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Effect of a neuromuscular electrical stimulation muscle strength training intervention on muscle force and mass, physical health and quality of life in people with spinal cord injury

Vanesa Bochkezanian
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Effect of a neuromuscular electrical stimulation muscle strength training intervention on muscle force and mass, physical health and quality of life in people with spinal cord injury

This thesis is presented for the degree of

Doctor of Philosophy

Vanesa Bochkezanian

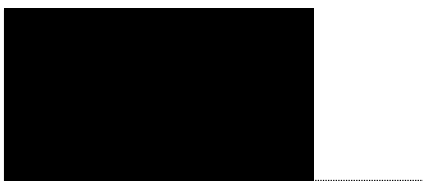
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School of Medical and Health Sciences
2017

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Abstract

Spinal cord injury (SCI) leads to significant deficits in muscle strength and mass, impacting negatively on physical health and quality of life (QoL). Physical rehabilitation techniques for people with SCI rely on constant updates and the accumulation of evidence regarding the efficacy of available and/or new physical interventions. Neuromuscular electrical stimulation (NMES) is already commonly used to activate skeletal muscles and subsequently reverse muscle atrophy, however NMES as a high-intensity “strength training” intervention appears to be a particularly promising technique for increasing muscle strength and mass and to subsequently improve physical health and quality of life (QoL) in people with SCI. Nonetheless, there are many factors limiting the use of standard NMES protocols, and further evidence pertaining to the use of high-intensity NMES strength training in clinical populations is warranted. The primary aim of the research described in this thesis was to examine the effects of NMES as a high-intensity muscle strength training intervention, specifically using wide-pulse width (1000 μ s), low-to-moderate frequency (30 Hz) NMES combined with tendon vibration, on muscle strength and mass, physical health, symptoms of spasticity and QoL in people with SCI. This thesis includes two cross-sectional studies examining the effects of patellar tendon vibration (55 Hz, 7 mm amplitude) superimposed onto wide-pulse width (1000 μ s) NMES (e.g. 30 Hz over 2 s) on the peak muscular (knee extensor) force and total impulse elicited by, and rate of recovery from, the intervention in healthy subjects (Study 1) and in people with chronic SCI (Study 2). The results of Study 1 revealed that superimposing tendon vibration onto wide-pulse width NMES leads to an increase in the muscle work performed before fatigue in only some individuals (i.e. positive responders, 50% of individuals in the current study), but decreases it in others (i.e. negative responders). However, it tends to reduce the voluntary force loss that was consistently experienced after a training session using high-intensity NMES, and may thus allow for additional exercise or rehabilitation work to be performed without ongoing voluntary muscle fatigue in healthy people. The results of Study 2 also identified positive and negative responders to tendon vibration in people with SCI, however the responses were less clear and a defined effect of tendon vibration superimposed onto NMES was not discerned. In Study 3, a 12-week (twice-weekly) high-intensity NMES strength training intervention was implemented in people with chronic SCI; based on results of Study 2, high-force contractions were evoked by NMES without superimposed tendon vibration. A significant increase in muscle mass (45%) and strength (tetanic evoked force; 31.8%), amelioration of spasticity symptoms, and improvement in some aspects of physical health and QoL were observed. Therefore, the use of high-intensity NMES strength training appears to be an effective rehabilitation tool to increase muscle force and mass, ameliorate symptoms of spasticity and improve physical and mental health outcomes in people with SCI.

List of publications

The following chapters have been accepted to be published in academic journals and have been or will be published at local and international conferences:

Chapter three (Study 1)

Bochkezanian, V., Newton, R.U., Trajano G.S., Vieira, A., Pulverenti, T., Blazeovich, A.J. (2017) Effect of tendon vibration during wide-pulse neuromuscular electrical stimulation (NMES) on the decline and recovery of muscle force. *BMC Neurology*, 17(1), 82. DOI: 10.1186/s12883-017-0862-x

Chapter three and four (Studies 1 and 2)

Bochkezanian, Newton R.U, Blazeovich, A.J. Effect of patellar tendon vibration superimposed on wide-pulse width neuromuscular electrical stimulation on quadriceps contractile impulse in uninjured individuals and people with spinal cord injury. 9th World Congress for NeuroRehabilitation (WCNR 2016); 5/2016.

Bochkezanian, Newton R.U, Blazeovich, A.J. Effect of patellar tendon vibration superimposed on wide-pulse width neuromuscular electrical stimulation on quadriceps contractile impulse in uninjured individuals and people with spinal cord injury. Annual Scientific Meeting of the Australian and New Zealand Spinal Cord Society (ANZSCoS) 2015, University of Western Australia (UWA), Perth, Australia; 11/2015.

Chapter five (Study 3)

Bochkezanian, Newton R.U, Trajano G.S., Blazeovich, A.J. Can a novel 12-week wide-pulse width NMES strength training intervention improve quadriceps muscle size, strength and spasticity in people with SCI? 11th Annual Congress of the International Society of Physical and Rehabilitation Medicine (ISPRM 2017). Buenos Aires, Argentina. 1-5th May, 2017.

Bochkezanian, Newton R.U, Trajano G.S., Blazeovich, A.J. Can a novel 12-week wide-pulse width NMES resistance training intervention improve quadriceps muscle size and strength in people with SCI? The 55th International Spinal Cord Society (ISCoS) Annual Scientific Meeting, Vienna, Austria. The attendance to this Conference was supported by Neurotrauma Research Program- Harry Perkins Institute of Medical Research travel grant; 9/2016.

Bochkezanian, Newton R.U, Trajano G.S., Blazeovich, A. Can a novel 12-week wide-pulse width NMES resistance training intervention improve quadriceps muscle size and strength in people with SCI? 3rd Annual Western Australian Sports and Exercise Physiology Research Symposium, University of Western Australia, Perth, Australia; 6/2016.

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List of Abbreviations

1H	One hour after the Intervention
48H	Forty-eight hours after the Intervention
ABRT	Activity-based restorative therapies
AD	Autonomic Dysreflexia
ANOVA	Analysis of variance
AIS	ASIA Impairment Scale
ASIA	American Spinal Cord Injury Association Impairment Scale
BMC	Bone mineral content
BMD	Bone mineral density
Ca²⁺	Calcium
ICC	Intra-Class Correlation
ICF	International Classification of Functioning, Disability and Health
CRP	Plasma C-Reactive Protein
CSA	Cross-sectional area
CSA_Q	Cross-sectional area quadriceps femoris
CNS	Central nervous system
CT	Computed tomography
DXA	Dual-energy X-ray absorptiometry
e.g.	exempli gratia (for example)
EI	Echo-intensity
EFOV	Extended field-of-view technique
EMG	Electromyography
FES	Functional electrical stimulation
HDL-C	High-density lipoprotein levels
i.e.	id est (that is)
IL	Interleukin
IMF	Intra-muscular fat
K⁺	Potassium
LDL-C	Low-density lipoprotein levels
LL-LBM	Lower limb- lean body mass
MANCOVA	Multiple analysis of variance

MRI	Magnetic resonance imaging
MVIC	Maximal voluntary isometric contraction
Na⁺	Sodium
NA	Noradrenaline
NMES	Neuromuscular electrical stimulation
$\tau_{tw,p}$	Maximal evoked force
$\tau_{tw,sub}$	Submaximal evoked force
PAR-Q	The Physical Activity Readiness Questionnaire
PRE	Before the intervention
POST	After the intervention
PIC	Persistent inward currents
PSSI	Polar strength-strain index
pQCT	peripheral Quantitative Computed Tomography
RF	Rectus femoris
QoL	Quality of life
SCI	Spinal cord injury
SCI-SET	Spinal Cord Injury-Spasticity Evaluation tool
5-HT	5 –hydroxytryptamine or Serotonin receptor
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
STIM	Neuromuscular electrical stimulation (wide-pulse width)
STIM+Vib	Neuromuscular electrical stimulation (wide-pulse width) superimposed onto tendon vibration
TVR	Tonic vibration reflex
TTI	Torque-Time Integral
Vib	Tendon vibration
QF	Quadriceps femoris
VL	Vastus lateralis
VM	Vastus medialis
wk	Week
WHO	World Health Organisation

Dedication

To my parents, Elba and Norberto Bochkezanian. I hope this work will contribute back for all the love and dedication you have given me. I am forever grateful, I am who I am thanks to you!

Dedicado a mi mama y papa: Elba y Norberto Bochkezanian. Espero este trabajo retribuya un poco de todo el amor y dedicacion que me han dado siempre. Les agradezco infinitamente, soy lo que soy gracias a Uds! Los amo!!

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To the reader:

I hope you find this thesis interesting and relevant to the field of neurological rehabilitation. I hope this PhD thesis becomes the first step of more to come, so we can continue improving the lives of people living with a neurological condition. This is my humble work and I hope you enjoy it as much as I did.

Chapter 1 Introduction

1.1 Overview

This doctoral thesis contains three research studies with the underlying focus of examining the efficacy of neuromuscular electrical stimulation (NMES)-based muscle “strength training” with and without superimposition of tendon vibration for improving quadriceps femoris muscle force and mass, physical health, symptoms of spasticity and quality of life (QoL) in people with spinal cord injury (SCI). All studies included in this thesis were controlled experimental studies. The first two studies were cross-sectional studies and aimed to examine the individual and cumulative effects of trains of NMES (e.g. 30 Hz over 2 s, 1000 μ s) and blocks of tendon vibration (55 Hz, 7 mm amplitude) on the peak force and total impulse prior to ‘fatigue’ as well as the rate of recovery of force in healthy subjects (Study 1) and in people with chronic spinal cord injury (SCI; Study 2). Specifically, Study 1 was developed in healthy people to obtain feedback about pain and discomfort, which may cause adverse effects in people with SCI. Study 2 was then developed to determine whether tendon vibration superimposed onto wide-pulse width NMES under standard clinical conditions elicits a greater peak muscle force with less muscle fatigue (i.e. a greater total impulse) when compared to NMES applied without tendon vibration in people with SCI. In study 3 a 12-week high-intensity NMES strength training intervention, with the protocol characteristics being determined after consideration of the results of Study 2, was implemented in people with chronic SCI. This study examined the effects of NMES strength training using a low-to-moderate-frequency wide-pulse width (30 Hz and 1000 μ s) NMES intervention (i.e. standard clinical conditions) on muscle force and mass, physical health, symptoms of spasticity and QoL in people with SCI. Improving musculoskeletal function can impact on functional activities and physical health and thus improve QoL in people with SCI.

1.2 Background

Spinal cord injury (SCI) leads to dramatic deficits in muscle strength and mass, impacting negatively on the physical health and QoL of people with SCI (Hosseini et al., 2012; Tewarie et al., 2010). Among the various physical exercise interventions, strength training has been proven to enhance longevity and QoL in clinical populations (Andrade & da Silva, 2015; Caserotti et al., 2008; Clark & Goon, 2015; Orlando et al., 2016). Moreover, muscle strength improvements can be partly explained by increasing the quantity (i.e. absolute muscle volume) and quality (i.e. muscle/intra-muscular adipose content) of limb muscle mass (Schaap et al.,

2009; Srikanthan & Karlamangla, 2014). However, strength training represents an increasing challenge for people with limited ability, or are unable, to voluntarily activate their muscles, such as people with SCI. Thus, the use of neuromuscular electrical stimulation (NMES) as a strength training method appears to be a promising method for increasing muscle strength and mass and improving physical health and QoL in people with SCI (Gorgey, Mather et al., 2012; Ho et al., 2014; Mahoney, Bickel et al., 2005). However, many limiting factors, such as the rapid onset of fatigue caused by higher-intensities of NMES, impede the development of NMES as a strength training tool in the rehabilitation setting (Binder-Macleod & Snyder-Mackler, 1993). Therefore, the adaptations evoked by NMES when used as a strength training method, and more specifically the use of high-intensity NMES (hereafter used to refer to near maximal muscle evoked contractions) on muscle strength, mass, physical health and QoL in people with SCI needs to be further examined. Clinical recommendations for NMES propose the use of short pulse widths (100-200 μ s) and low-to-moderate pulse frequencies (30-50 Hz) (Allen & Goodman, 2014). However, these NMES characteristics cause rapid muscle fatigue due to the (non-physiological) high stimulation intensities required to elicit higher muscle forces as well as the non-orderly recruitment of motor units (Hortobagyi & Maffiuletti, 2011). The use of wide pulse widths (1000 μ s) might optimise motor unit activation through activation of spinal reflex pathways, and thus delay the onset of muscle fatigue when compared to standard clinical NMES (Collins, 2007; Collins et al., 2001). The use of long pulse durations aim to mimic voluntary contractions through the use of Ia afferents (reflex pathways) and that could potentially reduce peripheral muscle fatigue. This may produce less fatigue in motor units that are active (Gorgey et al, 2009) and also through an additional central response by the activation of persistent inward currents (PICs) (Collins et al, 2007).

Another method to increase muscle force production whilst potentially minimising muscle fatigue in people with SCI is tendon vibration (Cotey et al., 2009; Ribot-Ciscar et al., 2003), especially when coupled with wide-pulse width NMES (Magalhaes & Kohn, 2010). Superimposing patella tendon vibration onto the wide-pulse width NMES is speculated to elicit further increases in the contractile impulse evoked before significant fatigue by triggering an increase in the recruitment of motor units through sustained depolarisation of the motor neurone, leading to higher muscle forces being produced between trains of electrical stimuli (i.e. self-sustained motor unit firing) particularly of low-threshold, fatigue resistant motor units (Magalhaes & Kohn, 2010; Trajano et al., 2014). However, research is needed to explicitly test the hypothesis that superimposing tendon vibration onto high-intensity NMES produces higher levels of contractile force (higher stimulation intensities and isometric contractions, using wide-pulse width NMES), improves muscle force production after a period

of training, and increases the total muscle work performed prior to fatigue before it is implemented as an exercise training technique in people with SCI (Study 3).

1.3 Significance of the Research

This research will provide evidence for or against the use of NMES as a high-intensity strength training modality, including the potential beneficial effects of simultaneous tendon vibration, as a rehabilitation method for the purposes of increasing muscle force production and mass, ameliorating symptoms of spasticity, and improving physical health and QoL outcomes in people with chronic SCI. This research will contribute to a better understanding of the adaptive changes resulting from the use of high-intensity NMES- driven muscle strength interventions, particularly with regards to muscle strength and mass and physical health, symptoms of spasticity and QoL in people with SCI. The knowledge gained will assist health practitioners to prescribe safer and more effective neuromuscular electrical stimulation and tendon vibration muscle strength training interventions in clinical populations.

1.4 Purpose of the research

The overall purpose of the research presented in this thesis is to provide evidence with respect to the effects of NMES as a strength training modality (i.e. 30 Hz, 1000 μ s) with and without superimposing tendon vibration. The research will specifically examine the effects of superimposing tendon vibration onto high-intensity NMES on peak muscle force and the total impulse (force developed over time) evoked prior fatigue (defined as a 40% peak force loss) (Studies 1 and 2). Subsequently, the ‘best’ protocol identified in studies examining the above will be used as part of a 12-week strength training intervention to determine its effects on muscle cross-sectional area and bone mineral density, systemic inflammation and lipid profile, symptoms of spasticity and QoL in people with chronic SCI (Study 3).

1.5 Research questions and hypothesis

The research hypotheses tested in the three studies contained within this PhD thesis are as follows:

1.5.1 Study one (Chapter 3)

Question 1: What are the individual and cumulative effects of (1) a series of NMES trains (each 30 Hz over 2 s, 1000 μ s) and (2) superimposing tendon vibration (55 Hz, 7 mm amplitude) onto NMES, on the peak force and total impulse evoked prior to ‘fatigue’ and the

rate of recovery of force (measured by the MVIC, US and VAS scales taken immediately after, 1 h and 48 h after the implementation of the NMES protocols) after the intervention in healthy individuals?

Hypothesis 1: The use of tendon vibration superimposed onto wide-pulse width NMES (1000 μ s) will elicit a greater peak muscle force with less fatigue (i.e. an increase in torque-time integral, a faster rate of force recovery and less muscle soreness and damage) than NMES imposed without tendon vibration. Tendon vibration alone will elicit very low muscle forces.

1.5.2 Study two (Chapter 4)

Question 2: What are the effects of superimposing tendon vibration onto wide-pulse width NMES (as done in Study 1) on the peak force and total impulse prior to fatigue in people with chronic SCI?

Hypothesis 2: The superimposition of tendon vibration on wide-pulse width NMES will increase muscle force production, increase total impulse (i.e. reduce the rate of force decline or increase in sustained contractions between NMES-evoked contractions) and allow faster force recovery in people with chronic SCI.

1.5.3 Study three (Chapter 5)

Question 3: Does a 12-week high-intensity NMES-strength training intervention (frequency = 30 Hz, pulse width = 1000 μ s), performed with the protocol determined to be optimum after consideration of the results of study 2, provide meaningful benefits including increases in muscle force production and mass, amelioration of the symptoms of spasticity and improvements in physical health and QoL in people with chronic SCI?

Hypothesis 3: A 12-week high-intensity NMES-strength training intervention using wide-pulse width NMES, imposed with the protocol characteristics being determined after consideration of the results of study 2, will increase muscle force production and mass, ameliorate symptoms of spasticity and improve physical health and QoL in people with chronic SCI.

Chapter 2 Literature review

Spinal Cord Injury (SCI)

2.1 Introduction

People with neurological conditions, such as spinal cord injury (SCI), need physical rehabilitation to regain function and independence and to improve their physical health and QoL (World Health Organization, 2017). Physical rehabilitation consists of a set of interventions designed to optimise physical function and reduce disability in individuals with a health condition when interacting with their environment (World Health Organization, 2017). These physical interventions address impairments, activity limitations, participation restrictions and environmental factors, that have an impact on functioning (World Health Organization, 2017). The global need for physical rehabilitation is predicted to increase (World Health Organization, 2011), and it has been proven to be highly efficient in improving clinical outcomes by enhancing function and QoL in people with a neurological condition, such as SCI (Lemmi, Gibson et al., 2015). However, physical rehabilitation relies on constant updates and growing evidence regarding the efficacy of available and/or new physical interventions and assistive technologies to improve clinical outcomes, function and QoL (World Health Organization, 2017).

2.2 Prevalence and burden

SCI is a debilitating health condition that occurs worldwide (van den Berg et al., 2010). The prevalence rate of SCI in Australia was 681 per million of population over the period from 1986 to 1997 (O'Connor, 2005). The incidence of traumatic spinal cord injuries in Australia was reported to be 15 cases per million in 2007 (Jazayeri et al., 2015) with 362 new SCI cases reported between 2007 to 2008 (AIHW, Norton, 2010). If the injury rate continues, based on age-specific incidence rates, the predicted prevalence of SCI will be 11,871 by 2021 (O'Connor, 2005). Consequently, healthcare costs related to SCI are estimated to increase significantly (Walsh, 1988). Burden of disease describes the impact of a health problem on an area measured by financial costs, mortality, morbidity and other indicators (World Health Organization, 2004), and SCI represents a growing burden of disease with increasing healthcare costs in Australia and worldwide (Begg et al., 2008). Lifetime costs of a SCI were reported to be \$2.0 billion in 2010 in Australia and were expected to grow in the following years (Collie et al., 2010). Total healthcare costs for people with SCI were calculated to be \$201,145 in the first 6 years after the

incident (Collie et al., 2010). Thus, SCI produces extremely high social and economic costs, which warrant extensive research into physical rehabilitation to improve the levels of functioning in people with SCI and reduce the healthcare costs.

2.3 Classification and causes of SCI

A SCI is a lesion to the spinal cord caused by a trauma, most commonly a motor vehicle trauma or a fall or work-related injury (Harvey, 2008). Other causes include sport and water-based activities and war-related injuries. It can also be the consequence of a disease (including congenital) or infection (Harvey, 2008). SCI interrupts the connection between the brain and upper spinal regions and the lower spinal regions and the periphery (Tewarie et al., 2010). This reduces the voluntary activation of the muscles below the lesion level, which can reduce muscle force production (and thus impair physical function) and profoundly compromise physical health and QoL (Hosseini et al., 2012; Oyster et al., 2011; Tewarie et al., 2010).

This reduction in muscle force can be explained by the two phases of the various clinical symptoms observed after suffering a SCI. These two phases are an initial phase initiated by the mechanical trauma to neurones, glial cells and the surrounding vasculature, and a second (expansive) phase resulting from the invasive degeneration of the surrounding spinal cord tissue (Sandrow-Feinberg & Houle, 2015). Some of the events that occur at the cellular level include apoptosis of neurones and oligodendrocytes, axon retraction, glial scarring with recruitment of inflammatory cells and demyelination and aberrant sprouting of spared nerve fibres (Fitch & Silver, 2008; Jones et al., 2005). There are also alterations to the electrophysiological properties of the neurones due to excitotoxicity and a release of pro-inflammatory factors (Jones et al., 2005).

The various clinical presentations after a SCI are based on the severity and degree of motor and/or sensory deficits and the location and extent of damage to the spinal cord tissue. One classification is based on the body part that is affected and classifies into tetraplegia and paraplegia. Tetraplegia refers to an injury that affects the cervical region of the spinal cord and all four limbs are affected, whereas paraplegia refers to an injury in the thoracic, lumbar and sacral areas and only the lower limbs are affected (Kirshblum et al., 2011). Another important classification is based on the level(s) of injury in the spinal cord and this is expressed as cervical, thoracic, lumbar or sacral, followed by the vertebral number, for example C₆ or T₁₁. Finally, SCI can be classified into “complete” and “incomplete” lesions depending on the clinical presentation and severity of the injury. A “complete” spinal injury classification means that all functions (motor and sensory) below the injured area are completely affected, usually due to a transection through the spinal cord resulting in a complete interruption or separation

between spinal segments. An “incomplete” injury refers to some or all functions below the injury being unaffected due to preservation of motor and sensory function and is usually the result of a contusion or bruising of the spinal cord, with some tissue sparing (Maynard et al., 1997). This classification is clinically determined based on the ability of the person with SCI to contract the anal sphincter voluntarily or to feel a pinprick or touch around the anus (sacral segments S₄₋₅) (Kirshblum et al., 2011). This is because the nerves in this area are connected to the very lowest region of the spine, the sacral region, and retaining sensation and thus function in these parts of the body indicates that the spinal cord is only partially damaged, this is classified as an incomplete lesion (Ho et al., 2007). The most used method of SCI classification is called The American Spinal Injury Association (ASIA) impairment scale, which is one of the preferred tools used widely among clinicians (El Masry et al., 1996). The ASIA scale divides spinal cord injuries into five categories, with optional clinical syndromes:

- A - complete
- B - incomplete: sensory but not motor function is preserved below the neurological level and includes the sacral segments S₄₋₅
- C - incomplete: motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3 strength
- D - incomplete: motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more strength
- E – normal sensory and motor function

After the initial trauma, there is at least some spontaneous recovery in an incomplete SCI. This recovery is evidenced by adaptive changes or plasticity observed as a change in neuronal properties (Lee et al., 2007), collateral sprouting (Fouad & Tetzlaff, 2012), changes in cortical maps (Dietz & Fouad, 2014) or changes to spinal networks associated with the central pattern generator (Edgerton et al., 2004). The degree of spontaneous recovery is difficult to predict, but can be estimated based on the level and extent of the injury. However, this recovery is not substantial and additional physical interventions are needed to maximise functional recovery in all grades of severity of spinal cord lesion. Although functional recovery may not be possible in AIS A with the present advances in science and technology, muscles need to be activated to increase the chances to utilise future technologies that may allow people with different levels of lesion in the spinal cord to have some of their functional activities restored or improved. Moreover, the latest research in neuroplasticity shows that the combination of therapeutic strategies, such as locomotor training and electrical stimulation can induce biochemical changes at the cellular level of the spinal cord correlating with sensorimotor recovery in people with complete levels of SCI (AIS A) (Edgerton et al., 2004). Therefore, interventions are needed to optimise recovery in all levels of injury, including complete lesions (i.e. AIS scale A).

2.4 Neuromuscular changes after SCI

After suffering a SCI, depending on the completeness of the lesion, the volitional (afferent and efferent) drive from the central nervous system (CNS) to the spinal motor neurones located below the level of injury is decreased or absent due to axonal disruption and/or demyelination (Dimitrijevic et al., 2012; Thomas et al., 2014). Specifically, the spinal segments may not receive any descending and/or ascending inputs, leading to a significant loss of motor and sensory function (Dimitrijevic et al., 2012; Thomas et al., 2014). Different clinical presentations following SCI are mostly explained by alterations of motor, sensory control due to total or partial disconnection between brain and spinal cord and a disruption of the autonomic nervous system (Kern et al., 2005). The connection between brain and spinal cord is driven through ascending and descending neurological tracts (Marieb & Hoehn, 2010). The pyramidal tracts originate within the motor cortex (area 4 posterior portion of frontal lobe, precentral gyrus) and pass through the spinal cord within the corticospinal tracts. The spinal cord carries not only motor and sensory nerves but also autonomic nerves, so dysfunctions after SCI are also accompanied by autonomic and sensory dysfunction. Sympathetic nerves exit the vertebral canal via thoraco-lumbar spinal nerves, and parasympathetic nerves exit via craneo-sacral spinal nerves, the cranial aspect is through the vagus cranial nerve (nerve number 10) (Marieb & Hoehn, 2010). Consequently, patients with cervical lesions lose supraspinal control of the entire sympathetic nervous system and of the sacral part of the parasympathetic nervous system (Harvey, 2008). Therefore, many neuromuscular changes occur after the initial mechanical spinal cord injury.

Among the neuromuscular changes, a reduction or lack of muscle activation is the most predominant one that needs to be addressed by physical interventions. After the primary mechanical injury, a cascade of secondary injury processes produce further loss of tissue and function (Yang et al., 2005), with an upregulation of pro-inflammatory cytokines, which may result in ion channel (Na^+ and K^+) conductance pathogenesis and demyelination (Kuwabara et al., 1999; Waxman, 1998). There is also an axonal dysfunction induced by SCI, which involves multifactorial complex interactions between ischaemia and decentralisation, with a subsequent inactivity and disuse atrophy (Lee et al., 2015). Thus, motor neurons and roots are affected two to four segments below the level of injury and myelomalacia may be developed due to ischaemia (Lin et al., 2007). Mostly because of loss of motor control and a dysfunction in the sympathetic nervous system, there is reduction in voluntary muscle activation which can affect the skeletal muscle tissue and directly affect the muscle force production (Tewarie et al., 2010) in people with SCI.

2.4.1 Changes in skeletal muscle tissue after SCI

Many changes in skeletal muscle tissue occur after SCI and this affect muscle force production. The loss of muscle force production is evidenced by the low levels of muscle force and work output in people with SCI (Greve et al., 1993; Levy et al., 1990; Rabischong & Ohanna, 1992). The altered muscle performance resulting from a decreased or absent muscle activation leads to dramatic changes in muscle properties (Biering-Sorensen, Kristensen et al., 2009). Muscle wasting or muscle atrophy, which is evidenced by the loss of muscle mass, is a large contributor to this altered muscular performance. The loss of muscle mass and force may be strongly related to the loss, or reduction in size and/or number, of muscle fibres (Malisoux et al., 2007), and loss of functioning motor units (Hunter & Ashby, 1984). The reduction in muscle size is observed as a smaller fibre cross-sectional area (CSA) found in the muscles below the level of injury, in particular in functionally important muscles such as the quadriceps femoris (Bajd et al., 1989; Greve et al., 1993; Martin et al., 1992). By way of example, the CSA of the thigh was reported to be 33% smaller at 6 weeks post-injury than controls (Gorgey & Dudley, 2007). There was also a 26% increase in intra-muscular fat after 12 weeks, indicating that the remaining muscle volume may contain less contractile tissue; this atrophy is less pronounced in incomplete compared to complete SCI (Shah et al., 2006).

The loss of muscle mass can be present as denervation atrophy or disuse atrophy resulting from a reduced number and size of the muscle fibres that remain. Denervation atrophy is the consequence of an injury to motor neurones in the spinal cord or the motor nerves (Allen et al., 2008) situated in the ventral roots from where they exit; whilst disuse atrophy is the result of muscle activation loss after disruption to the central and segmental synaptic drive onto the surviving spinal motor neurones (Gordon & Pattullo, 1993; Solandt & Magladery, 1942). The cell bodies of the motor neurones may be fatally damaged and ventral and dorsal roots may be indirectly injured, thus the muscles supplied by those roots may suffer denervation. However, there is often only a small proportion of denervated muscle(s) after a spinal cord injury. The most common form of atrophy is disuse atrophy, which is the consequence of muscle inactivity due to the loss of synaptic input from central pathways and from spinal cord segments to spinal motor neurones (Solandt & Magladery, 1942). This muscle atrophy is often specifically attributed to the transformation into type II (fast glycolytic) fibres of the motor neurone paralysed muscles (Burnham et al., 1997). This transformation into fast fatigable fibres commences in the early stages of the injury (Lotta et al., 1991). During the first months after the injury type IIa fibre atrophy is predominant, followed by type I fibre atrophy and their transformation into IIx type fibres in the later stages (Lotta et al., 1991; Scelsi et al., 1982). This transition from slow (type I) to fast fibres (first IIa and later to IIx) has been attributed to muscle unloading, denervation and cross-reinnervation (Pette & Staron, 2000). As an example,

fibre transformation into type II starts between 7 to 9 months after injury in the quadriceps muscle (Biering-Sorensen et al., 2009).

The loss of muscle mass can subsequently trigger alterations in metabolic and contractile protein profiles (Andersen et al., 1996; Crameri et al., 2002; Rochester et al., 1995). Some metabolic alterations, such as a reduced oxidative metabolic capacity, result from lower levels of succinate dehydrogenase activity and decreased capillary/fibre ratio, with a shift towards a fast-glycolytic metabolic profile (Martin et al., 1992; Rochester et al., 1995). There is also an activation of protein degradation and a decrease in signalling networks to protect muscle degradation in the days post-SCI (Urso, et al., 2007). Therefore, muscle atrophy, which is related to the loss of activation and subsequent unloading and alterations in fibre length and composition, could impair the force generating capacity with detrimental consequences for people with SCI (Castro et al., 1999). This reduction of muscle force can increase muscle fatigue due to (a) preferential atrophy (or complete loss) of type I (slow) fibres, which are more capable of maintaining an energy balance over repeated contractions (Martin et al., 1992; Sargeant, 1994), and (b) the requirement for the remaining musculature to work at a higher proportion of its maximum during submaximal activities. The increased susceptibility to muscle fatigue in people with SCI can clearly result from a reduced number of fatigue-resistant motor units in the paralysed muscles, which reduces the oxidative capacity of muscle (Gordon & Pattullo, 1993; Martin et al., 1992; Roy et al., 1991). This ultimately affects the motor output necessary to perform functional activities, such as transfers from the bed to the wheelchair (Noreau et al., 1993) in people with SCI. Therefore, people with SCI typically produce lower voluntary and electrically evoked muscle forces and experience a faster rate of muscle fatigue in comparison to uninjured individuals.

