplitude, area, duration and traditional jiggle. For the assessment of MUP stability, used to reflect neuromuscular transmission variability, MUPs that represent the isolated activity of a single motor unit are automatically selected by the DQEMG algorithms. The sets of automatically selected isolated MUPs were manually inspected and any MUPs found to be significantly contaminated by the activity of other motor units were removed and replaced. The DQEMG technique described has been shown to possess similar test-retest values within individuals\textsuperscript{28}, and high degrees of intra- and inter-rater reliability in
control and clinical populations\textsuperscript{29,30}. The investigator was blinded to the status of the participant (DPN patient vs control) during off-line analysis.

4.2.4 Novel Near Fibre Motor Unit Potential Parameters

4.2.4.1 Near Fibre MUP Template

The MUP template provided by the DQEMG algorithms is high-pass filtered using a second ordered low-pass differentiator\textsuperscript{17,31}. The second order filter equation is:

\[ x_t = y_{t+2} - y_{t+1} - y_t + y_{t-1} \]

Where \( y_t \) is the sampled raw signal and \( x_t \) is the sampled filtered signal\textsuperscript{31}. Due to the spatial low-pass filtering properties of volume conduction, the resulting near fibre MUP template waveform, or NF MUP, is primarily comprised of contributions from fibres that are ‘near’ (within ~350 µm) to the detection surface of the needle electrode.

The NF MUP can be used to focus on characteristics of the near fibres and is defined as a MUP contributed to by the fibers that are close to the detection surface of the needle electrode. In contrast, a NF contribution is the specific contribution of an individual NF (an individual muscle fibre) to a NF MUP. The temporal relationships of the near fibre contributions may be more easily determined than relationships among all contributing fibres as would be the case when analyzing MUPs collected using only traditional Butterworth filtering. This is due to the spatial filtering used to create NF MUPs, which filters out more distant volume conducted MU activity that could potentially reduce the ability to detect individual muscle fibre. As such, by focusing on the near fibre contributions measures of the temporal dispersion of these near fibre contributions can be obtained. These measures in turn can be used to reflect the relative conduction times of the muscle fibre action potentials to the detection surface of the electrode. Assuming similar conduction velocities across the fibres of a motor unit which may not be true in disease, the temporal dispersion of NF contributions can therefore reflect the relative conduction distances of the axonal and muscle fibre action potentials. With presumed similar conduction distances along the muscle fibres, temporal dispersion can reflect the degree of axonal sprouting in the motor unit and provide evidence of
reinnervation. Figure 4.1 displays a MUP and its associated NF MUP as well as important aspects of the NF MUP parameters described below.

4.2.4.2 Near Fibre Parameters

Near fibre duration (NF Dur) is the time between the onset and end positions of the near fibre MUP. The NF MUP onset and end positions are calculated by applying the same criteria used to calculate the MUP onset and end positions. Near fibre area (NF Area) is the sum of the absolute area under the curve of the NF MUP between the onset and end positions multiplied by the sampling interval (i.e. 1/ (sampling rate)).

Near fibre count (NF fiber count) is the number of detected near fiber contributions to the NF MUP. A positive turn detected in the NF MUP with sufficient symmetry and amplitude is considered a distinct near fiber contribution. The NF fiber count reflects the density of fibers in the motor unit.

Near fibre dispersion (NF dispersion) can be used to assess reinnervation, and is the time interval between the first and last detected fiber contribution to the NF MUP.

The maximum near fibre interval (max NF Interval) is the maximum time between consecutive detected near fiber contributions. Large max NF interval values may indicate long reinnervating axonal sprouts. Large max NF intervals values may relate to traditionally identified satellite potentials.

Near fibre jiggle (NF jiggle) is a statistic that measures the variability of consecutive isolated NF MUPs of a MUP train. The statistic is the same as introduced by Stalberg applied to the MUPs of a MUP train\textsuperscript{10}. The NF MUPs of a MUP train are created by high-pass filtering each MUP using the same second ordered low-pass differentiator used to create the NF MUP template. Isolated NF MUPs are selected as described previously\textsuperscript{17}.

Near fibre jitter refers to the mean consecutive difference value measured for a pair of detected distinct fiber contributions within a NF MUP across the NF MUP train\textsuperscript{10,17}. Suitable muscle fibre pair tracking was confirmed by visual inspection. In
addition, % blocking was assessed for all pairs of detected fiber contributions across the sets of isolated NF MUPs. % blocking is indicative of the rate at which an individual muscle fibre fails to propagate an action potential following the activation of its motor neuron. This is measured by counting the number of intermittent absences of an individual NF contribution to the NF MUPs of the set of isolated NF MUPs analyzed\textsuperscript{10}.

4.2.5 Inter-Rater Reliability of DQEMG NF Jiggle Analysis

To provide an assessment of the inherent variability of DQEMG-derived NF jiggle, a second examiner performed the DQEMG NF MUP analysis described above on a pseudo-random subset of the collected data (6 controls, 6 DPN patients). A reliability assessment of NF jiggle was appropriate as there is some subjective decision-making regarding the inclusion and exclusion of NF MUPs for stability analysis due to signal contamination from adjacent motor units. Data analysis by each examiner was performed independently, and examiners were blinded to participant status during analysis. Each rater remained blind to the results of the other examiner until data collection and respective analyses were complete for all participants.
Figure 4.1 Electromyographic tracings of motor unit potential and near fibre motor unit potential

Electromyographic tracings from decomposition based quantitative electromyography (DQEMG) of a sample (A) motor unit potential (MUP), and (B) the corresponding near fibre (NF) MUP. On the NF MUP tracing (B), numbers 1, 2, 3 and 4 represent NF contributions, providing the NF fibre count. NF dispersion is calculated as the duration between 1 and 4. Maximal NF interval is calculated as the duration between 2 and 3.
4.2.6 Statistics

Mean values ± standard deviations are presented in the text. Normally distributed data were analyzed using a one-way ANOVA; the level of significance was set at $P \leq 0.05$. Normality was assessed using a Shapiro-Wilk test. Non-normally distributed data were analyzed using a Kruskal-Wallis one-way ANOVA on ranks. Relationships among variables were tested using Pearson’s Product Moment Correlation. To represent the degree of differences between groups, effect sizes between group MUP and NF MUP parameter values were calculated using Cohen’s D. Relative inter-rater reliability was tested using a Model 2 (two-way random, absolute agreement) single measure intra-class correlation coefficient (ICC). ICC point estimates were considered ‘poor’ (<0.50), ‘moderate’ (0.50 – 0.75) or ‘good’ (>0.75)\(^3\). Data analyses were performed using SigmaPlot software (Version 12.2; Systat Software, Chicago, Illinois, US) excluding the ICC calculation which was performed using SPSS (Version 20.0; SPSS, Chicago, Illinois, US).

4.3 Results

Participant characteristics are presented in Table 4.1. No significant differences were detected for age ($p>0.05$) or height ($p>0.05$) between groups. DPN patients had significantly greater body mass ($p<0.05$) and BMI ($p<0.05$) than controls. Data characterizing the clinical history and status of the DPN patient group are also presented in Table 4.1.

Dorsiflexion strength and standard MUP parameters and MUNE values\(^7\) are presented in Table 4.2. The DPN patients had smaller CMAPs compared to controls (6.8 vs 5.0 mV; $p<0.05$), however the mean CMAP value for the patient group was not below our clinical laboratory’s lower limit of normal (4.0 mV). DPN patients had fewer motor units (-45%) and were weaker (-35%) than controls ($p<0.05$), but no group differences were detected with respect to traditional jiggle ($p>0.05$). Both DPN patients and controls were able to achieve near-maximal (>95%) voluntary activation during MVCs as
assessed using the ITT. Finally, the DPN group had larger (+47% MUP area) MUPs with significantly more turns (+21%) compared to controls (p<0.05).

NF MUP parameter values are presented in Table 4.3. DPN patients had greater: NF area (+40%), NF duration (+24%), NF dispersion (+38%) and maximal NF interval (+22%) compared to controls (p<0.05). Additionally, NF jiggle was found to be greater in DPN patients versus controls (+21%; p<0.05), and NF jiggle was found to have a ‘good’ level of inter-rater reliability (ICC = 0.81). In comparison with controls, DPN patients had increased NF fibre counts (+31%) and greater NF jitter values (+34%; p<0.05). In comparing how DPN patients differed from controls in standard MUP parameters and NF MUP parameters, differences in NF MUP parameters tended to have greater effect sizes (Tables 4.2 and 4.3). The largest magnitude of effects were found for NF fibre count and max NF interval (d = 1.76 and 1.51, respectively; Table 4.3). In comparison, most standard MUP parameters had effect sizes less than 1.0 (Table 4.2).
### Anthropometric Parameters

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<th>Control (n = 12)</th>
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<td>BMI (kg/m²)</td>
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### Diabetic Characteristics

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<td>Duration of Diabetes (years)</td>
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<tr>
<td>Duration of DPN (years)</td>
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### Nerve Conduction Studies

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<td>TA CMAP amplitude (mV)</td>
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<tr>
<td>Fibular nerve CV (m/s)</td>
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</table>

#### Table 4.1 Participant Characteristics

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

SNAP – sensory nerve action potential; TA – tibialis anterior; CMAP – compound muscle action potential; CV – conduction velocity; DPN – diabetic polyneuropathy. # SNAP responses were absent in 10 out of 12 patients.
<table>
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<th>DPN Patients (n=12)</th>
<th>% Difference</th>
<th>Effect Size</th>
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<tr>
<td>Dorsiflexion MVC Strength (Nm)</td>
<td>34.1 ± 9.5</td>
<td>22.3 ± 7.2*</td>
<td>-35%</td>
<td>1.39</td>
</tr>
<tr>
<td>%MVC RMS EMG</td>
<td>23.2 ± 4.2</td>
<td>25.2 ± 3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMG Intensity (pps)</td>
<td>74.3 ± 20.2</td>
<td>55.6 ± 19.2*</td>
<td>-25%</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean MU FR (Hz) at 25% MVC</td>
<td>12.10 ± 1.80</td>
<td>10.2 ± 1.2*</td>
<td>-15%</td>
<td>1.24</td>
</tr>
<tr>
<td>MUP Vpp (µV)</td>
<td>810.4 ± 217.0</td>
<td>1164.3 ± 493.0*</td>
<td>+43%</td>
<td>0.92</td>
</tr>
<tr>
<td>MUP Duration (ms)</td>
<td>12.3 ± 2.1</td>
<td>14.4 ± 2.2*</td>
<td>+17%</td>
<td>0.97</td>
</tr>
<tr>
<td>MUP Area (µVms)</td>
<td>1436.5 ± 503.6</td>
<td>2117.5 ± 991.1*</td>
<td>+47%</td>
<td>0.86</td>
</tr>
<tr>
<td>AAR (ms)</td>
<td>1.77 ± 0.22</td>
<td>1.8 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape Width (ms)</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turns (#)</td>
<td>4.2 ± 0.7</td>
<td>5.1 ± 0.9*</td>
<td>+21%</td>
<td>1.11</td>
</tr>
<tr>
<td>Traditional Jiggle (%)</td>
<td>36.4 ± 21.0</td>
<td>25.4 ± 20.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP (mV)</td>
<td>6.8 ± 0.9</td>
<td>5.0 ± 1.7*</td>
<td>-26%</td>
<td>1.32</td>
</tr>
<tr>
<td>mSMUP (µV)</td>
<td>77.0 ± 20.0</td>
<td>116.0 ± 61.0*</td>
<td>+51%</td>
<td>0.85</td>
</tr>
<tr>
<td>MUNE (#)</td>
<td>99 ± 19</td>
<td>54 ± 25*</td>
<td>-45%</td>
<td>2.02</td>
</tr>
</tbody>
</table>

**Table 4.2** Tibialis Anterior Strength, MUP and MUNE Parameters

* Denotes significant difference between groups (p<0.05). Effect size calculated as Cohen’s D. MVC – maximal voluntary contraction; RMS – root mean squared; EMG – electromyography; pps – pulses per second; MU – motor unit; MUP – motor unit potential; Vpp – peak to peak voltage; AAR – area to amplitude ratio; CMAP – compound muscle action potential; mSMUP – mean surface motor unit potential; MUNE – motor unit number estimate; DPN – diabetic polyneuropathy
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=12)</th>
<th>DPN Patients (n=12)</th>
<th>% Difference</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td># of MUPTs</td>
<td>23.4 ± 1.6</td>
<td>26.4 ± 3.4</td>
<td>+13%</td>
<td>1.13</td>
</tr>
<tr>
<td>Contractions (#)</td>
<td>4.4 ± 0.5</td>
<td>6.2 ± 2.5</td>
<td>+42%</td>
<td>0.99</td>
</tr>
<tr>
<td>NF Area (kV/s²·ms)</td>
<td>6.9 ± 1.8</td>
<td>9.7 ± 3.0*</td>
<td>+40%</td>
<td>1.13</td>
</tr>
<tr>
<td>NF Duration (ms)</td>
<td>3.8 ± 0.7</td>
<td>4.7 ± 0.9*</td>
<td>+24%</td>
<td>1.12</td>
</tr>
<tr>
<td>NF Dispersion (ms)</td>
<td>1.7 ± 0.4</td>
<td>2.4 ± 0.9*</td>
<td>+38%</td>
<td>1.01</td>
</tr>
<tr>
<td>Max NF Interval (ms)</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.2*</td>
<td>+22%</td>
<td>1.51</td>
</tr>
<tr>
<td>NF Fibre Count (#)</td>
<td>2.5 ± 0.4</td>
<td>3.3 ± 0.5*</td>
<td>+31%</td>
<td>1.76</td>
</tr>
<tr>
<td>NF Jiggle (%)</td>
<td>33.8 ± 3.7</td>
<td>40.9 ± 6.3*</td>
<td>+21%</td>
<td>1.18</td>
</tr>
<tr>
<td>NF Jitter (µs)</td>
<td>37.6 ± 9.0</td>
<td>50.6 ± 14.2*</td>
<td>+34%</td>
<td>1.09</td>
</tr>
<tr>
<td>% of MUPTs w/ blocking</td>
<td>0.0 ± 0.0</td>
<td>17.1 ± 7.9*</td>
<td>+17%</td>
<td>3.06</td>
</tr>
<tr>
<td>% Blocking</td>
<td>0.0 ± 0.0</td>
<td>3.7 ± 1.3*</td>
<td>+4%</td>
<td>4.02</td>
</tr>
</tbody>
</table>

Table 4.3 Neuromuscular Transmission Stability and Near Fibre Parameters

* Denotes significant difference between groups (p<0.05). Effect size calculated as Cohen’s D.

MUPT – motor unit potential train; NF – near fibre; DPN – diabetic polyneuropathy
NF MUP size parameters were positively related to MUP area, the conceptually analogous standard MUP size parameter. NF area was positively related to MUP area in both controls (r = 0.67; p<0.05) and DPN patients (r = 0.55; p<0.05). NF duration was positively related to MUP area in controls (r = 0.45; p<0.05) and DPN patients (r = 0.35; p<0.05). Also, in both controls (r = 0.64; p<0.05) and DPN patients (r = 0.81; p<0.05), NF dispersion (a measure of fiber contribution dispersion) was positively associated with MUP area.

NF MUPs with greater numbers of fibre contributions tended to be larger in size. This is supported by the result that NF area was positively related (controls, r = 0.60; DPN patients, r = 0.51; p<0.05) with the number of distinct muscle fibres detected within that NF MUP (NF count) (Figure 4.2). Furthermore, NF jiggle was not related to MUP area (p>0.05), indicating acute neuromuscular instability was not associated with a traditional metric of motor unit size. However, NF jiggle was positively associated with NF fibre count (Figure 4.3; r = 0.46; p<0.05).