2.4.2 Skeletal muscle fatigue in SCI

Multiple factors can influence the muscle's ability to sustain a given level of force over time (i.e. muscle fatigue). Among these factors, the metabolic profile and fibre type composition of the muscles play an important role (Lieber, Friden et al., 1986; Lieber, Johansson et al., 1986). Muscles with predominantly type I (often slow-twitch) fibres are fatigue resistant due to the presence of a higher proportion of slow oxidative fibres whereas, in contrast, "fast-twitch" muscles contain mostly type II (IIa or IIx) fibres and are more susceptible to fatigue due to their low oxidative capacity and high glycolytic enzyme activity (Gordon & Mao, 1994). Paralysed muscles have been observed to have a reduced number of fatigue-resistant motor units and thus are more susceptible to muscle fatigue in comparison to healthy muscles (Enoka, 1988; Roy et al., 1991; Yang, Stein et al., 1990). Due to the above-mentioned changes in muscle's properties, the early onset of muscle fatigue is a continuous

issue in people with SCI (Binder-Macleod, Halden et al., 1995; Binder-Macleod & Snyder-Mackler, 1993; Estigoni et al., 2014). Muscle fatigue is defined as an exercise-induced reduction in muscle force generating capacity, and it may arise due to peripheral changes occurring at the muscle level (peripheral fatigue) and/or changes at the spinal or supraspinal levels, which fail to drive the motor neurones adequately (Gandevia, 2001). However, in people with a complete SCI the fatigue is essentially peripheral and, due to a reduced sensory feedback to prevent failure, this muscle fatigue represents an important issue (Mizrahi, 1997). However, in an individual with incomplete SCI, central fatigue also plays an important role and may particularly have an influence in people classified as ASIA B and C, due to some sensory and motor preservation below the level of injury.

Peripheral fatigue, as defined in this review, is a decline in muscle performance due to mechanisms at or distal to the neuromuscular junction (Allen et al., 2008), including increases in metabolite concentrations P_i and H^+ that affect different sites in the muscle cell leading to inefficient excitation-contraction (E-C) coupling during muscle contraction (Allen et al., 2008; Fitts, 1994). These increased metabolite concentrations negatively affect the release of Ca^{2+} from the sarcoplasmic reticulum (Allen et al., 2008) and reduce myofibrillar Ca^{2+} sensitivity (Allen et al., 2008; Wilson et al., 1998). Consequently, this excess of metabolites can negatively affect the rate of force development, the active maximal cross-bridge force output, and the force relaxation rate (Allen et al., 1995; Fitts, 2008).

Central fatigue, as defined in this review, is the progressive exercise-induced reduction in the voluntary activation of skeletal muscle due to a mechanism proximal to the neuromuscular junction (Gandevia, 2001). Reduction in the output from the upper motor neurones (i.e. corticospinal tract) to activate skeletal muscle result from changes in the intrinsic properties of descending tracts and spinal motor neurone responsiveness (Gandevia, 2001). Some of these changes include modifications in reflex excitatory and inhibitory inputs (Gandevia, 2001), changes in cerebral oxygenation (Rasmussen et al., 2010) and changes in neurotransmitter concentrations, such as serotonin, noradrenaline and dopamine (Meeusen et al., 2006). The neuromodulators noradrenaline and serotonin act primarily on the dendritic regions of motor neurones to generate persistent inward currents (PICs) that can also act to amplify the excitatory synaptic input and increase the firing rate of motor neurones (Heckman et al., 2008; Heckman et al., 2009; Heckmann et al., 2005), especially during high-intensity muscle contractions (Dean et al., 2007) where it helps to delay muscle fatigue in the presence of substantial peripheral fatigue. The concentration of serotonin during prolonged exercise would promote lethargy and negatively impact on mood and drive and thus result in fatigue while noradrenaline and dopamine will impact positively in mood, attention and motivation

and thus delay fatigue (Meeusen & Roelands, 2017; Meeusen et al., 2006). However, the mechanisms underpinning the changes in the central nervous system during fatiguing exercise are still not perfectly described, especially after injury to the spinal cord where the balance between excitatory and inhibitory inputs is inconsistent and very different to the 'normal' response in people with an intact spinal cord (D'Amico et al., 2014). Importantly, peripheral and central fatigue can prevent motor axons from responding to rehabilitation techniques (Lee et al., 2015), and therefore finding an appropriate and effective mode of exercise for paralysed muscles is challenging.

2.5 Physical health after SCI

In addition to the changes in skeletal muscle tissue that can affect the muscle force production, muscle quality of limb muscle mass is also affected (i.e. muscle/intra-muscular adipose content) (Schaap et al., 2009; Srikanthan & Karlamangla, 2014). The muscle atrophy and increased intra-muscular fat usually observed in paralysed muscles (Gorgey & Dudley, 2007) can significantly impact in the physical health and thus, decrease longevity (Goodpaster et al., 2006). Although the overall physical health in people with SCI has increased in recent years, poor health in the years after injury is still problematic (McColl et al., 1997) and mortality rate is still higher than the general population (Garshick et al., 2005). For example, an estimate of 70% survival in people with complete tetraplegia, 84% for complete paraplegia and 92% in incomplete SCI after the age of 25 in comparison with the general population was reported in Australia (Yeol et al., 1998). Cardiovascular diseases are a major cause of death and, thus, many cardiovascular risk factors such as metabolic syndrome, obesity and diabetes are prevalent in people with SCI (Bauman & Spungen, 2008; Krum et al., 1992).

The lack of ability to voluntarily recruit skeletal muscle for movement, and thus the reduction in physical activity levels after suffering a SCI, can trigger changes in body composition, including decreases in fat-free mass and increases in fat mass. These are secondarily associated with lipid and cholesterol disorders (Bauman & Spungen, 2000, 2008) including increases in subcutaneous and ectopic fat (i.e. visceral and intramuscular) and decreases in both lean tissue mass and bone mineral density (Bauman & Spungen, 2008; Castro et al., 1999; Gorgey & Dudley, 2007; Wilmet et al., 1995). These changes are associated with an increased risk of cardiovascular disease and metabolic disorders (e.g. type 2 diabetes mellitus) that are characteristic of people with SCI (Bauman & Spungen, 2008; Castro et al., 1999; Gorgey & Dudley, 2007; Wilmet et al., 1995). The prevalence of type 2 diabetes mellitus, impaired glucose tolerance and insulin resistance are three times higher than in the able-bodied population (Bauman & Spungen, 2008; Elder et al., 2004) and the risk of

dyslipidaemia is characterised by a depression in high-density lipoprotein levels (HDL-C) and elevation of cholesterol, triglycerides and low density lipoprotein levels (LDL-C) (Bauman & Spungen, 2008; Gater, 2007). The low levels of HDL cholesterol, elevated compensatory levels of angiotensin II, insulin resistance and carbohydrate and lipid metabolism disorders increase the already-high risk of developing coronary heart disease in people with SCI (Bauman & Spungen, 2008). For example, cardiovascular diseases were reported to be the primary cause of death in people with SCI with more than 30 years of injury (46% of all deaths) and among those over 60 years old (35% of all deaths) (Whiteneck et al., 1992). Therefore, physical interventions that target a broad decrease in cardiovascular risk factors are needed in the SCI population.

Another physical health disorder in people with SCI is obesity. This greater tendency to become obese results from reductions in the thermic effect of physical activity, which greatly depends on skeletal muscle mass and can change based on the mode, intensity, duration and frequency of the physical activity performed (Gater, 2007). Adipose tissue accumulated in excess has severe consequences related to hyperlipidaemia, hypertension and thromboembolism (Grundy, 2004). Adipose tissue has also been observed to secrete a large volume of pro-inflammatory cytokines (cell-derived proteins), including IL-6, which stimulates the hepatic production of C-reactive protein (CRP) and is associated with vascular inflammation, ultimately leading to vascular endothelial cell injury and apoptosis (Blake & Ridker, 2001; Kern et al., 1995). CRP levels have also been reported to be in the high-risk range for people with SCI (Lee et al., 2005; Manns et al., 2005). Thus, in conjunction with the elevation of triglycerides and LDL-C and a diminished HDL-C, an atherogenic environment is created throughout the vascular tree (Verges, 2005) and this is highly detrimental for the physical health of people with SCI. Thus, physical interventions targeting the reduction of adipose tissue are needed in order to decrease cardiovascular risk and improve longevity in people with SCI.

Another physical health problem after SCI is the development of osteoporosis, which is characterised not only by bone loss but also alterations in bone structure and microstructure (Jiang et al., 2006). Osteoporosis results from a combination of mechanical, neural and hormonal factors (Qin et al., 2010). The reduction in bone mineral content (BMC) and bone mineral density (BMD) in people with chronic SCI has been extensively documented and is associated with an increased risk of fractures (Biering-Sorensen et al., 1988; Griffiths & Zimmerman, 1973; Lazo et al., 2001; Sabo et al., 2001). An incidence of 1 to 34% of lower extremity fractures has been reported among people with SCI (Jiang et al., 2006), which is higher than the general population. Moreover, the rapid acceleration of the loss of bone mineral

density has been reported to last from one to three years after the injury at a rate of 2-4% per month (Shields, 2002; Wilmet et al., 1995); this bone mass loss is more severe in complete SCI lesions than in incomplete (Demirel et al., 1998). The development of osteoporosis predominates in the long bones of the lower limbs and with greater bone demineralisation being observed in the distal and proximal epiphyses of the femur and tibia (Dolbow, Gorgey et al., 2011; Jiang et al., 2006) (Biering-Dørensen et al., 1990; Garland et al., 1992). Mechanical unloading due to motor function loss plays an important role in the pathogenesis of the bone loss and muscular loading of the bones can influence bone density after SCI (Jiang et al., 2006). Thus, physical interventions can help to prevent or decelerate the degree of osteoporosis and the use of electrical stimulation (Hangartner et al., 1994), specifically at higher intensities for at least 3 months, was reported to increase BMD by 18% in people with SCI (Bloomfield et al.; Jiang et al., 2006). Therefore, physical strategies such as muscle electrical stimulation are needed to reduce the rate of loss of bone and/or to improve bone structure by increasing bone mineral density in people with SCI.

2.6 Spasticity and contractures after SCI

Spasticity is a common sequela resulting from injury to the descending tracts and spinal motor neurones within the central nervous system (Sheean, 2002) and can be defined as a state of sustained increase in muscle tone elicited by muscle stretch and caused by augmented excitability of the muscle stretch reflex arc (Chakravarty & Mukherjee, 2010). Spasticity is characterised by involuntary muscle activity, also referred as spasms, hyperreflexia, clonus and co-contraction (Pandyan et al., 2009). The development of spasticity in people with SCI occurs in the second stage of the development of the spinal cord injury. The first stage is referred to as “spinal shock” and it is characterised by severe muscle paralysis, flaccid muscle tone and loss of reflexes and sensation below the level of injury (Ditunno et al., 2004). This period is characterised by the disappearance of persistent inward currents (PICs) at the motor neurones and is coupled with hyperpolarization of the motor neurone membrane potential, an increased pre-synaptic inhibition and decreased background synaptic and gamma motor neurone drive, which result in an unexcitable motor neurone and spinal circuit for the first days and weeks after the injury (D'Amico et al., 2014). The second stage of the injury is characterised by the recovery of motor neurone PICs, which contributes to both recovery of motor function and the development of spasticity (D'Amico et al., 2014). In response to brief, or low-levels of, depolarising synaptic drive after SCI both calcium (CaPIC) and sodium (NaPIC)-mediated regenerative firing allow motor neurones to respond with a prolonged and involuntary firing (i.e. spasticity) (D'Amico et al., 2014). The modulation of PICs is not only voltage-sensitive but also requires the activation of serotonergic (5-HT) or noradrenergic (NA)

receptors located at the motor neurones (D'Amico et al., 2014; Heckmann et al., 2005; Murray et al., 2010). In chronic SCI the activation of these monoaminergic receptors occurs even though there is a marked disappearance of both 5-HT and NA fibres emerging from the locus coeruleus and raphe nuclei, respectively, in the brainstem, and thus a reduction in monoaminergic action (Andén et al., 1964). This appears to be an important strategy by which the injured spinal cord can regain its lost excitability (D'Amico et al., 2014), but unfortunately this is also associated with an increase in symptoms of spasticity. Ultimately, the development of involuntary muscle spasms results from the long excitatory post-synaptic potentials and self-sustained firing that are uncontrolled due to the loss of descending and intrinsic inhibition of the motor neurone (D'Amico et al., 2014; Heckmann et al., 2005). Pre-synaptic inhibition of Ia afferents and post-activation depression after repetitive activation of Ia afferents are presumably reduced and thus may contribute to the development of spasticity after SCI (D'Amico et al., 2014). Thus, it seems reasonable that physical strategies may be developed to excite the spinal cord with patterned activities that mimic inputs produced during natural movement, through stimulation of Ia afferents, to keep the inhibitory mechanisms viable and reduce symptoms of spasticity (D'Amico et al., 2014).

Another common sequela after SCI is the development of contractures, probably due to changes in the muscle and joint (Diong et al., 2012). This becomes prominent after the pronounced muscle atrophy and remodelling of non-muscle tissue occur, such as atrophic myofibres being replaced by adipocytes, collagen and other amorphous substances (Olsson et al., 2006). Contractures affect mobility and physical function leading to pain and a reduced effectiveness of physical rehabilitation techniques, such as locomotor training (Diong et al., 2012; Grover et al., 1996). Thus, strategies to control and diminish levels of spasticity and contractures are in demand in the physical rehabilitation setting.

2.7 Psychological and quality of life changes after SCI

A spinal cord injury brings not only physical but also psychological and social changes in every area of everyday life for the affected individual. 'Quality of life' (QoL) is defined as a person-oriented outcome parameter and a multi-dimensional construct, which is determined by the subjective evaluation of objective living conditions in different areas of life (Fuhrer, 2000). QoL in people with SCI is lower when compared to the general population values (Fuhrer, 2000) and it is primarily affected by functional impairments, such as inability to walk and loss of independence in every-day life activities (Anneken et al., 2010). However, QoL was found to be independent of level of injury and the degree of impairment (DeVivo & Richards, 1992; Fuhrer et al., 1992). Nonetheless, among the factors that can influence QoL,

spasticity represents one of the major complications affecting functional ability, and therefore in people with SCI (Westerkam et al., 2011). Ultimately, QoL in people with SCI is affected directly by the imposed changes in living conditions that can influence their satisfaction with life (Anneken et al., 2010; Fuhrer, 2000), and indirectly as for instance when spasticity interferes negatively with QoL (Adams & Hicks, 2005).

Among the factors that can positively affect QoL physical exercise plays an important role, as it can contribute to self-determination and autonomy, representing a major contributor to the success of the rehabilitation process (Noreau & Shephard, 1995; Tasiemski et al., 2005), helping to minimise or prevent depression (Lawlor & Hopker, 2001) and many other health conditions (Faulkner & Taylor, 2005). Thus, physical exercise has a great impact on QoL because it provides for a mobility advancement, which ultimately contributes to self-determination and autonomy (Noreau & Shephard, 1995). Physically active people have reported better QoL than inactive people with SCI (Anneken et al., 2010) and, thus, finding an effective physical rehabilitation technique to improve physical health and function should have a positive impact on QoL in people with SCI. Finding a physical exercise intervention that can ameliorate symptoms of spasticity and have a positive impact on the QoL is of great interest in physical rehabilitation in people with SCI.

2.8 Importance of physical exercise and muscle strength training in people with SCI

Physical exercise is well known to provide many health benefits to the general adult population (Cowan, 2016), as well as in clinical populations such as people with SCI (Galea, 2012; Panisset et al., 2016). People with SCI have functional capacity deficits, including in motor tasks such as wheelchair-to-chair or -bed transitions and standing endurance, due to weakness of the muscles innervated via damaged spinal segments and the general reduction of physical activity (Dimitrijevic et al., 2012; Modlesky et al., 2004; Thomas et al., 2014). Thus, physical exercise interventions targeting these weak muscles below the level of injury are mandatory for the improvement of muscle strength and mass. These improvements can then be translated into improvements in physical capacity, general health and function in people with SCI.

Among physical exercise interventions, muscle strength training has been found to be the most effective intervention to improve muscle strength and mass (Binder et al., 2005; Hanson et al., 2009; Mangine et al., 2015) with some evidence in people with SCI (Harvey et al., 2009). Moreover, in some functionally important muscles like the quadriceps femoris, muscle strength has been proved to be a strong predictor of mortality (Newman et al., 2006). Muscle

strength may be defined as the ability to develop force against an unyielding resistance in a single contraction of unrestricted duration, and it is the consequence of the interaction of neural (i.e. recruitment and modulation of discharge frequency of motor units), muscular (i.e. cross-sectional area and structure of the muscle) and mechanical (i.e. moment arms associated with different forces) factors (Enoka, 1988). Musculoskeletal adaptations to strength training are determined by the quantity and quality of muscle mass and to neural adaptation, which is the extent of muscle mass activated by the nervous system (Sale, 1988). Muscle strength training seems to be a promising physical rehabilitation method to improve muscle strength and mass and obtain physical health benefits in people with SCI (Bickel et al., 2003; Glinsky, Harvey et al., 2007; Harvey, Fornusek et al., 2010). However, many factors need to be considered before its implementation in clinical practice.

Among the factors to be considered to use muscle strength training in the rehabilitation of people with SCI, the role of neuroplasticity needs to be considered. Physical exercise rehabilitation after SCI has evolved in recent years, where initially researchers and clinicians focused on developing compensatory strategies to improve physical independence, and thus concentrating on strengthening the muscles above the lesion level (Kirshblum & O'Connor, 2000). However, in recent years, the focus in physical exercise rehabilitation has moved towards improving and maintaining optimum health and to target systems below the level of injury (Galea, 2012). This new focus is based on the concept of plasticity of the central nervous system (including the spinal cord) and raises the possibility of eliciting changes in motor and sensory function through changes in motor behaviour (Behrman et al., 2006). Neuroplasticity refers to the changes at anatomical and physiological levels, which reflect the reorganisation of the central nervous system (Dietz & Fouad, 2014) and denote the adaptation of the sensorimotor system, which includes changes in synaptic formation and strength (Rioult-Pedotti et al., 2007), changes in the intracellular properties (Murray et al., 2010) and axonal sprouting (Bareyre et al., 2004). Many physical exercise rehabilitation programs are underpinned by this new concept of neuroplasticity and focus on enhancing any remaining motor and/or sensory function in the lower limbs (Teeter et al., 2012). Some of these new rehabilitation programs include activity-based restorative therapies (ABRT), which are a group of multimodal interventions that provide activation of the neuromuscular system below the level of the lesion, with a view to retraining the central nervous system to recover a specific motor task (Edgerton & Roy, 2009; Sadowsky & McDonald, 2009). These therapies are based on three types of interventions: 1) patterned motor activation, such as locomotor training or functional electrical stimulation-induced cycling; 2) non-patterned motor activation, such as recruitment and strengthening task specific training; and 3) sensory stimulation, such as muscle, tendon or whole-body vibration (Sadowsky & McDonald, 2009). The “Neuromoves

Spinal Cord Injury Recovery Program”, for example, is an ABRT that focuses on the improvement of sensory and motor function and is individually designed to assist a person with a SCI to maximise their functional recovery using voluntary and involuntary muscle contractions (Edgerton & Roy, 2009; Sadowsky & McDonald, 2009). The muscle strength component in the ABRTs, such as the Neuromoves program, includes non-patterned voluntary as well as a patterned involuntary motor activation that involves task-specific training recruitment and strengthening (Sadowsky & McDonald, 2009). However, the use of muscle strength training for the lower limbs in most of these physical rehabilitation programs does not include a strength training intervention in the lower limbs to effectively improve muscle strength and mass. Therefore, these rehabilitation approaches might benefit from the incorporation of a strength training modality in the lower limbs that follows the principle of neuroplasticity, which could provide benefits in physical health outcomes in people with SCI.

Strength training can also facilitate neurological improvements in people with a neurological condition. The addition of sufficient external resistance whilst practicing strength training on the lost or impaired movements that occur after SCI can stimulate sensorimotor systems that may restore functional abilities (Dietz & Fouad, 2014; Edgerton et al., 2004). Additionally, since lost patterned activation of the spinal cord leads to a decrease in the inhibitory control of sensory transmission and an increased excitability of the motor neurones, generating higher levels of spasticity (D'Amico et al., 2014), the activation of synaptic inputs using strength training on the muscles below the level of injury may help recover the lost inhibition, and thus decrease levels of spasticity in people with SCI (D'Amico et al., 2014). Therefore, muscle activation of paralysed or paretic muscles by strength training may stimulate the spinal networks (i.e. sensorimotor systems) and thus promote the restoration of descending input (Dietz & Fouad, 2014), decreasing spasticity levels (D'Amico et al., 2014) and ultimately improving function and physical health outcomes in people with SCI.

Some of the physical health benefits derived from muscle strength training relate to the increased muscle mass, since this is a crucial factor to maintain the strength capacity (Mayer, Scharhag-Rosenberger et al., 2011). Muscle mass can be influenced by physical activity, nutritional status, hormonal status and chronic inflammation (Rolland et al., 2008). Increasing muscle mass may positively impact on the physical health profile of people with SCI. The contractile activity of the muscle mass involved during muscle strength training has been proven to release cytokines (i.e. myokines, such as IL-6) that promote glucose uptake and fat oxidation and have various effects on the liver and adipose tissue (Pedersen et al., 2007). These myokines appear to act against chronic low-grade systemic inflammation and protect against cardiovascular disease and type 2 diabetes as well as attenuate dyslipemia (Pedersen et al.,

2007; Pedersen & Febbraio, 2008; Sasaki et al., 2014). For example, during prolonged lower-limb exercise there is a significant acute increase of IL-6 in the circulation, which mediates the exercise-associated adaptive changes in the metabolic system, promoting fat oxidation, insulin-stimulated glucose uptake and anti-inflammatory effects (Pedersen et al., 2007; Pedersen & Febbraio, 2008). However, high resting levels of IL-6 are associated with physical inactivity and obesity, which might relate to a feedback mechanism resulting in impaired IL-6 signalling during chronic inflammatory conditions (Pedersen et al., 2007). This can be explained by the mediating role of IL-6 between the hepatic glucose output and the usage of blood glucose by skeletal muscles, thus IL-6 acts as a hormone released by the active skeletal muscle (Steensberg, van Hall et al., 2000). Long-term strength training can affect the basal levels of CRP and IL-6, released by adipocytes or infiltrated immune cells in the adipose tissue, and play an important role in low-grade inflammatory diseases (Calle & Fernandez, 2010). Muscle strength training might also decrease CRP levels through the elicited increases in muscle mass (Mayer et al., 2011) and might then have a positive impact on energy expenditure and insulin sensitivity (Calle & Fernandez, 2010). Thus, strength training ameliorates systemic inflammation, preventing the development of low-grade systemic inflammatory-related diseases (Calle & Fernandez, 2010). In contrast, high levels of systemic inflammatory markers have been associated with increased mortality and morbidity and higher IL-6 levels are associated with physical disability (Cohen et al., 1997; Harris et al., 1999). Therefore, the use of muscle strength training in the lower limbs might be incorporated in physical rehabilitation programs in order to obtain the physical benefits derived from a healthy musculoskeletal system.

Developing a healthier musculoskeletal system through the use of strength training may also have other long-term advantages in people with chronic SCI. For instance, having a strong (and healthy) musculoskeletal system is important if people with SCI might want to utilise new treatments, such as stem cell therapies (Zhang & He, 2014), epidural stimulation (Angeli et al., 2014), the use of exoskeletons (Cruciger et al., 2014), and many other promising strategies aiming to repair the injured spinal cord (Buchli & Schwab, 2005; Fawcett, 2006; Rowland et al., 2008). Having a healthier musculoskeletal system will allow people with SCI to take advantage of these future, innovative therapies and help them improve functional outcomes in the lower limbs, which may allow them to recover greater physical function.

2.8.1 High-intensity strength training in people with SCI

High-intensity strength training refers to repeated sessions of relatively brief, intermittent exercises performed at or near the maximum level of muscle contraction capacity (Nelson et al., 1994). The mechanical strain elicited by high-intensity strength training is believed to be

a major stimulus for the creation of an anabolic environment (i.e. release of auto-, para- and endocrine hormones) that enhances the hypertrophic response and generates gains in muscle strength and mass (Goto et al., 2004; Goto et al., 2005; Raastad et al., 2000). The main aim of high-intensity strength training is to progressively increase the external resistance in order to stimulate optimal increases in muscle strength (Goto et al., 2004; Goto et al., 2005) and thus generate muscle adaptations to obtain the physical health benefits derived from improvements in muscle strength (Ahtiainen et al., 2003).

High-intensity strength training has been proved to elicit adaptations in the muscular (i.e. muscle hypertrophy and architectural changes) (Seynnes et al., 2007) and nervous systems (i.e. movement execution and control and synaptic efficacy) (Aagaard et al., 2002; Carroll et al., 2001). Many studies have reported the positive effects of high-intensity strength training on maximum strength and the underlying neuromuscular adaptations in healthy subjects, including an increase in maximal isometric muscle strength, recruitment of the available motor unit pool, motor unit firing frequency, efficiency at submaximal loads and muscle oxidative metabolism (Aagaard et al., 2002; Carroll et al., 2001; Hakkinen et al., 1985; Komi et al., 1978; Sale, 1988). High-intensity strength training has also been reported to improve muscle strength and function, decrease disability in other clinical populations such as nonagenarians and people recovering from a stroke (Fiatarone et al., 1990; Ouellette et al., 2004), and improve QoL in cancer survivors (De Backer et al., 2007). Thus, high-intensity strength training would theoretically be a highly efficient form of resistance exercise for improving muscle strength, eliciting neuromuscular adaptations and improving physical and mental health in people with SCI.

However, muscle strength improvement represents a major challenge in the SCI population. A person is weak due to paralysis or paresis after SCI and relies on the neurally intact muscles to perform everyday tasks. Thus, the upper limb muscles can be trained voluntarily and muscle strength training plays an essential role in functional outcomes such as transfers from the wheelchair to a bed (Thomas et al., 1997). This type of training has been shown to improve muscle strength with positive correlations to functional outcomes (Dallmeijer et al., 1997; Davis & Shephard, 1990). An improvement of 19.0-34.0% in upper body strength was reported after a 9-month intervention of twice-weekly progressive strength training (70-80% of one-repetition maximum) (Hicks et al., 2003) and increases of 11.9-30.0% were reported after a 12-week circuit training intervention (3 times per week) (Jacobs et al., 2001). However, muscle strength training of the paretic or paralysed lower limbs represents a challenge due to limited, or lack of, activation of muscles below the level of injury. Therefore, other strategies such as the use of neuromuscular electrical stimulation (NMES) are mandatory for strength training to be implemented in people

with SCI. For example, an 8-week block of progressive resistance training in combination with an NMES intervention was reported to increase voluntary strength in comparison to a control group in people with incomplete lesions (C₃-L₂, ASIA C and D) (Harvey et al., 2010). Thus, the use of muscle electrical stimulation appears to be a valid strategy to improve muscle strength in the lower limbs of people with SCI.

2.9 Exercise using neuromuscular electrical stimulation (NMES)

Neuromuscular electrical stimulation (NMES) is a commonly-used intervention in rehabilitation programs for increasing muscle recruitment and thus muscle force production in individuals with some loss of motor function (i.e. SCI) (Barbeau et al., 2002; Harvey et al., 2010; Thrasher et al., 2013). Movement or functional capacity can be improved using different NMES modalities because activities of daily living require a minimum of muscle force production that is usually higher than that possessed by non-exercising people with SCI (Souza et al., 2005). NMES consists of the “application of intermittent electrical stimuli to superficial skeletal muscles, aiming to trigger visible muscle contractions by activating the intramuscular nerve branches” (Hultman et al., 1983). The electrical stimuli are delivered by positioning skin-based electrodes on the proximity of motor points and the stimulation is delivered by pre-programmed electrical stimulation units (Maffiuletti, 2010). Two electrodes are required for the production of an electrical current flow, and bipolar stimulation is the most common configuration used in neuro-rehabilitation programs because it creates a more localised electrical field resulting in greater selectivity of muscles (Grandjean & Mortimer, 1986). NMES induces the same action potential as by natural physiologic means after reaching the “stimulus threshold”, which is the lowest level of electrical charge required to generate an action potential (Sheffler & Chae, 2007). To generate muscle contractions through electrical stimulation the stimulus can be applied from the origin of the nerve to its motor point where it connects with the muscle (Ragnarsson, 2008), thus it is necessary to have an intact lower motor neurone including the neuromuscular junction (Ragnarsson, 2008).

The use of NMES has been documented to induce many health benefits in people with SCI. For instance, a 12-week NMES twice-weekly strength training intervention using a 450 μ s pulse width resulted in significant skeletal muscle hypertrophy and improvements in lipid metabolism and insulin profile (Gorgey, Mather, et al., 2012). NMES has also proved to be effective for decreasing spasticity and improving physical health (D'Amico et al., 2014). For example, an 8-week NMES protocol yielded a significant decrease in spasticity and a significant increase in lean body mass in a cohort of adults with SCI (Carty et al., 2013).

Therefore, NMES seems to be an essential rehabilitation tool for improving muscle force production and obtaining physical health benefits in people with SCI.

NMES is usually performed at low (current) intensities during a functional task, and is referred as functional electrical stimulation (FES). FES has been used for physical rehabilitation of people with SCI by sequentially stimulating the paralysed muscles to produce cyclical leg motion, i.e. FES cycling (Peng et al., 2011). Commonly, the leg pedalling power output is modulated by a controlled intensity of the current using fixed values of pulse width (200 and 300 μ s) and frequency in a range of 10-50 Hz (Peng et al., 2011; Rabischong & Ohanna, 1992). Some research studies have used this modality to show the positive effects on muscle strength and endurance adaptations and functional capacity improvements in people with SCI (Belanger et al., 2000; Crosbie et al., 2009; Crosbie et al., 2014; Thrasher et al., 2013). Other studies have documented lower extremity muscle hypertrophy, improved metabolic profiles and decreases in fat mass after FES interventions (Gorgey, Mather, et al., 2012; Gorgey & Shepherd, 2010; Griffin et al., 2009). However, this type of NMES uses low-intensity currents evoking low levels of force during muscle evoked contractions and cannot optimally stimulate muscular strength and mass improvements, which requires the imposition of a higher load to the muscle to obtain higher force output (American College of Sports Medicine, 2009). Moreover, there is still insufficient evidence to support the use of FES to increase skeletal muscle recruitment, and thus significantly increase muscle force production (Gater et al., 2011), to improve carbohydrate and lipid disorders (Carlson et al., 2009) and/or to reverse or decelerate the rapid and linear bone mineral loss in adults with SCI (Gater et al., 2011). Therefore, the use of NMES as a strength training tool may be a more effective physical rehabilitation method than FES to obtain significant increases in muscle force production along with the physical health benefits that may derive from its use.