NF dispersion, but not stability, was associated with whole muscle denervation. Specifically, NF jiggle values were not significantly related to dorsiflexion strength or TA MUNE values (p>0.05) (Figure 4.4A and 4C; p>0.05); whereas, in DPN patients, NF dispersion was negatively related to MUNE (Figure 4.4D; r = -0.63; p<0.05) but not strength (Figure 4.4B; p>0.05). Finally, no significant relationships were found between NF MUP parameters and duration of DM (p>0.05), duration of DPN (p>0.05) or glycemic control values (HbA1c levels) (p>0.05).
Figure 4.2 Relationship between near fibre (NF) fibre count and NF motor unit potential area

Relationship between near fibre (NF) fibre count and NF Area in the tibialis anterior of controls (grey circles; $r = 0.60$ [grey line]) and diabetic polyneuropathy (DPN) patients (black circles; $r = 0.51$ [black line]). In both groups, NF motor unit potentials (MUPs) with greater NF fibre counts had larger NF MUP areas.
Figure 4.3 Relationship between near fibre (NF) fibre count and NF jiggle

Relationship between near fibre (NF) fibre count and NF Jiggle in the tibialis anterior of controls (grey circles) and diabetic polyneuropathy (DPN) patients (black circles; $r = 0.46$). In DPN patients, increased fibre counts were associated with increased NF instability (black line). No relationship was detected between these variables in controls (grey line).
Figure 4.4 Relationships between tibialis anterior health, strength and neuromuscular transmission stability

Relationships between (A) dorsiflexion maximal voluntary contraction (MVC) strength and NF Jiggle; (B) dorsiflexion MVC strength and NF dispersion; (C) motor unit number estimates and near fibre (NF) Jiggle; and (D) motor unit number estimates and NF dispersion (DPN $r = -0.63$) in controls (grey circles) and diabetic polyneuropathy (DPN) patients (black circles). Relationships for control participants are denoted by grey lines; relationships for DPN patients are denoted by black lines.
4.4 Discussion

The main findings of this study were: (1) patients with DPN had larger (NF area, NF duration), more complex (NF dispersion, NF fibre count) and less stable NF MUPs (NF jiggle, NF jitter), compared to controls; (2) NF dispersion was positively related to whole muscle denervation (MUNE) but not strength, whereas NF MUP stability (NF jiggle) was not related to either strength or whole muscle denervation; (3) NF MUPs with a greater number of distinguishable near fibre contributions tended to be less stable, although no relationship was found between NF jiggle and NF area; (4) NF parameters were not significantly related to metrics of disease duration or glycemic control.

4.4.1 Near Fibre versus Traditional MUP Parameters in Diabetic Polyneuropathy

In the present study, patients with DPN had clear evidence of DM and DPN through their clinical assessment, elevated glycosylated hemoglobin (HbA1c) levels and reduced or absent sural sensory nerve action potential amplitudes (SNAPs) (Table 4.1). Our traditional MUP (Table 4.2) and NF MUP (Table 4.3) results show increased neuromuscular transmission instability and fibre density in patients with DPN. Although this finding is consistent with a prior report that used traditional single-fibre EMG\(^5\), the present study extends these observations by using novel quantitative concentric needle-derived EMG measures (NF MUP parameters).

In the present study, traditional jiggle was not different between groups (p>0.05) whereas NF jiggle was significantly greater in patients with DPN compared to controls (Table 4.3; p<0.05). These divergent findings in two metrics purported to assess the same variable (i.e. neuromuscular transmission instability) support the idea that when analyzing intramuscular EMG signals detected using concentric needle electrodes during moderate level (25% MVC) contractions, NF MUP parameters may be more robust than standard MUP parameters\(^17\). Traditional jitter measured using concentric needle detected MUPs have been reported before in other neuropathic conditions\(^33,34\), and its advantages over SFEMG have been outlined\(^34\). However, our study appears to be the first to perform
this technique with DQEMG software, to present concentric needle acquired jiggle data, and to report NF MUP parameters in a chronic neuropathic condition.

The lack of any significant differences in traditional jiggle values between groups in this study is surprising given our other results showing increased NF MUP instability (NF jiggle) in DPN patients (Table 4.3), and the conclusions of previous investigations\(^5\). However, one possible contributing factor to explain our finding may be the different degrees of intensity of MUPs detected during the sustained voluntary contractions in controls compared to patients with DPN (75 vs 55 pps respectively; Table 4.2). As a consequence of their neuropathy, individuals in the DPN group have significantly enlarged and fewer MUs compared to controls (Table 4.2)\(^7\). Thus, during a sustained dorsiflexion contraction at 25% MVC, DPN patients are activating fewer MUs, and those activated are discharging at lower firing rates (Table 4.2) leading to less intense electromyographic interference pattern activity (-25% less pps; Table 4.2). The reduced firing rates in DPN patients may reflect the slowing of muscle contractile properties\(^35\) or preferential loss of fast motor units\(^35\). These group differences in recruitment patterns are influential when examining MUP raster plots for stability analysis, as higher amounts of electrophysiological ‘contamination’ from nearby MUs is apparent in the control data versus that of the DPN patient group. This drives the traditional jiggle values artificially higher in controls such that they may not reflect the true amount of neuromuscular transmission instability. Compared to traditional jiggle, NF jiggle may be less affected by this contamination as the focus is placed on MUs with fibers near (within ~350 μm) the electrode detection surface, with minimal contributions from more distal fibres. This technical limitation could be reduced by using lower voluntary contractile intensities\(^5,10\) or low-frequency, evoked contractions\(^33\). However performing contractions at 25% of MVC provides two important advantages. Firstly, this contractile intensity includes a greater, more representative sample of MUs from the muscle being examined, thus allowing for a more comprehensive assessment of the muscle’s entire motor unit pool. In contrast, other methods of measuring neuromuscular transmission instability have been limited to low threshold motor units, at relatively low firing rates\(^5,9\). Secondly, performing the protocol at 25% of MVC when using DQEMG allows for the simultaneous calculation of a valid MUNE\(^25\), which is another important and useful
measure related to neuromuscular health\textsuperscript{36,6,7}. Indeed, reduced MUNEs in the DPN group had one of the largest effect sizes across all parameters (Table 4.2), supporting the inclusion of MUNEs as a useful clinical indicator. The specific MUNE results presented in this study have been reported and discussed previously\textsuperscript{7}.

In comparing NF MUP parameters, other than NF jiggle, with their respective analogous traditional MUP parameters there is a stronger degree of correlation. NF area and NF duration were significantly positively related to MUP area and MUP duration, respectively. This indicates these NF MUP parameters are positively associated with traditional metrics representing MU size. Additionally, NF area was positively related to NF fibre count which indicates the NF MUP size is partially determined by the number of near muscle fibres innervated by that particular motor unit (Figure 4.2). Similar to a previous report in DPN examining traditional jitter, we found increased NF jitter in DPN patients compared to controls (Table 4.3)\textsuperscript{5}. It is important to note when comparing the traditional MUP and NF MUP parameter results for the present study that the apparent ability to discriminate between controls and DPN patients is greater using the NF MUP parameters. Specifically, the majority of the NF MUP parameters had greater effects sizes than standard MUP parameters (Tables 4.2 and 4.3). Indeed, the two parameters with the largest effect sizes were NF fibre count (d = 1.76) and max NF interval (d = 1.51). Thus, NF MUP parameters may prove more useful than standard MUP parameters in distinguishing neuropathic from non-neuropathic MUs, however further study is required.

4.4.2 Physiological Interpretation and Clinical Relevance of Near Fibre MUP Parameters

Our data showing increased NF jiggle and NF jitter in DPN patients relative to controls may be reflective of pathological alterations in neuromuscular action potential propagation, or neuromuscular transmission instability, due to DPN (Table 4.3). Increased NF jiggle and NF jitter values primarily reflect the denervation-reinnervation process acutely\textsuperscript{10}. Specifically, increased neuromuscular transmission instability is thought to occur in association with partial loss of innervation by a damaged motor axon, or incomplete reinnervation occurring between orphaned muscle fibres and their adopted axonal sprout during the process of collateral reinnervation\textsuperscript{10}. In this instance, the concept
of acute reinnervation is contrasted by chronic reinnervation in which mature neuromuscular junctions have formed, and neuromuscular transmission variability has recovered. Additionally, our data show an increased amount of neuromuscular transmission failure (i.e. % blocking) in DPN patients compared to controls (Table 4.3). A previous study in DPN using SFEMG did not include blocking as a measured parameter. Indeed, a greater contraction intensity would be more likely to challenge the neuromuscular system and induce neuromuscular transmission failure. This intermittent transmission failure reflects the underlying dysfunction in processes related to neuromuscular transmission, and would also directly lead to increases in NF jiggle and NF jitter. However, it is unclear how potential dysfunction occurring at the site of distal axonal branches; the neuromuscular junction; muscle fibre action potential propagation; or some combination thereof, contribute to increased instability. Previous studies, mainly relying upon animal models, provide evidence that DPN-related neuromuscular instability could be due to a range of factors including: nerve demyelination and reduced axonal calibre; axon terminal dysfunction; ion channel alterations; or structural changes at the muscle fibre, among other possible mechanisms. Any of these putative changes within the neuromuscular system could differentially affect muscle fibre action potential conduction velocity, consequently affecting MUP shape stability. Assuming these diabetes-related neuromuscular changes would not occur uniformly, nor have a consistent effect on action potential propagation across terminal axon branches or muscle fibres within a single motor unit, they could result in increased NF jiggle and NF jitter. In comparison, our results showing increased NF dispersion, max NF interval and NF fibre count in DPN are most likely representative of prior compensatory reinnervation that has occurred following muscle fibre denervation.

We did not find any relationships between neuromuscular transmission instability (NF jiggle) and whole muscle denervation (MUNE) or muscle strength (Figures 4.4C and 4.4A; p>0.05). NF jiggle and MUNE may not have been related to one another as they reflect different aspects of the denervation-reinnervation process. Whereas increased NF jiggle may indicate acute, or on-going, denervation or reinnervation, a decreased MUNE represents denervation that is complete. Therefore it may be possible that individuals who have lost many motor units may possess fewer, enlarged, but relatively stable motor
units. Indeed, no significant relationship was found between NF jiggle and MUP area 
(p>0.05). Notwithstanding, there was a tendency for MUPs with greater numbers of 
distinguishable near fibre contributions to be less stable than their counterparts with 
fewer distinguishable near fibre contributions (Figure 4.3). This occurs perhaps because a 
motor unit that is sprouting axons to reinnervate denervated fibres will have a greater 
chance of having immature or nascent neuromuscular junctions which in turn will be 
unstable and lead to higher jitter and therefore contribute to higher jiggle. NF jiggle and 
strength did not correlate as increased neuromuscular transmission instability does not 
necessitate concurrent muscle atrophy or functional decrement. In contrast, in the DPN 
patients, NF dispersion was negatively related to MUNE (Figure 4.4D). This is likely due 
to increased NF dispersion representing prior motor unit remodeling that occurs 
following denervation (or a decrease in MUNE). Given these relationships, our results 
indicate the assessment of NF jiggle may be most useful in identifying patients in the 
early stages of neuromuscular pathology, prior to the development of detectable motor 
unit loss or decreases in strength.

Our NF MUP stability parameters were not significantly related to the duration of 
DM or DPN, or glycemic control as expressed by HbA1c values. This is in contrast to 
one previous finding in which traditional jitter, derived from SFEMG, correlated 
positively with HbA1c\(^5\). The difference in findings may be attributed partially to the 
complex, multi-factorial nature of DPN. Some factors that may interact and effect 
neuromuscular transmission instability include: disease duration, disease severity, 
glycemic control\(^5\), adult aging, physical activity history, and natural biological variability. 
The effects and importance of these, and other factors, likely will require studies with 
greater numbers of participants.

4.4.3 Conclusions

We have demonstrated concentric needle-derived DQEMG can be used to detect 
increased neuromuscular transmission instability and motor unit remodeling in patients 
with DPN. Additionally, in a chronic neuropathic condition, we present NF MUP 
parameters and support their inclusion as potentially useful metrics concerning 
neuromuscular stability and motor unit remodeling relative to controls. Indeed, NF MUP
parameters may have enhanced clinical utility in detecting neuropathic abnormalities compared to standard MUP parameters. Our results show NF MUPs with greater numbers of distinct near fibre contributions are less stable. Changes in neuromuscular transmission instability do not appear to closely associate with whole muscle denervation; perhaps as a result of the development of stable motor axon-muscle fibre synapses as the collateral reinnervation process progresses. The presence of neuromuscular transmission blocking in DPN patients may have consequences concerning neuromuscular function under conditions that stress the capacity of the system, such as fatiguing contractions. Finally, NF jiggle may be useful in identifying patients in the early stages of neuromuscular disease, prior to the loss of motor units or onset of muscle weakness.
References


Chapter 5

Skeletal muscle morphology and contractile function in relation to muscle denervation in diabetic neuropathy

5.1 Introduction

Diabetes mellitus (DM) and its common complication, diabetic polyneuropathy (DPN) are associated with changes in the neuromuscular system and motor dysfunction. Motor dysfunction as a result of DPN can manifest broadly as muscle atrophy, weakness, and increased susceptibility to fatigue. These changes may be due to a combination of DM-induced alterations to the α-motor neuron, neuromuscular junction, and skeletal muscle fibres. Despite many studies conducted using diabetic animal models, relatively few have attempted to directly quantify neuromuscular and motor unit properties in humans with DPN. Thus the current study was aimed at investigating alterations in contractile properties and morphology of skeletal muscle in patients with DPN in association with changes in motor unit properties and denervation.

In rodent models of DM, weakness, as measured through induced tetanic stimulation, has been attributed partially to muscle atrophy, particularly in fast-twitch fibres. However, reduction in strength persists when muscle is compared relative to weight or cross-sectional area. Studies investigating DPN in humans have reported length-dependent atrophy of skeletal muscle using magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DXA) imaging techniques, in association with weakness. Strength loss in DPN has also been linked to excess fat infiltration of skeletal muscle and preferential loss of type II muscle fibres related perhaps to their selective denervation. Other studies using experimental rodent models of DPN report

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distal motor axon or neuromuscular junction deficits, as well as excitation-contraction uncoupling and disruption of Ca\(^{2+}\) handling as contributing factors to strength loss\(^7,14,15\). Thus, there is no consensus on which factors, or combination of key factors explains DM or DPN-associated weakness in humans or rodents. In humans with DPN, a potential unexplored source and complication of muscle weakness may be due to increased antagonist coactivation. In other neurological disorders (i.e. stroke, muscular dystrophy, Parkinson’s disease), increased levels of coactivation have been reported to contribute to muscle weakness\(^{16,17}\) which may occur as a compensatory strategy to maintain joint stability. In addition to weakness, reductions in ankle plantar and dorsiflexion isokinetic muscle power\(^{12}\) and rate of torque development\(^{13}\) have been reported in DPN patients\(^{12,13}\). These changes have been associated with decrements to balance and physical function. To date, the relationships between muscle morphological changes and reduction in rate of torque development with a quantification of denervation, or motor unit loss, have not been explored in DPN patients.

Whereas a loss of power production in DPN patients may be accounted for partly by a loss in strength, a reduction in contractile velocity could also play an important role. Studies investigating neuromuscular function in healthy adult aging have explored this link\(^{18}\). In diabetic rodent skeletal muscle, reduced contractile speed of muscle fibres has been shown\(^9,10,15,19\), however these studies have utilized rodent models of diabetes, and mechanically skinned single-muscle fibre preparations, and thus the translation of these results to humans is unclear. A study in the tibialis anterior (TA) of humans reported no differences in evoked contractile properties between patients with DM and controls\(^{20}\), but it is important to note the patient group studied featured DM, and not DPN. The presence of DPN could reflect a longer duration or more severe history of DM, and indeed DPN can result directly in greater muscle atrophy, weakness and neuromuscular remodeling than DM alone\(^{21,22}\). Indeed, DPN has been associated with reduced motor unit number estimates (MUNEs)\(^{23,24}\), although how this chronic process of muscular denervation may affect muscle morphology has not been comprehensively explored.

Additionally, the potential slowing of muscle contractile properties has been purported to be associated with reduced motor unit (MU) firing rates\(^{25}\). Previous
investigations in rodent and human muscle have found muscle fibre contractile velocities match the firing rate properties of the motor neurons supplying their innervation\textsuperscript{26,27}. This has been reported for some muscles in humans in relation to adult aging, including the TA\textsuperscript{28}. It is conceivable that the lower maximal motor unit firing rates reported in DPN patients may be linked with slowed contractile properties relative to controls. However, although contractile slowing has been reported numerous times in rodent models of DM and DPN, this slowing has not been shown in humans with DPN.

The purpose of this study was to assess the neuromuscular contractile properties of the TA in patients with DPN in comparison to age and sex-matched controls. The TA was selected because it is generally observed to be affected by DPN earlier and to a greater extent than more proximal muscles\textsuperscript{29}, and it has a more important functional role in everyday tasks compared to more distal muscles (i.e. intrinsic muscles of the feet). Our specific objectives were three fold: (1) to investigate evoked twitch and voluntary contractile properties of dorsiflexors, including antagonist (plantar flexor) co-activation; (2) to determine peak TA muscle cross-sectional area (CSA), normalized strength (strength per unit of muscle tissue) and relative contractile and non-contractile tissue; (3) and to determine the relationship of the morphological measures stated above as they relate to TA denervation. We hypothesized patients with DPN would have slowed contractile properties compared to controls. Conjunctly, we hypothesized slowed contractile properties in DPN patients would be positively associated with lower mean firing rates (relative to controls) in TA motor units during a sustained, low intensity dorsiflexion contraction. We hypothesized patients with DPN would be weaker and demonstrate lower maximal rates of torque development during maximal isometric voluntary contractions (MVCs), and that these decrements would be positively related to increased antagonist co-activation. Additionally, we hypothesized DPN patients would possess smaller TA CSAs with greater relative amounts of non-contractile tissue which would be negatively related to the number of functioning motor units estimated in the TA.
5.2 Methods

5.2.1 Participants

With ethical approval from the local university’s Research Ethics Board, informed oral and written consent was obtained from twelve patients (7 men, 5 women; ages 32-78 years) with DPN. These patients met the criteria for diagnosis of type 2 non-insulin dependent DM with clinical and electrophysiological features of confirmed DPN. The majority of DPN patients were being treated with medications pertaining to glycemic control (i.e. metformin and/or insulin) and antihypertensives. Additionally, they had a thorough history, and clinical and electrophysiological examination by an experienced neurologist with specialized training in neuromuscular disease to exclude other causes of nerve injury and or neuropathy (i.e., compressive mononeuropathies or radiculopathies), and other neurological, metabolic or vascular conditions unrelated to DM or DPN. To provide electrophysiological evidence of neuropathy all DPN patients underwent motor nerve conduction studies of the fibular nerve while recording from the TA, as well as sensory nerve conduction studies of the sural nerve while recording from the ankle. Data regarding blood lipid profiles and glycemic control were obtained through chart review. The DPN patient group was compared with twelve healthy, age and sex-matched controls (7 men, 5 women; ages 29-77 years) who were recruited from the community. Control participants were recreationally active, living independently in the community, free from medications and were screened by physicians (neurologists) to ensure they met inclusion criteria.

5.2.2 Evoked Contractile Properties and Strength of Tibialis Anterior

To assess voluntary and electrically stimulated properties of the TA, participants were seated in a custom isometric dorsiflexion dynamometer. The right ankle was positioned at 30° of plantar flexion, while both knee and hip angles were maintained at 90°. Movement at the hip was minimized by securing a padded, C-shaped brace to the distal aspect of the right thigh. Inelastic straps were wrapped over the dorsum of the foot to secure the foot to the dynamometer.
All testing was performed on the right (dominant) leg. Maximal evoked twitch and compound muscle action potential (CMAP) responses of the TA were obtained by supramaximal, percutaneous electrical stimulation of the fibular nerve just distal to the fibular head. Stimulation was performed through a bar stimulating electrode using single, 100 µs square wave pulses via a constant-voltage electrical stimulator (Digitimer stimulator, model DS7AH; Digitimer Ltd., Welwyn Garden City, UK). Supramaximal dorsiflexion twitches were evoked prior to the assessment of voluntary strength. Participants performed three dorsiflexion maximal voluntary contractions (MVCs), with at least 3 min of rest between attempts. Participants were instructed to contract as hard and fast as possible to ensure maximal torque, and rate of torque development were achieved. Each MVC was held for approximately 3-4 seconds. Participants were provided with real time visual feedback of their torque on a computer monitor and were verbally encouraged throughout the contraction. Voluntary activation during the 2nd and 3rd MVC attempts was assessed using the interpolated twitch technique. This technique involves supramaximal electrical stimulation of the fibular nerve before, during and after a voluntary MVC. The amplitude of the interpolated torque electrically evoked from a single 100µs stimulus during the plateau of the MVC was compared with a single (100µs) resting twitch evoked ~1s following the MVC. Voluntary activation was calculated as a percent using the following equation: \[1 - \left(\frac{\text{interpolated twitch}}{\text{resting twitch}}\right)\] \times 100. The twitch evoked following the MVC was used to evaluate post-activation potentiation (PAP) torque. The peak torque of the three MVC attempts was taken as the maximal torque for the participant. All torque signals were collected and sampled online at 500 Hz using Spike2 software (Version 7.11; Cambridge Electronic Design Ltd., Cambridge, United Kingdom) and analyzed off-line to determine voluntary isometric torques (strength) and voluntary rate of torque development (RTD) (Nm/sec).

Surface electromyography (EMG) was collected from the TA and soleus (SOL) using self-adhering Ag-AgCl electrodes (1 cm x 3 cm; GE Healthcare, Helsinki, Finland). For the TA, the active electrode was placed over the motor point, approximately 7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia; whereas the reference electrode was placed over the distal tendon of the TA. For the
SOL, the active electrode was placed 2 cm below the gastrocnemii border, along the longitudinal axis over the soleus; whereas the reference electrode was placed over the distal tendon of the triceps surae. Electrode placement was adjusted to maximize the TA and SOL compound muscle action potential (CMAP) negative peak amplitudes as necessary. Two ground electrodes were placed over the patella. Electrically evoked CMAPs were elicited from the SOL using a bar electrode held in the distal portion of the popliteal fossa between the origins of the heads of the gastrocnemius to stimulate the tibial nerve. Surface EMG signals were preamplified (x100), amplified (x2), bandpass filtered (10 Hz to 1 kHz), converted by a 12-bit analog-to-digital converter (Power 1401, Cambridge Electronic Design, Cambridge, UK), and sampled online at 2,000 Hz. To calculate the level of coactivation, a 0.5 second period of EMG was analyzed from the SOL corresponding to the point of peak torque during a dorsiflexion MVC. The mean root mean square (RMS) of this EMG signal was calculated and normalized to the maximal SOL CMAP (% antagonist coactivation = [mean MVC SOL RMS EMG/peak RMS SOL CMAP] x 100).

5.2.3 Decomposition-based Quantitative Electromyography (DQEMG) of Tibialis Anterior

To obtain motor unit number estimates (MUNE) and mean MU firing rates in the TA, decomposition-based quantitative electromyography (DQEMG) data were acquired using decomposition enhanced spike triggered averaging (DE-STA) software, described in detail elsewhere. Intramuscular EMG signals were recorded via a disposable concentric needle electrode (Model N53153; Teca Corp., Hawthorne, NY) inserted into the TA, 5-10 mm distal to the active surface electrode. The surface and intramuscular EMG signals were bandpass filtered at 5 Hz to 1 kHz and 10 Hz to 10 kHz, respectively. Surface EMG was sampled at 3 kHz; intramuscular EMG was sampled at 30 kHz. Participants matched a target line of 25% MVC, visible on a computer monitor, for all isometric dorsiflexion contractions while the intramuscular needle electrode was inserted and manipulated in the muscle. This contraction intensity has been shown to be the most effective intensity for obtaining a representative motor unit number estimation (MUNE) in the tibialis anterior (TA) based on the limitations of the technique. Surface and intramuscular EMG were collected while participants sustained a steady target torque for
each contraction held for ~30 s. Between contractions, the concentric needle electrode was repositioned in order to obtain a sample from different motor units. These procedures were repeated until at least 20 suitable trains of motor unit potentials (MUPs) and their respective surface-motor unit potentials (S-MUPs) were collected.

Decomposed EMG signals were reviewed off-line to determine the acceptability of the needle-detected MUP trains and their corresponding S-MUPs. A computer algorithm aligned the negative onset markers for all accepted S-MUPs and created a mean S-MUP template based upon their data-point by data-point average. All MUP and S-MUP markers were subsequently reviewed visually by the operator. A MUNE was derived by dividing the negative-peak amplitude of the CMAP by the negative peak amplitude of the mean S-MUP (MUNE = [CMAP/mean S-MUP]). In addition to MUNEs, the DQEMG software measures firing rates (Hz) of individual motor unit action potential trains (MUAPTs), and provides overall mean firing rates per participant at a relative target level of contraction. The investigator was blinded to the status of the participant during off-line analysis.

5.2.4 Magnetic Resonance Imaging of Dorsiflexors

Magnetic resonance images were acquired via serial axial plane in a 3.0 Telsa magnet (Verio MRI, Siemens; Erlangen, Germany). Proton density images were acquired using the following parameters: 1500ms repetition time (TR), 14ms echo time (TE), 256x192 matrix, 243x325mm field of view, 5mm slice thickness with slice separation of 2mm. Participants were inserted into the magnet bore feet first, in the supine position, with the motor point on their right TA isocentred to the bore of the magnet. To ensure minimal movement the feet and knees were strapped together using inelastic, Velcro straps. The entire musculature of the leg from the tibial plateau to the malleoli was imaged.

From the images, total TA CSAs were calculated pixel-wise using a combination of manual and semi-automated techniques with open-source OsiriX image processing software (version 4.1, Geneva, Switzerland). Muscle CSAs were measured at the slice with the largest CSA. Analysis began proximally from the first slice, in which the TA
appeared to the most distal slice containing the TA. A region of interest (ROI) was manually outlined on the dorsiflexor compartment with the brush tool and repeated every five slices; missing ROIs on the skipped slices were automatically interpolated. With the TA outlined, all pixels outside the ROIs were set to zero. To quantify the contractile only tissue, a three-dimensional threshold-growing tool was used to ensure only muscular tissue was included in the ROIs (excluding non-contractile tissue and septal spaces). Any errors produced by the automatic generation were corrected manually. The software calculated muscle CSA and volume for the ROIs. Sample images of a leg of a control participant and DPN patient are depicted in Figure 5.1. The investigator was blinded to the status of the participant during off-line analysis. Previous research has shown a high degree of intra- (ICC=0.997) and inter-rater reliability (ICC=0.997) with this analysis technique. Normalized strength was calculated by relating MVC torque values to TA CSA (Nm/cm²).

Figure 5.1 Sample magnetic resonance imaging (MRI) images

Sample MRI images of age-matched (~65 year old) male control (A) and diabetic polyneuropathy (DPN) patient (B) leg. The anterior compartment of the leg is outlined in white. Note: the greater amounts of intramuscular fatty infiltration and non-contractile tissue found in the DPN patient leg compared to the control leg (also see Table 5.3).
5.2.5 Statistics

Mean values ± standard deviations are presented in the text. Normally distributed data were analyzed using a one-way ANOVA (group); the level of significance was set at \( p \leq 0.05 \). Normality was assessed using the Shapiro-Wilk normality test; the only variable found to have a non-normal distribution was mean SMUPs. Non-normally distributed data were analyzed using a Kruskal-Wallis one-way ANOVA on ranks. Relationships among variables of interest were testing using Pearson’s Product Moment Correlation. Data analyses were performed using SigmaPlot software (Version 12.2; Systat Software, Chicago, Illinois, US).

5.3 Results

5.3.1 Participant Characteristics

Participant characteristics are presented in Table 5.1. No significant differences were detected for age (\( p=0.42 \)) or height (\( p=0.29 \)) between groups. DPN patients had significantly greater body mass (\( p=0.008 \)) and BMI (\( p=0.02 \)) than controls. Data characterizing the clinical history and status of the DPN patient group are also presented in Table 5.1.

5.3.2 Dorsiflexor Contractile Properties

Dorsiflexor contractile properties are presented in Table 5.2. DPN patient MVCs were 35% lower with 48% slower rates of maximal voluntary RTD compared to controls (\( p=0.0001 \)), although these differences did not appear to be due to any differences in voluntary activation (\( p=0.57 \)). DPN patients featured weaker and slower evoked twitch responses as evidenced by lesser peak twitch torque (~\( 35\% \); \( p=0.02 \)), lesser average rate of twitch rise (~\( 40\% \); \( p=0.01 \)), greater half-relaxation time (~\( 35\% \); \( p=0.001 \)) and greater twitch contraction duration (~\( 22\% \); \( p=0.003 \)). Additionally, the twitch response of DPN patients potentiated significantly less (~\( 40\% \); \( p=0.01 \)) following a 3 second MVC compared to controls.
### Anthropometric Parameters

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 12)</th>
<th>DPN Patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>7/5</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.5 ± 14.7</td>
<td>65.6 ± 14.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
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<tr>
<td>Weight (kg)</td>
<td>73.8 ± 6.1</td>
<td>83.1 ± 7.4*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 3.1</td>
<td>28.9 ± 3.7*</td>
</tr>
</tbody>
</table>

### Diabetic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Range or Limit of Normal</th>
<th>DPN Patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Diabetes (years)</td>
<td>-</td>
<td>14.1 ± 11.2</td>
</tr>
<tr>
<td>Duration of DPN (years)</td>
<td>-</td>
<td>9.2 ± 8.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>&lt;6.0</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.77-1.7</td>
<td>1.9 ± 1.3</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.0-5.0</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>0.9-2.0</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>2.0-3.0</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Total Cholesterol:HDL Ratio</td>
<td>&lt;5.0</td>
<td>3.9 ± 0.9</td>
</tr>
</tbody>
</table>

### Nerve Conduction Studies

<table>
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<tr>
<th></th>
<th>Range or Limit of Normal</th>
<th>DPN Patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sural Nerve SNAP Amplitude (µV)</td>
<td>&gt;5</td>
<td>1.3 ± 2.5*</td>
</tr>
<tr>
<td>TA CMAP amplitude (mV)</td>
<td>&gt;4.0</td>
<td>5.0 ± 1.7</td>
</tr>
<tr>
<td>Fibular nerve CV (m/s)</td>
<td>&gt;40.0</td>
<td>41.4 ± 10.4</td>
</tr>
<tr>
<td>FDI CMAP amplitude (mV)</td>
<td>&gt;10.0</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td>Ulnar nerve CV (m/s)</td>
<td>&gt;53.0</td>
<td>49.2 ± 3.4</td>
</tr>
</tbody>
</table>