2.9.1 High-intensity NMES strength training

The effects of alterations in NMES parameters (e.g. pulse frequency, pulse width, current intensity) need to be fully examined before NMES can be considered for use as a high-intensity (i.e. near maximal evoked muscle forces) strength training modality in people with SCI. In order to optimally use high-intensity NMES strength training (hereafter referred as near-maximal evoked muscle forces), there are some differences between evoked (i.e. by NMES) versus voluntary muscle contractions that need to be considered. These differences include the motor unit activation being orderly versus non-selective, the contraction intensity being near-maximal versus submaximal, the muscle activation being synergistic versus targeted, and the physiological origin of the contraction being internal versus external during NMES-based

training (Hortobagyi & Maffiuletti, 2011). Also, electrical stimulation using standard parameters typically evokes a (spatial) recruitment pattern of motor units from large to small, which is opposite to that in voluntary contractions, and thus activates fast-fatigable motor units before fatigue-resistant motor units (Rabischong & Ohanna, 1992). NMES also imposes a synchronous temporal recruitment pattern, whilst motor units are recruited asynchronously during voluntary contractions (Adams et al., 1993). Spatial recruitment is limited because NMES imposes a continuous contractile activity to the axonal branches in proximity to the stimulation electrodes and this fixed recruitment diminishes proportionally as distance increases from the electrode in able-bodied populations (Vanderthommen et al., 1997, 2003). However, using conservative parameters of NMES to evoke isometric muscle contractions in the paralysed quadriceps femoris can elicit higher levels of muscle damage and a greater relative muscle activation than in able-bodied people (Bickel, Slade, & Dudley, 2004). In paralysed quadriceps femoris increases in current intensity would activate more muscle fibres and increase the likelihood of excitability of more nerves closer to firing threshold and thus, more muscle fibres would be activated. Thus, if NMES produces a tetanic, fused muscle contraction, muscle fibres are activated maximally and thus the only way to increase muscle force would be to increase the current intensity with the consequent muscle damage effect in paralysed muscles (Bickel et al., 2004). Therefore, the use of NMES using standard stimulation parameters may elicit early muscle damage and may not be optimum for eliciting broad ranging muscular adaptations, and thus for increasing muscle force production and physical health benefits in functionally important muscles. Further research is required in order to more completely describe the adaptive processes resulting from NMES under different stimulation conditions.

Similar training programming principles need to be followed when using NMES as a high-intensity strength training modality as when using voluntary strength training. Muscle force generation varies according to the level of motor unit activation, and the magnitude of strength enhancement with voluntary strength training is known to be highly dependent on muscle action, intensity, volume, exercise selection and order, rest periods and frequency of exercise (Kraemer & Ratamess, 2004). To utilise NMES as a strength training modality, therefore, basic principles of progressive strength training also need to be followed, such as imposing progressively heavier loads to elicit stronger muscle contractions (Ploutz et al., 1994) and a progression of the other parameters during training, such as current intensity, evoked force and training volume (Maffiuletti, 2010; Maffiuletti et al., 2009). The force of the muscle contractions and the number of nerve fibres activated during NMES depend on parameters such as the amplitude and duration of the electrical stimulation (Maffiuletti, 2010), and this can affect the effectiveness of the NMES strength training program. However, it is important

to note that the muscle force elicited by NMES not only depends on these external controllable factors, but also on intrinsic anatomical properties of the individual and a considerable inter-individual variation response to NMES exists (Lloyd et al., 1986). Muscle force production during electrical stimulation is also influenced by multiple factors, such as inherent length-tension characteristics of the muscle and volume conduction of the current (Sheffler & Chae, 2007). Nonetheless, the key factor influencing the long term adaptive response to NMES training is the muscle tension developed during each training session, which is the level of evoked force as a proportion of the maximal force capacity of the muscle (Lieber & Kelly, 1991) and this can be maximised by appropriately manipulating the frequency and intensity of the NMES trains (Maffiuletti, 2010). Current recommendations to maximise muscle tension during NMES in healthy subjects are to use biphasic rectangular pulses of 100-400 μ s delivered at stimulation intensities of 50-100 Hz (Vanderthommen & Duchateau, 2007), with the highest tolerated current intensity and in a static loading condition (i.e. isometric) to control the level of evoked force. However, standard parameters of NMES have a significant drawback, which is the rapid onset of muscle fatigue.

The rapid onset of muscle fatigue is a common problem when using NMES in clinical populations, such as people with SCI. Factors influencing the rate of muscle fatigue onset include the random motor unit recruitment pattern elicited by NMES (rather than orderly recruitment observed in voluntary contractions and in accordance with Henneman's size principle, i.e. from fatigue resistant type I motor units to more fatigable type II units), non-physiologically high stimulation frequencies, and consequent muscle damage (Bickel, Gregory et al., 2011; Fouré, Nosaka et al., 2014; Mizrahi, 1997). This muscle fatigue is exacerbated in paralysed muscles due to the loss of type I, slow-fatiguing motor units and their higher proportion of more fatigable type II motor units (Adams et al., 1993; Bickel et al., 2011; Bickel et al., 2004; Burnham et al., 1997; Pelletier & Hicks, 2011), accumulation of metabolites and problems with excitation-contraction coupling (Biering-Sorensen et al., 2009; Pelletier & Hicks, 2011; Shields, 1995). One of the characteristics of the rapid onset of muscle fatigue is the prompt decline in performance during repetitive activity (Binder-Macleod et al., 1995; Gregory et al., 2007). This fatigue has been documented in people with SCI as a decline in peak torque that is 1.7 times higher than in healthy control subjects, which remained reduced by 22% after evoked isometric contractions of the quadriceps femoris (Bickel, Slade, & Dudley, 2004). Additionally, since muscle recovery from fatigue after NMES takes longer than in healthy subjects (Mahoney et al., 2007), the intensity and duration of stimulation that can be applied to paretic or paralysed muscles in an exercise session are reduced, thus limiting the potential for muscular adaptation (Black & McCully, 2008b; Bouchard et al., 2011;

Cramer et al., 2002; Daussin et al., 2008). Therefore, different parameters of NMES need to be considered in order to maximise muscular adaptations and reduce or delay muscle fatigue.

NMES can be applied directly over the muscle to elicit muscular contraction via direct activation of motor axons that overlie the muscles, and/or via indirect activation through Ia reflex pathways (Collins, Burke et al., 2002). Current clinical guidelines for NMES do not consider alternative pulse sequences that could elicit greater muscle force production whilst minimising muscle fatigue. They currently recommend the use of relatively short pulse widths (100-200 μ s) and low-to-moderate pulse frequencies (30-50 Hz), and such protocols are commonly used in research studies (Allen & Goodman, 2014; Gorgey, Cho et al., 2013). Altering NMES parameters has been documented to influence motor unit recruitment and muscle fatigue in healthy and clinical populations (Chou et al., 2008; Gorgey et al., 2009; Gorgey & Dudley, 2008; Gorgey, Mahoney et al., 2006; Kesar et al., 2008). Increasing the amplitude of the current and pulse duration (i.e. pulse width) have been proved to increase skeletal muscle recruitment without interfering with the rate or extent of muscle fatigue (Chou et al., 2008; Gorgey et al., 2009; Gorgey & Dudley, 2008; Gorgey et al., 2006; Kesar & Binder-Macleod, 2006). Longer pulse duration, but not stimulation duration, has been shown to increase evoked torque when compared to shorter pulse durations. Indeed, increasing from 150 μ s to 500 μ s resulted in an increase in torque probably due to recruitment of large motor units or high-threshold, usually larger, motor units that added non-linearly to the force, however increasing the frequency of the pulse stimulation increased torque but not the activated area (Gorgey & Dudley, 2008; Gorgey et al., 2006; Troiani et al., 1999). Increasing the pulse width increases the number of recruited fibres and thus increases muscle force, whereas increasing the frequency drives the already-active motor units to higher force levels, but therefore also induces more rapid fatigue (Chou et al., 2008; Dean et al., 2007; Gregory et al., 2007; Kesar & Binder-Macleod, 2006). An important point to reiterate here is that increasing activation frequency does not increase the amount of muscle recruited. However, the different effects of pulse width are not always apparent. In one study, the use of short (< 350 μ s) versus wider (>350 μ s) pulse widths during FES cycling showed no difference in the oxygen uptake, energy expenditure or time to fatigue in people with SCI (Gorgey, Poarch et al., 2014). Nonetheless, NMES as a strength training protocol using wider pulse widths (e.g. \geq 1000 μ s) with low-to-moderate frequencies (20-30 Hz) has not been studied in this population. Therefore, the best NMES parameters to use during ‘strength training’ are not currently known, and therefore it is not yet possible to recommend parameters that will elicit the greatest increases in muscle mass and strength in people with SCI.

Higher intensity strength training induces greater muscular adaptive responses in healthy and clinical populations, such as stroke patients, in comparison to low-intensity strength training (Sanchez et al., 2005; Weiss, Suzuki et al., 2000), and indeed voluntary high-intensity training has been proven to significantly increase muscle mass and strength in healthy older people (Candow et al., 2011; Fiatarone et al., 1990). Therefore, the use of NMES as higher-intensity, resistive-type exercise may be a good rehabilitation method to increase muscle mass and strength in people with SCI, who may not voluntarily activate their muscles sufficiently to trigger an optimum response. Nonetheless, as described above, a major problem associated with standard NMES protocols is the rapid onset of fatigue, which is mainly caused by the simultaneous activation of the same motor units in repeated contractions (Adams et al., 1993; Bickel et al., 2011; Bickel et al., 2004). The early onset of muscle fatigue represents a major problem in the clinical setting because it limits the achievement of an optimum training response. This is especially a problem when applying NMES as a high-intensity strength training modality due to the inability to follow the overload principle by using progressively higher stimulation intensities, given that strength gains are obtained at higher contraction forces (Lai et al., 1988; Selkowitz, 1985) than the forces elicited by prolonged, low intensity stimulation protocols (e.g. FES). Another issue when applying NMES in long term paralysed muscles is the susceptibility of contraction induce muscle damage (Bickel et al., 2004). Thus, to overcome some of the problems associated with standard NMES protocols and use it as a high-intensity strength training modality, the effect of increasing the intensity and frequency of stimulation on muscle force have been examined (Black & McCully, 2008a; Chou et al., 2008). In particular, wide-pulse width NMES (1000 μ s) has been used in an attempt to recruit motor units through central (i.e. spinal) pathways and increase muscle force production (Chou et al., 2008; Dean et al., 2007). Electrical pulses with a longer duration tend to activate sensory axons that project to the spinal cord, thus exciting descending projections back to the muscle (Bergquist, Wiest et al., 2012). This effectively allows signals from the spinal cord to activate the muscles, in addition to their direct axonal activation (Bergquist et al., 2012). This can provide synchronous motor unit activation through the stimulation of H-reflexes (Bergquist et al., 2012) as well as asynchronous motor activation through the triggering of persistent inward currents (PIC) or post-activation potentiation of neurotransmitter release (Bergquist et al., 2012; Collins et al., 2001, 2002). This method could speculatively elicit greater peak muscle forces at lower stimulation intensities and with less fatigue and discomfort (Bergquist et al., 2012; Bergquist, Wiest et al., 2014; Collins et al., 2001, 2002). Moreover, wide-pulse width NMES can also increase afferent input, which seems to be the key to triggering neural adaptations similar to those in voluntary contractions (Hortobagyi & Maffiuletti, 2011). However, these NMES parameters were used during high-frequency stimulations (80-100 Hz) in healthy muscles and this would represent a problem if being implemented into the highly

fatigable paralysed muscles, which require lower frequencies of stimulation to reduce muscle fatigue and limit contraction-induced muscle damage (Binder-Macleod et al., 1995; Black & McCully, 2008a; Shields & Chang, 1997). Thus, changes in parameters may need to be done progressively and safely in people with SCI, to avoid any unwanted side effects, such as spasticity or autonomic dysreflexia. High-intensity, wide-pulse width NMES delivered at low-to-moderate frequencies (i.e. 30 Hz) may then be an effective strength training modality for in people with SCI, but the optimum parameters remain to be elucidated.

2.9.2 Tendon vibration

Tendon vibration may be another promising tool for the triggering of greater force production whilst minimising muscle fatigue (Cotey et al., 2009; Ribot-Ciscar et al., 2003). Tendon vibration may generate trains of Ia afferent signals to the spinal cord that induce a progressive excitation of homonymous motor neurones via the development of persistent inward calcium (Ca^{2+}) or sodium (Na^{+}) currents (PIC) at their dendritic trees, thus evoking a tonic vibratory reflex (TVR) that can include both spinal and supraspinal pathways (Ribot-Ciscar et al., 2003). The development of PICs at the motor unit level could amplify and prolong synaptic input and create a sustained depolarisation leading to an increased recruitment of motor units (i.e. self-sustained firing), thus increasing muscle force production (Magalhaes & Kohn, 2010). However, the sole use of tendon vibration would evoke a weak TVR (Bongiovanni & Hagbarth, 1990), and thus the addition of wide-pulse width NMES may help recruit further motor units through central pathways (Collins, 2007; Collins et al., 2001) and these may then exhibit self-sustained firing when PICs are developed by the tendon vibration stimulus. Importantly, antidromic activation of motor neurones does not occur during tendon vibration (Bongiovanni & Hagbarth, 1990), as it might during wide-pulse width NMES. Therefore, superimposing tendon vibration onto wide-pulse width NMES at low-to-moderate frequencies (e.g. 30 Hz) may induce motor neurone discharge in synchrony with the stimulus.

Tendon vibration can stimulate the muscle spindles and presumably, via polysynaptic projections from those spindles to the homonymous muscle, generate supraspinal facilitation (Burke et al., 1976; Desmedt & Godaux, 1978; Gillies, Burke, & Lance, 1971). This response may be enhanced if tendon vibration is applied during isometric muscle contractions due to the co-activation of the fusimotor systems elicited by the corticospinal descending pathways (Burke et al., 1976).

Therefore, the use of such a technique could maximise the input from reflex pathways, activating fatigue-resistant motor neurones (Behrman et al., 2006; Edgerton & Roy, 2009). Nonetheless, despite several researchers examining the influence of tendon vibration on

muscle activity in people with SCI (Cotey et al., 2009; Ribot-Ciscar et al., 2003), to the author's knowledge no research has investigated its effects in combination with wide-pulse width NMES on peak force production or rate of muscular fatigue during isometric muscle contractions. Also, the possibility of increased activation of afferents, pain fibres might also be associated with pain and discomfort which needs to be monitored in future research. Therefore, it is not known whether it provides an optimum method for high-intensity strength training in healthy or clinical populations, such as in people with SCI.

2.10 Summary and conclusions

Physical rehabilitation for people with neurological conditions is essential for the regeneration of muscle function, physical and mental health and QoL. Thus, growing evidence in this area is mandated. A SCI is a lesion to the spinal cord mainly caused by a trauma and is a devastating condition that affects the musculoskeletal system considerably. SCI can be classified according to the location of the lesion in the spine and according to the completeness of the lesion (complete or incomplete). The main consequence for the neuromuscular system of suffering a SCI is the reduction in motor function (i.e. voluntary muscle activation) which can affect muscle force production in paretic or paralysed muscles; the loss of motor function is evidenced by the low levels of muscle force and work output. Muscle atrophy, or loss of muscle mass, is a major consequence that affects the ability to perform functional activities, and paralysed muscles suffer from a reduced number of fatigue-resistant motor units and therefore are more susceptible to muscle fatigue. Poor health in the years after injury is problematic (McColl et al., 1997) and the mortality rate is higher than in the general population (Garshick et al., 2005). Among the physical health problems after SCI, cardiovascular risk factors, such as metabolic syndrome, high cholesterol levels, diabetes type II and osteoporosis, are highly prevalent. Other physical consequences affecting people with SCI are the development of spasticity and contractures, which interfere negatively in everyday life activities and ultimately decrease QoL.

Physical exercise, including muscle strength training, can be an effective tool for improving physical and mental outcomes in people with SCI. High-intensity strength training in particular holds promise as an effective tool to increase muscle strength and mass, stimulate systemic changes to improve physical health, and improve QoL in people with SCI. However, voluntary muscle strength training represents a major challenge for these people and the use of NMES is commonly used to activate skeletal muscles. Clinical recommendations for NMES suggest short pulse widths (100-200 μ s) and low-to-moderate pulse frequencies (30-50 Hz) (Allen & Goodman, 2014). However, this type of NMES causes rapid muscle fatigue due to

the (non-physiological) high stimulation intensities, non-orderly recruitment of motor units, and the fixed recruitment order. To overcome these issues in the clinical setting, superimposing tendon vibration onto wide-pulse width (1000 μ s) NMES might be used to improve motor unit activation through spinal pathways, delay the onset of muscle fatigue, and thus increase total muscle workload (i.e. impulse). Since tendon vibration elicits a weak muscle contraction through reflex pathways, superimposing tendon vibration onto wide-pulse width NMES is speculated to elicit further increases in impulse. Therefore, NMES may be used as a higher-intensity, resistive-type exercise and superimposing tendon vibration onto this type of NMES may elicit further increases in muscle force and mass. This may lead to significant physical health benefits, such as increases in bone mineral density, decreases in systemic inflammation, an improved lipid profile, ameliorated symptoms of spasticity and an improved QoL.

The current research will contribute much-needed evidence for the effectiveness of tendon vibration superimposed onto wide-pulse width NMES, used as a high-intensity strength training mode, to improve muscle force and mass. These three studies will yield insights into how tendon vibration combined with high-intensity NMES strength training might improve muscle force and mass, physical health (increases in bone mineral density, decreases in systemic inflammation and an improved lipid profile), symptoms of spasticity and QoL in people with SCI. The knowledge gained will assist health practitioners to prescribe safer and more effective muscle strength interventions, using NMES with or without superimposed tendon vibration, in clinical populations.

Chapter 3 Study one

Effect of tendon vibration during wide-pulse neuromuscular electrical stimulation (NMES) on the decline and recovery of muscle force

3.1 Abstract

Introduction: Neuromuscular electrical stimulation (NMES) is commonly used to activate skeletal muscles and reverse muscle atrophy in clinical populations. Clinical recommendations for NMES suggest the use of short pulse widths (100-200 μ s) and low-to-moderate pulse frequencies (30-50 Hz). However, this type of NMES causes rapid muscle fatigue due to the (non-physiological) high stimulation intensities and non-orderly recruitment of motor units. The use of both wide pulse widths (1000 μ s) and tendon vibration might optimise motor unit activation through spinal reflex pathways and thus delay the onset of muscle fatigue, increasing muscle force and muscle mass in people with chronic SCI. Thus, the objective of this study was to examine the acute effects of wide-pulse width (1000 μ s) knee extensor electrical stimulation (NMES, 30 Hz) superimposed onto patellar tendon vibration on peak muscle force, total impulse before “muscle fatigue”, and the post-exercise recovery of muscle function.

Methods: Tendon vibration (Vib), NMES (STIM) or NMES superimposed onto tendon vibration (STIM+Vib) were applied in separate sessions to 16 healthy adults. Total torque-time integral (TTI), maximal voluntary contraction torque (MVIC) and indirect measures of muscle damage were tested before, immediately after, and 1 h and 48 h after each stimulus.

Results: TTI increased ($145 \pm 127\%$) in STIM for positive responders to tendon vibration (8/16 subjects), but decreased in negative responders to tendon vibration ($-43.5 \pm 25\%$). MVIC (-8.7%) and rectus femoris electromyogram amplitude (RF EMG) (-16.7%) decreased after STIM (group effect) for at least 1 h, but not after STIM+Vib. No changes were detected in indirect markers of muscle damage in any condition.

Conclusions: Tendon vibration superimposed onto wide-pulse width NMES increased TTI in only 8 of 16 subjects, but reduced voluntary force loss (fatigue) ubiquitously. Negative responders to tendon vibration may derive greater benefit from wide-pulse width NMES alone.

Significance: Tendon vibration use during NMES only increases the muscle work performed before fatigue in some people, but decreases it in others. However, it tends to reduce the voluntary force loss after a training session, and may thus allow for additional exercise or rehabilitation work to be performed.

Keywords: muscle stimulation, muscle strength, muscle function, muscle fatigue, muscle damage, neuro-rehabilitation

3.2 Introduction

Muscular strength is a major predictor of mortality in clinical populations, and this appears to be partly explicable by the quantity (i.e. absolute muscle volume) and quality (i.e. muscle/intra-muscular adipose content) of limb muscle mass (Schaap et al., 2009; Srikanthan & Karlamangla, 2014). Improvements in muscle mass are also observed to be beneficial for functional mobility and quality of life as well as preventing functional decline, cardiovascular disease and hospitalisation (Solberg et al., 2013). Strength training is commonly used to stimulate gains in muscle strength and has been proven to enhance longevity and quality of life in a variety of clinical populations (Andrade & da Silva, 2015; Caserotti et al., 2008; Clark & Goon, 2015; Orlando et al., 2016). However, strength training poses an increasing challenge for people with a neurological condition, such as people with spinal cord injury (SCI) who have limited ability, or are unable, to voluntarily activate their muscles.

Due to this limitation, neuromuscular electrical stimulation (NMES) has been conventionally used in clinical practice, particularly in the form of functional electrical stimulation (FES), i.e. a continuous, prolonged stimulation at low-to-moderate frequencies (30-50 Hz) paired simultaneously or intermittently with a functional task (e.g. cycling) (Thrasher et al., 2013). FES exercise has been shown to slow muscle weakening or even increase muscle strength as well as reduce the rate of skeletal muscle atrophy and weakness and improve physical health in people with a SCI (Gregory & Bickel, 2005; Griffin et al., 2009; Harvey et al., 2010; Hillegass & Dudley, 1999). However, such interventions evoke only low relative muscle forces (Gordon & Mao, 1994; Griffin et al., 2009) and therefore may not optimally stimulate neuromuscular strength and mass increases (Bersch et al., 2015). Instead the imposition of a higher load on the muscle with intermittent rest periods to allow continuous higher force output would be preferable (American College of Sports Medicine, 2009).

Possible reasons for the lack of clinical use of high-intensity (hereafter used to refer to near maximal evoked muscle contractions), intermittent NMES protocols include a lack of scientific exploration of its efficacy and long term functional effect (Hortobagyi & Maffiuletti, 2011), and their propensity to elicit rapid muscle fatigue and (possibly) muscle damage (Gorgey et al., 2006; Gregory & Bickel, 2005; Hillegass & Dudley, 1999; Ibitoye et al., 2016). Moreover, this muscle fatigue is exacerbated in people with SCI due to the loss of type I, slow-fatiguing motor units and their high proportion of more fatigable type II motor units (Bickel et al., 2011; Bickel, Slade, & Dudley, 2004; Burnham et al., 1997; Pelletier & Hicks, 2011). Whilst the muscle damaging effects can be reduced with repeated exposures (i.e. repeated bout effect), the rapid muscle fatigue induced by NMES is an ongoing issue (Aldayel, Jubeau et al., 2010b; Gorgey, Poarch, et al., 2014; Ibitoye et al., 2016; Karu et al., 1995). This rapid fatigue

partly results from the use of short-pulse widths ($< 300 \mu\text{s}$; commonly used in standard NMES protocols), which activate muscle fibres largely through depolarisation of motor axons, and which typically leads to a random motor unit recruitment pattern and therefore a substantial recruitment of fast-fatiguing type II motor units, rather than orderly recruitment as observed in voluntary contractions and in accordance with Henneman's size principle (i.e. from fatigue resistant type I motor units to more fatigable type II units) (Henneman et al., 1965; Maffiuletti, 2010; Vanderthommen et al., 2003). Fatigue may also result the use of non-physiologically high stimulation frequencies (e.g. $\geq 80 \text{ Hz}$) (Bickel et al., 2011; Bigland-Ritchie, Jones et al., 1979; Fouré et al., 2014; Karu et al., 1995; Mizrahi, 1997) and the simultaneous activation of the same motor units in repeated contractions (Adams et al., 1993; Allen & Goodman, 2014; Bickel et al., 2011; Gorgey et al., 2013). Therefore, the level of muscle force developed and duration of stimulation that can be applied to muscles in an exercise session are reduced, thus limiting the potential for muscle force capacity, muscle mass and musculoskeletal function adaptations (Black & McCully, 2008a; Bouchard et al., 2011; Crameri et al., 2002; Daussin et al., 2008).

To overcome some of these problems, wide-pulse width NMES (i.e. $\geq 1000 \mu\text{s}$) appears to be a promising tool for use in clinical populations as it appears to recruit motor units through central (i.e. indirect) pathways (Bergquist, Clair et al., 2011; Bergquist et al., 2014; Clair-Augier, Collins et al., 2012; Collins, 2007; Lagerquist & Collins, 2010; Neyroud, Armand et al., 2015). Wide-pulse width NMES can elicit asynchronous motor unit activation through the reflexive recruitment of motor neurones (Bergquist et al., 2012), identified by the presence of spinal H-reflexes, or asynchronous motor unit activation through the triggering of persistent inward currents (PIC) via repetitive activation of Ia afferents or post-activation potentiation of neurotransmitter release (Bergquist et al., 2012; Collins et al., 2001, 2002). However, the contribution of wide-pulse width NMES to asynchronous motor unit activation through central recruitment is shown mostly during higher frequencies of stimulation ($>80 \text{ Hz}$) and appears to have minimal effect at lower frequencies (i.e. $\leq 20 \text{ Hz}$), which may preclude its use in a clinical context (Dean, Clair-Augier et al., 2014). Also, when used at higher stimulation intensities the contribution of H-reflexes to muscle activation may be minimised by orthodromic-antidromic collision, and indeed recent evidence has indicated that wide-pulse width NMES may exacerbate muscle fatigue at the higher frequencies of stimulation that may be required to elicit higher levels of muscle force (Clair-Augier, Lagerquist et al., 2013; Neyroud et al., 2014). Thus, there is a need to consider different strategies of activating motor units in a more physiological manner.

One promising method is the application of tendon vibration during muscle stimulation. Tendon vibration evokes a tonic vibration reflex through both spinal and supraspinal pathways via repetitive activation of Ia afferent fibres and possibly triggers the development of persistent inward currents at the motor neuron level (Cotey et al., 2009; Ribot-Ciscar et al., 2003; Trajano et al., 2014). Tendon vibration could amplify and prolong synaptic input and create a sustained depolarisation leading to an increased physiological recruitment of motor units, and thus increasing muscle force output (McPherson et al., 2008). Since tendon vibration can excite only low-threshold motor units (fatigue-resistant) (Bongiovanni & Hagbarth, 1990), an additional excitation of the fatigue resistant motor units may be elicited if it is coupled with wide-pulse width NMES, and thus may result in an additional increase in muscle force output (Behrman et al., 2006; Edgerton & Roy, 2009; Magalhaes, de Toledo et al., 2013; Trajano et al., 2014). Moreover, in some functionally important muscles, such as the quadriceps femoris, the use of wide-pulse width NMES alone may not be effective in recruiting motor units through “central pathways” (Bergquist et al., 2012), so the addition of tendon vibration might help to recruit Ia afferent fibres and thus increase muscle force production. Such a phenomenon has already been demonstrated in healthy people in the plantar flexors (Magalhaes & Kohn, 2010).

Importantly, antidromic activation of motor neurones does not occur during tendon vibration (Burke & Schiller, 1976; Fornari & Kohn, 2008) as it might during NMES. Therefore, superimposing tendon vibration onto wide-pulse width NMES at low-to-moderate frequencies (e.g. 30 Hz) may induce motor neurone discharge in synchrony with the electrical stimulus and that may help to get the best potential benefit from tendon vibration. However, it is still uncertain whether the imposition of tendon vibration onto wide-pulse width NMES could increase the peak force production and reduce the rate of muscle fatigue for a given current level of NMES. It is also not known whether tendon vibration might increase potential muscle damaging effects due to the higher evoked forces, or instead reduce the risk by eliciting a more normal physiological excitation of the motor neurone pool. To our knowledge, no research has examined the effects of superimposing tendon vibration onto wide-pulse width, low-to-moderate frequency (30-50 Hz) NMES on peak force production and rate of muscle fatigue to determine whether greater muscular forces can be elicited with less (or no additional) fatigue.

Therefore, the main purpose of this study was to examine the effects of patellar tendon vibration superimposed onto an acute bout of wide- pulse width NMES (1000 μ s) of low-to-moderate frequency (30 Hz) on peak muscle force, impulse performed before “fatigue”, and the post-exercise recovery of muscle function when compared to wide-pulse width NMES applied without tendon vibration in healthy individuals. This study will help to stablish the feasibility of using long pulse width NMES and understanding the normal physiological

response in able-bodied individuals before applying to people with SCI. These individuals also provided feedback about pain and comfort levels during the NMES protocols, so that these types of interventions might be better understood before their use in future clinical research studies in people with SCI.

3.3 Methods

3.3.1 Subjects

Sixteen healthy subjects (6 women, 10 men) with no neurological or musculoskeletal disorders volunteered for the study (mean \pm SD, age: 28.6 ± 7.5 y; height: 165.1 ± 27.8 cm; body mass: 77.4 ± 24.5 kg BMI: 24.1 ± 2.2 kg/m²). The subjects were physically active individuals who typically performed structured physical activity 2 to 4 times a week (i.e. recreationally trained). We chose to study the effects of our interventions in healthy individuals who can provide feedback regarding the pain and discomfort experienced, because such stimuli may trigger spasticity in clinical populations such as people with spinal cord injury, stroke, brain damage or other neurological disorders (Rabchevsky & Kitzman, 2011). Prior to the study, the participants were given detailed information about the procedures and risks of participation and they all signed an informed consent document. The participants completed the Physical Activity Readiness Questionnaire (PAR-Q) to ensure safe exercise participation and refrained from vigorous exercise (48 h) and alcohol (24 h) and stimulant consumption (e.g. caffeine, energy drinks, 6 h) prior to testing. Twelve of the 16 participants were also measured at 1 h and 48 h after the intervention to assess muscle force recovery and markers of muscle damage (details on Section 2.4.3). This study was approved by the University's Human Ethics Committee.

3.3.2 Procedures

All participants attended the laboratory on four testing sessions spread out over one month, one session per week at the same time of day with a minimum of 7 days between sessions. One week prior to the first experimental session, participants attended a full familiarisation session where each participant received patellar tendon vibration as well as NMES with and without patellar tendon vibration, and performed maximal voluntary isometric contractions (MVIC) of the knee extensors to ensure they could tolerate the protocols. All participants tolerated the NMES and tendon vibration protocols well. The subsequent three sessions were used to complete the following three experimental protocols in a random order without replication: 1) NMES only (STIM); 2) NMES superimposed onto tendon vibration (STIM+Vib); and 3) Vibration only (Vib). All participants ($n = 16$) were tested immediately before (PRE), immediately after (POST)

and a subset of participants ($n = 12$; four participants were unable to attend all follow-up testing) were also tested 1 hour (1H) and 48 hours (48H) after each experimental session. A standardised warm-up protocol was performed at the beginning of every session, which consisted of six consecutive concentric knee extension contractions with resistance provided by the inertia of an isokinetic dynamometer (Biodex System 3 Pro Ronkonkoma, NY) and then one repetition of isometric knee extensions at 30%, 50%, 70% and 90% of perceived maximal effort before performing a series of knee extension MVICs.

In experimental sessions, three MVICs were performed at each time point (PRE, POST, 1H and 48H) separated by 1 min of rest, with a fourth completed if a difference in peak torque of $\geq 3\%$ was observed between the best two attempts. The participants were seated with hip and knee joint angles of 85° and 90° , respectively ($0^\circ =$ full knee extension), with the thigh and trunk secured to the dynamometer chair and the knee joint was aligned with the centre of rotation of the dynamometer. Peak isometric knee extension torque was quantified during MVIC. Participants were instructed to produce a force against the dynamometer arm by extending the knee as fast and hard as possible for 3 s. Verbal encouragement and visual feedback was provided during all MVICs.