**Table 5.1** Participant Characteristics

SNAP – sensory nerve action potential; TA – tibialis anterior; CMAP – compound muscle action potential; CV – conduction velocity. * SNAP responses were absent in 10 out of 12 patients. * Denotes significant difference between groups (p<0.05).
<table>
<thead>
<tr>
<th>Muscle Property</th>
<th>Control (n = 12)</th>
<th>DPN (n = 12)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC (Nm)</td>
<td>34.1 ± 9.5</td>
<td>22.3 ± 7.2*</td>
<td>-34.6%</td>
</tr>
<tr>
<td>Maximal MVC RTD (Nm/s)</td>
<td>180.5 ± 49.9</td>
<td>93.9 ± 33.1*</td>
<td>-47.9%</td>
</tr>
<tr>
<td>Voluntary Activation (%)</td>
<td>98.3 ± 1.9</td>
<td>97.6 ± 2.1</td>
<td>-</td>
</tr>
<tr>
<td>Peak twitch tension (Nm)</td>
<td>5.4 ± 1.9</td>
<td>3.5 ± 1.3*</td>
<td>-35.2%</td>
</tr>
<tr>
<td>Twitch average rate of rise (Nm/ms)</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01*</td>
<td>-40.0%</td>
</tr>
<tr>
<td>Time to peak tension (ms)</td>
<td>105.3 ± 17.1</td>
<td>113.6 ± 25.6</td>
<td>-</td>
</tr>
<tr>
<td>Half relaxation time (ms)</td>
<td>121.8 ± 16.6</td>
<td>164.3 ± 32.1*</td>
<td>+34.8%</td>
</tr>
<tr>
<td>Twitch Rate of Relaxation (Nm/ms)</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01*</td>
<td>-50.0%</td>
</tr>
<tr>
<td>Twitch contraction duration TPT + HRT (ms)</td>
<td>227.1 ± 24.6</td>
<td>277.9 ± 43.9*</td>
<td>+22.0%</td>
</tr>
<tr>
<td>Potentiated twitch tension (Nm)</td>
<td>8.8 ± 2.7</td>
<td>4.8 ± 1.5*</td>
<td>-45.5%</td>
</tr>
<tr>
<td>% Potentiation</td>
<td>65.4 ± 22.1</td>
<td>39.7 ± 17.3*</td>
<td>-39.3%</td>
</tr>
</tbody>
</table>

**Table 5.2** Dorsiflexion (tibialis anterior) contractile properties in controls and patients with diabetic polyneuropathy (DPN)

MVC – maximal voluntary contraction; RTD – rate of torque development. * Denotes significant difference between groups (p<0.05).
5.3.3 Tibialis Anterior Cross-Sectional Area and Normalized Strength

Both groups featured similar peak absolute TA total size (p=0.21), contractile tissue (p=0.18), and non-contractile tissue (p=0.14; Table 5.3). However, as a relative percentage of total size, DPN patients featured 5% less contractile tissue and 5% more non-contractile tissue (p=0.0003). When normalized to total TA CSA DPN patients were 30% weaker than controls, although total TA CSA contains both contractile and non-contractile tissue (p=0.008; Figure 5.2). However, DPN patients were also found to be weaker when strength was normalized to maximal TA contractile tissue CSA alone (p=0.03; Figure 5.2).

<table>
<thead>
<tr>
<th>Tibialis Anterior Parameter</th>
<th>Control (n = 12)</th>
<th>DPN (n = 12)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CSA (cm$^2$)</td>
<td>10.3 ± 2.3</td>
<td>9.5 ± 2.4</td>
<td>-</td>
</tr>
<tr>
<td>Contractile tissue CSA (cm$^2$)</td>
<td>9.6 ± 2.0</td>
<td>8.3 ± 2.2</td>
<td>-</td>
</tr>
<tr>
<td>Contractile tissue CSA (%)</td>
<td>93.2 ± 2.8</td>
<td>87.8 ± 4.3*</td>
<td>-4.8%</td>
</tr>
<tr>
<td>Non-contractile CSA (cm$^2$)</td>
<td>0.8 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Non-contractile CSA (%)</td>
<td>7.7 ± 2.8</td>
<td>12.2 ± 4.5*</td>
<td>+74.3%</td>
</tr>
<tr>
<td>Strength normalized to total CSA (Nm/cm$^2$)</td>
<td>3.5 ± 0.8</td>
<td>2.5 ± 0.9*</td>
<td>-28.6%</td>
</tr>
<tr>
<td>Strength normalized to contractile CSA (Nm/cm$^2$)</td>
<td>3.8 ± 0.6</td>
<td>2.8 ± 0.9*</td>
<td>-26.3%</td>
</tr>
</tbody>
</table>

Table 5.3 Tibialis anterior cross-sectional area and normalized dorsiflexion strength

CSA – cross sectional area; DPN – diabetic polyneuropathy. * Denotes significant difference between groups (p<0.05).
5.3.4 Relationships between Muscular Denervation, Function and Morphology

DPN patients featured 25% smaller TA CMAPs (p=0.002), 50% larger mSMUPs (p=0.03), and 45% reduced MUNEs (p=0.0001) when compared to controls (Table 5.4). Between groups, no differences were found in antagonist co-activation during MVCs (p=0.07). In DPN patients, TA MUNEs were negatively related to the proportion of TA non-contractile tissue (p=0.007, r=0.72; Figure 5.3); whereas no significant relationships were found between TA MUNEs and contractile/non-contractile tissue in the control group (p=0.10, r=0.49; Figure 5.3). A significant negative relationship was found between dorsiflexion twitch half-relaxation time and tibialis anterior MUNEs in the DPN patient group (p=0.04; r = -0.61), but no significant relationship was found in the control group (p=0.32, r = -0.31) (Figure 5.4). In the DPN patient group, a negative relationship was found between antagonist co-activation and dorsiflexion MVC strength (p=0.03, r = -0.64) but not RTD (p=0.24, r = -0.36). During 25%MVC dorsiflexion contractions, mean TA firing rates were significantly lower in patients with DPN (10 vs 12 Hz; p=0.009). Finally, within the patient group, mean firing rates were negatively correlated with twitch half relaxation time (p=0.009, r = -0.77), whereas no significant relationship was found within the control group (p=0.29, r = -0.36).
<table>
<thead>
<tr>
<th>Electrophysiological Parameter</th>
<th>Control (n = 12)</th>
<th>DPN (n = 12)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA CMAP NP (mV)</td>
<td>6.8 ± 0.9</td>
<td>5.0 ± 1.7*</td>
<td>-26.5%</td>
</tr>
<tr>
<td>TA mSMUP NP (µV)</td>
<td>77.0 ± 20.0</td>
<td>116.0 ± 61.0*</td>
<td>+50.6%</td>
</tr>
<tr>
<td>TA MUNE (#)</td>
<td>99.0 ± 19.0</td>
<td>54.0 ± 25.0*</td>
<td>-45.5%</td>
</tr>
<tr>
<td>Mean TA Firing Rate at 25%MVC (Hz)</td>
<td>12.2 ± 1.7</td>
<td>10.1 ± 1.2*</td>
<td>-17.2%</td>
</tr>
<tr>
<td>MVC % Antagonist (Plantar Flexor) Coactivation</td>
<td>2.7 ± 0.9</td>
<td>4.4 ± 2.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.4 Needle and surface electromyography of tibialis anterior and plantar flexors

CMAP – compound muscle action potential; NP – negative peak amplitude; mSMUP – mean surface detected motor unit potential; MUNE – motor unit number estimation; MVC – maximal voluntary contraction; DPN – diabetic polyneuropathy. * Denotes significant difference between groups (p<0.05).
**Figure 5.2** Dorsiflexion specific strength

Dorsiflexion strength normalized to total tibialis anterior (TA) cross-sectional area (CSA) in controls (solid grey) and diabetic polyneuropathy (DPN) patients (solid black) and TA contractile tissue CSA in controls (striped grey) and diabetic polyneuropathy (DPN) patients (striped black). * denotes significant difference between groups (p<0.05).
Figure 5.3 Relationships between relative tibialis anterior (TA) non-contractile tissue with TA motor unit number estimations (MUNE)

Controls (open symbols) and diabetic polyneuropathy (DPN) patients (closed symbols).
Figure 5.4 Relationships between dorsiflexion twitch half-relaxation time and tibialis anterior (TA) motor unit number estimates (MUNE)

Controls (open symbols) and diabetic polyneuropathy (DPN) patients (closed symbols).
5.4 Discussion

The main findings of this study were: (1) patients with DPN had significantly weaker and slowed evoked and voluntary contractile properties of the dorsiflexors; (2) no differences between groups were found in antagonist co-activation during voluntary contractions; (3) DPN patients featured less dorsiflexion post-activation potentiation; (4) DPN patients had less relative contractile tissue and greater relative non-contractile tissue in the TA than controls; (5) compared to controls, DPN patients featured less specific strength when torque was related to total TA CSA or contractile TA tissue CSA; (6) MUNEs were positively related to relative TA contractile tissue CSA (and negatively related to TA non-contractile tissue CSA) in DPN patients, but not controls.

In the present study, our patient group featured clear evidence of DM and DPN through elevated glycosylated hemoglobin (HbA1c) levels and reduced or absent sural nerve sensory nerve action potential amplitudes (SNAPs) (Table 5.1). The slowed dorsiflexor contractile properties in patients with DPN are consistent with previous findings reported in rodent models of experimental diabetes. This contractile slowing is evidenced by a decreased twitch average rate of rise (-40%), prolonged evoked half relaxation times (+35%) and contraction durations (+22%), as well as decreased maximal RTD during voluntary MVCs (-50%) (Table 5.3). In addition to slowing, DPN patients featured ~40% less post-activation potentiation (PAP) compared to controls (Table 5.3). Our results may be due to a variety of non-mutually exclusive factors, both myogenic and neurogenic in nature, and these factors may be directly related to mechanisms underlying decreases in strength and muscle quality.

5.4.1 Muscle Strength, Quality and Denervation

In humans, it has been well established that DM and DPN are associated with a loss of muscle mass and strength. Indeed, our DPN group featured ~35% weaker dorsiflexion strength than the control group (Table 5.2). In DPN, this weakness is related to the severity of the neuropathy and is thought to be caused by motor axonal degeneration and subsequent muscle fibre atrophy. In addition to the present study, previous reports have shown patients with DM have reduced muscle quality (torque per
unit of muscle) when compared to healthy controls (Figure 5.2)\textsuperscript{11,37}. Thus, the present study importantly extends and focuses these previous findings by investigating patients with DPN, and relating neurogenic changes (i.e. motor axon or motor unit loss) with changes to muscle morphology. From our results a greater loss of motor units is associated with greater proportions of non-contractile intramuscular tissue ($r = -0.72$) and proportional loss of contractile tissue ($r = 0.72$) in patients with DPN (Figure 5.3). This process may be caused by the diabetes-induced denervation of muscle fibres and insufficiency of surviving motor neurons in providing collateral reinnervation, thus failing to rescue these orphaned muscle fibres\textsuperscript{23,24,38}. If denervated muscle fibres fail to acquire a new source of innervation, they may atrophy, die and subsequently the vacated space may be replaced by intramuscular fat deposits or other non-contractile tissue\textsuperscript{39–41}.

Based on our results, strength and muscle quality differences cannot be attributed to differences in voluntary activation of the TA, as both groups were able to activate their muscle similarly, and near maximally (>95%). Additionally, no differences were found between groups for antagonist co-activation during voluntary MVCs (Table 5.4). However within the DPN group, there was a significant negative relationship ($r = -0.64$) between antagonist co-activation % and strength, thus perhaps with greater disease severity antagonist co-activation may become more pronounced to help stabilize the ankle joint during contraction. Alternatively, this pattern of weakness and increased co-activation may be related to a loss of proprioception associated with DPN\textsuperscript{42}. In either case, changes in agonist-antagonist co-activation in DPN patients seem comparable to contraction strategies used in other patient populations with neurological disorders such as stroke, muscular dystrophy and Parkinson’s disease\textsuperscript{16,17}. Finally, myocellular gene expression of key contractile and regulatory proteins are markedly reduced in diabetes, particularly in the absence of insulin (and presumably established insulin resistance as occurs in type 2 DM)\textsuperscript{43}. Some of these down-regulated genes control the synthesis of myosin heavy chain polypeptide, myosin light chain, actin, troponin, tropomyosin and calmodulin proteins \textsuperscript{43}. In a single myocyte, reduced transcription of these genes could result in less contractile (e.g. actin, myosin) and contractile-supporting proteins (e.g. troponin, tropomyosin), potentially resulting in less tension produced per muscle fibre.
5.4.2 Muscle Contractile Slowing and DPN

Conflicting results have been reported from studies that have explored the impacts of diabetes on muscle fibre type composition, which is important pertaining to contractile speed and post-activation potentiation (PAP). Using an experimental animal model of diabetes, one investigation showed slow oxidative muscle fibres were least affected by DM, whereas fast oxidative and fast glycolytic muscle fibres were reduced in proportion and size\cite{44}. In contrast, using human cross-sectional studies, patients with non-insulin-dependent DM (type 2 DM) have been reported to possess lower proportions of type I muscle fibres and greater proportions of type II fibres (especially type IIb) in skeletal muscle (vastus lateralis) compared to healthy controls\cite{45,46}. It is important to note that the animal model of experimental diabetes\cite{44} represented type 1 DM in humans, whereas the muscle fibre composition studies in humans investigated patients with type 2 DM. Thus the latter may better reflect the physiological status of the patients included in the present study, who had type 2 DM. Given this information, faster contractile properties might be expected to accompany DM, contrary to the results presented in this study. However, it is possible changes in excitation-contraction (EC) coupling and Ca\textsuperscript{2+} handling supersede the effects of muscle fibre type and MHC expression on contractile speed, or they are competing factors which are differently expressed depending on the muscle or disease severity. Indeed, it is important to note the durations of diabetes in the studies that tested human vastus lateralis (VL) muscles\cite{45,46} are unknown, and the VL is a relatively proximal muscle that may be less affected (versus TA) by the length-dependent neurogenic changes associated with DPN. The muscle fibre compositions in the patients included in those prior studies with more recent disease onset may be more a reflection of the risk of development of DM as opposed to the effects of DM on potential muscle fibre type changes\cite{47}. Additionally, studies investigating muscle fibre composition and DM have not explored the impacts of DPN on this relationship.

Although motor unit or axon loss is a feature of DPN\cite{2,23,24,38} it is not known whether there is a preferential loss of larger, faster (type 2) motor units. If type 2 motor units are lost preferentially, this could drive the observed slowing of muscle contractile
properties via the collateral reinnervation of orphaned muscle fibres by slow, type 1 motor neurons. Subsequently, these reinnervated muscle fibres acquire the characteristics of their new parent motor neuron\textsuperscript{48}. Indeed, there is some evidence to suggest this may be the case in DPN. For example, our results show a negative relationship between MUNEs and dorsiflexion twitch half-relaxation time ($r = -0.61$). This relationship could reflect a slowing of muscle properties due to a preferential loss of faster motor units associated with muscular denervation (Figure 5.4). The significantly lesser degree of PAP in muscles from DPN patients reported in this study may also reflect a greater proportional loss of fast, type 2 motor units because muscle potentiation is known to be heavily influenced by fibre type composition\textsuperscript{49}. Also, motor unit firing rates are reduced in DM and DPN patients at a given contractile intensity in both the TA (Table 5.4)\textsuperscript{23} and vastus lateralis\textsuperscript{25} which may indicate a preferential loss of faster motor units, or slowing of the firing rates of surviving motor units. This process matches the contractile slowing reported in the present study and corroborates findings in healthy development and aging\textsuperscript{27,28}. Additionally, within the DPN group, prolonged twitch half-relaxation times were negatively associated with decreases in mean firing rates ($r = -0.77$), however it is not known if one alteration is necessarily dependent on the other.