3.3.3 Neuromuscular electrical stimulation (NMES) and tendon vibration protocols

Following the MVICs, and to habituate participants to the electrical stimulations, two electrical square-wave stimuli (two 1000 μs square-wave pulses separated by 5 ms) were delivered to the dominant (stronger leg) every 20 s while the stimulation current was increased from 30 to 99 mA in 10-mA increments until a plateau in the maximum peak twitch torque was observed. Subsequently, trains of NMES were delivered by a high-voltage constant-current electrical stimulator (400 V, DS7A, Digitimer Ltd., Welwyn Garden City, UK) through four self-adhesive stimulation electrodes (Axelgaard, PALS, USA) placed over the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM). Two 5×10 cm electrodes were placed over RF and one 5×5 electrode was placed on each of the VM and VL approximately at their motor points. The electrodes were placed to elicit the greatest twitch response with a low stimulation current level of NMES, as determined in the familiarisation session, and were marked with an indelible ink on the skin to ensure identical electrode placement at subsequent sessions. The NMES protocol consisted of repeated 30-Hz trains of 58 wide-pulse width (1000 μs) symmetric biphasic pulses (0.033-s inter-pulse interval). A single train duration was 2-s and the inter-train interval was 2-s (i.e. 2-s on and 2-s off). 2-s contractions were used because extensive pilot testing revealed that shorter-duration contractions (i.e. 1 s) failed to evoke a torque plateau (i.e. maximal activation) during each train of stimulation, and that longer-

duration contractions (i.e. ≥ 3 s) tended to elicit a rapid muscular fatigue. Symmetric biphasic NMES, which employed currents with balanced positive and negative phases (polarity), was used due to its superior efficacy (in contrast to monophasic) to produce tetanic contractions and its demonstrated therapeutic benefits in clinical practice (Field-Fote, Anderson et al., 2003; Laufer, Ries et al., 2001). The stimulation current level of NMES was chosen to elicit 20% of the level of force developed during the muscle contraction on the best MVIC recorded during PRE measurements for each experimental session (henceforth referred to as the ‘target torque’) by delivering three single trains of the NMES protocol with increasing stimulation current level of NMES separated by one minute. Whilst the contribution of afferent pathways to motor unit activation occurs mainly at a high frequency (i.e. $>80\text{Hz}$) and at low stimulation force levels (i.e. 10% MVC)(Collins et al., 2001, 2002; Dean et al., 2014; Magalhaes & Kohn, 2010), a higher force level (i.e. $\geq 20\%$ MVIC) and low-to-moderate frequencies (20-30 Hz) (i.e. standard clinical conditions) were chosen to elicit higher forces that should stimulate significant changes in muscle force and mass in training interventions in clinical populations. Moreover, the use of 20% MVC has been previously investigated in the plantarflexors with a clear recruitment of motor units through the reflex arc during bouts of tendon vibration (Trajano et al., 2014).

Patellar tendon vibration was applied with a vibration device (Deep Muscle Stimulator, Las Vegas, NV, USA) to mechanically vibrate the tendon at 55 Hz and amplitude of 7 mm (determined by direct measurement using high-speed video capture). The tip of the vibration device was maintained at a steady pressure in a fixed position on the tendon immediately distal to the inferior border of the patella. This position was marked on the skin, and covered by a thin (1 mm thickness) soft pad to minimise pain or abrasion (refer to Figure 3.5).

The three experimental interventions were:

STIM: electrically-evoked muscle contractions were elicited by delivering the NMES protocol until the torque was reduced to $\leq 60\%$ of the target torque (i.e. 20% MVIC) in one electrically-evoked contraction, which was defined as ‘target fatigue’.

STIM+Vib: electrically-evoked contractions delivered as in STIM, but were superimposed with patellar tendon vibration which was applied for at least 5 s before NMES and after target fatigue was reached.

Vib: continuous patellar tendon vibration for one minute.

3.3.4 Data collection and analysis

3.3.4.1 Peak torque, impulse, fatigue index and number of contractions

The peak voluntary isometric knee extensor torque assessed during the MVIC was used to normalise the torque elicited by NMES during the sessions. Peak voluntary isometric knee extensor torque was defined as the maximum torque produced over a 500-ms window and included the plateau phase after at least a 250 ms rise time above baseline. The torque-time integral (TTI) was used to provide a measure of the total exercise stimulus received by the muscle in each condition (Figure 3.1). TTI was calculated as the product of torque and time calculated from the onset of the first stimulation train (STIM) or vibration onset (STIM+Vib) to the end of the final evoked contraction at the point of target fatigue (defined on Section 3.3.3). Peak evoked torque was defined as the highest torque value obtained after the onset of the first stimulation train for both STIM and STIM+Vib. TTI and peak evoked torque were compared between STIM and STIM+Vib. However, some participants responded with a greater TTI after STIM+Vib (positive responders to tendon vibration) whilst others showed a lower TTI after STIM+Vib (negative responders to tendon vibration), thus a second analysis was performed after separating participants into positive and negative responder to tendon vibration groups (described in Section 3.3.3). Positive and negative responders were identified not only during the training sessions, but also during pilot testing sessions where different NMES protocols with superimposed tendon vibration were utilised. Although this pilot data was not presented during this PhD thesis, this information reinforced the identification of positive and negative responders to tendon vibration during at least one to two pilot sessions and provided confidence in the interpretation of results. Total number of contractions was measured as the number of contractions performed from the beginning of the first evoked contraction (i.e. not including up to 3 contractions used to establish the current that elicited the target torque) reaching the target torque until the last contraction before reaching the target fatigue (defined on Section 3.3.3).

3.3.4.2 Muscle activity (EMG)

Vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) electromyograms (EMG) were recorded using bipolar electrode configurations sampled at analogue-to-digital conversion rate of 2,000-Hz (bandwidth 25 - 450 Hz) using a Wave wireless EMG system (Cometa Systems, Bareggio, Italy). The skin was carefully prepared by shaving, gently abrading and cleaning with alcohol prior to electrode placement. A bipolar electrode set (DE-2.1 single differential surface EMG sensor) with a 1-cm inter-electrode distance was attached to the skin over the belly of each muscle parallel to the predicted direction of muscle fibres,

following the SENIAM recommendations (Hermens, Freriks et al., 2000). Muscle activity was expressed as the root mean square of the EMG amplitude (applying a symmetric moving average with filter window = 500 ms) over the same time as the torque measurements, and the peak EMG was retained for analysis. Torque and EMG data were simultaneously recorded using LabChart version 8.0.2 Software (PowerLab System, ADInstruments Pty. Ltd, NSW, Australia) at the same analog-digital conversion_rate.

3.3.4.3 Muscle fatigue and muscle damage

Muscle fatigue was determined immediately post-intervention (POST) in all participants and at 1 (1H) and 48 h (48H) in a subset of 12 participants. Muscle fatigue was calculated as the percent decrement in MVIC torque. Pain and comfort scales were measured immediately after the training to report the pain and comfort during the training. Pain was measured for two different outcomes; one measure was to reflect the comfort of the different NMES protocols and the second measure was to reflect pain for muscle damage. Although muscle fatigue normally recovered rapidly, ongoing force depression might cause muscle and connective tissue damage resulting from the training and thus, force reduction is known to be strongly related to the degree of muscle damage (Nosaka et al., 2011). To determine whether muscle damage may have been elicited and thus contributed to the fatigue, indirect muscle damage markers were assessed POST, 1H and 48H after the intervention. Ultrasound imaging of RF and VI muscle thickness, defined as the distance between the subcutaneous fat layer and deep muscle border were measured using B-mode axial-plane imaging (Aloka SSD- α 10, Aloka Co., Ltd., Tokyo, Japan) (Nosaka & Clarkson, 1995). Muscle thickness changes are considered to be an indicator of the osmotic fluid shift that results in muscle swelling subsequent to muscle damage (Nosaka & Clarkson, 1995). The use of B-mode ultrasound imaging to detect increases in muscle thickness or volume resulting from muscle swelling, or changes in echo intensity, resulting from changes in muscle fibre integrity, is a common method used in previous research studies as an indirect marker of muscle damage after the application of electrical stimulation (Howell, Chleboun, & Conatser, 1993; Nosaka et al, 2011). The assessor performing ultrasonography was the PhD candidate, who received proper training by the PhD supervisor and practised the method for 3 months before data collection started. The participants were seated on a plinth with hip and knees at 90°. The same examiner obtained images at the 50% distance between the anterior superior iliac spine and superior border of the patella. The probe was placed in a marked area in a perpendicular position using a spirit level attached to the probe. The mean of three images of the RF and VI muscle thickness measurements at the same level was obtained for each condition and time. Perception of muscle soreness was assessed using the visual analogue scale and palpation. Participants were

asked to rate on a line from 0-100 mm (with “no pain” at 0 mm and “unbearable pain” at 100 mm) the soreness of the muscle after performing three bodyweight squats to approximately a 90° knee angle. The palpation assessment of muscle soreness consisted of the application of digital pressure using three fingers for approximately 3 s against the middle part of RF (Ohrbach & Gale, 1989). These tests have been extensively used in previous research studies evaluating indirect markers of muscle damage (Nosaka & Clarkson, 1996; Ohrbach & Gale, 1989).

Pain and comfort levels were also measured immediately after the completion of each NMES protocol (STIM and STIM+Vib). Subjects indicated the rate of perceived pain and comfort on a 1-10 scale based on how comfortable and painful the different protocols were perceived to be, with 1 being “comfortable and pain free” and 10 being “unbearable and extremely painful”.

3.3.5 Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) was used to compare changes in all variables between conditions (STIM, STIM+Vib and Vib) over time (PRE, POST, 1H and 48H) in the subset of 12 participants. A second two-way repeated measures ANOVA was used to compare STIM, STIM+Vib and Vib between PRE and POST in the full participant sample ($n = 16$). Repeated measures ANOVAs were used to compare EMG amplitude (RMS) in all individual muscles (RF, VM and VL) for STIM and STIM+Vib for PRE, POST, 1H and 48H. Pairwise t-tests were performed when significant interaction effects were found. Pearson’s product moment coefficients were computed to quantify the linear association between torque-time integral (TTI), peak torque and total number of contractions during STIM condition, and a binomial logistic regression analysis was performed to ascertain the ability of the torque-time integral (TTI) and total number of contractions in STIM to predict positive and negative responders to tendon vibration (i.e. TTI difference between STIM and STIM+Vib). Statistical significance was set at an alpha level of 0.05 and values were reported as mean \pm SD.

3.4 Results

No significant changes were observed in any measure after Vib, thus the subsequent analysis focused on the changes in response to STIM and STIM+Vib conditions. Mean values for MVIC peak torque and surface EMG amplitudes for the Vib condition are presented in Table 3.1.

3.4.1 Torque-time integral (TTI), peak evoked torque and total number of contractions

No statistical differences in peak evoked torque ($p = 0.94$), TTI ($p = 0.56$) or total number of contractions ($p = 0.49$) were observed between STIM and STIM+Vib. Nonetheless (as described in Section 2 and shown in Figure 3.1 the response to STIM+Vib was clearly greater in eight participants (50% of sample) but lesser (i.e. negative) in the other eight. Thus, a positive versus negative responder to tendon vibration analysis was undertaken where positive responders to tendon vibration were defined as participants who responded with a greater TTI after STIM+Vib and negative responders to tendon vibration as participants who showed lower TTI after STIM+Vib compared to STIM (TTI: positive responders: STIM: 1201.1 ± 321 Nm·s; STIM+Vib: 2757.2 ± 1329.7 Nm·s; negative responders: STIM: 2402.6 ± 497.7 Nm·s; STIM+Vib: 1344.0 ± 674.6 Nm·s). This analysis revealed a group \times condition interaction effect ($p < 0.001$) indicating a significant $145.0 \pm 127.7\%$ increase in TTI in STIM+Vib compared to STIM for positive responders to tendon vibration ($p = 0.014$) (see Figure 3.3A), indicating an increase in the total cumulative force produced by the muscle in STIM+Vib. A significant decrease in TTI ($-43.5 \pm 25.0\%$) was observed in STIM+Vib ($p = 0.002$) in the negative responders to tendon vibration (see figure 3.3B). The mean peak evoked torque for positive responders to tendon vibration for STIM was 49.3 ± 16.8 Nm and 52.1 ± 15 Nm for STIM+Vib, whilst for negative responders for tendon vibration under STIM was 51.7 ± 20.5 Nm and 48.1 ± 21.9 Nm under STIM+Vib. The mean total number of contractions for positive responders to tendon vibration for STIM was 16.2 ± 5.1 and for STIM+Vib was 29.1 ± 19.0 , whilst the means for negative responders were 39.8 ± 25.0 for STIM and 17.1 ± 7.3 for STIM+Vib.

Subsequent analyses of participants' responses in STIM were undertaken to determine if the likelihood of having a positive or a negative response in STIM+Vib could be predicted. This involved examination of TTI, peak torque and total number of contractions evoked by STIM, as well as the difference in TTI between STIM and STIM+Vib. A strong and statistically significant negative correlation was observed between TTI measured in STIM ($r = -0.72$, CI 90%: -0.44 to -0.88) and the difference between the TTI measured in STIM versus STIM+Vib. Also, a correlation of -0.45 (CI 90%: -0.03 to -0.74) between TTI in STIM and the difference between STIM and STIM+Vib for the total number of contractions was observed, whilst for peak torque a correlation of -0.27 was found (CI 90%: 0.18 to -0.62). Given the strong negative relationships observed between the difference in TTI in STIM+Vib and both TTI and total number of contractions in STIM, a binomial logistic regression analysis was performed to predict the likelihood of having a positive or a negative response to

STIM+Vib. The logistic regression model was statistically significant for torque-time integral ($\chi^2 = 17.845$, $p < 0.0005$), explaining 89% (Nagelkerke R^2) and predicting 87.5%. For the total number of contractions ($\chi^2 = 10.515$, $p < 0.0005$) the model explained 64% (Nagelkerke R^2) and predicted 81.3% of positive and negative responders to tendon vibration. Based on these results, torque-time integral and total number of contractions in STIM can be used to determine whether an individual will be a positive or negative responder to STIM+Vib in 87.5% and 81.3% of cases. Under the conditions of the present study a positive responder to tendon vibration would perform ≤ 16 contractions whilst a negative responder to tendon vibration would perform > 16 contractions until target fatigue. An example of the response of a positive responder to tendon vibration to STIM and STIM+Vib is shown in Figure 3.2.

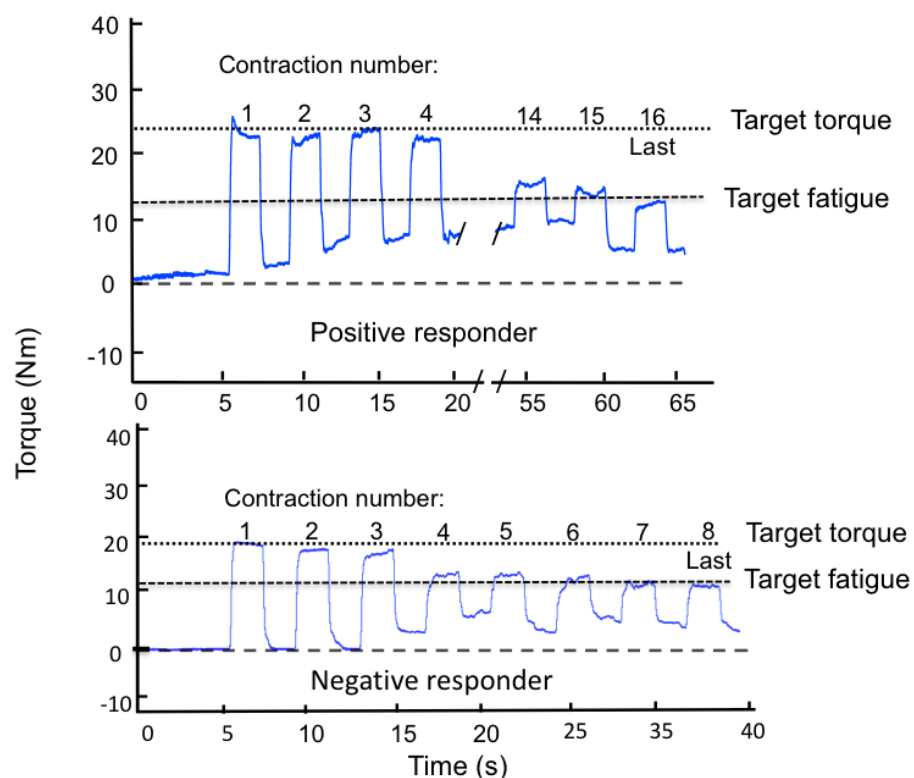


Figure 3.1 Torque production STIM+Vib

Examples of torque production (Nm) for a positive and a negative responder during STIM+Vib. A greater torque production, greater torque-time integral (TTI) and more contractions can be visualised in the positive responder graph in comparison to the negative responder during STIM+Vib. Also, an ongoing force production (i.e. typically a non-zero force) between trains of stimuli can be visualised in the positive responder to tendon vibration starting from the first train of electrical stimuli.

Last: last contraction before target fatigue. Target torque= 20% MVIC. Target Fatigue = 60% of target torque. Last: last contraction before target fatigue. Target torque= 20% MVIC. Target Fatigue = 60% of target torque.

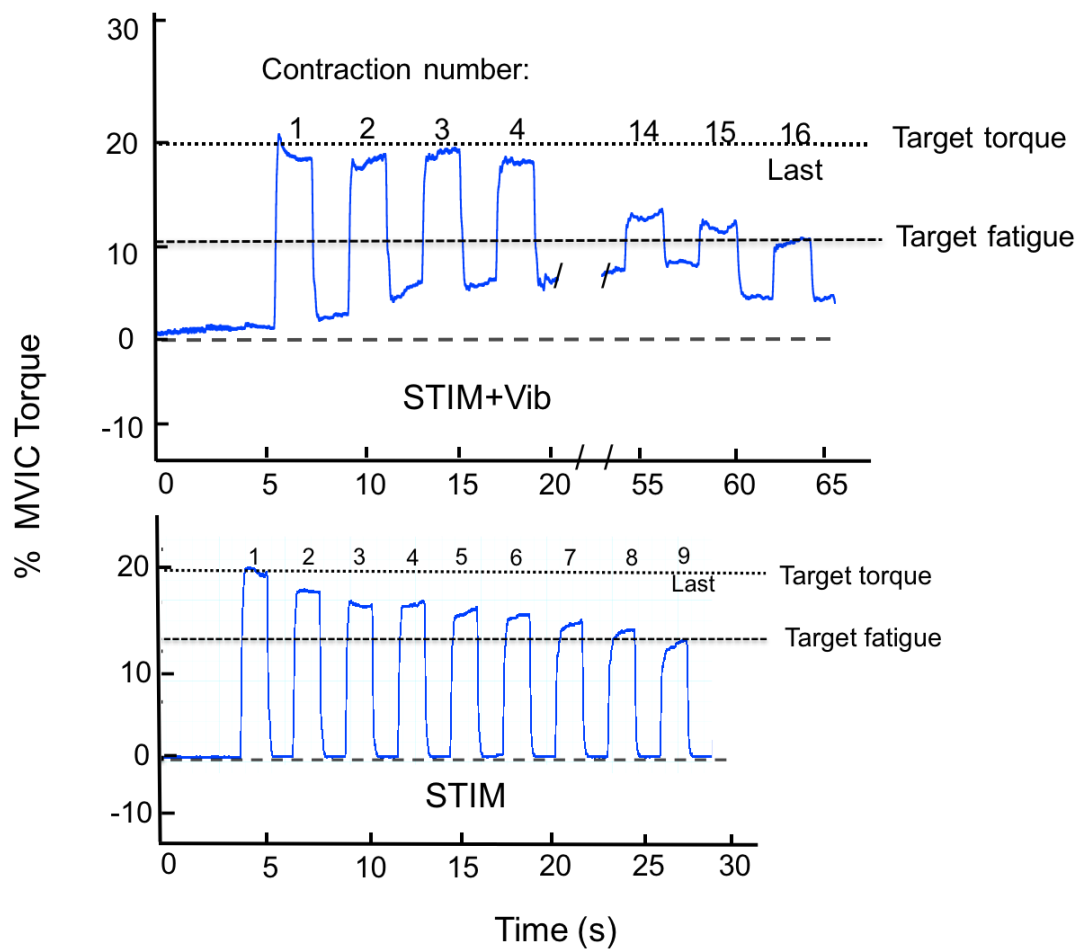


Figure 3.2 Torque production (% torque MVIC) and number of contractions positive responder to tendon vibration

Examples of torque production (Nm) for a positive responder to tendon vibration during STIM+Vib and STIM. A higher torque time integral (TTI) and greater number of contractions can be visualised in a positive responder to tendon vibration during STIM+Vib in comparison to STIM.

Last: last contraction before target fatigue. Target torque = 20% MVIC. Target Fatigue = 60% of target torque.

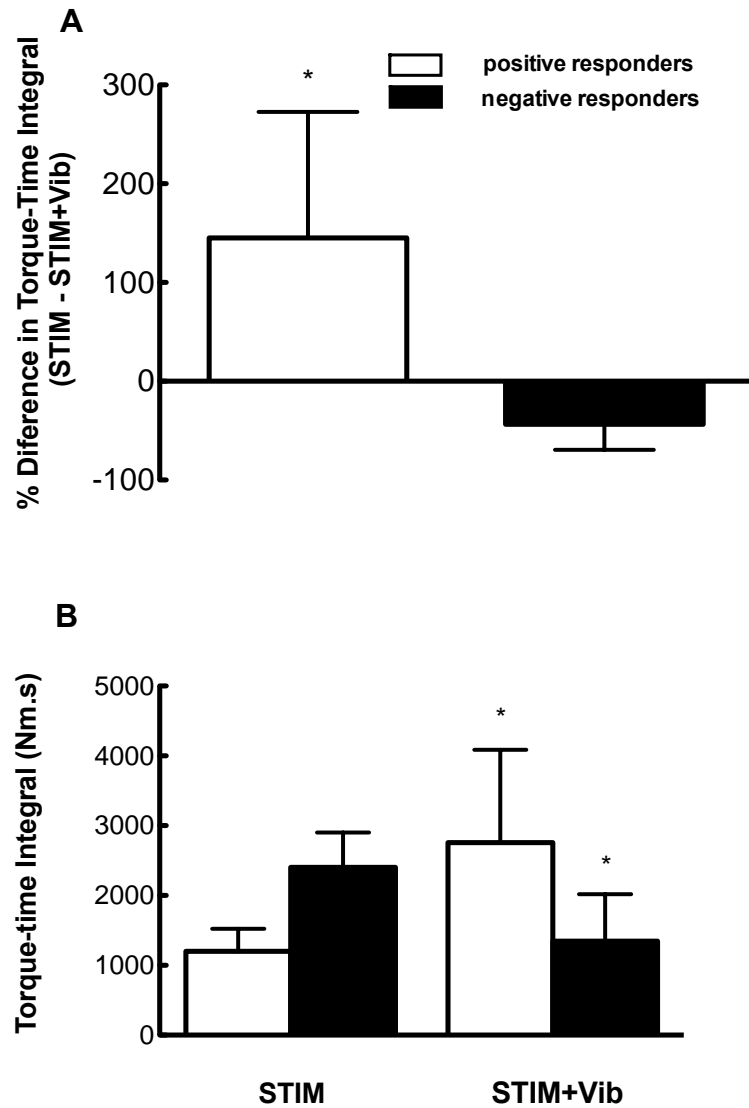


Figure 3.3 (A) Percentage difference between STIM and STIM+Vib in Torque-Time Integral (B) Mean Torque-Time Integral

- A) Percent difference in torque-time integral (Nm·s) for positive and negative responders ($145.0 \pm 127.7\%$ and $-43.5 \pm 25.7\%$). A significant increase in TTI in STIM+Vib compared to STIM for positive responders to tendon vibration was observed ($p = 0.014$).
- B) Mean torque-time integral (TTI; Nm·s) for positive and negative responders for STIM (1201.2 ± 321.9 Nm·s and 2402.6 ± 497.7 Nm·s) and STIM+Vib (2757.2 ± 1329.8 Nm·s and 1344.0 ± 674.6 Nm·s). A significant decrease in TTI ($-43.5 \pm 25.0\%$) was observed in STIM+Vib ($p = 0.002$) in the negative responders to tendon vibration.
- * Significant difference from STIM ($p < 0.05$).

3.4.2 Peak voluntary isometric contraction (MVIC) torque

As shown in Figure 3.4, a time \times condition interaction effect ($p = 0.016$) was observed for MVIC with a significant decrease in STIM observed from PRE (237.8 ± 90.1 Nm) to POST (219.1 ± 90.0 Nm; $p < 0.001$) and from PRE to 1H (215.8 ± 7.0 Nm; $p = 0.007$), but no change in STIM+Vib at any point. MVIC peak torques for STIM, STIM+Vib are shown in Figure 3.4.

The percentage change in MVIC from PRE to POST was -8.7% for STIM and -3.3% for STIM+Vib, as shown inset of Figure 3.4. However, a subgroup analysis for peak voluntary isometric contraction between positive and negative responders to tendon vibration did not reveal any statistically significant difference ($p = 0.30$ for condition \times time \times group interaction). It is important to note that these results were not influenced by the values obtained prior (MVIC PRE) to the application of the electrical stimulation protocols, as these values were not statistically different ($p = 0.19$) and were reliable between days (ICC = 0.95).

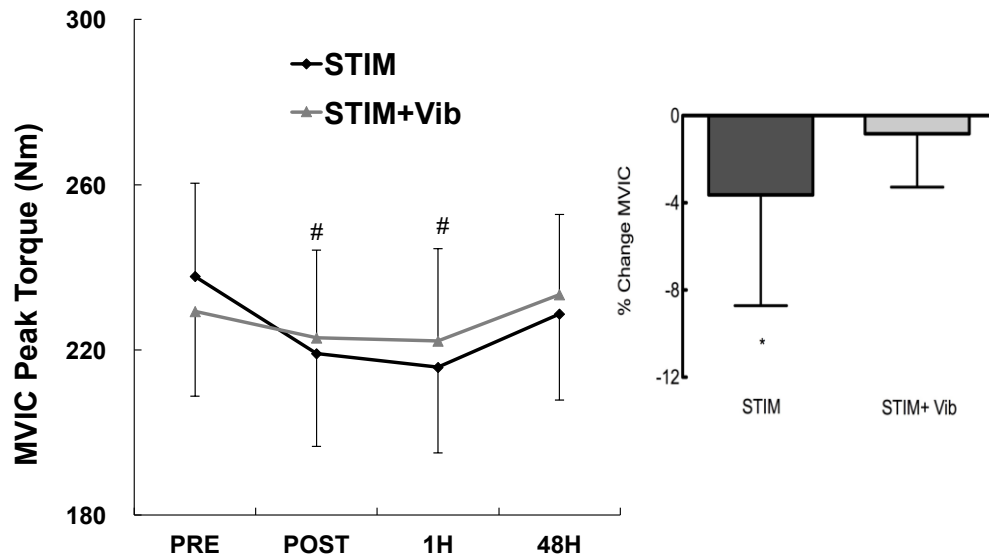


Figure 3.4 MVIC Peak torque and percentage change

Changes in peak isometric voluntary contraction torque (MVIC) across time (PRE, POST, 1H and 48H). A significant decrease in STIM from PRE (237.8 ± 90.1 Nm) to POST (219.1 ± 90.0 Nm; $p < 0.001$) and from PRE to 1H (215.8 ± 7.0 Nm; $p = 0.007$), but no change in STIM+Vib at any point was observed.

Significant difference from PRE ($p < 0.05$) for STIM. Mean values \pm standard error (SE). Inset: % change in MVIC from PRE to POST in STIM and STIM+Vib conditions. * Significant difference from PRE ($p < 0.05$). Mean change \pm SD.

3.4.3 Muscle activity (EMG) during MVIC

An interaction effect ($p = 0.006$) was observed for RF EMG amplitude during MVIC, with a significant decrease (16.7%) in the RF EMG amplitude after STIM from PRE (0.39 ± 0.16 mV) to POST (0.32 ± 0.14 mV; $p < 0.01$), but no differences for VM and VL EMG in STIM or any muscle in STIM+Vib. MVIC peak isometric torque and surface EMG amplitudes data during peak voluntary isometric torque at PRE, POST, 1H and 48H for STIM, STIM+Vib and Vib are presented in Table 3.1.

Table 3.1 MVIC peak torque and surface EMG amplitudes(Mean (\pm SD, 95% CI)) at PRE, POST (n: 16), 1H and 48H (n: 12) for STIM, STIM+Vib and Vib conditions

Measure	PRE		POST		1H		48H	
	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI
MVIC PT (N)								
STIM	237.8 \pm 90.2	189.7 - 285.8	219.1 \pm 90.0*	171.1-267.0	216 \pm 72.0*	170.1-261.4	228.7 \pm 72.2	182.8 - 274.6
STIM+Vib	229.3 \pm 82.0	185.6 - 273.0	222.9 \pm 84.8	177.7 - 268.0	222.1 \pm 77.3	173.0-271.3	233.4 \pm 67.2	190.6 - 276.0
Vib	214.2 \pm 63.4	174.0 - 254.5	212 .8 \pm 59.0	175.4 - 250.5	224.6 \pm 73.8	177.7 - 271.5	230.5 \pm 68.1	187.2 - 273.8
QUAD EMG (mV)								
STIM	1.27 \pm 0.60	0.94 - 1.61	1.14 \pm 0.48	0.88 - 1.40	1.20 \pm 0.53	0.90 - 1.51	1.19 \pm 0.48	0.85 - 1.52
STIM+Vib	1.32 \pm 0.51	1.05 - 1.60	1.29 \pm 0.52	1.01 - 1.56	1.38 \pm 0.54	1.03 - 1.72	1.4 \pm 0.49	1.09 - 1.71
Vib	1.14 \pm 0.62	0.75 - 1.54	1.16 \pm 0.60	0.78 - 1.54	1.16 \pm 0.52	0.83 - 1.50	1.23 \pm 0.64	0.82 - 1.63
RF EMG (mV)								
STIM	0.39 \pm 0.16	0.30 - 0.48	0.32 \pm 0.14 *	0.25 - 0.40	0.35 \pm 0.19	0.23 - 0.47	0.33 \pm 0.17	0.22 - 0.44
STIM+Vib	0.38 \pm 0.17	0.29 - 0.47	0.38 \pm 0.19	0.28 - 0.49	0.39 \pm 0.23	0.25 - 0.53	0.39 \pm 0.15	0.29 - 0.48

Measure	PRE		POST		1H		48H	
	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI
Vib	0.32 \pm 0.2	0.20 - 0.45	0.33 \pm 0.19	0.21 - 0.45	0.33 \pm 0.17	0.22 - 0.44	0.37 \pm 0.22	0.23 - 0.51
VM EMG (mV)								
STIM	0.50 \pm 0.44	0.27 - 0.74	0.48 \pm 0.38	0.28 - 0.68	0.51 \pm 0.39	0.26 - 0.76	0.52 \pm 0.37	0.28 - 0.75
STIM+Vib	0.47 \pm 0.33	0.29 - 0.64	0.46 \pm 0.34	0.28 - 0.64	0.51 \pm 0.38	0.27 - 0.75	0.61 \pm 0.43	0.34 - 0.88
Vib	0.35 \pm 0.34	0.14 - 0.57	0.35 \pm 0.33	0.14 - 0.56	0.34 \pm 0.30	0.15 - 0.53	0.34 \pm 0.33	0.13 - 0.56
VL EMG (mV)								
STIM	0.38 \pm 0.22	0.26 - 0.50	0.33 \pm 0.19	0.23 - 0.43	0.34 \pm 0.24	0.19 - 0.50	0.35 \pm 0.30	0.16 - 0.54
STIM+Vib	0.48 \pm 0.30	0.32 - 0.64	0.45 \pm 0.28	0.30 - 0.60	0.47 \pm 0.36	0.24 - 0.70	0.40 \pm 0.27	0.23 - 0.57
Vib	0.46 \pm 0.37	0.23 - 0.69	0.48 \pm 0.38	0.24 - 0.72	0.49 \pm 0.36	0.26 - 0.72	0.51 \pm 0.42	0.25 - 0.78
% from PRE (baseline MVIC PT)								
STIM	-	-	-8.72 \pm 5.79 *	-11.80 - -5.64	-6.79 \pm 7.26	-11.40 - -2.17	-0.67 \pm 11.39	-7.91 - 6.57
STIM+Vib	-	-	-3.28 \pm 6.44	-6.71 - 0.15	-1.61 \pm 6.61	-5.80 - 2.59	4.52 \pm 4.86	1.43 - 7.60
Vib	-	-	0.05 \pm 5.76	-3.61 - 3.71	4.42 \pm 11.61	-2.95 - 11.80	7.93 \pm 12.56	-0.06 - 15.91

MVIC: maximal voluntary isometric contraction; PT: peak torque; VM: vastus medialis muscle; VL: vastus lateralis muscle; RF: rectus femoris muscle; EMG_{RMS}: root mean square EMG amplitude; SD: standard deviation; 95% CI: 95% Confidence Interval * Significant difference from PRE (p < 0.05)

3.4.4 Indirect markers of muscle damage: muscle thickness and muscle soreness scales; pain and comfort scale

No changes were detected in combined RF and VI muscle thickness ($p = 0.66$) or muscle soreness scales either upon palpation ($p = 0.33$) or when performing bodyweight squats ($p = 0.37$) immediately after. Thus, no indications of muscle damage or soreness were observed in any condition at any time points (POST, 1H, 48H).

No statistically significant differences were found in the pain and comfort scales between STIM and STIM +Vib conditions (STIM: 4.1 ± 2.1 STIM+Vib: 5.0 ± 2.3 ; $p = 0.21$) immediately after the NMES protocols. Thus, both protocols elicited only “light-to-moderate” levels of pain and discomfort.

3.5 Discussion

The main finding of the present study was that the torque-time integral (TTI) measured at the point of fatigue (i.e. 60% of initial evoked torque) was not statistically different between STIM and STIM+Vib. Based on these results, the tendon vibration superimposed onto the wide-pulse width NMES did not appear to provide any additional benefit that might not have been derived from stimulations alone under the current conditions (30 Hz, 20% MVIC in quadriceps femoris muscle). However, a significantly greater TTI was observed in a subgroup ($n = 8$) of “positive responders” to tendon vibration. Thus, in 50% of the present participants, the addition of the tendon vibration allowed for a greater total muscular work to be performed, but this was not consistent among the participants.

Another notable, and practically relevant, finding was that a significant (-8.7%) reduction in maximal voluntary torque was evoked by STIM, which persisted for at least one hour and was associated with a reduction in RF EMG amplitude; thus, the wide-pulse width NMES elicited a notable fatigue response that persisted for at least 1 hour after the session and which could affect post-training movement capacity. Nonetheless, reductions in voluntary force and muscle activity were not observed when vibration was superimposed onto the NMES, even in negative responders to STIM+Vib. Therefore, the application of tendon vibration attenuated the fatigue response and allowed NMES to be used without a persisting voluntary muscle fatigue. These results are consistent with previous studies where the use of tendon vibration superimposed onto weak-to-moderate voluntary contractions was observed to attenuate the fatigue-induced decline in motor output, as assessed using standard surface EMG techniques during maximal voluntary contraction (e.g. tibialis anterior) (Bongiovanni & Hagbarth, 1990).

Regarding the lack of effect of vibration alone (Vib) on the outcome variables, this was predictable as the vibration stimulation only recruits the lowest threshold motor units (Bongiovanni & Hagbarth, 1990) and would not be sufficient to evoke strong muscle contractions, since larger, higher threshold motor units contribute more to higher force levels (Henneman et al., 1965).

The finding of an increased torque-time integral being produced when tendon vibration was superimposed onto NMES in the positive responders to tendon vibration (8 of 16 participants; for example see Figure 3.2) shows that an increase in total muscle contractile work was achieved. This could be considered advantageous in clinical practice as it would allow the muscle to produce a greater tension for longer, and thus may better evoke chronic increases in muscle strength and mass (Ahtiainen et al., 2003; Bax, Staes et al., 2005) and potentially improve muscle performance in people with limited voluntary muscle activation capacity (e.g. stroke, spinal cord injury, brain injury) (Smith et al., 2003). This result in positive responders was similar to previous observations of higher force levels (up to 50% maximal voluntary contraction) elicited by tendon vibration applied simultaneously with electrical stimulation in healthy participants (Magalhaes & Kohn, 2010). These greater muscular forces might possibly be attributed to the development of persistent inward currents (PIC), which could amplify and prolong the synaptic input and generate a sustained depolarisation of α -motor neurons leading to an increased recruitment of fatigue resistant motor units, maximising the use of reflexive pathways and thus increasing muscle force production (Bergquist et al., 2011; Collins et al., 2001, 2002; Dean et al., 2007; Magalhaes & Kohn, 2010). The augmented torque-time integral may also be attributed to the development of tonic vibration reflexes (TVR) occurring between muscle evoked contractions only when superimposed tendon vibration is applied (see Figure 3.2).

Nonetheless, 8 of 16 participants (negative responders to tendon vibration) showed a decrease in their TTI when tendon vibration was superimposed onto the wide-pulse width NMES, indicating that tendon vibration may reduce the ability to produce force and decrease the total muscle contractile work during high-intensity NMES contractions (i.e. near maximal evoked muscle contractions), thus representing a disadvantage in this subgroup of participants. This negative response may speculatively have been caused by the stimulation of Golgi tendon organs by the low-to-moderate frequency (55 Hz) of vibration applied during the contraction (Fallon & Macefield, 2007). Alternatively, the additional synaptic input provided by tendon vibration might have exacerbated fatigue mechanisms (e.g. ion channel function and neurotransmitter depletion), particularly for those individuals for whom the wide-pulse width NMES has already successfully recruited the lower threshold motor units.

In this case, we can infer that negative responders to tendon vibration might benefit from the sole application of (possibly wide-pulse width) NMES (STIM) based on the similar response in total amount of work (i.e. TTI) and total number of contractions performed under STIM in comparison to positive responders to tendon vibration under STIM+Vib (see Figure 3.3B). So, if a lower TTI is found after STIM then the application of tendon vibration would likely improve performance to approximately equally to the negative responders, whilst if a higher TTI is found after STIM then tendon vibration would likely reduce the TTI to similar levels to those found in negative responder". Thus, it appears that negative responders to tendon vibration will show a decrease in total muscle contractile work if tendon vibration is added and in these cases tendon vibration superimposed onto wide-pulse width NMES may represent a disadvantage, and thus the use of NMES alone would be more beneficial to elicit a high muscle force production. Whether this group has derived benefits from the wide-pulse width NMES as compared to standard (i.e. narrow pulse widths) remains to be explicitly investigated in future studies. The large inter-individual variability observed in our study is consistent with previous studies using wide-pulse width NMES, where substantial individual variability exists regarding its magnitude of effect (Neyroud et al., 2015; Neyroud et al., 2014; Regina Dias Da Silva et al., 2015; Wegrzyk et al., 2015a). Due to this large inter-individual variability, clinicians may need to test individual responses to tendon vibration before its implementation in clinical practice.

Additionally, given that TTI and total number of contractions measured in STIM could be used to predict 87.5% and 81.3% of the positive and negative responders to tendon vibration, respectively, the measurement of TTI or the number of contractions during wide-pulse width NMES might be a clinically relevant method to predict whether a patient would benefit from superimposed tendon vibration (i.e. a positive response to tendon vibration). In that case, clinicians might determine whether to use tendon vibration on their patients based on their response to STIM alone. However, since measuring TTI in clinical practice may not be practically feasible in some cases, using total number of contractions, for example by visually counting until reaching a pre-determined torque level (representing muscle fatigue), might be used to identify patients who will benefit from additional tendon vibration. Under the same conditions of this study a positive responder to tendon vibration would perform ≤ 16 contractions whilst a negative responder to tendon vibration would perform >16 contractions until target fatigue and this would be accurate in 81.3 % of the cases. However, due to high variability of the response to tendon vibration, this method may need to be used as an additional tool and further assessments may be required to ascertain the positive or negative response to tendon vibration.

A secondary finding of the present study was that a significant decrease in maximal voluntary force production was observed for at least 1 hour in STIM but not STIM+Vib. It is important to note that these results were not influenced by the values obtained prior (MVIC PRE) to the application of the electrical stimulation protocols, as these values were not statistically different ($p = 0.19$) and were reliable between days ($ICC = 0.95$). This result showed an advantage of the superimposed tendon vibration onto the electrical stimulation that prevented the significant fatigue-induced decline in MVIC. Thus, in the clinical context, tendon vibration may provide a benefit of reduced voluntary muscle fatigue when compared to moderate-frequency, wide-pulse width NMES that could allow for further rehabilitation work or improved performance of activities of daily living and occupational tasks in the hours after a rehabilitation session. It is not clear from the present data how the vibration provided a fatigue-attenuation benefit. Speculatively, it may have reduced the synchrony of the motor unit activity during NMES, which may have then reduce the rate of muscle fatigue (Ribot-Ciscar et al., 2003; Ribot-Ciscar et al., 1998). This might occur if ongoing facilitation of fatigue-resistant motor units was provided due to the generation of trains of Ia afferent signals into the spinal cord, inducing an excitation of homonymous motor neurons through the development of persistent inward calcium (Ca^{2+}) or sodium (Na^{+}) currents (PIC) at their dendritic trees (Ribot-Ciscar et al., 2003; Ribot-Ciscar et al., 1998). Such a mechanism would evoke a TVR influencing both spinal and supraspinal pathways (Ribot-Ciscar et al., 2003; Ribot-Ciscar et al., 1998). Tendon vibration-induced primary muscle spindle endings (i.e. Ia afferent activation) might also substitute for the fusimotor-driven Ia discharge and α -motor output decline that usually occurs during sustained voluntary contractions (Bongiovanni & Hagbarth, 1990; Macefield et al., 1991). This would have attenuated the muscle fatigue response observed in our study by continuing the Ia afferent activation response. Regardless of the potential mechanism, there seems to be a reversal of central drive failure when tendon vibration is superimposed onto wide-pulse width NMES, but further tests are needed to confirm this theory. However, the levels of muscle voluntary isometric fatigue observed in the present study (-8% after STIM) were somewhat smaller than the 22-30% reported by other studies (Aldayel et al., 2010b; Jubeau et al., 2008; Nosaka et al., 2011). This discrepancy may be attributed to the use of biphasic wide-pulse width NMES, the use of a lower stimulation frequency (30 vs. 75 Hz) or different duty cycle ratio (2-2 vs. 5-15 s), or that muscles were activated to only 20% of MVIC (with 'fatigue' being 60% of this value) in comparison to maximal tolerable levels of MVIC used by others (Aldayel et al., 2010b; Jubeau et al., 2012; Nosaka et al., 2011). Further explanation of these possibilities is required to accurately explain the differences in voluntary fatigue.

Another important finding was a reduced RF EMG amplitude observed during MVIC in STIM but not STIM+Vib, indicating that the loss of central drive to the muscle was minimised or eliminated with the application of tendon vibration. This selective decline in activation on RF at POST and not on the other muscles may be attributed to the higher activity of RF during isometric knee extension at 90° of knee flexion (Watanabe et al., 2009) influencing the activation of the bi-articular RF over the vastii muscles (VM and VL) during STIM (Maffiuletti et al., 2003; Matta et al. 2015) and the higher EMG fatigue experienced on RF due to its bi-articular nature (Ebenbichler et al., 1998). It would be of interest to determine whether the decrease in EMG during MVIC and its “rescue” when tendon vibration is imposed onto NMES is observed in other skeletal muscles, or whether it is unique to RF or other biarticular muscles (Ebenbichler et al., 1998).

Of final note, no evidence for muscle damage or soreness was found in either condition at any time point, and levels of reported pain and comfort were “light-to-moderate” and not statistically different between conditions (mean for STIM = 4/10, STIM+Vib = 5/10). Therefore, the muscle stimulation and vibratory stimuli could be applied without concern for ongoing muscle fatigue or damage, and with reasonable levels of pain and comfort, at least in healthy individuals. The muscle damaging effects of electrically evoked isometric contractions have been previously attributed to the disruption of the muscle fibres and their surrounding connective tissue (Aldayel et al., 2010a; Mackey et al., 2008; Mackey et al., 2011; Nosaka et al., 2011), which causes a prolonged loss of muscle force generating capacity. The lack of damage in the present study might be explained by the fact that muscles were activated to only 20% of MVIC, whilst maximal tolerable levels of muscle contraction were evoked in previous studies (Aldayel et al., 2010a; Mackey et al., 2008; Mackey et al., 2011; Nosaka et al., 2011). Finally, being able to exercise regularly without ongoing soreness or force loss may have broad clinical relevance since pain can trigger life-threatening episodes in some clinical conditions such as autonomic dysreflexia in people with spinal cord injury (Rabchevsky & Kitzman, 2011). Finally, since the levels of pain and discomfort were “light-to-moderate” and not different between the conditions, these NMES protocols can be considered safe for implementation in future clinical studies.

Limitations of this study are that our results are only pertinent under specific conditions of NMES (1000 μ s, 30Hz) at relatively higher torque levels than previously been investigated (Collins et al., 2001, 2002; Dean et al., 2014; Magalhaes & Kohn, 2010). Thus, a different response may result under different NMES conditions. Further studies using relatively higher intensities (i.e. 20% MVIC) and the same parameters of NMES as used in this study should be performed to confirm our results.

3.6 Conclusion

Based on the present results, the imposition of tendon vibration onto moderate-frequency wide-pulse width NMES may allow for a greater amount of muscular work to be performed, and thus for a more optimum training response to be achieved, in a proportion of participants who respond positively. However, a lesser response might be elicited in those individuals who respond negatively (50% in the current study) and in these cases tendon vibration superimposed onto wide-pulse width NMES may represent a disadvantage and thus, the use of NMES alone would be more beneficial to elicit a high muscle force production. Nonetheless, the use of tendon vibration superimposed onto wide-pulse width NMES appeared to minimise the voluntary fatigue experienced after the training session and might therefore allow for additional rehabilitation work to be performed or for the trained muscle groups to be more effectively used for locomotion (i.e. crutches use) and activities of daily living after the session for both positive and negative responders to tendon vibration. Finally, since muscle damage and soreness were not observed, and levels of pain and discomfort were light-to-moderate after both NMES conditions, the application of these methods appear to be sufficiently safe to be used in clinical populations, such as in people with SCI. This is important as some clinical populations may be susceptible to high levels of muscle fatigue and muscle damage or might respond negatively to painful stimuli. Nonetheless, replication of these findings in a larger sample is encouraged before this type of NMES protocol is recommended in clinical practice.

Chapter 4 Study two

Effect of tendon vibration during wide-pulse neuromuscular electrical stimulation (NMES) on muscle force production in people with spinal cord injury

4.1 Abstract

Introduction: Neuromuscular electrical stimulation (NMES) is commonly used to activate skeletal muscles in people with spinal cord injury (SCI). Clinical recommendations for NMES suggest the use of short pulse widths (100-200 μ s) and low-to-moderate pulse frequencies (30-50 Hz). However, this type of NMES causes rapid muscle fatigue due to the (non-physiological) high stimulation intensities and non-orderly recruitment of motor units. With respect to overcoming these issues in the clinical setting, the use of wide pulse widths (1000 μ s) might speculatively optimise motor unit activation through spinal reflex pathways and thus delay the onset of muscle fatigue and increase total contractile impulse improving muscle force production. Moreover, superimposing patella tendon vibration onto the wide-pulse width NMES is speculated to elicit further increases in impulse.

Methods: Nine people with SCI received two NMES protocols with and without superimposing tendon vibration on different days (i.e. STIM and STIM+vib), which consisted of repeated 30 Hz trains of 58 wide-pulse width (1000 μ s) symmetric biphasic pulses (0.033-s inter-pulse interval; 2 s stimulation train; 2-s inter-train interval) being delivered to the dominant quadriceps femoris. Starting torque was 20% of maximal doublet-twitch torque and stimulations continued until torque declined to 50% of the starting torque. Total knee extensor impulse was calculated as the primary outcome variable.

Results: Total knee extensor impulse increased in four subjects when patella tendon vibration was imposed ($59.2 \pm 15.8\%$) but decreased in five subjects ($-31.3 \pm 25.7\%$). However, there were no statistically significant differences between these sub-groups or between conditions when the data were pooled.

Conclusions: Based on the present results there is insufficient evidence to conclude that tendon vibration provides a clear benefit to muscle force production or delays muscle fatigue during wide-pulse width, moderate-intensity NMES in people with SCI.

4.2 Introduction

Spinal cord injury (SCI) is most commonly caused by trauma and interrupts the connection between supra-spinal and spinal regions of the CNS (Tewarie et al., 2010). This leads to a reduction of the voluntary activation of muscles below the lesion level, reducing muscle force production, impairing physical function, and profoundly compromising physical health and quality of life (QoL) (Hosseini et al., 2012; Oyster et al., 2011; Tewarie et al., 2010). Furthermore, the reduction in muscle force production is a major predictor of mortality risk and seems to be partly attributable to the quantity (i.e. absolute muscle volume) and quality (i.e. muscle density) of muscle mass (Schaap et al., 2009; Srikanthan & Karlamangla, 2014).

A common method to increase muscular force production and muscle mass is the use of muscle strength training. Particularly, the high-intensity muscular contractions have been proven to enhance longevity and QoL in different clinical populations (Andrade & da Silva, 2015; Caserotti et al., 2008; Clark & Goon, 2015; Orlando et al., 2016). However, muscle strength training poses an increasing challenge for people with a neurological condition, such as people with SCI. Alternatively, neuromuscular electrical stimulation (NMES) is a commonly used intervention in rehabilitation programs with the aim of increasing muscle recruitment and thus muscle force production, especially in individuals with a complete loss of motor function (Barbeau et al., 2002; Harvey et al., 2010; Thrasher et al., 2013). NMES has been conventionally used in clinical practice as functional electrical stimulation (FES), i.e. a prolonged and low levels of evoked force NMES exercise paired simultaneously or intermittently with a functional task (Thrasher et al., 2013). However, such interventions cannot optimally stimulate muscular strength and mass improvements, which require the imposition of a higher load to the muscle to obtain higher force output (Ahtiainen et al., 2003; American College of Sports Medicine, 2009) and according to the overload training principles (Enoka, 2002), to obtain musculoskeletal changes in paralysed muscles.

The use of high intensity (hereafter used as near maximal evoked muscle contractions) intermittent NMES training as a strength training method has not been adopted clinically, probably due to the lack of evidence from long-term intervention trials documenting its physiological effects (Alon, 2003; Hortobagyi & Maffiuletti, 2011) but mainly because these types of protocols generate rapid muscle fatigue and thus prevent the development of repeated high-intensity muscle contractions (Gorgey et al., 2006; Gregory & Bickel, 2005; Hillegass & Dudley, 1999; Ibitoye et al., 2016). This muscle fatigue is exacerbated in people with SCI due to the loss of low-threshold (fatigue-resistant) motor units and their higher proportion of fast fatigable motor units (Adams et al., 1993; Bickel et al., 2011; Bickel et al., 2004; Burnham et al., 1997; Pelletier & Hicks, 2011), making the rapid muscle fatigue induced by NMES a

continuing issue (Gorgey, et al., 2014; Karu et al., 1995). Additionally, lack of knowledge about the stimulation parameters and the different response to NMES are the main factors why NMES is not widely used among clinicians. It is because of these reasons that early muscle fatigue represents a problem because if clinicians are not aware of the best stimulation parameters, then the NMES training session would likely produce early muscle fatigue that would prevent the optimum dose response to obtain musculoskeletal adaptations in paralysed muscles.

One promising method to enhance force production whilst minimising muscle fatigue is the application of tendon vibration (Cotey et al., 2009; Ribot-Ciscar et al., 2003). Tendon vibration mechanically generates trains of Ia-afferent signals to the spinal cord that may induce a progressive excitation of homonymous motor neurones and promote the development of persistent inward calcium (Ca^{2+}) or sodium (Na^+) currents at their dendritic trees (Collins, 2007; Gondin et al., 2010; Wegrzyk et al., 2015a). Thus, this method could amplify and prolong synaptic input and create a sustained depolarisation (i.e. self-sustained firing) leading to an increased recruitment of motor units and increase muscle force production, especially when coupled with wide-pulse width (e.g. 1000 μs) NMES (Magalhaes & Kohn, 2010; Trajano et al., 2014). Importantly, however, vibration tends to preferentially excite low-threshold (i.e. fatigue resistant) motor units (Bongiovanni & Hagbarth, 1990), which exhibit significant fatigue resistance and are therefore likely to contribute to a great total muscular work being completed before muscular forces are notably reduced (i.e. before fatigue is induced). In previous studies the positive effects of vibration were observed during wide-pulse width NMES applied both at relatively low (~5% MVC force; Magalhaes & Kohn, 2010) and high ~20% MVC force; Trajano et al., 2014) levels of muscle force production, suggesting that vibration may augment muscle contraction force and allow a greater total muscular effort before fatigue during wide-pulse NMES even when relatively high contraction intensities are used, such as those required for strength training. Evidence of the effects of wide-pulse width NMES into the generation of more fatigue-resistant muscle contractions (i.e. through central pathways) in people with SCI has been previously documented (Clair-Augier et al., 2013). However, it is unknown whether the imposition of tendon vibration onto wide-pulse width NMES promotes such benefits in people with SCI. In fact, it is also not known whether tendon vibration might elicit an additional excitation of the motor neurone pool through the same (central) pathways as wide-pulse width NMES (Collins, 2007; Gondin et al., 2010; Neyroud et al., 2015; Wegrzyk et al., 2015a,b) and therefore instead promote a greater muscle fatigue in partially or completely paralysed muscles.

In Study 1 of this thesis, tendon vibration during wide-pulse width NMES resulted in an increased total muscle work performed in “positive responders” to tendon vibration only, but

a reduced total work in others. Nonetheless, within the whole study cohort the application of tendon vibration tended to minimise the voluntary muscle fatigue caused by the NMES, and would therefore have allowed the participants to immediately perform other physical activities without a notable negative impact on performance. Thus, it is of great interest to determine whether similar effects would be obtained in people with SCI. The purpose of the present study, therefore, was to determine whether tendon vibration superimposed onto wide-pulse width NMES under standard clinical conditions elicits a greater peak muscle force with less muscle fatigue (i.e. a greater total impulse) when compared to NMES applied without tendon vibration in people with SCI.

4.3 Methods

4.3.1 Subjects

Nine subjects with SCI (3 females, 6 males) were recruited from the Spinal Cord Injuries Australia (SCIA) Activity-based therapy exercise program “Neuromoves” and the Perth community (mean \pm SD, age: 39.4 ± 10.6 y; height: 176.2 ± 9.7 cm; body mass 80.6 ± 9.6 kg). Four subjects were classified as complete spinal cord injury (ASIA A) and five were classified as incomplete (ASIA B-D). All subjects were recruited by word of mouth or email and had a SCI of more than 6 months. Prior to the study, subjects were given detailed information about the procedures and risks of participating in the study and they all signed a written consent form. Subjects completed the physical Activity Readiness Questionnaire (PAR-Q) to ensure safe exercise participation and they refrained from vigorous exercise (48 h) and alcohol (24 h) and stimulant consumption (e.g. caffeine, energy drinks for 6 h) prior to testing. This research study was approved by the University’s Human Ethics Committee. Subject characteristics are detailed in Table 4.1.

Table 4.1 Subject characteristics for Study 2

Subject levels of injury, completeness of lesion, time since injury, ASIA scale score, medication type and response to tendon vibration (i.e. whether a tendon vibration reflex response is detectable).

Subject	Level of injury	Complete (C)/ Incomplete (I)	Time since injury (y)	ASIA	Medication	Positive responder to tendon vibration	Negative responder to tendon vibration
A	T ₇	I	3	B	Baclofen		x
B	T ₆	C	6	A	Oxybutin		x
C	T ₆	C	5	A	Baclofen		x
D	C ₆ -C ₇	C	4	A	Baclofen		x
E	T ₁₂	I	2	D	Baclofen		x
F	C ₇	C	1	A	Baclofen	x	
G	T ₅	I	20	D	Baclofen	x	
H	T ₃	I	2	B	Baclofen	x	
I	L ₃	I	4	D	N/A	x	

4.3.2 Procedures

All subjects attended the Neuromuscular Physiology Laboratory at Edith Cowan University on three occasions on different days (one day per week for the duration of 4 weeks) with a minimum of 7 days between sessions. One week before starting data collection, the subjects attended a full familiarisation session where the NMES protocol was applied to the dominant quadriceps femoris with and without simultaneous patellar tendon vibration. Each subject received 1 min of tendon vibration and 3 min of NMES to ensure they could tolerate the intervention; all subjects tolerated the NMES and tendon vibration protocols well. The subsequent two sessions were used to complete the following two experimental protocols in a random order without replication: 1) NMES only (STIM); and 2) NMES superimposed onto tendon vibration (STIM+Vib).

In familiarisation and experimental sessions, the subjects were asked to produce a voluntary knee extension contraction of 50% of perceived maximal intensity while seated on an isokinetic dynamometer (Biodex System 3 Pro Ronkonkoma, NY). If any voluntary contractions were visualised and recorded then a standardised warm-up protocol was performed, consisting of

isometric knee extensions at 30%, 50%, 70% and 90% of perceived maximal effort, before they performed a series of three knee extension MVICs. However, if no voluntary contraction was recorded then three attempts of maximal voluntary contractions (MVICs) were instructed without warm-up efforts. The subjects were seated with hip and knee joint angles of 85° and 90°, respectively (0° = full knee extension), with the thigh and trunk secured to the dynamometer chair and the knee joint aligned with the center of rotation of the dynamometer. All subjects were instructed to produce a force against the dynamometer arm by extending the knee as fast and hard as possible for 3 s, and verbal encouragement and visual feedback were provided during all MVICs irrespective of the subject's ability to voluntarily activate their lower limbs. This method was implemented to be consistent with the procedures among all participants

4.3.3 Electrical stimulation and tendon vibration protocols

NMES was delivered by a high-voltage constant-current electrical stimulator (400 V, DS7A, Digitimer Ltd., Welwyn Garden City, UK) through four self-adhesive stimulation electrodes (Axelgaard, PALS, USA) placed over the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM); two 5×10 cm electrodes were placed over RF and one 5×5 electrode was placed on each of the VM and VL, approximately at their motor points. The electrodes were placed to elicit the greatest twitch response at a low stimulation intensity, as determined in the familiarisation session. The electrode positions were marked on a plastic sheet for each subject and indelible ink was used to mark these positions on the skin to ensure identical electrode placement at subsequent sessions.

Following the MVICs, and to habituate the subjects to the electrical stimulations, two electrical square-wave stimuli (1000 μ s square-wave pulses separated by 5 ms) were delivered to the dominant leg (determined in familiarisation session using NMES) every 20 s while the stimulation current was increased from 30 to 99 mA in 10-mA increments until a plateau in the maximum peak twitch torque was observed. This was defined as maximal peak twitch torque ($\tau_{tw,p}$) and was used as the “target torque”. A second, submaximal peak twitch torque ($\tau_{tw,sub}$) recording was obtained at 40 mA and retained for analysis of changes in submaximal torque, to assess the ability of the muscle to contract under submaximal conditions, which are often used in a clinical context. Subsequently, a maximum of three trains of NMES (described below) were performed at different stimulation current intensities until reaching the closest value to the target torque. The NMES protocol consisted of repeated 30 Hz trains of 58 wide-pulse width (1000 μ s) symmetric biphasic pulses (0.033-s inter-pulse interval). A single train duration was 2 s and the inter-train interval was 2 s (i.e. 2-s on and 2-s off). The level of evoked torque was set equal or close to the maximal peak twitch torque ($\tau_{tw,p}$).

Patellar tendon vibration was applied with a vibration device (Deep Muscle Stimulator, Las Vegas, NV, USA) that mechanically vibrated the tendon at 55 Hz and amplitude of 7 mm, as determined by direct measurement using high-speed video capture. The two test conditions were:

STIM: Electrically-evoked muscle contractions evoked by delivering the NMES protocol until torque was reduced to $\leq 50\%$ of the target torque (i.e. $\tau_{tw,p}$) in one electrically-evoked contraction, which was defined as “target fatigue”.

STIM+Vib: Electrically-evoked contractions delivered as in STIM, but superimposed with patellar tendon vibration which was applied for at least 5 s before NMES and after target fatigue was reached.

4.3.4 Data collection and analysis

4.3.4.1 Peak evoked torque, torque-time integral and number of contractions

Two measures of peak twitch (evoked) torque were analysed: one submaximal ($\tau_{tw,sub}$) evoked at a 40-mA current, and one maximal ($\tau_{tw,p}$) obtained from the peak of the torque-current relationship. These were obtained both before and after the NMES protocols were delivered. The total torque-time integral (TTI) was used to provide a measure of the total exercise stimulus received by the muscle in each condition. TTI was calculated as the product of torque and time calculated from the onset of the first stimulation train (STIM) or vibration onset (STIM+Vib) to the end of the final evoked contraction when “target fatigue” was reached (as defined in Section 4.3.3). Peak evoked torque was defined as the highest torque value obtained after the onset of the first stimulation train for both STIM and STIM+Vib. TTI and peak evoked torque were compared between STIM and STIM+Vib. The post-study data analysis revealed that some subjects responded with a greater TTI after STIM+Vib (i.e. positive responders to tendon vibration) whilst others showed no difference or a lower TTI after STIM+Vib (i.e. negative responders to tendon vibration), as described in Results (Section 4.4). Therefore, a second analysis was performed after separating subjects into positive and negative responders to tendon vibration groups (described in Section 4.4.1). Positive and negative responders were identified not only during the training sessions, but also during pilot testing sessions where different NMES protocols with superimposed tendon vibration were utilised. Although this pilot data was not presented during this PhD thesis, this information reinforced the identification of positive and negative responders to tendon vibration during at least one to two pilot sessions and provided confidence in the interpretation of results. The total number of contractions was measured as the number of contractions from the beginning

of the first evoked contraction reaching the target torque until the last contraction when reaching 50% of the target torque (“target fatigue”).