Changes in EC coupling and Ca\textsuperscript{2+} handling may partially be responsible for slowing of contractile properties of patients with DPN. It has been reported previously that baseline myocellular Ca\textsuperscript{2+} levels are elevated in diabetic rats\textsuperscript{50}. Even relatively minor increases in myocellular Ca\textsuperscript{2+} concentrations have been demonstrated to cause attenuation in the rate of Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) during contraction\textsuperscript{51,52}, and therefore a lower rate of SR Ca\textsuperscript{2+} release could directly lead to a decrease in RTD in evoked or voluntary contractions. Additionally, muscles from diabetic rodents may possess elevated SR Ca\textsuperscript{2+} ATPase activity levels and increased microsomal Ca\textsuperscript{2+} uptake capacity\textsuperscript{53}. This increased sequesterization and reuptake of Ca\textsuperscript{2+} may impose further limitations on Ca\textsuperscript{2+} interaction with the actin-myosin contractile complex, resulting in the reduced RTD in the muscles of the DPN group compared with controls found in our study. However, increased reuptake of Ca\textsuperscript{2+} by the sarcoplasmic reticulum would presumably result in an increased rate of relaxation, which is contrary to the results reported in the present study.
Additional considerations regarding the reported reductions in strength and slowed contractile properties in patients with DPN include alterations in muscle architecture and tendon mechanical properties. Whereas aspects of muscle architecture, such as pennation angle, fascicle length and sarcomere number and orientation, were not directly measured in this study, the increased proportion of non-contractile tissue in the TA of DPN patients may reflect broader architectural changes. Heterogeneous development of intramuscular non-contractile tissue and fatty infiltration\textsuperscript{12} may not only reduce muscle CSA, but it may also disrupt normal fascicular organization. Structurally, an important determinant of whole muscle contractile speed is the number of sarcomeres in series\textsuperscript{54}, and fascicular disruption via non-contractile tissue could result in contractile slowing. With regard to tendon mechanical properties, previous studies have found the tendons of DM and DPN patients appear to show signs similar to those undergoing accelerated aging\textsuperscript{55,56}. Specifically, these changes include altered tendon fibril shape, increases in fibril packing density, and increased tendon stiffness\textsuperscript{55,57}. However, these DM-related changes in tendon properties are unlikely to lead to a slowed or weaker contractile response as a stiffer tendon would be conducive to a faster RTD. Slowed contractile properties as a result of DPN may be related to important functional decrements in conjunction with strength loss; specifically impaired muscle power generation. As power is a function of both muscle torque generating capacity and contractile velocity, a decrease in either or both aspects will lead to reductions in power. Indeed, previous reports show patients with DM feature a loss of isokinetic power generation relative to controls, and the degree of power loss is greater than reductions in torque generating capacity\textsuperscript{12,36}. Additionally, this loss of power in DPN patients may be more important than strength loss alone in terms of functional consequences in dynamic muscle activities and the onset of physical disability\textsuperscript{58}. Finally, one possible confound related to our findings regarding contractile slowing is the potential difference between groups in physical activity levels. A previous study has shown that with adult aging, more sedentary individuals demonstrate greater contractile slowing than those who are more physically active\textsuperscript{59}. We do not report a direct quantification of physical activity history of our participants, however both our controls and nearly all of our DPN patients were living independently in the community with no mobility issues (2 DPN patients had
impaired mobility and utilized a wheelchair or a cane). We do not believe there were substantive differences in everyday physical activity levels between the two groups. Thus, whereas a lesser engagement in physical activity may be responsible for some of the slowing observed in our patient group, we believe the primary reason underlying the reported contractile differences is due to DPN itself, not reduced physical activity levels.

5.4.3 Summary

The present study reports contractile slowing, loss of muscle CSA, loss of strength and reduced contractile quality of lower limb muscles in association with DPN in humans. We propose these neuromuscular alterations may be caused by a combination of factors related to both myogenic and neurogenic changes that are known to occur with DPN. However, the relationships between muscle morphology and contractile slowing with denervation (MUNE) suggests changes in muscle properties may be secondary to neurogenic processes. With regards to functional consequences, these findings may represent key underlying mechanisms leading to DPN-related impairments in gait\textsuperscript{11,60}, impaired balance and increased fall risk\textsuperscript{61,62}, and reduced walking speed\textsuperscript{62}.  


References


CHAPTER 6

Increased fatigability associated with severe diabetic neuropathy is attributed partially to neuromuscular transmission failure\(^5\)

6.1 Introduction

Diabetic polyneuropathy (DPN), a common complication of diabetes mellitus (DM), can be associated with dysfunction of the neuromuscular system. Some DPN-related neuromuscular changes include: muscle atrophy\(^1\), weakness\(^2\), muscle contractile slowing\(^3\), reduced motor unit firing rates\(^4\)–\(^6\), and motor unit loss\(^5\),\(^7\),\(^8\). However, at present, little is known regarding how these alterations may affect the performance of the neuromuscular system when its capacity is stressed during a fatiguing task and subsequent recovery. Challenging the compromised system will permit the identification and functional relevance of key underlying factors contributing to DPN.

Neuromuscular fatigue can be defined as the loss of voluntary force-producing capacity during physical activity\(^9\). In the context of this investigation, this is differentiated from “experienced fatigue” which refers to a general sense of tiredness, lack of energy or chronic exhaustion often reported clinically\(^10\),\(^11\). Neuromuscular fatigue is well known to be task-dependent and can be affected by a wide array of factors including age\(^12\), physical training status\(^13\) and neurological disease\(^14\).

One previous study that explored muscle fatigue in insulin dependent (type 1) DM, concluded DM patients have greater relative muscle endurance than controls during repeated, maximal isokinetic contractions of the ankle plantar and dorsiflexors\(^15\). However, in that report, the majority of DM patients either did not have symptoms of DPN or had a neuropathy with minimal motor involvement\(^15\). Additionally, no measure

\(^{5}\) A version of this chapter has been submitted for publication. Used with permission from the American Physiological Society.

Allen, M.D., Kimpinski, K., Doherty, T.J., Rice, C.L. Increased fatigability associated with severe diabetic neuropathy is attributed partially to neuromuscular transmission failure. J Appl Physiol (undergoing revision)
of voluntary activation was made, therefore it is not known whether participants were producing maximal efforts during their isokinetic contractions\textsuperscript{15}. It has been shown previously that individuals with metabolic disease without neuropathy (e.g. type 1 DM)\textsuperscript{16}, vascular disease (e.g. chronic heart failure)\textsuperscript{17}, or nerve disease (e.g. amyotrophic lateral sclerosis [ALS])\textsuperscript{18}, fatigue more quickly than controls.

Greater fatiguability in various other clinical populations when compared with controls has been attributed to a range of factors including: insufficient blood flow\textsuperscript{17}, impaired neuromuscular transmission\textsuperscript{19}, and central activation failure\textsuperscript{20}. Indeed, even during minimally fatiguing contractions (25\% MVC for 30 seconds), neuromuscular conduction failure has been reported in DPN patients (Chapter 4). In contrast, healthy adult aging has been associated with greater resistance to fatigue in most isometric tasks\textsuperscript{12,21}, but not all isometric tasks and muscle groups\textsuperscript{22}. It is not entirely clear whether slowed contractile and metabolic functions often reported with aging necessarily provide greater endurance\textsuperscript{23}, in any case, slowed contractile properties also have been reported in DPN patients which could theoretically impart the same effect on fatigability\textsuperscript{3}. However, because DPN can involve concurrent pathophysiological changes to metabolism, vasculature, and nerve and muscle, the impact of altered contractile properties may be mitigated. Therefore, DPN provides an interesting model to study various factors contributing to fatigue as well as to improved understanding about the functional impact of DPN.

Investigating the recovery of neuromuscular properties following fatigue also can provide insight into underlying physiological processes, although it is typically less well studied than fatigue per se. Metabolic disturbances\textsuperscript{24}, blood flow impairments\textsuperscript{25} and dysfunction in myocellular processes related to clearing metabolites and restoring homeostasis\textsuperscript{26,27} could conceivably impair or delay recovery in DPN patients.

The present study used a sustained, isometric dorsiflexion maximal voluntary contraction (MVC) to induce fatigue to a predetermined task termination point (i.e. 60\%MVC). Dorsiflexion was selected because the dorsiflexors (i.e. tibialis anterior) are known to be affected in DPN\textsuperscript{3,5,7}, and participants are readily able to maximally activate
the dorsiflexors, with minimal familiarization or training. A sustained, isometric MVC was selected to provide a relatively simple model that would substantially stress the neuromuscular system. Finally, a pre-determined task termination point (60%MVC) was utilized to ensure all participants fatigued to the same degree relative to their maximal strength, thus providing an opportunity to assess recovery.

Thus the main objective of this study was to investigate neuromuscular fatigue in patients with DPN compared to age-matched controls using a sustained, dorsiflexion MVC. We also aimed to determine whether impairments in neuromuscular transmission or voluntary activation were associated with fatiguability in the DPN group. An additional objective was to assess the short-term (i.e. 2 minute) neuromuscular recovery of DPN patients compared to controls. We hypothesized individuals with DPN would fatigue more slowly than controls due to reduced absolute strength and slowed muscle contractile properties. Additionally we hypothesized both groups would be able to achieve near maximal voluntary activation (~95%) while fatiguing to 60% of MVC torque. Finally, we hypothesized DPN patients would recover more slowly than controls following the fatigue protocol due to DPN-related dysfunction in metabolite clearance and blood flow.

6.2 Methods

6.2.1 Participants

Ten patients (6 males) with DPN (aged 64 ± 11 yrs) and ten age- and sex-matched controls (aged 62 ± 12 yrs) were recruited for participation in this study. All participants provided informed, written consent prior to participation. The study was approved by the local university’s Research Ethics Board and conformed to the Declaration of Helsinki. The patients with DPN met accepted clinical criteria for diagnosis of DM and DPN. The presence of DPN was confirmed via clinical and electrophysiological examination performed by a trained neurologist, who also confirmed the absence of other causes of nerve injury or neuropathy. Data outlining some of the neuromuscular impacts of DPN (e.g. loss of motor units, muscle slowing, and neuromuscular transmission instability) in these patients have been previously reported elsewhere. Patients with metabolic,
neurological or vascular conditions unrelated to DM or DPN were excluded from participation. Data regarding glycemic control were obtained through clinical chart review. Control participants were living independently in the community, free from medications and were screened by physicians (neurologists) to ensure they met inclusion criteria. Participants in both groups were not engaged in systematic physical exercise regimens.

6.2.2 Evoked Contractile Properties and Maximal Strength of Dorsiflexors

The experimental set-up has been described in detail in previous reports\(^3\,^5\). Participants were seated in a custom isometric dorsiflexion dynamometer to assess voluntary and electrically evoked properties of the dorsiflexor muscles\(^3^2\). In all participants the right leg was tested. The ankle was positioned at 30° of plantar flexion; both knee and hip angles were positioned at 90°. Hip joint movement was minimized by securing a padded, adjustable brace over the distal aspect of the right thigh. Inelastic straps were wrapped over the dorsum of the foot to secure it to the dynamometer\(^3^3\). The great toe was left uncovered by the straps to minimize torque contributions from extensor hallucis longus.

Maximal evoked single twitch and compound muscle action potential (CMAP) responses from the dorsiflexors (tibialis anterior muscle) were obtained using supramaximal electrical stimulation of the fibular nerve just distal to the fibular head. Percutaneous nerve stimulation was performed using a bar electrode which transmitted the single, 100 \(\mu\)s square wave pulses triggered from a constant-voltage electrical stimulator (Digitimer stimulator, model DS7AH; Digitimer Ltd., Welwyn Garden City, UK). Twitches were analyzed off-line for: peak twitch tension (Nm), time to peak tension (ms), and half-relaxation time (ms). From these data twitch contraction duration (CD [ms]; time to peak tension + half-relaxation time) and twitch rate of torque development (RTD (peak twitch tension/time to peak twitch tension)) were calculated. Accompanying CMAPs were analyzed for maximal peak to peak voltage (mV).

At least 3 dorsiflexion, maximal voluntary contractions (MVCs), and no more than 5, were performed by each participant, and each MVC attempt was separated by at
least 3 minutes to minimize the development of any fatigue before the actual fatigue test. Participants were verbally instructed to perform their MVC as hard and as fast as possible to ensure maximal torque and rate of torque development were produced. MVCs were held for ~3-4 seconds during each attempt, during which strong verbal encouragement was given. Real time visual feedback of the torque signal was provided to the participant on a computer monitor throughout the experimental protocol. The interpolated twitch technique (ITT) was used to assess voluntary activation (VA) during the 2\textsuperscript{nd} and 3\textsuperscript{rd} MVC attempts to ensure adequate muscle activation was achieved\textsuperscript{34}. The ITT involves supramaximal electrical stimulation of the fibular nerve before, during and immediately after a voluntary MVC. The amplitude of the interpolated torque electrically evoked during the plateau of the MVC was compared to the twitch elicited immediately following the MVC. VA\% was calculated as: [1 - (interpolated twitch /resting twitch)] x 100. The peak torque measured during the 3 MVC attempts was recorded as the maximal torque for that participant. All torque signals were collected and sampled online at 500 Hz using Spike2 software (Version 7.11; Cambridge Electronic Design Ltd., Cambridge, United Kingdom). Torque was later analyzed off-line to determine voluntary isometric torques (strength) and maximal voluntary rate of torque development (RTD (Nm/s)). Maximal RTD was obtained by transforming the torque signal into its first derivative and recording the peak value for a given contraction.

Throughout the experimental protocol surface electromyography (EMG) was recorded from the TA and soleus (SOL) muscles using self-adhering Ag-AgCl electrodes (1 cm X 3 cm; GE Healthcare, Helsinki, Finland). For the TA, the active electrode was placed over the motor point, approximately 7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia; the reference electrode was placed distally over the tendon of the TA. For the SOL, the active electrode was placed 2 cm below the gastrocnemii border, along the longitudinal axis over the soleus; the reference electrode was placed distally over the tendocalcaneus. Minor adjustments to active electrode placement over the TA were made until the optimal CMAPs (greatest amplitude and fastest rate of rise) were obtained from percutaneous, supramaximal nerve stimulation. Ground electrodes were placed over the patella. Maximal SOL CMAPs were obtained by electrically stimulating the tibial nerve between the origins of the heads of the
gastrocnemii muscles. Surface EMG signals were pre-amplified (x100), amplified (x2), bandpass filtered (10 Hz to 1 kHz), converted by a 12-bit analog-to-digital converter (Power 1401, Cambridge Electronic Design, Cambridge, UK), and sampled online at 2,000 Hz.