4.3.5 Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) was used to compare changes in all variables between conditions (STIM, STIM+Vib) over time (PRE, POST). A Wilcoxon test was conducted to compare STIM, STIM+Vib between PRE-and POST in positive and negative responders to tendon vibration whilst using assessments at PRE as the covariates. Pairwise t-tests were performed when significant interaction effects were found. A chi-square test for independence was used to assess whether an association existed between subjects with complete and incomplete SCI and the likelihood of being a positive or negative responder. Statistical significance was set at an alpha level of 0.05 and values are reported as mean \pm Standard deviation (SD).

4.4 Results

4.4.1 Peak evoked torque, torque-time integral (TTI) and total number of contractions

No statistical differences in peak evoked torque ($p = 0.43$; Fig. 4.1A), torque-time integral (TTI; $p = 0.39$; Fig. 4.1B) or total number of contractions ($p = 0.78$) were observed between STIM and STIM+Vib. Nonetheless, the response to STIM+Vib (based on TTI) was clearly greater in some subjects (40% of sample) but lesser (or negative) in others. Thus, an additional comparative analysis of positive versus negative responders to tendon vibration analysis was undertaken, where positive responders to tendon vibration were defined as those subjects who responded with a greater TTI in STIM+Vib when compared to STIM. This analysis revealed no statistical difference in TTI between STIM and STIM+Vib for positive or negative responders (Fig. 4.2), or a difference between STIM and STIM+Vib for the whole cohort collectively. However, the between-condition difference was dissimilar between the responder groups when using $\tau_{tw,p}$ at PRE for both conditions as a covariate ($p = 0.02$); TTI was $59.2 \pm 15.8\%$ greater in STIM+Vib than STIM in positive responders to tendon vibration ($p = 0.13$) but decrease of $31.3 \pm 25.7\%$ in STIM+Vib compared to STIM was observed in negative responders ($p = 0.14$), as shown in Figure 4.2. Also, there was no clear relationship between completeness of lesion (complete or incomplete SCI) and the frequency of positive or negative responders to tendon vibration ($\chi^2 (1, n = 9) = 1.10, p = 0.70$). Finally, no significant differences were found between the conditions (STIM and STIM+Vib) for total number of contractions, despite a trend being observed; the mean total number of contractions for positive responders to tendon vibration for

STIM was 56.5 ± 60.5 and for STIM+Vib was 70.2 ± 68.9 , whilst the means for negative responders to tendon vibration were 41.6 ± 37.4 for STIM and 33.8 ± 30.9 for STIM+Vib.

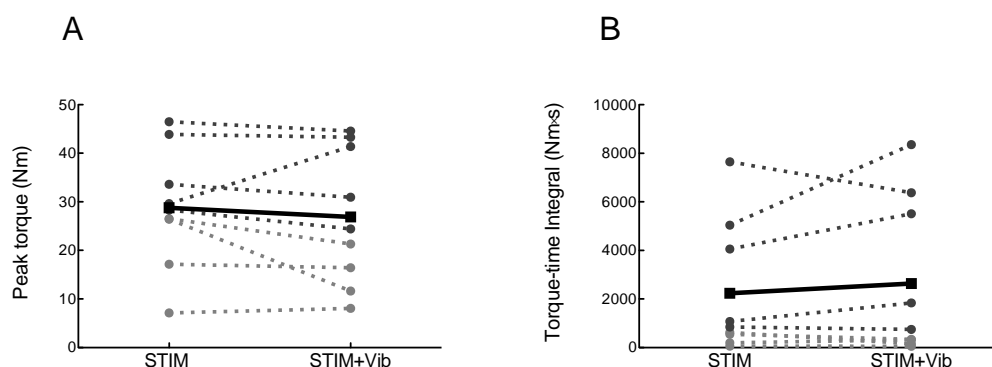


Figure 4.1 Peak torque and Torque-Time Integral (TTI) in STIM and STIM+Vib conditions

A) Peak torque production (Nm) in STIM and STIM+Vib conditions for all subjects. No statistically significant differences were found between the two conditions B) Torque-time integral (TTI; Nm.s) in STIM and STIM+Vib conditions for all subjects. No statistically significant differences were found between the two conditions. Grey dashed lines represent individual subjects and the black solid line represents the group mean. Darker grey dots indicate subjects with incomplete spinal cord injury (SCI) and light grey dots indicate subjects with complete SCI.

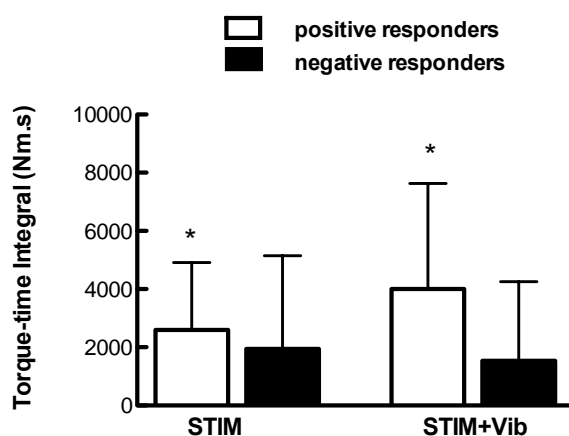


Figure 4.2 Torque-Time Integral (TTI; Nm.s) in STIM and STIM+Vib

TTI recorded in STIM and STIM+Vib conditions for positive and negative responders to tendon vibration). Significant increases of $59.2 \pm 15.8\%$ in TTI in STIM+Vib compared to STIM for positive responders to tendon vibration ($p = 0.13$) and decreases of $-31.3 \pm 25.7\%$ in STIM+Vib for negative responders to tendon vibration ($p = 0.14$) were observed, however TTI was not statistically different between conditions.

* Significantly different between positive and negative responders ($p < 0.05$).

4.4.2 Muscle force measures: Maximal evoked torque ($\tau_{tw,p}$) and submaximal evoked torque ($\tau_{tw,sub}$)

There was a significant effect of time, as the relative maximal force ($\tau_{tw,p}$; $p < 0.001$) and submaximal force ($\tau_{tw,sub}$; $p < 0.001$) torque decreased from PRE to POST in both conditions (STIM and STIM+Vib). However, a similar pattern was observed in STIM+Vib and STIM conditions, as there were no significant effects of condition ($\tau_{tw,p}$: $p = 0.21$; $\tau_{tw,sub}$: $p = 0.13$) and no significant condition \times time interaction ($\tau_{tw,p}$: $p = 0.98$; $\tau_{tw,sub}$: $p = 0.77$). Submaximal twitch torque ($\tau_{tw,sub}$) declined to $40.4 \pm 4.7\%$ and maximal torque ($\tau_{tw,p}$) declined to $27.0 \pm 5.0\%$ of baseline in STIM, whilst $\tau_{tw,sub}$ declined $45.0 \pm 4.2\%$ and $\tau_{tw,p}$ declined to $30.6 \pm 5.0\%$ of baseline in STIM+Vib (Fig. 4.3).

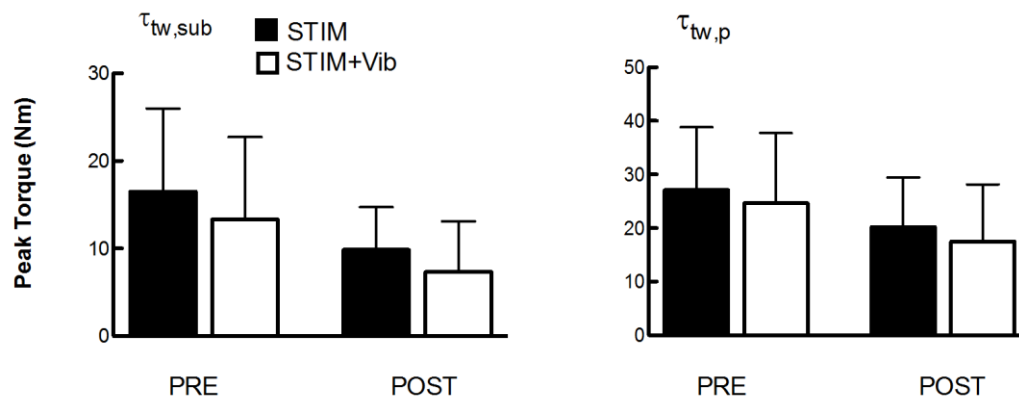


Figure 4.3 Submaximal ($\tau_{tw,sub}$) and maximal ($\tau_{tw,p}$) peak twitch torque recorded before (PRE) and after (POST) STIM and STIM+Vib

Submaximal ($\tau_{tw,sub}$: 40 mA) and maximal ($\tau_{tw,p}$) electrical stimulation peak twitch torques recorded before (PRE) and after (POST) STIM and STIM+Vib. Submaximal twitch torque ($\tau_{tw,sub}$; top panel) declined $40.4 \pm 4.7\%$ and maximal force ($\tau_{tw,p}$; bottom panel) declined $27.0 \pm 5.0\%$ of baseline in STIM, whilst $\tau_{tw,sub}$ declined $45.0 \pm 4.2\%$ and $\tau_{tw,p}$ declined $30.6 \pm 5.0\%$ of baseline in STIM+Vib. However, no statistically significant differences were found between the two conditions.

4.5 Discussion

The purpose of this study was to determine whether tendon vibration superimposed onto wide-pulse width NMES would elicit greater peak muscle force production and/or induce less muscle fatigue (i.e. increase the total impulse before fatigue) than wide-pulse width NMES alone in people with spinal cord injury (SCI). The main finding was that the torque-time integral (TTI) measured at the point of fatigue (i.e. 50% of initial evoked torque) was not statistically different between STIM and STIM+Vib conditions, although a significantly greater TTI was found in four (of nine) “positive” responders to tendon vibration ($59.2 \pm$

15.8%). Thus, in four of the present participants, the addition of the tendon vibration allowed for a greater total muscular work to be performed, but a lower total muscular work was completed in the other five subjects ($-31.3 \pm 25.7\%$). However, the TTI produced by the cohort as a whole not statistically different between conditions. Moreover, as observed in Figure 4.1, there was no evidence that completeness of lesion (complete vs. incomplete SCI) was a factor influencing the likelihood of being a responder. Therefore, whilst the imposition of tendon vibration during wide-pulse width NMES may allow for a greater impulse to be provided before fatigue in some individuals, it is not apparently clear who might benefit from the application of tendon vibration in people with SCI based on our results.

The use of tendon vibration superimposed onto wide-pulse width NMES was hypothesised to allow for a greater training impulse prior to fatigue, and thus it might evoke greater chronic increases in muscle strength and mass as well as improve muscle performance after a period of training (Bax et al., 2005; Dudley-Javoroski, 2008), however this appears to be possible only in those individuals who showed a positive response to tendon vibration. The ability to produce greater forces after a period of training may allow for higher volumes of muscle work to be performed during a physical rehabilitation session and thus generate early improvements in physical performance and physical health benefits in people with SCI; this hypothesis should thus be tested using a longitudinal study design. A reflex response induced by tendon vibration may facilitate the initiation, maintenance and strength of any residual voluntary contraction in people with incomplete SCI. The use of tendon vibration in those with a complete lesion can help to regulate the reflex response, which is highly altered after a complete SCI, by stimulating reflex pathways. Previous research has shown that peripheral input continues to flow in a modified manner after a complete SCI and that there is a potential to generate motor patterns if this peripheral input is stimulated by the use of methods such as NMES and tendon vibration (Edgerton & Roy, 2009; Edgerton et al., 2004). The mechanisms of action of tendon vibration relate to the tonic vibration reflex (TVR), elicited by the application of tendon vibration, which may potentially help recruit more motor units and thus increase muscle force production when used in combination with NMES (Magalhaes & Kohl, 2010). Greater muscular forces elicited by the tendon vibration when superimposed to NMES were found in previous studies in healthy people (Magalhaes & Kohn, 2010; Bochekezanian et al, 2017) and were attributed to the generation of persistent inward currents, recruiting higher-threshold motor units through reflexive pathways and increasing muscle force production (Bergquist et al., 2011; Collins et al., 2001). On the other hand, the reduced training impulse prior to fatigue observed in the negative responders to tendon vibration suggested that tendon vibration may be detrimental for some people and that in these people the use of (wide-pulse width) NMES alone may be more beneficial. This response observed in negative responders

to tendon vibration may speculatively be caused by the low-to-moderate frequency (55 Hz) vibration resulting in a stimulation of Golgi tendon organs, which can cause autogenic inhibition and potentially decrease the motor output (Fallon & Macefield, 2007). Therefore, the present, individual-specific results cannot confirm the hypothesis that superimposing tendon vibration onto wide-pulse width NMES would elicit an additional greater peak muscle force with less muscle fatigue (i.e. a greater total impulse) when compared to NMES applied without tendon vibration in most people with a SCI.

These variable responses to tendon vibration may also be explained by the highly individual functional deficits found between individuals with incomplete SCI, where the transmission of the sensory-motor information is altered at the synaptic level (Lieberman, 1981; Xia & Rymer, 2005) and there is a muscle spindle afferent dysfunction after both complete and incomplete lesions to the spinal cord (Lieberman, 1981; Xia & Rymer, 2005). A similar inter-individual variability in response to wide-pulse width NMES has also been suggested to originate from a difference in monoamine levels between individuals (Collins, 2007) and could be one reason for the different response to tendon vibration superimposed onto wide-pulse width NMES observed in the current study. This difference in monoamine levels could be exacerbated by the altered neuromuscular system in people with spinal cord injuries (Biering-Sorensen et al., 2009), such as changes in the excitability of the motor neurone (Kim et al., 2015), fibre type transformation towards fast-fatigable fibres (Biering-Sorensen et al., 2009) and high levels of muscle atrophy (Gordon & Mao, 1994). Moreover, the increased TTI in positive responders to tendon vibration might be attributable to the development of tonic vibration reflexes (TVR) which increase muscle force contributions between the evoked muscular contractions (Magalhaes et al., 2013; Trajano et al., 2014). However, the activation of already hyper-excitabile sensory pathways by the use of tendon vibration in people suffering from a spinal cord injury may have either triggered episodes of intrinsic phasic spasticity in some participants (Adams & Hicks, 2005) or attenuated spasticity symptoms in others (D'Amico et al., 2014), and thus may have increased the variability in the response to wide-pulse width NMES and tendon vibration observed in the present study. These hypotheses will need to be explored in further studies investigating explicitly the pathophysiological responses (i.e. spasticity) of paralysed and paretic muscles to tendon vibration superimposed onto wide-pulse width NMES.

Another important finding of the current study was that the decline in evoked muscle force was not attenuated by the application of tendon vibration. Muscle fatigue experienced after both STIM and STIM+Vib could be attributed to the “peripheral fatigue” (i.e. contractile alterations) induced by NMES, due to the repeated activation of the same muscle fibres,

especially due to the absent sensory feedback from the muscles to the spinal cord to prevent failure after a SCI (Binder-Macleod & Snyder-Mackler, 1993; Kent-Braun, 1999; Mizrahi, 1997). It may also be possible that tendon vibration activated not only excitatory but also inhibitory interneurons and thus negated the possible positive effects of tendon vibration on muscle fatigue (Eklund et al., 1982). Accordingly, results from the present study are inconclusive regarding the effects of superimposing tendon vibration onto wide-pulse width NMES in people with SCI and future research studies are needed to investigate the mechanisms of action of tendon vibration in chronically paralysed muscles. Moreover, the present results remain to be verified in future studies in a larger cohort of people with SCI, whilst also considering the potentially-confounding factors of age, level of injury, ASIA classification (complete vs. incomplete), time since injury, spasticity and medication use, which may impact in the measured outcomes.

4.6 Conclusion

The imposition of tendon vibration onto moderate-frequency, wide-pulse width NMES may allow for a greater amount of muscular work (muscle force production) to be completed before fatigue in a proportion of participants (i.e. positive responders to tendon vibration) with SCI. However, a lesser response may also be elicited in participants who respond negatively to tendon vibration (60% in the current study) and thus, for these individuals, wide-pulse width NMES alone may provide greater benefits. It is also notable that the use of tendon vibration superimposed onto wide-pulse width NMES did not minimise the (peripheral) fatigue elicited by the training session. Based on the present results there is insufficient evidence to conclude that tendon vibration improves muscle force production when superimposed onto wide-pulse width NMES in people with SCI. Nonetheless, replication of these findings is mandated before decisions as to whether to implement the strategy in clinical practice can be made.

Chapter 5 Study three

Can high-intensity NMES strength training improve muscle strength and mass and indicators of health and quality of life in people with spinal cord injury?

5.1 Abstract

Introduction: Muscle force production is usually impaired in people with spinal cord injury (SCI). However, the use of high-intensity NMES strength training can help promote metabolically active lean muscle mass and thus, increase muscle mass and provide physical health and quality of life (QoL) benefits. Nonetheless, NMES is usually used at low-stimulation intensities (e.g. functional electrical stimulation) and there is limited evidence regarding the effects of high-intensity NMES strength training for increasing muscle force capacity and mass, ameliorating symptoms of spasticity or improving physical health markers and quality of life (QoL) in people with SCI.

Methods: Five individuals with SCI completed five 10-repetition sets of high-intensity isometric knee extension NMES strength training sessions twice a week for 12 weeks. Training was performed on both right (R) and left (L) quadriceps muscles. Before and after the intervention, quadriceps femoris isometric knee extensor torque was measured on a dynamometer and cross-sectional area (CSA_{QF}) and muscle ultrasound echo-intensity (EI) were measured with extended-field-of-view ultrasonography. Bone mineral content (BMC) and density (BMD) of whole body and both legs, lean body mass, fat mass and body fat percentage were measured using dual-energy X-ray absorptiometry (DXA). Bone parameters using peripheral quantitative computed tomography (pQCT) at 4% and 33% of femur length (diaphyseal slices) included total bone CSA, cortical bone CSA, cortical thickness, cortical BMD and polar strength-strain index. Venous blood samples were collected for blood lipid profiling and C-Reactive Protein (CRP) analyses. The Spinal Cord Injury Spasticity Evaluation Tool (SCI-SET) was used to assess symptoms of spasticity and the quality of life index (QLI) SCI version III was used for QoL measures.

Results: Following the intervention, QF tetanic isometric knee extensor torque increased on average by 35% (2 - 92%) and CSA_{QF} increased by 47% (14 - 145%). Statistically

significant increases in BMC and decreases in BMD were observed, but were not sufficiently large to be deemed clinically relevant. A significant increase in the HDL/LDL cholesterol ratio ($p < 0.001$) was also found. A mean significant improvement of $4.8\% \pm 2.3\%$ (absolute value = 0.26) in SCI-SET score was observed, whilst QoL showed a trend towards improvement in the health & functioning domain (15.0 ± 4.2 ; 17.3 ± 5.1 ; $p = 0.07$).

Conclusions: Findings of increases in muscular strength and CSA_{QF} , improvements in several physical health markers, decreases in symptoms of spasticity and trends towards improvements in some aspects of QoL highlight the efficacy of high-force NMES strength training in people with SCI. Whilst further examination is required, clinicians might consider using high-intensity NMES strength training to improve muscle strength and mass as well as physical and mental health.

5.2 Introduction

Spinal cord injury (SCI) is a devastating lesion that leads to significant muscular changes below the level of the spinal lesion. It pre-emptly a marked reduction in muscle force production and commonly evoke symptoms of spasticity, which can profoundly impair physical health and quality of life (QoL) (Chakravarty & Mukherjee, 2010; Harvey, 2008; Tewarie et al., 2010; Westerkam et al., 2011). Muscle force production capacity is clearly influenced by skeletal muscle mass (Trezise et al., 2016; Collier et al., 2016), which is reduced up to 50% when compared to able-bodied controls (Castro et al., 1999; Gorgey & Dudley, 2007) and plays a crucial role in reducing the risk of premature all-cause mortality (Dankel, Loenneke et al., 2016). In addition to skeletal muscle atrophy, there is an increase in intramuscular fat in people with both complete and incomplete SCI (Castro et al., 1999; Gorgey & Dudley, 2007), which is associated with an increase in diabetes risk (Addison, Marcus et al., 2014). Thus, people with SCI are at a higher risk of developing cardiovascular diseases, dyslipidemia (Bauman & Spungen, 2000; Yekutieli et al., 1989), osteoporosis (Jiang et al., 2006) and metabolic syndrome (Bauman et al., 1999), and are thus faced with decreased life expectancy (De Vivo et al., 1999; Whiteneck et al., 1992).

Muscle strength training promotes numerous benefits and it is probably one of the most effective tools to stimulate gains in muscle mass (which can be measured by ultrasound locally and as whole body lean mass using DXA scan) and strength, reducing systemic inflammation and enhancing longevity and QoL (Andrade & da Silva, 2015; Orlando et al., 2016; Pedersen et al., 2007; Pedersen & Febbraio, 2012; Sievanen et al., 1994). More specifically, high-intensity strength training, which refers to the imposition of sufficient loading to require the production of maximal or near maximal muscle force, can achieve (near-)maximal motor unit activation (Ahtiainen & Hakkinen, 2009). Thus, a strong mechanical stimulus from the generation of high, as compared to lower muscular forces, may enhance the hypertrophic response and generate optimum gains in muscle strength (Goto et al., 2004; Goto et al., 2005). Based on this, most effective strength training protocols involve the use of slow-speed strength training, and particularly (near-)isometric and eccentric modes where muscular forces are high in accordance with the force-velocity relationship for skeletal muscles (Higbie et al., 1996; Noorkoiv et al., 2014, 2015; Norrbrand et al., 2008; Roig, O'Brien et al., 2009; Seger & Thorstensson, 2005). The main aim of high-intensity strength training is to progressively increase the resistance in order to stimulate optimal increases in muscle strength (Carpinelli, 2008) and thus generate adaptations leading to physical health benefits that are derived from the attainment of muscle strength (Ahtiainen et al., 2003). Therefore, it is of interest to examine the impact of strength training under, for example, isometric contractions using higher levels

of force than typically evoked using low-intensity NMES (i.e. FES training), hereafter named “high-intensity NMES strength training”. This type of NMES training may provoke systemic changes and thus positively affect muscle force and mass (including intramuscular-fat), physical health, symptoms of spasticity and QoL in people with SCI.

Since muscle strength training poses a great challenge for people with SCI, NMES has been used in clinical practice, commonly in the form of functional electrical stimulation (FES), to overcome muscle weakness and aim to improve muscle strength and mass with the consequent benefits in physical health (Cramer et al., 2002; Dudley-Javoroski, 2008; Gorgey, Harnish et al., 2012; Gorgey et al., 2006; Gorgey, Mather, et al., 2012; Gorgey, Poarch et al., 2010; Gorgey & Shepherd, 2010; Griffin et al., 2009; Harvey et al., 2010). Nonetheless, the use of NMES as a strength training modality has previously been shown to stimulate quadriceps muscle hypertrophy between 35% and 75% after 8-16 weeks of training (Bickel, Yarar-Fisher et al., 2015; Dudley et al., 1999; Gorgey, Mather, et al., 2012; Mahoney et al., 2005), improved skeletal muscle oxidative capacity (Erickson et al., 2016) as well as to possibly produce small decreases in visceral adipose tissue and increases in lean body mass (Gorgey, Mather, et al., 2012) and muscle and bone changes in people with SCI (Dudley-Javoroski, 2008; Hangartner et al., 1994; Shields et al., 2006). Yet, the use of NMES as high-intensity strength training mode has not been extensively investigated and, due to the limited evidence supporting its use for increasing muscle strength (Glinsky et al., 2007; Harvey, 2016), is not commonly used in clinical practice. Importantly, some essential outcomes, such as muscle force and physical health improvements, muscle and bone plasticity (Dolbow et al., 2011; Gater et al., 2011), effects on intramuscular fat (Gorgey & Dudley, 2007; Gorgey & Shepherd, 2010) and symptoms of spasticity and QoL, have not been extensively explored in people with SCI after NMES strength training interventions (D'Amico et al., 2014; Dudley-Javoroski, 2008; Glinsky et al., 2007; Harvey et al., 2009; Pedersen et al., 2007; Pedersen & Febbraio, 2008) and specifically in response to high-intensity muscle strength training. A larger body of work is therefore required to more clearly define the adaptations to NMES-based strength training to allow for clearer cost-benefit decisions to be made by clinicians.

Therefore, the purpose of the present study was to investigate the effects of high-intensity strength training performed under isometric conditions using low-to-moderate-frequency (30 Hz) NMES (i.e. standard clinical conditions) on muscle force and mass, physical health (including bone structure), symptoms of spasticity and QoL in people with SCI.

5.3 Methods

5.3.1 Subjects

Twenty people with chronic SCI expressed interest in participating in the study and eight subjects were initially recruited based on the inclusion criteria: age 18-65 years; SCI longer than 6 months that led to complete or incomplete paraplegia or tetraplegia; level of injury between C₂ and L₅; ASIA (American Spinal Cord Injury Association) A, B, C or D; have medical permission to enrol an intensive exercise program; and able to participate in the program over a 14-week period. Exclusion criteria also were applied, including acute phase of injury (less than 6 months from injury); ventilator dependent, other associated neurological disease; and complications such as severe urinary infection, pressure ulcers, previous lower-limb fractures or any other health condition that may constrain the participation in an exercise program. Of these eight subjects, five subjects (4 males, 1 female) completed 12-week intervention; one subject decided not to commence the training intervention period after completing the initial assessments (i.e. 2-week period) and two subjects dropped out after the third week of the intervention period (after sessions 4 and 6) due to time constraints-. Three subjects (subject A-C; see Table 5.1) were already enrolled in an activity-based-therapy exercise program that included FES and other (individual-specific) exercise-training modalities (Spinal Cord Injuries Australia Activity-based therapy exercise program;- SCIA-Neuromoves) whilst two (subjects D, E) were living in the local community but not currently involved in a formal clinical rehabilitation program; these subjects also had no prior experience with electrical stimulation-based exercise. The mean (\pm SD) age, height, body mass and body mass index of the subjects were: 33.8 ± 6.5 y; 174.4 ± 9.7 cm; 69.6 ± 16.4 kg; 20.2 ± 22.7 kg/m²) (see Table 5.1). Prior to the study, the subjects were given detailed information about the procedures and risks of participation and they all signed an informed consent document. The subjects completed the Physical Activity Readiness Questionnaire (PAR-Q), provided a medical certificate to ensure safe exercise participation, and refrained from vigorous exercise (48 h), alcohol (24 h) and stimulant consumption (e.g. caffeine, energy drinks, 6 h) prior to testing. This study was approved by the University's Human Research Ethics Committee.

Table 5.1 Subject characteristics for Study 3

Subject	Level of injury	Complete (C)/ Incomplete (I)	Time since injury (y)	ASIA	Medication	Wheelchair user	Community walker (crutches)	Use of FES routinely
A	T ₃	I	2	B	Baclofen	x		x
B	T ₇	I	3	B	Baclofen	x		x
C	T ₁₂	I	2	D	Baclofen		x	x
D	T ₁₀	C	7	A	None	x		
E	T ₁₀	C	2	A	Baclofen	x		

5.4 Experimental design

The study duration was 14 weeks and assessments were performed identically on three different occasions at approximately the same time of the day (± 2 h) and under the same experimental conditions. In the first two weeks of the study (i.e. between measurements at weeks -1 and 0) the subjects completed a control (reliability) phase (Häkkinen et al., 1998), where the subjects did not perform any experimental training but continued their regular physical activities. This followed a familiarisation session that was performed in the week before this period. The following 12 weeks of the study constituted the experimental phase. Training was performed twice a week, every second day for 12 weeks. All assessments were completed at -1 (hereafter named “Control period”), 0 weeks (0 wk) and 12 weeks (12 wk), except for resting blood samples which were taken at 0 wk and 12 wk only. Post- training assessments were taken 4-6 days after the last training session (session 24; see Figure 5.1 for timeline) to allow for recovery of the acute, residual effects of intense exercise.

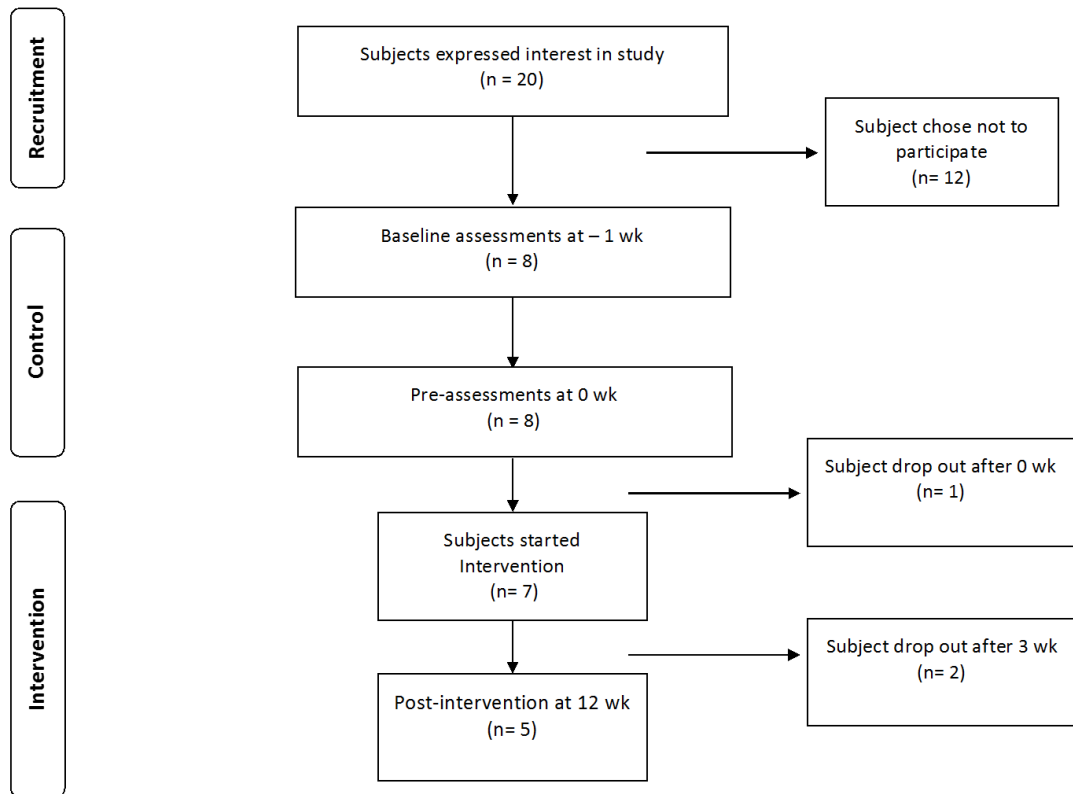


Figure 5.1 Timeline of subjects involved in Study 3 during recruitment, control and intervention phases

Subject recruitment and completion numbers for the study. Twenty subjects originally volunteered for participation, 8 subjects met inclusion criteria and were able to commence the study and 5 subjects completed the training period and measurement sessions.