6.2.3 Fatigue Protocol

Following the assessment of maximal dorsiflexion strength participants rested for 10 minutes to ensure any fatigue or post-activation potentiation had dissipated. The fatigue protocol in the present study involved a single, sustained, isometric dorsiflexion MVC. The MVC was maintained until the participant could no longer sustain 60% of their pre-fatigue MVC torque. For the fatigue protocol, participants were instructed to contract as hard and as fast as possible, and as long as possible. Additionally participants were instructed to minimize any extraneous movements or contractions aside from dorsiflexion; also they were asked to continue breathing throughout the fatigue protocol. During the fatigue task, participants were provided with continuous and strong verbal encouragement. Throughout the protocol visual feedback of their torque was provided on a computer monitor. In addition to torque output, the computer monitor displayed a horizontal lines demarcating 100% of MVC and 60% MVC. Task termination occurred when the torque dropped and remained below the 60% MVC level despite further verbal encouragement. Participants were not informed that the line on the computer monitor demarcating 60% of MVC torque represented their task termination point. Prior to instructing the participant to cease the fatiguing MVC, an interpolated twitch was applied to assess VA% at task termination.

Similar to baseline measures, fatigue protocol torque and EMG data were analyzed offline. Time to task termination was determined as the time from which the participant reached their maximal torque until they could no longer produce torque greater than 60% of their baseline MVC. Torque and TA EMG data from the fatigue protocol were subsequently binned into increments of 10% of their time to task termination (i.e. a fatigue protocol lasting 60 seconds would consist of 10 x 6 second bins). Average torque and TA RMS EMG data for these 10% bins were calculated for each participant. Binned torque and RMS EMG data were subsequently normalized to
MVC torque and maximum RMS EMG data, respectively. This served to provide profiles of torque output and neural activation of the TA over the course of the fatigue protocol. Antagonist (soleus) co-activation was quantified during the dorsiflexion MVC fatigue protocol in similar manner to TA activation, but was normalized to soleus RMS EMG during baseline MVCs. The area under the curve of the torque tracing was measured to assess the angular impulse (Nm x sec) for each participant. This served as an analogue for the parameter of work, which is often calculated for dynamic muscle contractions. VA% at the cessation of the fatigue protocol was calculated similarly to baseline MVCs, described above. A sample fatigue protocol is depicted in Figure 6.1.

6.2.4 Assessment of Recovery

Immediately (~1 second) following cessation of the fatigue protocol a supramaximal electrical stimulus was applied to the fibular nerve to help assess the degree of central and peripheral fatigue induced by the fatigue protocol. Subsequently, participants performed MVCs with ITT at 30 seconds and 2 minutes post-fatigue protocol. Prior to these MVCs, additional maximal dorsiflexion twitches were elicited to provide an assessment of recovery of the twitch and CMAP properties following fatigue. MVCs were assessed for VA% using the ITT technique. Recovery MVC torques and TA EMG data were analyzed similarly to baseline MVCs.
Figure 6.1 Raw torque and EMG tracings from fatigue and recovery protocol

Sample torque, tibialis anterior root-mean-squared electromyography (RMS EMG) and plantar flexor RMS EMG during a sustained, isometric dorsiflexion maximal voluntary contraction (MVC) fatigue protocol, and subsequent recovery at 30 seconds and 2 minutes post-task termination.
6.2.5 Statistical Analysis

Mean values ± standard deviations are presented in the text, tables, and figures, except where noted. The level of significance was set at $p \leq 0.05$ for all statistical tests. All baseline data, and time to task termination of the fatigue protocol were analyzed using a one-way ANOVA (group). Binned torque and EMG values for fatigue data, as well as recovery data were analyzed using a mixed design (split-plot) ANOVA. For these mixed design ANOVAs the between-subjects factor was group (control or DPN patient), with repeated measures over time. Fatigue and recovery data were analyzed both as absolute values, as well as relative to baseline. Mauchly’s sphericity test and Levene’s test were used to ensure there were no violations of sphericity or homogeneity of variance assumptions, respectively. Main and interaction effects were determined by Pillai’s trace ($p \leq 0.05$). Following each ANOVA in which a significant main effect or interaction was found, post-hoc analysis was performed using the modified Bonferroni correction method. Relationships among variables of interest were testing using Pearson’s Product Moment Correlation. Data analyses were performed using IBM SPSS Statistics software (Version 20.0; IBM SPSS, Arnock, New York, USA).

6.3 Results

6.3.1 Participant Characteristics and Baseline Measures

Participant characteristics are presented in Table 6.1. No differences between groups were found for age ($p>0.05$), height ($p>0.05$), or weight ($p>0.05$). Baseline neuromuscular measures are presented in Table 6.2. DPN patients were found to have 40% weaker ($p<0.05$) MVCs with 47% slower maximal RTD ($p<0.05$). Strength differences were not due to differences in VA% ($p>0.05$). Compared with controls, the DPN group twitches were ~36% smaller ($p<0.05$), and ~25% slower (i.e. longer contraction durations) ($p<0.05$) with ~40% smaller CMAP peak to peak amplitudes ($p<0.05$).
### Participant Characteristic

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>DPN Patients (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females</td>
<td>6/4</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.2 ± 12.1</td>
<td>64.7 ± 11.3</td>
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<tr>
<td>Height (m)</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
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<tr>
<td>Weight (kg)</td>
<td>71.4 ± 5.6</td>
<td>78.9 ± 8.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
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</tr>
</tbody>
</table>

**Table 6.1** Participant Characteristics

DPN – diabetic polyneuropathy

### Torque Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>DPN Patients</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC Torque (Nm)</td>
<td>33.7 ± 8.1</td>
<td>20.2 ± 9.2*</td>
<td>-40%</td>
</tr>
<tr>
<td>MVC RTD (Nm/s)</td>
<td>170.2 ± 41.5</td>
<td>89.1 ± 37.9*</td>
<td>-47%</td>
</tr>
<tr>
<td>Voluntary Activation (%)</td>
<td>98.8 ± 2.1</td>
<td>97.2 ± 3.7</td>
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</tr>
<tr>
<td>Peak Twitch Torque (Nm)</td>
<td>5.1 ± 1.7</td>
<td>3.2 ± 1.8*</td>
<td>+36%</td>
</tr>
<tr>
<td>Time to Peak Twitch (ms)</td>
<td>106.2 ± 17.1</td>
<td>116.2 ± 28.6</td>
<td>-</td>
</tr>
<tr>
<td>Twitch RTD (Nm/s)</td>
<td>45.0 ± 17.0</td>
<td>28.0 ± 14.0*</td>
<td>-42%</td>
</tr>
<tr>
<td>Half-Relaxation Time (ms)</td>
<td>122.0 ± 15.0</td>
<td>170.2 ± 25.0*</td>
<td>+40%</td>
</tr>
<tr>
<td>Contraction Duration (ms)</td>
<td>228.0 ± 23.0</td>
<td>286.0 ± 42.0*</td>
<td>+25%</td>
</tr>
</tbody>
</table>

**Table 6.2** Dorsiflexion neuromuscular parameters at baseline

MVC – maximal voluntary contraction; RTD – rate of torque development; contraction duration = time to peak twitch + half-relaxation time; EMG – electromyography; CMAP – compound muscle action potential. * Denotes significant difference between groups (p<0.05).
6.3.2 Fatigue

Fatigue profiles of torque over time are presented as absolute values in Figure 6.2. The mean time to task termination of the fatigue protocol was ~21% shorter in DPN patients (56.4 ± 14.2 sec) compared to controls (71.1 ± 11.7 sec; p = 0.001). Additionally, DPN group angular impulse was ~50% less than controls (1637.4 ± 639.7 vs 852.4 ± 410.5 Nm*s; p<0.05). There was a main effect for time (p<0.05) and an interaction effect for group and time (p<0.05) for the changes in relative torque calculated from the binned time intervals. Both groups featured decreases in torque output relative to their baseline MVCs over the course of the fatigue protocol (p<0.05). Pairwise comparisons between groups over binned time intervals showed the DPN group had lesser decreases in relative torque output from 50-90% of the time to task termination compared to controls (p<0.05). Just prior (1-2 s) to task termination VA% was >95% in both groups (p>0.05). No significant relationships were found between glycemic control (i.e. HbA1c %) or severity of neuropathy (i.e. TA CMAP amplitude) and time to fatigue.

In both groups over the course of the fatigue protocol, TA RMS EMG normalized to MVC RMS EMG values are expressed over 10% binned time intervals in Figure 6.3. For these variables a main effect of time (p<0.05) and an interaction effect between time and group (p<0.05) were detected. From 0-30% of the time to task termination, the DPN group featured less relative EMG activity from the TA compared with controls. For the remainder of the fatigue protocol no differences were detected in relative TA RMS EMG between groups (p>0.05). There were no group differences (p>0.05) in % antagonist soleus coactivation during the fatigue protocol, however there was an effect of time (p<0.05) with both groups featuring decreased % antagonist coactivation over the fatigue protocol (results not shown).

6.3.3 Recovery

Absolute data pertaining to neuromuscular recovery following the fatiguing protocol are found in Table 6.3. At thirty seconds post-fatigue both DPN patients and control groups featured MVCs that were significantly weaker (~10%) compared to baseline (p<0.05; Table 6.3), but these relative changes were not different between
groups (p>0.05). At 2 minutes post-fatigue, the MVC for both had recovered to baseline levels (p>0.05). Similarly, MVC RTD was significantly reduced (~20%) in both groups 30 seconds following fatigue (p<0.05; Table 6.3), however both groups were not significantly different from their respective baselines at 2 minutes post-fatigue (p>0.05). VA% was near maximal (>95%) in both groups during MVCs at 30 seconds and 2 minutes post-fatigue (p>0.05).

The twitch evoked immediately following task termination was 15-20% weaker in both groups relative to their respective baseline twitches (p<0.05; Table 6.3), however the relative changes were not different between groups (p>0.05; Figure 6.4A). Peak twitch torque recovered to baseline levels in both groups at 30 seconds post-fatigue (p>0.05). Twitch half-relaxation time (HRT) was prolonged ~20% compared to baseline in both groups immediately following the fatigue protocol (p<0.05; Table 6.3), and at 30 seconds post-fatigue twitch HRT recovered in both groups.

Peak to peak CMAP amplitude was significantly reduced by ~12% in DPN patients immediately following the fatigue protocol (p<0.05; Figure 6.4B); whereas for controls there was no change (p>0.05; Figure 6.4B). By 30 seconds post-fatigue, the DPN group CMAPs had recovered to baseline (p<0.05).
Mean absolute dorsiflexion torque (Nm) produced during a sustained, isometric dorsiflexion maximal voluntary contraction (MVC) fatigue protocol in controls (open symbols; time to task termination = 71 s) and diabetic polyneuropathy (DPN) patients (closed symbols; time to task termination = 56 s). * denotes significant difference between groups in time to task termination (p<0.05). Controls produced significantly greater torque throughout the protocol (p<0.05).

**Figure 6.2** Fatigue protocol: absolute torque over time
### Table 6.3 Absolute recovery of maximal twitch, MVC and CMAP

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Immediate Post Fatigue</th>
<th>30 sec Post Fatigue</th>
<th>2 min Post Fatigue</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Controls</td>
<td>DPN Patients</td>
<td>Controls</td>
<td>DPN Patients</td>
</tr>
<tr>
<td>Twitch Torque (Nm)</td>
<td>5.1 ± 1.7</td>
<td>3.2 ± 1.8*</td>
<td>4.1 ± 1.3$§</td>
<td>2.8 ± 1.7*§</td>
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<tr>
<td>Twitch HRT (ms)</td>
<td>122 ± 15</td>
<td>170 ± 25*</td>
<td>145 ± 21$§</td>
<td>203 ± 23*§</td>
</tr>
<tr>
<td>MVC (Nm)</td>
<td>33.0 ± 8.0</td>
<td>20.0 ± 9.0*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MVC RTD (Nm/s)</td>
<td>170.2 ± 41.5</td>
<td>89.0 ± 37.9*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CMAP Peak to Peak Amp (mV)</td>
<td>6.6 ± 0.7</td>
<td>4.6 ± 0.9*</td>
<td>6.2 ± 0.7</td>
<td>4.0 ± 0.5*§</td>
</tr>
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DPN – diabetic polyneuropathy; HRT – half-relaxation time; MVC – maximal voluntary contraction; RTD – rate of torque development; CMAP – compound muscle action potential. * denotes significant difference between groups; § denotes significant difference from baseline (p<0.05)
Figure 6.3 Tibialis anterior electromyography activity during fatigue task

Tibialis anterior relative root-mean-squared electromyography (RMS EMG) during a sustained, isometric dorsiflexion maximal voluntary contraction (MVC) fatigue protocol in controls (open symbols) and diabetic polyneuropathy (DPN) patients (closed symbols). * denotes significant difference between groups (p<0.05).
Figure 6.4 Twitch torque and compound muscle action potentials following fatigue protocol.

Electrically evoked dorsiflexion relative twitch torques at baseline, immediately post-task termination, 30 seconds post-task termination and 2 minutes post-task termination in controls (open symbols) and diabetic polyneuropathy (DPN) patients (closed symbols). § denotes significant difference from baseline. (4B) Relative peak to peak compound muscle action potential (CMAP) amplitudes (mV) at baseline, immediately post-task termination, 30 seconds post-task termination and 2 minutes post-task termination in controls (open symbols) and diabetic polyneuropathy (DPN) patients (closed symbols). § denotes significant difference from baseline; * denotes significant difference between groups (p<0.05).
6.4 Discussion

The main findings of this study were: (1) during a sustained isometric dorsiflexion MVC, those with DPN had less endurance compared to controls; (2) the differences in fatigability between groups did not appear to be due to deficiencies in VA%; (3) DPN patients featured evidence of possible neuromuscular transmission failure during and immediately following the fatiguing task, whereas controls did not; (4) at 2 minutes post-fatigue, the maximal evoked and voluntary contractile properties of the dorsiflexors in both groups had recovered similarly to baseline levels.

6.4.1 Increased Fatiguability in DPN

In agreement with other studies, baseline neuromuscular properties of the dorsiflexors related to strength (MVC, MVC RTD, twitch torque) and CMAP amplitude were decreased in patients with DPN compared to controls (Table 6.2)\textsuperscript{3,5,35,36}. However novel to the present study, when tasked with maintaining a sustained, isometric dorsiflexion MVC, those with DPN fatigued significantly more quickly (~21%) compared to controls. Furthermore, the difference in fatigability between groups did not appear to be due to differences in VA, as both groups maintained greater than 95% VA (p>0.05) as measured via the interpolated twitch technique at task termination. The time to task failure of the control group was similar to that reported previously using this same task and protocol (71 vs 85 s)\textsuperscript{29}, but the greater endurance of the control group did not seem to be due to any differences in voluntary activation, as both groups achieved >95% voluntary activation just prior to task termination. Moreover, the greater muscle endurance in the control group does not appear to be due to a pacing strategy as their RMS EMG indicates a maximal or near-maximal effort and follows the expected pattern for a sustained MVC (Figure 6.3).

DPN patients fatigued significantly more quickly (~21%; Figure 6.1), while producing lower absolute torques, and substantially lesser total angular impulse (~50%) compared to controls. However, our results stand in contrast to those previously reported in a study investigating fatigue in a mixed population of insulin-dependent (type 1) DM, with and without DPN in which DM patients had greater muscular endurance than
controls\textsuperscript{15}. The divergent findings could be accounted for by several key differences in experimental design. Andersen (1998)\textsuperscript{15} investigated fatigue in a group of insulin-dependent DM patients, many of whom reported no symptoms of DPN or featured a mild DPN with minimal motor involvement. The present study included non-insulin-dependent DM patients (type 2 DM), all with substantial neuromuscular impairment of the dorsiflexors\textsuperscript{3,5}. Moreover, there are important differences in the underlying alterations related to DPN and metabolism in insulin-dependent versus non-insulin-dependent DM\textsuperscript{37}. Additionally, the prior study used a metric of fatigue that combined plantar and dorsiflexor performance which may have affected the results given the greater resistance of the plantar flexors to DPN-related changes relative to the dorsiflexors. Finally, the previous work used repeated isokinetic contractions which are difficult to assess in terms of voluntary activation and their intermittent nature could attenuate the development of any substantive neuromuscular transmission failure.

There are several possible mechanisms underlying the increased fatiguability observed in DPN patients, which are not mutually exclusive. One possible mechanism is the presence of neuromuscular transmission failure, which may occur at the motor axon, neuromuscular junction or muscle fibre membrane. Evidence supporting transmission failure is reflected by the decreased relative surface EMG activity during fatigue in the DPN group compared to controls (Figure 6.3), and a reduction in CMAP size immediately following fatigue in DPN but not in controls (Figure 6.4B). Failure to propagate action potentials at any point in the neuromuscular system would fail to cause contractions of the associated muscle fibres, thus limiting the system’s force generating capacity. Demyelination or axonal damage could lead to a reduced safety factor and action potential propagation failure in the motor axon in a manner similar to frequency dependent conduction block observed in other demyelinating neuropathies\textsuperscript{38,39}. Furthermore, given the significant reduction in motor units in DPN patients\textsuperscript{5,7,8} any neuromuscular transmission failure occurring at the level of the motor axon may have exacerbated decrements in force production compared to other demyelinating conditions because each remaining motor unit innervates a greater relative proportion of muscle fibres following motor unit loss and subsequent collateral reinnervation. DPN-related pathological alterations at the neuromuscular junction (e.g. reduced numbers of synaptic
vesicles) or muscle fibre (e.g. T-tubule disruption) levels could also contribute to failed neuromuscular transmission\textsuperscript{40}. In addition, dysfunctional axonal Na+/K+ pumps\textsuperscript{27,41} and myocellular Ca\textsuperscript{2+} channels\textsuperscript{42,43} may contribute to any conduction failure in DPN, as previously postulated\textsuperscript{41}. Moreover, neuromuscular transmission failure has been previously reported in DPN during sustained (i.e. 30 seconds) low intensity (25\% MVC) contractions (Chapter 4). Thus it is possible the sustained maximal contraction used in the present study could result in equal or greater transmission failure, reducing force generation capacity in DPN patients more quickly than controls during a sustained contraction.

Another potential cause underlying the increased fatiguability in DPN observed in the present study is related to abnormal muscle energy metabolism. A previous report has documented that myocellular PCr loss and pH decrease occur significantly faster during low intensity plantar flexion exercise, in skeletal muscle of type 2 DM compared to healthy controls\textsuperscript{24}. Additionally, deoxygenation during exercise occurred more quickly in type 2 DM than controls\textsuperscript{24}. Greater PCr loss, increased myocellular inorganic phosphate concentrations and decreased pH in DPN patients compared to controls could lead to an earlier reduction in actomyosin cross-bridge force production during a sustained effort (see Westerblad et al 1998 for review\textsuperscript{44}). However, it is important to note that the aforementioned report discussing skeletal muscle energetics in DM during exercise used repeated dynamic contractions during a protocol that lasted ~7 minutes\textsuperscript{24}, which is a very different exercise duration and contraction type compared to the present study. Indeed, the task-dependent nature of fatigue has been well-described (see Enoka and Duchateau 2008 for review\textsuperscript{45}) therefore it is not completely clear how these previous findings may relate to the results presented here.

An additional consideration regarding DPN and skeletal muscle endurance is related to blood supply. Adequate blood flow is necessary to engage in oxidative metabolism, and if this blood flow is insufficient during muscular contraction, metabolism and the ability to remove metabolic by-products which inhibit force production are limited\textsuperscript{44}. It has been reported that DM and DPN are associated with dysfunction in the macro- and microvasculature\textsuperscript{25,46–49}, as well as oxygen kinetics during
Although blood flow to the dorsiflexors may not present a major limitation to force production in healthy individuals, it should be noted that an impaired ability to deliver oxygen and clear metabolites from contracting muscle could contribute to increased fatiguability in DPN patients.

6.4.2 Neuromuscular Recovery Post-Fatigue in DPN

DPN patients performed a fatigue protocol of shorter duration (~21%; Figure 6.2), at lower absolute levels of torque output (~40%; Figure 6.2), and therefore markedly less total angular impulse (~50% less angular impulse) compared to controls. Two minutes following fatigue evoked twitch and MVC properties had returned to baseline values in both groups. However, immediately following the fatigue protocol, DPN patients and controls both featured evoked twitch responses that were similarly weaker than their respective baseline twitches (Figure 6.4A). Additionally, 30 s post-task termination both groups had similar deficits in MVC torque (~10%) and MVC maximal RTD (~20%). The similar recovery profiles between patients and controls are surprising giving the substantial disparity in total angular impulse (i.e. work), and it may be interesting to test these groups with tasks of equal total angular impulse. Notwithstanding, whereas fatigue induced a similar relative change in twitch torque across groups, it appears that the reasons underlying the weaker twitch responses may be different. DPN patients featured a smaller CMAP compared to baseline, whereas the mean CMAP from the control group was unchanged immediately following fatigue (Figure 6.4B). This finding could indicate, as mentioned earlier, neuromuscular transmission failure may have occurred in DPN patients contributing to a decrease in strength; whereas twitch torque depression in controls is likely primarily due to accumulation of force depressing metabolites (e.g. inorganic phosphate, H+ ions). Another consideration is DPN patients have been shown to potentiate to a lesser relative extent (~40%) compared to healthy controls, which may be related to muscle atrophy or changes in fibre type composition. As individuals with DPN benefit less from post-activation potentiation, they may be more subject to the force-depressing effects of fatigue without gaining as much from the competing force-increasing effects of potentiation during recovery. In summary, to recover from a maximal, sustained fatiguing contraction of short duration, it seems DPN patients may
need to regain both neuromuscular transmission fidelity, as well as the clearance of metabolites, as evidenced by prolonged half-relaxation times.

6.4.3 Summary

The present study reports an increased susceptibility to neuromuscular fatigue in patients with diabetic neuropathy compared to controls, during sustained maximal contractions. Given our results, we propose this DPN-related greater fatiguability may be partially attributed to neuromuscular transmission failure and possibly pathological alterations in muscle metabolism affecting cross-bridge force production. Although the DPN group performed approximately 50% less angular impulse (an isometric analogue of work) than controls, post-fatigue, evoked and voluntary muscle strength properties recovered in both groups similarly. These findings may have functional relevance underlying abnormal gait and increased fall risk in patients with DPN\textsuperscript{56,57}. Finally, DPN-related fatiguability may warrant consideration for the design and implementation of exercise-based rehabilitation strategies for this patient population.
References


CHAPTER 7

7 General Discussion and Summary

7.1 General Discussion

This thesis provided substantial novel experimental evidence regarding the effects of diabetic polyneuropathy (DPN) on the neuromuscular system in humans. The first half of this thesis focused on DPN-related changes at the motor axon and neuromuscular signal transmission level. Indeed, my results support the concept of a DPN-related loss of motor units (Chapters 2 & 3) and neuromuscular transmission instability with intermittent signal propagation failure (Chapter 4). Moreover, the second half of this thesis investigated how these alterations at the motor axon and neuromuscular transmission levels affect skeletal muscle properties and function. I reported the degree of motor unit loss is related to increased non-contractile intramuscular tissue and muscle slowing (Chapter 5); and DPN is associated with increased fatiguability in the context of a sustained maximal contraction, which may be related to neuromuscular transmission failure (Chapter 6). These findings (1) build upon the understanding of how DPN may impact the neuromuscular system; (2) help explain DPN-related functional limitations; and (3) eventually may prove useful in the diagnosis and progressive assessment of DPN-motor system involvement in clinically or rehabilitative settings.

The studies included in this thesis confirm and extend the understanding of how DPN may affect the neuromuscular system. It has long been understood that DPN can cause neuromuscular dysfunction, and it has been presumed part of this dysfunction was due to neuromuscular remodeling and the loss of motor axons\textsuperscript{1,2}. Indeed, this notion forms the basis for using compound muscle action potential (CMAP) amplitude, and needle electromyography (EMG)-detected motor unit potential (MUP) parameters as electrophysiological markers of neuropathy in DPN patients\textsuperscript{1,3,4}. Moreover, one previous study reported DPN may be associated with motor unit loss in the extensor digitorum brevis (EDB), an intrinsic muscle of the foot\textsuperscript{2}. In this thesis, the results in Chapters 2 and
3 extend the concept of motor unit loss in DPN by exhibiting reduced MUNE\textsubscript{s} in the tibialis anterior (TA) in DPN patients versus controls. This finding is important as it shows DPN can impact motor unit numbers in a more proximal muscle, with a more fundamental functional role, compared to EDB. Also, studying the TA rather than EDB is warranted because it may be difficult to ascertain whether EDB motor units are damaged due to neuropathic stressors or mechanical damage caused by the altered gait associated with DPN\textsuperscript{5,6}. Furthermore, the results presented in Chapter 3 indicate DPN-related motor unit loss and remodeling may follow a similar distal to proximal pattern of progression as other aspects of DPN\textsuperscript{7}.

This thesis also builds upon previous work suggesting DPN patients could be susceptible to neuromuscular transmission failure (Chapter 4). Various studies using animal models of DPN have found dysfunction in motor axons, the neuromuscular junction, and muscle fibres, all of which may contribute to failed action potential propagation\textsuperscript{8-11}. Additionally, in humans, Na+/K+ pump dysfunction and reductions in nodal Na+ currents have been suggested as having the potential to trigger conduction failure\textsuperscript{12,13}. In Chapter 4 of this thesis, I report intermittent conduction failure in the form of % blocking, as well as unstable neuromuscular transmission, supporting the concept that transmission failure may occur in patients with DPN. Moreover, this susceptibility to transmission failure could have functional implications, specifically with regards to a reduced capacity to sustain intense efforts, as presented in Chapter 6.

The effect, if any, of DM or DPN on motor unit or muscle fibre type composition is not clear\textsuperscript{14-18}. Previous studies have suggested there is an increase in the relative proportion of type II or fast motor units in animal models or patients with DM\textsuperscript{14,15,18}. However those results may not reflect the chronic effect of DPN, but rather the physical inactivity associated with the development of diabetes mellitus (DM). Additionally those studies may have been influenced by technical limitations associated with the procedures used to assess muscle fibre type\textsuperscript{19}. The results in Chapter 4 of this thesis indicate the sensible possibility that DPN preferentially targets type II motor units. This is evidenced by: slowed evoked contractile properties, reduced voluntary rates of torque development, and reduced motor unit firing rates compared to controls (Chapter 4). This can be
interpreted to indicate that in terms of neuromuscular changes, DPN acts like an accelerated form of human aging\textsuperscript{20}. However, these aforementioned DPN-related alternations could be caused by a host of other factors (e.g. reduced numbers of sarcomeres in series or increased tendon compliance reducing muscle speed), and further study is required to parse out the physiological causes underlying these results.

DPN patients often feature various functional limitations which may affect their ability to live independently, and decrease their quality of life. These can include: altered or slowed gait\textsuperscript{5,21,22}, impaired standing and dynamic balance\textsuperscript{23}, increased fall risk\textsuperscript{29}, increased risk of injurious falls\textsuperscript{24}, an inability to do housework\textsuperscript{25}, and difficulty climbing stairs\textsuperscript{25}. These functional limitations have been associated with the presence and severity of neuropathy\textsuperscript{26}, however it is important to consider that dysfunction in the different compartments of the nervous system may have inter-related, synergistic effects. For example, lower limb muscle weakness (secondary to motor unit loss)\textsuperscript{2,7}, in conjunction with loss of sensation\textsuperscript{27} and deficits in proprioception\textsuperscript{28} likely all play contributing roles in altered gait patterns and increased prevalence of falls\textsuperscript{29}. Furthermore, patients with DPN with substantial dorsiflexor weakness and impaired sensation are at increased risk of developing ‘foot drop’\textsuperscript{5,6,21} and their foot drop is more likely to cause them to trip and fall\textsuperscript{29–31}. As outlined in this thesis, the notion that DPN is associated with reduced muscle speed\textsuperscript{16,32}, and hence reduced muscle power\textsuperscript{32,33}, may further exacerbate the impaired functional capacity of DPN patients, beyond strength loss alone\textsuperscript{34}. Additionally, the increased susceptibility to neuromuscular fatigue may impact the ability of this group to engage in sustained or repeated activity (i.e. climbing up many flights of stairs, engaging in an exercise-based rehabilitation or training program)\textsuperscript{35,36}. For example, some of the findings described in this thesis, particularly loss of muscle strength, speed and endurance, should be taken into consideration when developing or managing preventative and rehabilitative training programs. For example, in terms of rehabilitative strategy, a training program for those with DPN may feature a greater emphasis on maintaining both muscle mass and muscle power to help prevent the functional decline outlined above\textsuperscript{34,37–40}. 
Given the nature and extent of the potential DPN-related impacts on the neuromuscular system, early diagnosis may be integral to forming the optimal plan for treatment and prevention. Some of the findings presented in this thesis may prove helpful in contributing to the early identification and longitudinal assessment of DPN, especially in the context of neuromuscular involvement (Chapters 2-4). In Chapters 2 and 3, DPN was shown to be associated with a loss of motor units, which was correlated with a loss of strength. Interestingly, the majority of patients with DPN included in these studies had CMAPs greater than 4.0 mV and thus would not have met the electrophysiological clinical threshold for pathological motor involvement of the TA. However, when examining individual TA MUNEs in this group compared to healthy age matched controls, the DPN group generally appeared to possess substantially fewer motor units despite maintaining a clinically ‘normal’ or ‘healthy’ sized CMAP. Between groups, TA MUNEs had a larger magnitude of effect (or effect size) than CMAPs or needle EMG-derived measures of neuromuscular pathology (Chapter 3). Additionally, the NF MUP parameters introduced in Chapter 4 may prove useful in an EMG clinic setting, particularly when higher intensity contractions are performed during a clinical needle EMG study, which may be desirable to allow for a MUNE calculation. However, it is important to note the comparisons performed in Chapters 3 and 4 between MUNE, NF MUP parameters and more standard electrophysiological markers of disease are somewhat limited and biased. The results used to represent standard MUP parameters, for example, were not obtained using the same protocol that would be used in the EMG clinic. Additionally, the DPN patient group studied throughout this thesis featured a more severe neuropathy, with a more drastic motor component, than what would be expected in patients in the very early stages of DPN. Thus further study is warranted to better evaluate the novel electrodiagnostic parameters described herein to determine their potential diagnostic and prognostic utility.
7.2 Limitations

Many of the conclusions throughout this thesis were made based upon results obtained using electrophysiological methods, particularly analysis of DQEMG. Some of these conclusions could have been better supported or confirmed had I included muscle biopsies in my studies. For example, the analysis of muscle biopsies could have: supported the idea of neuromuscular remodeling (via fibre type grouping); given insight into any shift of muscle fibre type predominance; or provided greater understanding of muscle quality (via the assessment of single myofibre properties). However, the inclusion of muscle biopsies was not practical for these experiments. Moreover, the majority of my projects focused on the dorsiflexor muscle group, especially the TA, which is not considered a convenient muscle to biopsy compared with the knee extensors, for example. Obtaining and analyzing vastus laterali biopsies would provide a spurious means for comparison with the TA, given the length-dependent nature of DPN, and the different properties and functions of these muscle groups. Additional methodologies that could have added further insight into these studies are magnetic resonance spectroscopy (MRS) or near-infrared spectroscopy (NIRS). The use of MRS or NIRS would have been particularly helpful in Chapter 6, the study of DPN and fatigue, as these might have elucidated the accumulation of metabolites (inorganic phosphate, H+ ions etc) and level of deoxygenation throughout the fatigue and recovery protocols. However, the inclusion of these techniques was not a practical or feasible addition with the design of these original studies.