5.4.1 Assessments

5.4.1.1 Knee-extension torque measurements

Knee extension torque measurements were performed as described in Study 2. The subjects were seated with hip and knee joint angles of 85° and 90° , respectively (0° = full knee extension), with the thigh and trunk secured to the dynamometer chair and the knee joint aligned with the centre of rotation of the dynamometer. An initial contraction (or attempt) at 50% of perceived maximal voluntary knee extensors was performed by all subjects. If voluntary contractions were visualised and recorded, then a standardised warm-up protocol was performed consisting of one isometric knee extensions at 30%, 50%, 70% and 90% of perceived maximal effort before a series of three knee extension MVICs (one of five subjects met this criterion). However, if voluntary contraction was not observable then the subject was classified as motor complete and no further voluntary contractions were performed; four of five subjects met this second criterion. These four subjects were instructed to perform three maximal voluntary contractions (MVICs) attempts with no additional warm-up efforts.

Subsequently, two electrical square-wave stimuli (two 1000 μ s square-wave pulses separated by 5 ms) were delivered to the subjects' right legs every 20 s during which the stimulation current was increased from 30 to 99 mA in 10-mA increments until a plateau in the maximum peak twitch (doublet) torque was observed. This was defined as the maximal peak twitch torque ($\tau_{tw,p}$) and was used as the “target torque” during the subsequent training session (where the term ‘maximal’ refers to the use of the maximal stimulation intensity and ‘peak’ refers to the amplitude of the torque response). A second, submaximal peak twitch torque ($\tau_{tw,sub}$) recording was obtained at a current intensity of 40 mA and retained for analysis of changes in submaximal torque in order to assess the ability of the muscle to contract under submaximal conditions that are often used in a clinical context. Subsequently, a maximum of three NMES trains (described on Section 5.4.2) were provided at different stimulation current intensities until reaching the closest value to the target torque. The NMES protocol consisted of repeated 30 Hz trains of 58 symmetric biphasic pulses (0.033-s inter-pulse interval; 1000 μ s) and the inter-train interval was 2 s (i.e. 2-s on and 2-s off). The left leg was then tested using the same methodology. The peak tetanic torque was then recorded for each subject. An example of the knee extensor torque production obtained using these stimulation parameters can be seen in Figure 5.2. All subjects were instructed to consciously think about performing an MVIC in the knee extensors for 3 seconds and relax to be consistent among the whole cohort of participants. Some research studies showed that consciously thinking about moving a part of the body activates a part of the cerebral cortex and may have a repercussion in a better sensory integration of the information to the brain (Lee et al, 2017).

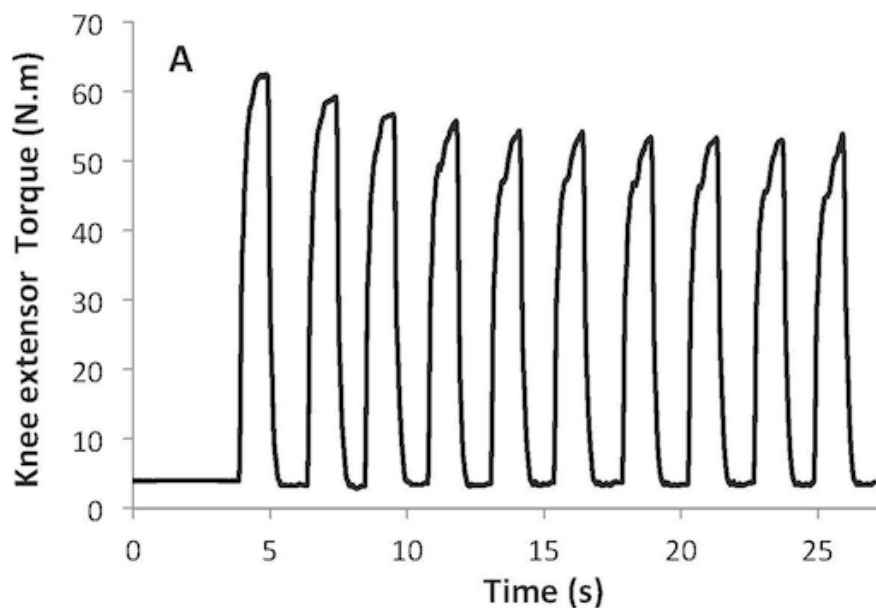


Figure 5.2 Example of knee extensor torque production.

Repeated 30 Hz trains of 58 symmetric biphasic pulses (0.033-s inter-pulse interval; 1000 μ s) and the inter-train interval was 2 s (i.e. 2-s on and 2-s off).

5.4.1.2 Muscle cross-sectional area (CSA) and intra-muscular fat (IMF)

Quadriceps femoris (QF, i.e. sum of VM, VL, VI and RF) CSA and ultrasound echo intensity (EI) were measured using B-mode axial-plane ultrasonography (Aloka SSD- α 10, software number 6.1.09, Aloka Co., Ltd., Tokyo, Japan). Subjects rested supine for 15 min prior to testing to minimise fluid shifts before images were captured with a 10 MHz linear-array probe (60 mm width) using the extended field-of-view technique (EFOV), as described previously (for example see figure 5.5) (Noorkoiv et al., 2010). A line from the central point of the patella to the medial aspect of the anterior superior iliac spine (ASIS) was marked to obtain the images (Noorkoiv et al., 2010). One line perpendicular to this was marked at 50% of the distance from the greater trochanter to the lateral epicondyle (Noorkoiv et al., 2010). A continuous single view was then obtained by moving the probe transversely across the thigh on the marked line. Two images were obtained consecutively. Minimal pressure was applied with the probe to avoid compression of the muscle. CSA_{QF} was measured using ImageJ digitising software (1.46r, Wayne Rasband, National Institutes of Health, USA) for each muscle (VM, VL, VI and RF) and for the whole quadriceps femoris (QF) with the mean of the two images taken as CSA. Intra-class correlation (ICC) analysis was performed for images obtained at -1 wk and 0 wk.

Ultrasound echo intensity (EI) was measured by computer-assisted greyscale analysis using the standard histogram function in Image-J (National Institute of Health, USA, version 1.42) after manually tracing around the muscle perimeters (i.e. whole muscles, rather than a region of interest, were examined). Mean EI measurements of the whole muscle were calculated twice (E₁ and E₂) using two images taken on two separate days (-1wk and 0 wk) and intra-class correlation (ICC) analysis was performed between these two images. The EI was expressed in values between 0 and 256 (0: black; 256: white); mean values between -1 and 0 wk and between E₁ and E₂ were calculated, resulting in a mean value at 0 wk, this mean value was then compared it to the values obtained at 12 wk. Under the current experimental conditions, EI changes were considered to be reflective of changes in intra-muscular fat (IMF) content because factors such as fibrosis and intra-muscular connective tissue concentration were considered to change little within the 12-week time frame of the intervention.

5.4.1.3 Body composition outcomes

The body composition outcomes of lean body mass, fat mass, body fat percentage and bone mineral density (BMD) of the whole body and both legs were measured using dual-energy X-ray absorptiometry (DXA, Hologic, Inc., Waltham, MA). Bone status at all sites was expressed in absolute units (g/cm² for BMD and g for BMC) and as a mean percent change (%)

across both limbs) score from 0 to 12 weeks. Prior to DXA assessments the subjects consumed 500 ml of water 30 min prior to scanning to standardise hydration status. The subject's legs were secured using non-elastic straps to prevent movement during the measurement. Quality assurance tests for DXA run daily in accordance with standard operating procedures.

Additional bone-based measurements were also performed on both right and left femurs using a peripheral quantitative computed tomography (pQCT) Stratec XCT 3000 scanner (Stratec Medical, Pforzheim, Germany), which linearly transforms the absorption of X-rays into hydroxyapatite (HA) densities, resulting in 0 mg HA for fat and 60 mg HA for water (Augat, Gordon et al., 1998). The manufacturer's software (XCT 550, Stratec Medizintechnik, Germany) was used to analyse the data. Scout views at the distal end of the femur were performed at 4% and 33% of the distance from the lateral epicondyle to the greater trochanter. The scanning speed of translation was $10 \text{ mm}\cdot\text{s}^{-1}$. The subjects were transferred to a height adjustable chair and placed in a seated or reclined position depending on level of spasticity. The selected leg was placed in the middle of the pQCT's gantry and the non-measured leg was abducted on a chair. Bone parameters obtained at 4% of femur length (epiphyseal slices) included total bone cross-sectional area (CSA), bone mineral content (BMC), trabecular BMD (BMD_{trab}) and total BMD. Total femur CSA was determined with a $150 \text{ mg}/\text{cm}^3$ density threshold. Bone parameters measured at 33% of femur length (diaphyseal slices) included total bone CSA, cortical bone CSA, cortical thickness, cortical BMD and polar strength-strain index. These parameters were calculated using a contour algorithm with a threshold of $280 \text{ mg}/\text{cm}^3$. A threshold of $710 \text{ mg}/\text{cm}^3$ was used to determine cortical BMD, cortical bone CSA and cortical thickness. Scan images were excluded from the analyses if extreme spasticity (spasms) provoked movement artefacts. The procedure was repeated if spasms were detected during the test until a good quality scan was obtained without movement artefacts.

5.4.1.4 Blood biomarkers for blood lipid profile and CRP concentration

Resting venous blood samples were collected from a superficial vein on the antecubital aspect of the arm. A needle and blood tube setup was used with the subject seated following a 12 h overnight fast at approximately 8:00 am, blood samples were collected at the same time of day on each testing occasion. Whole blood samples were collected in 5 ml serum separators (SST) blood tubes. The SST sample was centrifuged for 15 min at 5,000 rpm, with 500 μL aliquoted and stored at -80°C before being sent to a local pathology laboratory for blood lipid profiling and C-Reactive Protein (CRP) analysis.

5.4.1.5 Spasticity symptoms measures

The Spinal Cord Injury Spasticity Evaluation Tool (SCI-SET) (see Appendix A for full SCI-SET) was used to obtain subjective and objective measures of symptoms of spasticity and how these interfere with specific areas of life in people with SCI. This assessment has been validated in people with SCI (Adams, Ginis et al., 2007; Ansari, Kashi et al., 2016) and was administered at -1 wk, 0 wk and 12 wk of the study, immediately prior to the knee extension torque measurements. Subjects were instructed to answer 35 questions by choosing the answer that best described how spasticity symptoms had been affecting specific areas of their lives for the past seven days. Spasticity symptoms were referred as: a) Uncontrolled, involuntary muscle contraction or movement (slow or rapid; short or prolonged); b) Involuntary, repetitive, quick muscle movement (up and down; side to side); c) Muscle tightness; and/or d) What you might describe as “spasm”.

5.4.1.6 Quality of life (QoL) measures

The quality of life index (QLI) SCI version III (see Appendix B for full questionnaire) was used to obtain measures of both satisfaction and importance regarding various aspects of life. This questionnaire measures satisfaction with various life aspects and is reflected in four domains including health and functioning, family, social & economic and psychological & spiritual. A total score was also calculated with a possible range from 0 to 30. This questionnaire has been validated in people with SCI (May & Warren, 2002) and was administered before the knee extension torque measurements at -1 wk, 0 wk and 12 wk. Instructions were to answer 37 questions by choosing the answer that best described their satisfaction with life in specific area of their lives, and then answer another 37 questions by choosing the answer that best described how important each area of their lives was for them.

5.4.2 Muscle strength training intervention: electrical stimulation and training progression

NMES was delivered by a high-voltage constant-current electrical stimulator (400 V, DS7A, Digitimer Ltd., Welwyn Garden City, UK) through four self-adhesive stimulation electrodes (Axelgaard, PALS, USA) placed over the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM). Two 5×10 cm electrodes were placed over RF and one 5×5 electrode was placed on each of the VM and VL approximately at their motor points, using a split end cable, to increase the surface area of stimulation. The electrodes were placed to elicit the greatest twitch response with a low stimulation intensity, as determined in the familiarisation session and then adjusted at each session; however, the electrode positions were

marked on a plastic sheet for each subject and indelible ink was used to mark these positions on the skin to ensure identical preliminary electrode placement at subsequent sessions.

Each session commenced with a “warm-up” period consisting of paired electrical square-wave stimuli (two 1000 μ s square-wave pulses separated by 5 ms) followed by a maximum of three tetanic trains ($\tau_{t,40mA}$, see below) delivered to each leg separately every 20 s while the stimulation current was increased from 30 mA in 10-mA increments until a plateau in the maximum peak twitch torque was observed or the maximal current intensity was 99 mA. This plateau was defined as the maximal peak twitch torque ($\tau_{tw,p}$) and was used as the target torque during the training session. Subsequently, a tetanic train of NMES at 40 mA ($\tau_{t,40mA}$) was delivered followed by a maximum of three trains of NMES performed at different stimulation current intensities until reaching the closest value to the target torque. Each stimulation train and inter-train interval was 2 s (i.e. 2-s on and 2-s off), with 30 Hz trains of 58 symmetric biphasic pulses (0.033-s inter-pulse interval; 1000 μ s) being delivered. $\tau_{tw,p}$ was obtained at each time point (-1 wk, 0 wk and 12 wk), however $\tau_{t,40mA}$ was only obtained at 0 wk and 12 wk.; this was due to significant levels of spasticity being elicited initially (-1 wk) in most of the subjects, which prevented a reliable measurement of $\tau_{t,40mA}$. At 0 wk assessments, the subjects had completed a familiarisation session as well as the -1 wk assessments and, therefore, were accustomed to the NMES current. Thus, reliable measures of $\tau_{t,40mA}$ were obtained with little or no detectable spasms. 2-s contractions were chosen based on pilot testing observations revealing that shorter contractions (i.e. 1 s) failed to evoke a torque plateau (i.e. maximal activation) whereas longer-duration contractions (i.e. 3 s or greater) tended to elicit a rapid muscular fatigue.

After the warm-up period the NMES session commenced with electrically-evoked muscle contractions being elicited at the target torque for 5 sets of 10 repetitions on each leg, with a 1-min rest between sets. To determine the training intensity either one of two methods was used. The first method was by evoking the maximal peak (doublet) twitch torque ($\tau_{tw,p}$) and setting the current so the tetanic torque was equal to $\tau_{tw,p}$. However, on some days (particularly in weeks 3-12) the $\tau_{tw,p}$ tended to show a decrease when compared to previous sessions. In these cases, a second method was used whereby the starting current was set so that it was equal to the highest current used in the previous training session. Within each session, the current was increased by 2 mA per each set of 10 repetitions to maintain a high torque production as fatigue developed, thus if the second method was chosen (i.e. twitch torque was lower than in previous sessions) the current selected for set 1 was the same as that used in set 5 (the final set) of the previous session. Using this method, the torque produced in set 1 of training was

always higher than that performed in any set of the previous session and the evoked torque in each training session increased incrementally.

The training volume progression was based on total torque-time integral (TTI) over the 24 sessions and is shown in Figure 5.3. Maximum levels of torque evoked in the first contractions during the first week of training (i.e. between 0 and 1 wk) and in the last five contractions during the last week of training (between 11 and 12 wk) were calculated for analysis of training progression. In participants who could move their legs, instructions were to activate their muscles at the same time they were receiving the trains of stimuli whilst those with complete injuries were asked to think about contracting their muscles as they were visualising the muscle contraction evoked by the trains of stimuli by NMES. All training sessions were conducted by the same trained researcher and were additional to any other rehabilitation exercise completed by the subjects. The subjects were asked to keep their physical training routine consistent and FES parameters adopted by the three subjects in the SCIA-Neuromoves program were kept constant for the duration of the experiment.

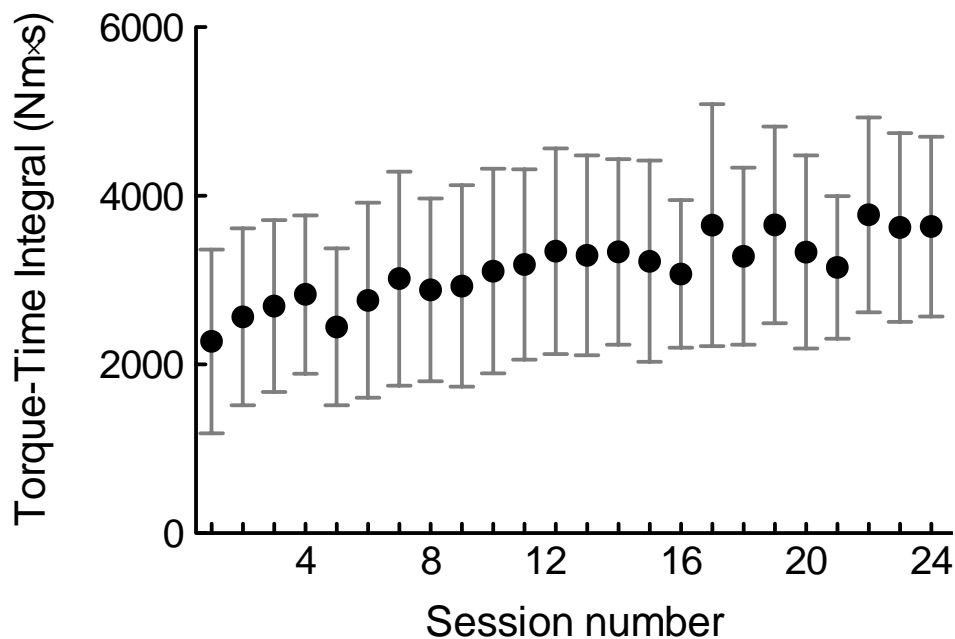


Figure 5.3 Training volume progression

Training volume progression over 12 weeks (24 sessions) measured by total torque-time integral (TTI). Mean TTI at session 24 was statistically greater than at week 1 (percentage difference: $126.8 \pm 131.2\%$; $p = 0.03$).

5.4.3 Statistical analysis

Wilcoxon non-parametric tests were used to compare changes in control and experimental periods (-1, 0 and 12 weeks) in peak twitch torque ($\tau_{tw,p}$), evoked tetanic torque ($\tau_{t,40mA}$), cross-sectional area (CSA), echo-intensity (EI), body composition, biochemical measures for lipid profile and CRP, symptoms of spasticity and QoL outcomes. Reliability of the outcome measures between the control period (-1 wk) and 0 wk was assessed using the intra-class correlation coefficient (ICC). Statistical significance was set at an alpha level of 0.05 and values are reported as mean \pm SD.

5.5 Results

5.5.1 Muscle strength: peak twitch torque($\tau_{tw,p}$) and evoked tetanic torque ($\tau_{t,40mA}$)

Although mean maximal peak knee extensor twitch torque ($\tau_{tw,p}$; sum of right and left quadriceps; QF) did not change significantly ($p = 0.08$) between 0 wk (50.4 ± 14.3 Nm) and 12 wk (44.8 ± 11.7), QF tetanic torque ($\tau_{t,40mA}$) increased significantly by $31.8 \pm 24.8\%$ ($p = 0.04$, Z score = -2.0, Figure 5.4) from 0 wk (44.2 ± 15.0 Nm) to 12 wk (56.7 ± 17.4 Nm). The intra-class correlation (ICC) coefficient for $\tau_{tw,p}$ for the right leg was 0.96 and for left leg 0.87 when assessed between -1 and 0 wk. The level of torque evoked during the first five contractions during the first week of training (between 0 and 1 wk: 29.1 ± 20.2 Nm) was equal or greater than the levels of evoked torque during the last five contractions during the last week of training (between 11 and 12 wk: 35.2 ± 27.9 Nm); i.e. torque developed during ‘fatigue’ at the end of the training period was statistically greater than the non-fatigued torque developed in week 1. Mean TTI at session 24 was statistically greater than week 1 (percentage difference: $126.8 \pm 131.2\%$; $p = 0.03$).

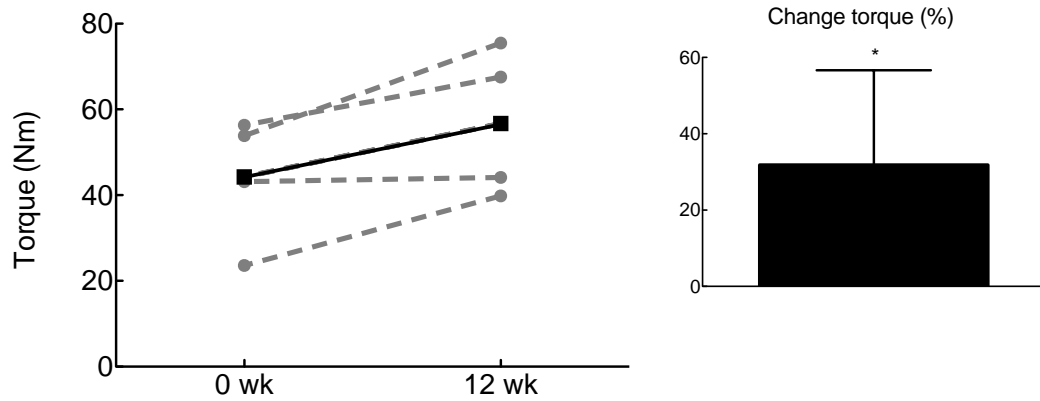


Figure 5.4 QF evoked tetanic torque ($\tau_{t,40mA}$) measured at 0 and 12 wk

Isometric knee extensor torque (QF; sum of right and left quadriceps) at weeks 0 and 12 (0 wk, 12 wk). QF evoked tetanic torque ($\tau_{t,40mA}$) showed a significant increase of $31.8 \pm 24.8\%$ between 0 and 12 wk.

Grey dashed lines represent individual subjects whilst the black solid line represents the group mean. Inset: Percentage change in evoked isometric knee extensor torque (QF) from 0 wk to 12 wk. * Significantly different between 0 and 12 wk ($p < 0.05$).

5.5.2 Muscle cross-sectional area (CSA_{QF}) and ultrasound echo-intensity (EI)

Mean quadriceps femoris CSA (CSA_{QF} , sum of right and left legs) increased by $45.0 \pm 25.8\%$ ($p = 0.04$, Z score = -2.0) from 0 wk ($80.0 \pm 33.8 \text{ cm}^2$) to 12 wk ($113.1 \pm 42.8 \text{ cm}^2$) (see Figure 5.5). Between-subject differences were qualitatively observed, where the greatest response (increase in CSA_Q of 145.4%, right leg), was observed in a subject E who had not been exposed to electrical stimulation training previously, whereas the least response (increase of 15.0%, right leg) was observed in subject C with an incomplete lesion (T_{12} , ASIA D) who was a community walker (walked with one crutch). The intra-class correlation (ICC) coefficients for $\tau_{tw,p}$ for both right and left leg separately were 0.99 between -1 wk and 0 wk.

Echo-intensity (EI) did not change significantly with the training (right leg: 0 wk: $173.4 \pm 26.1 \text{ a.u.}$, 12 wk: $174.0 \pm 15.5 \text{ a.u.}$, $p = 0.89$, Z score = -0.1); left leg: 0 wk: $170.16 \pm 28.6 \text{ a.u.}$; 12 wk: $174.0 \pm 15.5 \text{ a.u.}$, $p = 0.3$, Z score = -0.9). ICC coefficient for EI was 0.82 for left leg and 0.93 for right leg between -1 and 0 wk.

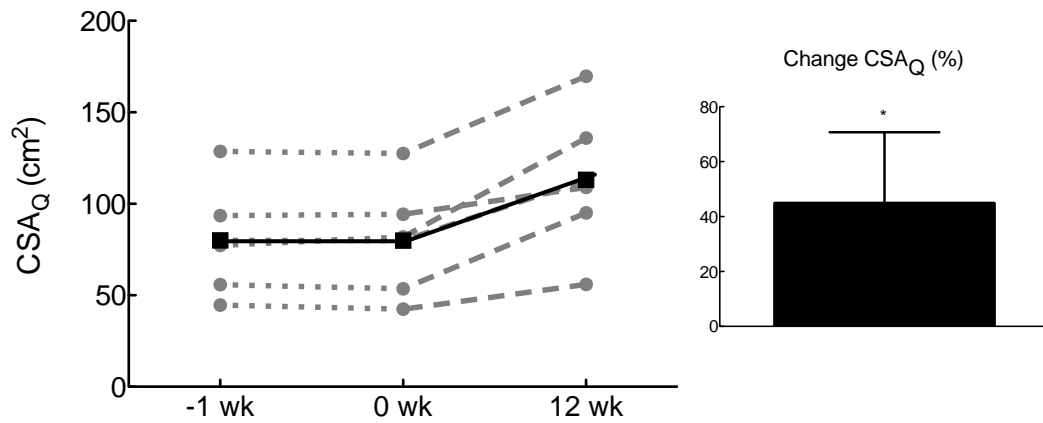


Figure 5.5 Cross-sectional area of the quadriceps (CSA_{QF})

Total cross-sectional area of the sum of right and left quadriceps at 0 wk and 12 wk. CSA_{QF} increased significantly ($p = 0.01$) by $45.0 \pm 25.8\%$ between 0 and 12 wk.

Grey, dashed lines represent individual subjects whilst black, solid line represents the group mean. Inset: Percentage change in CSA_{QF}. * Significantly different from 0 wk and Control ($p < 0.05$).

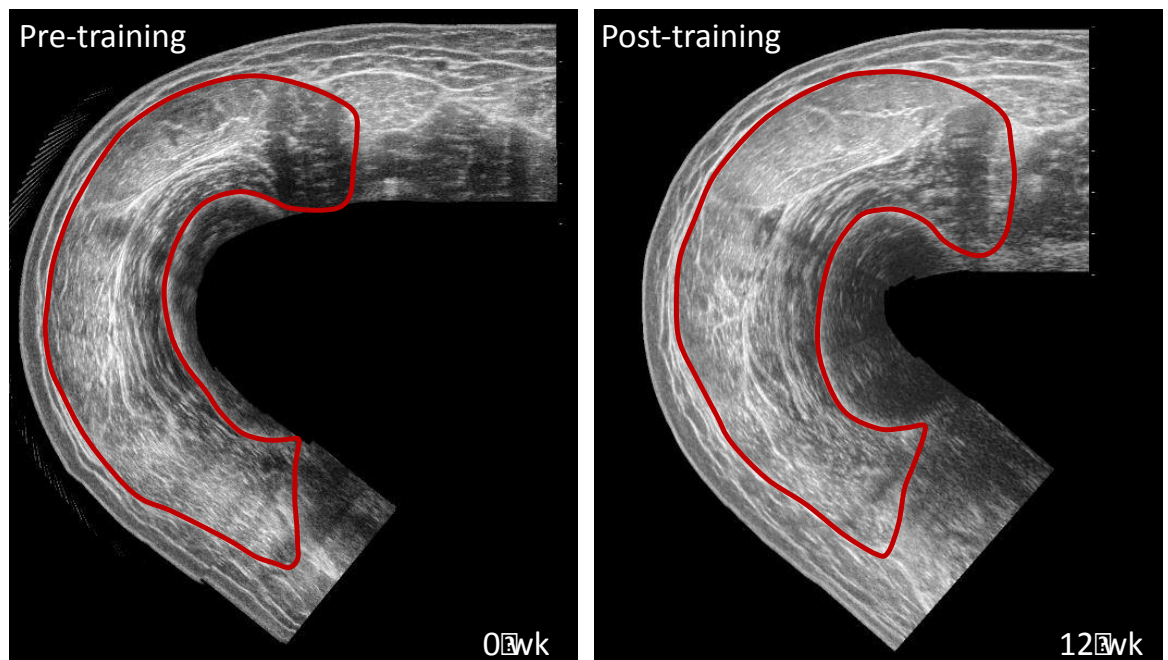


Figure 5.6 Cross-sectional area ultrasound image using extended-field of view technique

Example of the cross-sectional area (CSA) measurement of the (left) quadriceps measured in subject A at 0 wk and 12 wk using extended-field-of-view ultrasonography. A significant increase of $45.0 \pm 25.8\%$ was observed (mean \pm SD) in the group of 5 subjects.

5.5.3 Body composition outcomes

Bone mineral content (BMC) measured using DXA increased $1.6\% \pm 0.9\%$ ($p = 0.04$; Z score = -2.0) between 0 wk (307.0 ± 95.7 g) and 12 wk (311.3 ± 94.8 g) and bone mineral density (BMD) decreased by $1.8\% \pm 1.4\%$ ($p = 0.04$; Z score = -2.0) (0 wk: 1.01 ± 0.13 g/cm²; 12 wk: 0.99 ± 0.13 g/cm²; whilst total femur cross-sectional area (BMC/BMD; CSA_{femur}) increased by $3.5 \pm 2.0\%$ ($p = 0.04$; Z score = -2.0) see Table 5.2 for body composition data and Figure 5.7 for example images). However, no significant changes were found in lower-limb lean body mass (LL-LBM: 0 wk: 7002.6 ± 1355 g; 12 wk: 6664.0 ± 1384.0 g; ($p = 0.08$; Z score = -1.7), regional body fat (RBT: 0 wk: $34.1 \pm 8.0\%$; 12 wk: $34.7 \pm 8.3\%$; ($p = 0.5$; Z score = -0.6) or total body fat (TBF: 0 wk: $26.8 \pm 8.3\%$; 12 wk: $26.8 \pm 8.3\%$; ($p = 0.85$; Z score = -0.1).