Habitual physical activity levels play important roles in muscle strength, muscle speed, and perhaps the age-related rate of neuromuscular remodeling and motor unit loss. The DPN patient and control groups discussed in this thesis were not matched for physical activity levels, nor was physical activity level quantified using questionnaires, accelerometers or some other method. This must be considered when interpreting any of the results presented herein. If the control group was substantially more physically active than the DPN group, this difference could conceivably contribute to some of the divergence between the groups with respect to their neuromuscular properties and health. However, if the DPN group was substantially less active than the
controls, one would likely expect to find faster evoked muscle twitch properties in the DPN group often reported in models of disuse\textsuperscript{53,54} (Chapter 5). Rather, the twitch responses of the DPN patients were significantly slower than the controls (Chapter 5). Furthermore, anecdotally, the majority of patients with DPN appeared to maintain relatively ‘average’ physical activity levels for their age. Finally, although the control group may have engaged in more physical activity on a day-to-day basis than the DPN group, they were not engaged in systematic physical training and their activity levels likely were not so high as to cause a notable effect on neuromuscular properties.

Additionally, the mean participant ages were approximately 65 years old. The effects of natural adult aging on the neuromuscular system are thought to manifest detectably in most individuals in the 7\textsuperscript{th} decade of life\textsuperscript{55}. Thus, the majority of those in the DPN group studied in this thesis would not only be expressing DPN-related changes but also age-related changes to the neuromuscular system. This may affect the generalizability of the results obtained in this thesis, as DPN may differentially affect humans as they age, versus how DPN may affect a group of homogenously young participants. Furthermore, both experimental groups included similar equal numbers of males and females. Although the two groups were sex matched, the inclusion of both sexes may have introduced greater heterogeneity into our dataset. Unfortunately due to challenges with subject accrual, an insufficient number of DPN patients of both sexes in these studies precludes an examination of any sex-based differences in the effects of DPN on the neuromuscular system.

As mentioned earlier in this section, my conclusions regarding physiological mechanisms are generally derived from obligatory indirect measurements, including EMG, electrically evoked muscle properties, MRI, and dynamometry. In forming conclusions regarding DPN-related underlying changes to the neuromuscular system, such indirect measures are not necessarily ideal. However, although the specific mechanisms cannot be obtained with certainty using these techniques, this does not invalidate them or prevent their interpretation in the context of previous studies using animal models, reduced preparations or more invasive means in humans. Moreover, it
may emphasize the need for, and influence the direction of future studies which ultimately need to be explored in the human model.

7.3 Future Directions

There are a plethora of interesting and viable avenues for future research concerning the impacts of DPN on the neuromuscular system, the description of which will be necessarily somewhat limited in this section. Future studies may build upon my conclusion that DPN causes reduced motor unit firing rates and muscle slowing by recording maximal motor unit firing rates. This could be accomplished by recording motor unit firing rates intramuscularly using tungsten needle electrodes. Furthermore, tests of the force-velocity relationship, maximal contractile velocity and isotonic muscle power generation might greatly add to the understanding of DPN-related impacts on muscle function. This could be carried out using the isokinetic or, preferably, isotonic modes of a Cybex or Biodex multi-joint dynamometer. Additionally, such dynamic contractions used in the context of a fatigue protocol may allow for a better understanding of the limitations placed upon skeletal muscle affected by DPN. In conjunction with these measures, it would be helpful to include standard clinical measures of mobility or functional capacity, including sit-stand tests, shuttle walks, and tests of balance. Additionally, metrics, such as questionnaires, assessing physical activity history could provide useful information regarding the status of participants. Inclusion of such measures may help delineate the functional relevance of the various parameters tested in those with DPN in the neuromuscular laboratory.

To help identify and quantify the presence of neuromuscular transmission failure it would interesting to apply single motor unit recordings during sustained and fatiguing contractions in DPN patients. Including this measure could provide a more robust indicator of intermittent transmission failure of individual motor units, which would be more persuasive than relying upon the analysis of surface EMG or post-contraction CMAPs alone. Furthermore, this technique may be used during sustained, ramped contractions providing insight into the recruitment and de-recruitment patterns of patients
with DPN compared to controls. Repetitive nerve stimulation could also be used to test for neuromuscular transmission failure at rest or during fatigue.

Throughout this thesis, no limitations regarding voluntary activation during maximal efforts (including the fatigue protocol) were identified in controls or DPN patients. However, inclusion of transcranial magnetic stimulation (TMS) or cervicomедullary (CM) stimulation would provide further insight into any DPN-mediated changes at the supraspinal or spinal levels of excitability. Examining changes in the silent period following stimulation, or the amplitudes of motor evoked potentials (MEP) or cervicomедullary motor evoked potentials (CMEP) under various conditions would provide some insight into the influence of these important factors.

Finally, a longitudinal study of patients with DPN, including the neuromuscular parameters outlined in this thesis, could provide greater insight into the progression of DPN-related neuromuscular dysfunction. Concurrent assessments of the sensory and autonomic nervous system also would be helpful in determining how neuropathy progresses in the different aspects of the nervous system. Perhaps most interestingly, a longitudinal study would provide an opportunity to assess the ameliorative, restorative or preventative effects of exercise training. Previous studies have investigated the effects of resistance and/or aerobic training on other aspects of DM or DPN: including glycemic control, vascular function, development of neuropathy, autonomic dysfunction and balance and gait. However, the effects of training on neuromuscular impacts of DPN are less well understood, thus specifically it would be fascinating to assess how different training modalities and intensities affect the motor progression of DPN. In this context, to investigate how a resistance training regimen focused on training and restoring speed and power, in addition to strength, compares to a more traditional approach to exercise in DPN could be interesting. And finally, the potential neuroprotective effects of chronic exercise in DPN patients should be assessed.
7.4 Summary

Diabetic polyneuropathy (DPN) may cause many impactful physiological and functional alterations to the human neuromuscular system. My thesis consists of an integrated collection of novel and foundational studies concerning DPN. Specifically, it provides support for the importance of the progressive and accelerated loss of motor units underlying muscle atrophy and weakness in patients with DPN (Chapters 2 and 3). Indeed the accelerated loss of motor units is associated with the development of intramuscular non-contractile tissue, the loss of contractile muscle mass, and the slowing of muscle contraction (Chapter 5). In addition to the loss of motor units, my thesis indicates there is a loss of fidelity or stability in those motor units that remain (Chapter 4). This DPN-related neuromuscular instability may be associated with, and perhaps a precursor to, neuromuscular transmission failure during intense, sustained bouts of effort (Chapter 6). Overall these foundational explorations may help direct further useful studies and strategies to understand, and influence clinical support in those with DPN.
References


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Appendix A

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Tim Doherty  
Review Number: 18141  
Review Level: Full Board  
Approved Local Adult Participants: 60  
Approved Local Minor Participants: 0  
Protocol Title: Impacts of Diabetes on the Neuromuscular System in Humans  
Department & Institution: Clinical Neurological Sciences, London Health Sciences Centre  
Sponsor:  
Ethics Approval Date: August 17, 2011  
Expiry Date: May 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

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<td>including all instruments listed in section 8.1</td>
<td>2011/07/28</td>
</tr>
<tr>
<td>Letter of Information &amp; Consent</td>
<td>Healthy volunteers</td>
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This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB’s as defined in Division 5 of the Food and Drug Regulations.

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

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

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

This is an official document. Please retain the original in your files.
Appendix B

Custom-built hand apparatus used to study neuromuscular properties of the first dorsal interosseus muscle.
Curriculum Vitae

Matti D. Allen

Education:

Queen’s University: Aug 2014 – 2018 (expected)
Doctor of Medicine (MD)

The University of Western Ontario: 2011 – 2014
PhD Kinesiology: Clinical Neuromuscular Physiology
Thesis: Impacts of diabetic neuropathy on the neuromuscular system in humans
Advisor: Dr. Charles L. Rice, PhD

The University of Western Ontario: 2007 – 2009
MSc Kinesiology: Clinical Neuromuscular Physiology
Thesis: Effect of demyelinating ulnar nerve injury on strength and fatigue
Advisor: Dr. Timothy J. Doherty, MD, PhD

The University of Western Ontario: 2003 – 2007
BSc Honours Specialization in Kinesiology

Research Interests:

- Acute and chronic pathophysiological alterations to the nervous and neuromuscular systems as a result of neurological or neuromuscular disease
- Impacts of diabetes mellitus on the peripheral nervous system and skeletal muscle
- Effects of physical activity on the diseased or aging neuromuscular system
- Factors contributing to neuromuscular fatigue in static and dynamic activity
- Clinical and academic applications of electrodiagnostics and magnetic resonance imaging

Research Contributions

Summary
Articles in Peer Reviewed Journals: 17 (1st author: 8)
Abstracts in Peer Reviewed Conference Proceedings: 25
Published Commentaries (non-peer reviewed): 1
Submitted Manuscripts and/or Manuscripts in Preparation: 5 (1st author: 3)
Honours and Awards:

2014 – 2015  Ontario Graduate Scholarship (declined)
2013  Canadian Society for Exercise Physiology Poster, Doctoral poster competition winner
2013 – 2014  Ontario Graduate Scholarship
2012 – 2013  Ontario Graduate Scholarship
2012 – 2013  Queen Elizabeth Scholarship in Science and Technology (declined)
2012  UWO Kinesiology Graduate Board (KGB) Research and Service Award
2011  UWO Dean’s Honour Role
2010  Canadian Health Sciences Inquiry national commentary submission winner (Category A)
2007  UWO Dean’s Honour Role
2006  UWO Dean’s Honour Role
2004  UWO Dean’s Honour Role
Contributions to Research:

Articles Published in Refereed Journals


Presented and Published Abstracts


**Published Commentaries**


**Manuscripts under Review or Revision**


**Manuscripts in Preparation**


**Academic and Professional Experience:**

**Teaching Assistantships**

School of Kinesiology, The University of Western Ontario
Duties: Assist in teaching canoe skills and leading week-long canoe trip in Northern Ontario with approximately 30 Kinesiology undergraduate students

KIN 4430F/KIN 9430A: Exercise Physiology: Muscle Function and Metabolism (Fall 2011, 2013)
School of Kinesiology, The University of Western Ontario
Duties: Grading of assignments, providing teaching support after lectures and during office hours for undergraduate and graduate students

KIN 2230B: Introductory Exercise Physiology (Winter 2008, 2009)
School of Kinesiology, The University of Western Ontario
Duties: Instructing and supervising exercise physiology lab sections for undergraduate students; grading exams

**Academic Presentations**


1. **Allen MD** (2014) Nerves, muscles, aging and exercise: Are you fitter than a 95 year old? Bioscience Seminar, School of Kinesiology, University of Western Ontario, March 24th, 2014, London, ON, Canada

**Invited Academic Presentations**


Research Assistantships

Canadian Comprehensive Pain Program research assistant (2010 – 2014)
Toronto Western Hospital, University Health Network, Toronto, ON
Supervisor: Dr. Angela Mailis-Gagnon
Duties: Collected data through chart review; performed data analysis; assisted in writing of manuscripts

Musculoskeletal Imaging Lab research assistant (2010 – 2011)
Department of Medical Biophysics, University of Western Ontario, London, ON
Supervisor: Dr. Gregory D. Marsh
Duties: Analyzed MRI data; led writing of associated manuscripts

Neuromuscular Physiology research assistant (Winter/Spring 2011)
School of Kinesiology, University of Western Ontario, London, ON
Supervisor: Dr. Charles L. Rice
Duties: Assist senior PhD students in collecting data, led preparation and writing of manuscripts

Clinical Neuromuscular Physiology research assistant (Summer 2008)
Department of Clinical Neurological Sciences, London Health Sciences Centre, London, ON
Supervisor: Dr. Timothy J. Doherty
Duties: Troubleshoot malfunctioning equipment, recruit research participants, and collect data using electromyography and dynamometry techniques
External Research Consultations

McGill University Muscle Aging Diagnostics Laboratory (Fall 2012 – Summer 2013)
Primary Investigators: Dr. Russell Hepple and Dr. Tanja Taivassalo
Department of Kinesiology, McGill University, Montreal, QC
Duties: Perform advanced electrophysiological techniques (DQEMG) on special populations (older adults and older world class athletes); assist in synthesizing results with other data, writing relevant manuscripts

UWO Molecular Biology and Exercise Laboratory (Summer 2012)
Primary Investigators: Dr. Kevin Shoemaker and Dr. Earl Noble
School of Kinesiology, University of Western Ontario, London, ON
Duties: Perform motor nerve conduction studies on rodents to assess the impact of diabetes and exercise on nerve health

UWO NeuroBehavioral Laboratory (Winter 2011 – 2012)
Primary Investigator: Dr. Matthew Heath
School of Kinesiology, University of Western Ontario, London, ON
Duties: Assist electrical engineering graduate student (Ahmed Ali) in the development of a custom-designed electromyography system using MATLAB software and pre-amplified electrodes

University Hospital – LHSC: Clinical Neuromuscular Physiology Laboratory (Winter 2011 – Spring 2012)
Primary Investigators: Dr. Tim Doherty and Dr. Tom Overend
School of Physical Therapy, University of Western Ontario, London, ON
Duties: Instruct PhD candidate (Anuradha Sawant) in the application of clinical electrophysiological techniques and dynamometry in patients on dialysis

Professional Service:

Editor Positions

Managing Editor (News Department): Canadian Health Sciences Inquiry, 2011 – 2013
Duties: Led selection of News Department staff (~12 personnel); coordinated news article topic selection with Reporters; provided writing assistance and held ultimate editorial discretion on all news articles
Journal Peer Reviewer

*Muscle and Nerve*, since 2010 (6 manuscripts to date)

*Clinical Neurophysiology*, since 2014 (1 manuscript to date)

*American Journal of Physical Medicine and Rehabilitation*, since 2014 (1 manuscript to date)

*Physiotherapy*, since 2014 (1 manuscript to date)

*Physiotherapy Canada*, since 2012 (1 manuscript to date)

Elected Positions


Duties: Lead the interests of Kinesiology students to the SOGS University council and to discuss issues and aid in decision-making that affect graduate students across the University

Graduate Teaching Assistant (GTA) Union Representative (2011-2012)

Duties: Represent graduate student teaching assistants and address their concerns through their union (Public Service Alliance of Canada Local 610)

Committee Memberships


School of Graduate and Post-Doctoral Studies, University of Western Ontario

Duties: Organized and helped run UWO’s annual multi-disciplinary research forum (Western Research Forum); led fundraising for the Western Research Forum (secured ScotiaBank as a sponsor)
Professional and Academic Society Memberships

Ontario Medical Association (OMA)
   Student Member (2014 – present)

Canadian Medical Association (CMA)
   Student Member (2014 – present)

Canadian Association of Physical Medicine & Rehabilitation (CAPM&R)
   Student Member (2014 – present)

American Physiological Society (APS)
   Student Member (2013 – present)

Society for Neuroscience (SfN)
   Student Member (2013 – present)

Peripheral Nerve Society (PNS)
   Trainee Member (2012 – 2014)

American College of Sports Medicine (ACSM)
   Student Member (2011 – 2013)

Canadian Society of Exercise Physiology (CSEP)
   Graduate Student Member (2011 – present)