Table 5.2 Body composition measures characteristics (DXA)

Whole body and regional body composition measured by DXA at 0 wk and 12 wk and percent (%) change within the group (Mean \pm SD)

Body composition measures (DXA)	0 wk	12 wk	% Change
BMC (kg)	30.7 \pm 9.5	31.1 \pm 9.5 *	1.6 \pm 0.9
BMD (g/cm ²)	1.0 \pm 0.1	0.9 \pm 0.1*	-1.8 \pm 1.4
BMI	23.0 \pm 5.0	22.9 \pm 5.2	-0.7 \pm 3.6
LL-LBM (kg)	70.0 \pm 1.4	66.6 \pm 1.4	-4.9 \pm 4.5
RBT (%)	34.1 \pm 8.0	34.7 \pm 8.0	1.6 \pm 5.3
TBF (%)	26.8 \pm 8.3	26.8 \pm 8.3	-0.1 \pm 5.5
TM (kg)	70.7 \pm 22.1	69.8 \pm 22.3	-1.8 \pm 2.8

* Significant difference from 0 wk ($p \leq 0.05$)

BMC: bone mineral content; BMD: bone mineral density

BMI: body mass index; LL-LBM: Lower limb lean body mass;

RBT; regional body fat; TBF: total body fat;

TM: total mass

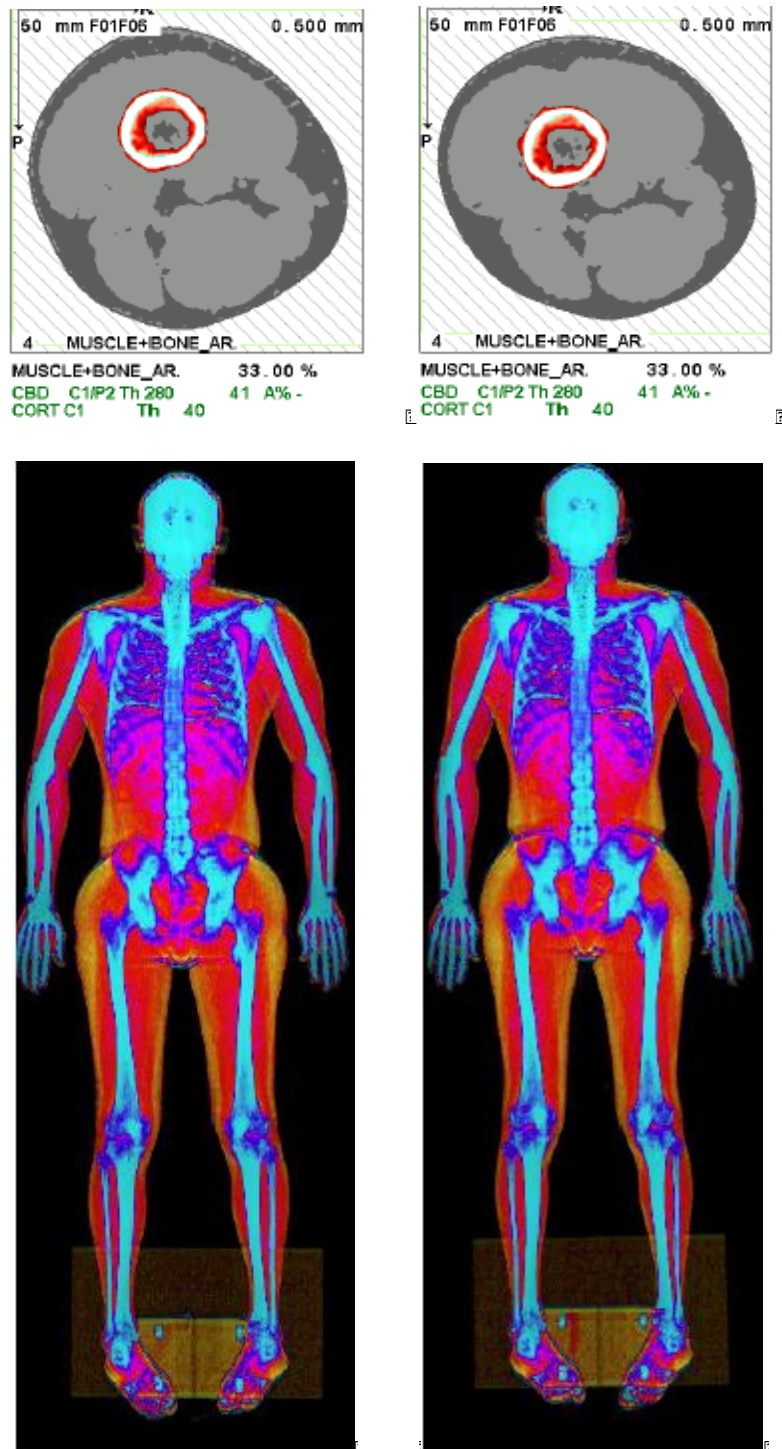


Figure 5.7 Example thigh-only pQCT (top) and whole-body DXA (bottom) images at 0 (left) and 12 wk (right)

Example of left quadriceps peripheral quantitative computed tomography (pQCT) and whole body dual-energy X-ray absorptiometry (DXA) images of the whole body in subject A at 0 wk and 12 wk. Significant changes in BMC and BMD were observed, however these were not considered clinically meaningful (see Discussion). No significant changes in other body composition measures were observed.

Bone strength indices measured using peripheral quantitative computed tomography (pQCT) including trabecular BMD ($p = 0.34$; Z score = -0.9), cortical BMD ($p = 0.5$; Z score = -0.6), polar strength-strain index (PSSI; $p = 0.22$; Z score = -1.2), and fracture force in longitudinal X ($p = 0.89$; Z score = -0.1), and vertical Y ($p = 0.50$; Z score = -0.6), directions, remained unchanged between 0 wk and 12 wk (see Figures 5.8 A, B and C).

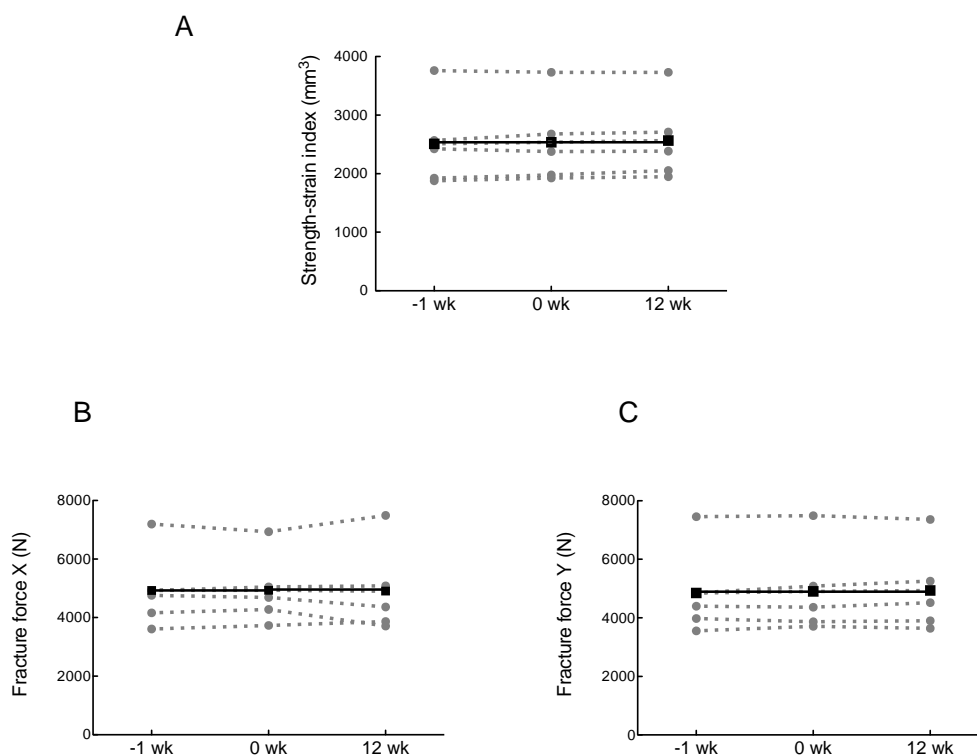


Figure 5.8 A) Strength-strain index; B) Fracture force in the longitudinal X direction and C) Fracture force in axial Y direction.

Bone strength indices measured by pQCT. No changes in bone ultrastructure were observed. Grey, dashed lines represent individual subjects, whilst the black, solid line represents the group mean. Measures obtained at -1 wk, 0 wk and 12 wk.

5.5.4 Blood biomarkers for blood lipid profile and CRP

A trend towards decrease in low density lipoprotein concentration ($p = 0.06$; Z score = -1.8) and significant increase in the cholesterol HDL/LDL ratio ($p = 0.04$; Z score = -2.0) were observed, whilst a trend towards a decrease in cholesterol/HDL ratio ($p = 0.08$; Z score = -1.7) was detected. Plasma C-reactive protein (CRP) concentration did not change significantly ($p = 0.50$; Z score = -0.6) however two subjects with CRP concentrations higher than the recommended levels showed clear reductions after the 12 wk of training (subject A: 6.8 mg·L⁻¹ at 0 wk, 1.8 mg·L⁻¹ at 12 wk, 73.3% decrease; subject E: 20.0 mg·L⁻¹ at 0 wk, 1.0 mg·L⁻¹ at 12 wk, 95% decrease). Mean changes in blood biomarkers are shown in Table 5.3.

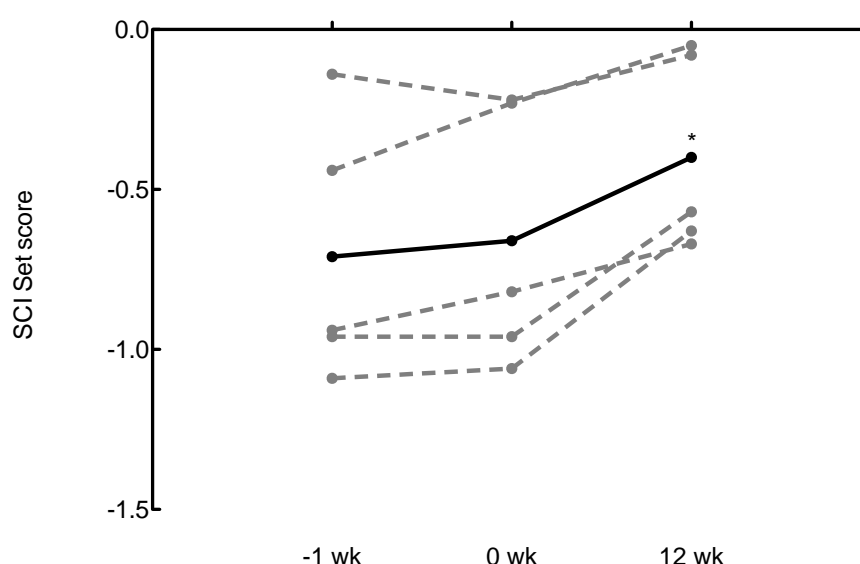
Table 5.3 Blood lipid profile and CRP concentrationBlood biomarkers at 0 wk and 12 wk and percent change within group (mean \pm SD).

Biochemical measure	0 wk	12 wk	% Change
Total plasma cholesterol (mmol/L)	4.9 \pm 1.3	4.6 \pm 1.0	-5.2 \pm 8.9
Triglycerides (TG) (mmol/L)	1.1 \pm 0.8	1.2 \pm 1.1	3.0 \pm 20.5
Low density lipoprotein (LDL) (mmol/L)	3.1 \pm 1.2	2.7 \pm 1.1 *	-14.0 \pm 10.5
High density lipoprotein (HDL) (mmol/L)	1.3 \pm 0.3	1.4 \pm 0.3	4.0 \pm 12.5
Cholesterol Chol/HDL ratio	3.7 \pm 0.7	3.4 \pm 0.7	-8.5 \pm 7.7
Cholesterol Chol/LDL ratio	1.6 \pm 0.2	1.8 \pm 0.4	10.5 \pm 8.2
Cholesterol HDL/LDL ratio	0.5 \pm 0.1	0.6 \pm 0.2 *	21.0 \pm 3.8
Plasma C-Reactive Protein (CRP) (mg/L)	6.6 \pm 7.8	2.4 \pm 1.6	-7.3 \pm 91.6

* Significant difference from 0 wk ($p \leq 0.05$)

5.5.5 Spasticity symptoms and quality of life (QoL)

Symptoms of spasticity measured using the spasticity evaluation tool (SCI-SET 7-day recall: positive vs negative effects of spasticity; -3-+3) were significantly reduced by $4.8\% \pm 2.3\%$ (0 wk: -0.7 ± 0.4 ; 12 wk: -0.4 ± 0.3 ; $p = 0.04$; Z score = -2.0; see Figure 5.9).

**Figure 5.9 Spasticity evaluation tool (SCI-SET) results**

Seven-day recall score (-3-+3) at -1, 0 and 12 weeks. A significant reduction of $4.8\% \pm 2.3\%$ was observed.

* Significantly different at 12 wk from 0 and -1 wk ($p < 0.05$). Grey, dashed lines represent individual subjects whilst black, solid line represents the group mean.

Quality of life was measured using the Quality of life index (QLI) for SCI version III. The total score (QLI) and subscales for health & functioning (HF), family (FAM), social & economic (SOC) and psychological & spiritual (PSP) domains did not change significantly from 0 wk to 12 wk. However, there was a trend towards an improvement in the health & functioning domain (0 wk: 15.0 ± 4.2 ; 12 wk: 17.3 ± 5.1 ; $p = 0.08$, Z score = -1.7). The intra-class correlation coefficients were 0.94 for QLI, 0.97 for HF, 0.85 for SOC, 0.87 for FAM and 0.97 for PSP when measured from -1wk and 0 wk. Subject C and D showed a change in QLI greater (20.0% and 16.0 %, respectively) than the mean percentage difference from -1 and 0 wk (12.0%) and thus showed notable responses. Subject C had an incomplete lesion (T₁₂, ASIA D) and was a community walker (walked with one crutch); subject D had a complete lesion T₁₀, ASIA A) and was physically active (i.e. involved in wheelchair basketball and mountain hand-bike).

5.6 Discussion

This is the first study to investigate the effects of a high-intensity NMES strength training on muscle force and mass, physical health, symptoms of spasticity and QoL in people with SCI. The main results of this study are that high-intensity NMES strength training induced substantial increases in evoked tetanic knee extensor torque (i.e. muscle strength) and quadriceps cross-sectional area (i.e. muscle size). More importantly, these changes were observed even in subjects who used other forms of electrical stimulation-based training (e.g. FES) regularly before and during the study. It was of specific interest that the mean evoked torque in the last contractions (i.e. at the point of fatigue) in the final week of training was either equal to or higher in all subjects than that during the first contractions (i.e. unfatigued) in the first week of training, because it clearly reveals a notable increase in muscle work capacity in paralysed muscles. The ability to produce greater forces even in fatigue than when before the training would speculatively allow for higher volumes of work to be completed during physical rehabilitation sessions and thus a more rapid improvement in physical function and health outcomes in the longer term. Another interesting observation was that tetanic muscle force (QF tetanic torque ($\tau_{t,40mA}$)) increased significantly whilst mean maximal peak twitch torque ($\tau_{tw,p}$) did not change significantly. One possibility is that $\tau_{tw,p}$ can be affected by factors such as series elastic component stiffness or changes in the relationship between Ca^{2+} release and force production in paralysed muscle, and thus may not be a reliable longitudinal measure of muscle force (Place et al., 2008; Taylor, 2009; Todd et al., 2003), particularly in clinical populations. Nonetheless, the substantial (mean = 31.8 %) increases in tetanic torque reveal a clear improvement in muscle force generating capacity after the training.

The training also stimulated a large (mean = 45.0%) increase in quadriceps CSA (CSA_{QF}), which is clinically important since the muscle atrophy that is typically associated with chronic SCI has detrimental effect on metabolic, cardiovascular and functional systems (Castro et al., 1999; Gorgey et al., 2014), which meaningfully impact life expectancy (Bauman & Spungen, 2008; Groah et al., 2011; Samsa et al., 1993). Previous researchers have reported less increases (20.0%) after an 8-week intervention (Dudley et al., 1999) and others reported similar large increases in CSA after NMES training (35.0% - 45.0%) after 12 to 24 weeks of training (twice weekly), albeit using different pulse widths (250/600 μ s) at similar frequencies (30-35 Hz) (Bickel et al., 2015; Gorgey, Mather, et al., 2012; Mahoney et al., 2005; Ryan et al., 2013). Thus, our study obtained the same increase in CSA (45%) in 12 weeks compared to that obtained after a 24-week, twice weekly NMES intervention (Bickel et al., 2015). This improvement in CSA_{QF} in a shorter period may speculatively be attributed to two important differences in the present study: (a) the use of isometric rather than concentric contractions, and (b) the use of near-maximal muscle contractions that could be performed with less fatigue due to the short duration (2-s) contractions. The use of isometric contractions was expected to have an advantage over concentric contractions for the triggering of muscle hypertrophy because muscle forces are higher at a given activation level, according to the force-velocity relationship of muscle, and therefore a greater mechanical load would have been imposed to stimulate muscle hypertrophy (Deley, Denuziller et al., 2015; Selkowitz, 1985). Based on previous evidence, the training was performed at a long length because this should provide the greatest hypertrophic benefit (Noorkoiv et al., 2014). Also, it was observed in pilot testing that short-duration contractions of ~1 s did not allow for peak tetanic torque to be evoked in many individuals, but that longer-duration contractions of 4-5 s caused a more rapid muscle fatigue than moderate-duration contractions (i.e. ~ 2 s). Thus 2-s contractions were used in the present study to allow for higher stimulation intensities to be used without the development of rapid muscle fatigue. Thus, the performance of 2-s isometric contractions at a long muscle length may speculatively be a main factor driving the positive outcomes found on this study; however, this hypothesis needs to be more explicitly examined in future studies by comparing adaptations to training using different exercise protocols.

An interesting observation was that no change in ultrasound echo intensity, which was used as an indicator of intra-muscular fat (IMF) content, was detected. This is important because IMF is significantly increased in people with SCI when compared to non-disabled controls (Elder et al., 2004) and is associated with an increased risk for metabolic disease and insulin resistance (Elder et al., 2004; Gorgey, Dolbow et al., 2014; Gorgey & Dudley, 2007). This finding may be partly attributed to the low reliability of the echo-intensity measures, as quantified from repeated observations between -1 wk and 0 wk (ICC for left leg: 0.82 and for

right leg: 0.93; mean absolute difference left leg: 20.6 ± 13.2 ; right leg: 8.3 ± 11.4). Nonetheless, only a single case study using NMES resistance training has reported a relative decrease in echo intensity (i.e. IMF) in thigh muscle groups and these researchers used magnetic resonance imaging (MRI) to estimate a decrease in intramuscular fat from $6.1\% \pm 5.5\%$ to $3.9\% \pm 2.0\%$, which occurred with a substantial increase in muscle CSA indicating that approximately the same total content of IMF existed before and after the training (Gorgey & Shepherd, 2010). Thus, it is still unclear as to whether NMES-based exercise training modalities significantly affect IMF in paralysed muscles and higher resolution methods to assess IMF, such as MRI or computed tomography (CT)-based methods or direct quantification from muscle biopsy samples, with larger sample sizes may be needed to more fully explore the role of NMES-based strength training in reducing intramuscular fat content.

Regarding physical health, it appears that the use of high-intensity NMES strength training had a largely positive effect on several physical health parameters. DXA assessments revealed significant increases in bone structure markers such as BMC (1.8%) and total femur CSA (1.6%; i.e. BMC/BMD) and a trend towards an increase in lower limb lean body mass ($p = 0.07$). Such results indicated that a consistent improvement in bone structure and lean body mass was elicited by the training and that such training might be clinically relevant to people with SCI. However, the changes in these bone variables were of small absolute magnitude and not of clear clinical benefit (Hangartner, 2007; Keil et al., 2016; Wilson & Smith, 2009), and they were less than the smallest significant changes that can be confidently reported, as emphasised in studies of the short-term measurement precision of DXA scanners (Baim et al., 2005; Hangartner, 2007; Keil et al., 2016; Shepherd & Lu, 2007). Nonetheless, our results showed an increase in BMC and a decrease in BMD and these consistent results of an increased BMC may suggest an increase in cortical cross-sectional area of the femur occurred, but with no change or a decrease in trabecular volumetric BMD (Sandini et al., 2005) and this may result in a better resistance to fracture (Seeman et al., 2001) in the osteoporotic bones in people with SCI (Jiang et al., 2006). This hypothesis would need further investigation to be confirmed. However, our marginal results in bone measurements could also be explained by the multifactorial pathogenesis of osteoporosis after SCI, and although muscle strain plays an important role in bone modelling, many other factors also contribute to the bone loss, such as endocrine disorders (i.e. hormonal changes) and the concomitant use of pharmacological interventions (Jiang et al., 2006; Watts, 1999). Other factors that may influence the mechanical function of the bones are the direction of the forces that deform bone structure (Lochmuller, Lill et al., 2002). Therefore, further studies should explicitly test the use of high-intensity NMES strength training under factors that could influence the rate of bone formation, such as positioning the subject to allow a greater mechanical loading (i.e. standing, using high dose

compressive loads) instead of adopting a closed chain position (i.e. seated, with less mechanical loading) (Dudley-Javoroski et al., 2012; Shields et al., 2006) and/or including pharmaceutical or dietary supplementation in addition to the NMES training (Morse et al., 2009). Thus, the current evidence suggests that high-intensity NMES strength training may be a useful stimulus for bone health, but adoption of other methodology, inclusion of other interventions and longer studies (> 6 months) (Dolbow et al., 2011) may be needed for clinically meaningful changes to be observed.

No changes in body fat and lean muscle mass were detected in the current study and these results are consistent with previous studies in people with SCI (Gorgey et al., 2015). Previous studies using NMES-based exercise in lower limbs showed similar results revealing modest (~ 2%) reductions in body fat (Bauman et al., 1994; Gorgey et al., 2015; Hjeltne et al., 1997), while some reported a lack of effects on whole body composition variables (Gorgey, Mather, et al., 2012) even after 24 weeks of training (Skold et al., 2002). One study revealed increases in lean muscle mass of 10.0% but no changes in bone structure after 10 weeks (Griffin et al., 2009). Therefore, NMES strength training of the quadriceps muscles only allowed for muscle-specific increases in lean muscle mass but was not sufficient to decrease body fat content. Other moderate-to-vigorous exercise modalities, probably using a larger active muscle mass, in combination with dietary interventions should be explicitly being tested in future research studies if the aim is to decrease body fat (Gater, 2007; Griffin et al., 2009), and thus improve longevity (Srikanthan et al., 2016) in people with SCI.

Changes in blood-based biomarkers of physical health were observed after the training including a significant decrease in LDL and an increase in HDL/LDL ratio. These improvements in physical health outcomes are thought to be associated with an increased life expectancy in people with SCI (Bauman & Spungen, 2008; Gater, 2007; Kocina, 1997). Similar findings of improvement in the lipid profile were previously reported after the performance of NMES-based strength training using concentric contractions (Gorgey et al., 2012; Ryan et al., 2013). However, in contrast with the findings of Gorgey et al. (2012), changes in total cholesterol levels and triglycerides were not found in the current study. As dietary intake was not strictly controlled, it is not possible to determine whether nutritional factors influenced this result, however it is also possible that the training of additional muscle groups by Gorgey et al. allowed for greater systemic (i.e. blood-based) changes to be elicited. Additionally, it is worth mentioning that two subjects with initial high CRP concentration levels obtained normal CRP levels after the intervention. This result might suggest the protective effect of the muscle contractions evoked by high-intensity NMES strength training, which promotes anti-inflammatory myokine release and may attenuate low-grade

inflammation, might reduce cardiovascular disease risk (Dhingra et al., 2007). However, CRP is a non-specific anti-inflammatory marker and other more specific markers, such as interleukin 6 (IL-6) (Pedersen & Febbraio, 2008) should be included in further studies to understand the metabolic response elicited by high-intensity NMES strength training. Thus, there is good evidence for modest improvements in blood-based biomarkers of systemic, physical health, but possibly interventions for longer periods or targeting more muscle groups (greater total work) in combination with a controlled diet intake may be important to evoke robust reductions in systemic inflammation.

Of final note, a very important and relevant finding of the present study was that the subjects reported improvements in their symptoms of spasticity, which were less negative (absolute values: pre: -0.7, post: -0.4; -4.8% change; less negative indicates that symptoms affected them less in daily life activities; the mean detectable change for the SCI-SET was reported to be 0.47; Adams et al, 2007). This relevant finding emphasises the potential benefits of high-intensity NMES strength training for improving the perception of spasticity symptoms, which clearly restricts the activities of daily living and represents a negative influence in QoL in people with SCI (Adams & Hicks, 2005). However, despite the reduced perceived problem of spasticity reported by the subjects, no overall improvements in QoL were reported and the change observed in the spasticity symptoms may not be clinically significant (Adams et al., 2007). Nonetheless, a trend was observed toward an increase in the health and functioning subscale of the QoL index (0 wk: 15.0 ± 4.2 ; 12 wk: 17.3 ± 5.1 ; $p = 0.07$) and two subjects showed a change in QLI score greater (20.0% and 16.0 %) than the mean percentage difference observed from -1 and 0 wk (12.0%), indicating clear improvements. One subject was ambulatory and most probably improved QoL because her muscle strength improvement could positively impact her daily physical function capacity. The other subject was very physically active and probably increased his QoL by perceiving fewer spasticity symptoms, which he reported were interfering with his daily physical activity routine (as he was not taking any anti-spasticity medication). These are relevant findings since symptoms of spasticity negatively influence quality of life by restricting activities of daily living and causing pain and fatigue (Adams & Hicks, 2005). Thus, the ultimate goal for any physical therapy program implemented in people with SCI is to enhance QoL by reducing the impact of the disease and getting expectations and experience of the physical limitations closer to each other (Carr et al., 2001). In future it will be important to better understand the perceptions of people with SCI of feeling physically active and experiencing muscle contractions of the paralysed muscles (Donovan-Hall et al., 2011). As an example of the positive effects of the high-intensity NMES strength training on the perception of their physical capacity, one subject who was a former competitive surfer expressed that he appreciated the opportunity to train his legs again, and

that he was enjoying having a “leg day at the gym” as he used to have before his injury. Another subject also mentioned that he had to buy new shorts as his legs were getting “too big” that his regular shorts no longer fitted. Thus, the present results provide evidence that the high-intensity NMES strength training intervention had a positive impact on the subject’s perceptions of their physical disability. Such programs may therefore improve QoL in addition of having the direct benefits of improved muscle force and mass and physical health markers associated with longer life expectancy.

There are some important limitations of the present study that should be noted. One was the absence of a non-training control group against which to compare the training outcomes. This results from the difficulty in recruiting people with SCI into a study where no intervention (i.e. opportunity for improvement) is given. Volunteers also did not agree to be studied through a 12-week control period before commencing the intervention. This is partly because transport issues make attendance at laboratory-based testing sessions difficult for people with SCI, and partly because the testing involved in this study required a significant time investment from the subjects (and carers). Another limitation was the small sample size, which also reflects recruitment difficulties. It would be of great scientific benefit if larger, controlled studies could be conducted in the future to test the findings of the current study.

5.7 Conclusion

This study showed that 12 weeks of high-intensity NMES strength training of the knee extensor muscle group resulted in increases in evoked tetanic knee extensor torque (i.e. muscle strength) and quadriceps cross-sectional area (i.e. muscle size). Despite the changes in quadriceps muscle CSA (CSA_{QF}), no changes in intra-muscular fat content were detected after the intervention (estimated from ultrasound echo intensity measurements). Positive and statistically significant changes in some bone metabolisms markers were detected, but these were not deemed to be of clinical significance; longer studies are required to fully determine the effect of the training intervention on bone architecture and strength. No changes in body fat or lean muscle mass were found, possibly because of the relatively small muscle mass involved in the training, and although a significant decrease in LDL and increase in HDL/LDL ratio was observed, no changes in total cholesterol and triglycerides were detected; it may be notable that CRP (low-grade inflammation) levels were markedly reduced and reached ‘safe’ levels in the two subjects who had high levels at the commencement of the study. An important and significant finding was the reduced symptoms of spasticity reported by the subjects after the intervention. Despite this, however, no overall improvement in QoL was reported, although a trend was observed toward an increase in the health and functioning subscale in the QLI and

positive, subjective comments were received from the subjects. This study provides strong evidence that high-intensity NMES strength training may be effective for improving muscle force and mass and decreasing perceived symptoms of spasticity, and that can be safely implemented in people with SCI. Some evidence also indicated improvements in physical health and quality of life. However, replication of these results in a larger sample of participants and with a non-training control group is necessary before its implementation in clinical practice.

Chapter 6 Discussion and conclusions

The overall aim of the research was to determine whether high-intensity NMES strength training is effective in increasing muscle strength and mass, improving physical health outcomes, ameliorating symptoms of spasticity and improving QoL in people with SCI. The first specific aim of the research was to examine the effects of tendon vibration superimposed onto wide-pulse width NMES as a high-intensity muscle strength training intervention, firstly by studying its acute effects when compared to NMES alone in young, healthy individuals without recent exposure to NMES (Study 1). The second aim of the research, after establishing the safety (minimum pain sensation, muscle ‘damage’ and soreness) of the protocols in Study 1, was to examine the effects of the same interventions in people with SCI (Study 2). The third aim was then to implement a 12-week high-intensity NMES-based muscle strength training intervention in people with SCI in order to examine its effects on muscle strength and mass, physical health, symptoms of spasticity and QoL; based on the results of Studies 1 and 2, the NMES training was performed without simultaneous use of tendon vibration.

The rationale for the methodology of Study 1 was supported by pilot data, collected before the start of this research by the principal researcher in healthy subjects, showing that wide-pulse width (1000 μ s), low-frequency (30 Hz) symmetric (balanced) biphasic NMES superimposed on tendon vibration elicited a larger ‘total work’ (i.e. contractile impulse) prior to fatigue. This occurred largely because an ongoing muscle force remains between series of NMES stimulation trains, probably due to the continued firing of low-threshold type I motor units (see Fig. 6.1; note the increase in torque in Fig. 6.1B). The pilot data suggested that this approach might enhance muscle force production and delay muscle fatigue. Subjects who volunteered for pilot testing also reported that this strategy was more tolerable (i.e. reduced perception of pain). Additional pilot data also showed that muscle activation via spinal pathways, evidenced by a clear EMG signal between NMES trains (not shown in figure), could be elicited even at low stimulation frequencies (e.g. ≤ 30 Hz), which is recommended for clinical populations. Results from Study 1, which tested the idea that tendon vibration might provide a superior acute exercise training stimulus than NMES alone, were important in terms of obtaining feedback from participants who had no recent exposure to NMES in the quadriceps (the target muscle group) about any adverse reactions, pain or discomfort experienced during the application of the NMES protocols. The lack of adverse reactions observed in subjects participating in Study 1 provided sufficient evidence that such interventions would be safe enough to be implemented in people with SCI, who had motor and sensory deficits and were unable to receive sensory information.

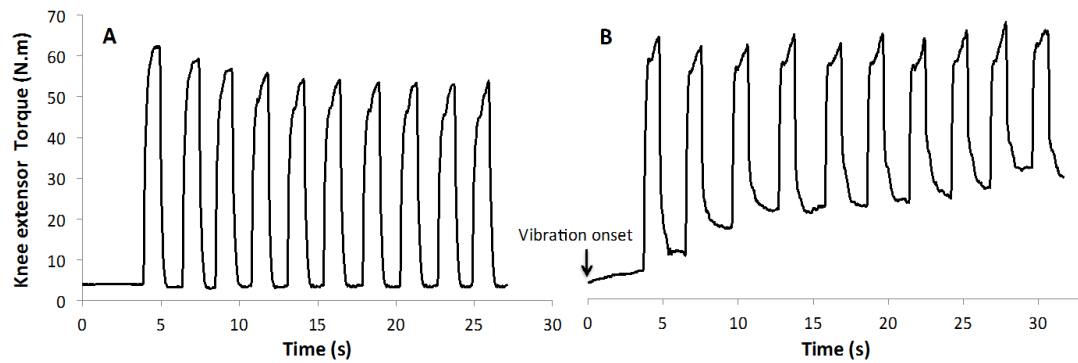


Figure 6.1 Torque responses during pilot testing

A: knee extensor torque elicited by biphasic, wide-pulse width NMES without simultaneous tendon vibration.

B: knee extensor torque elicited by biphasic, wide-pulse width NMES superimposed tendon vibration. A fatigue effect is visible in A, resulting in less total impulse or Torque-Time Integral (TTI) (535 Nm·s) than with vibration (B: 977 Nm·s).

Despite the positive pilot results, the results from Study 1 showed, in fact, that half of the subjects (i.e. positive responders to tendon vibration) benefited from the imposition of tendon vibration onto the wide-pulse width NMES in terms of increases in the torque-time integral (TTI) produced prior to ‘fatigue’ (defined as the peak tetanic torque [set to 20% of MVC torque at the onset of the exercise] decreasing to 60% of the starting torque). This implies that the use of tendon vibration may allow for a greater amount of muscular work to be performed, and thus for a more optimum training response to be achieved, only in some people. However, a lesser response was found in the other half of the subject cohort (negative responders to tendon vibration) and in those cases the use of tendon vibration may be detrimental to task performance, and it may be more beneficial to use wide-pulse width NMES (STIM) without tendon vibration. This is an interesting clinical finding because tendon vibration may augment the muscle force response in some people, however it may also diminish the response in others, so there is no conclusive evidence that this method might be more effective for improving muscle force and mass, at least in healthy people. Although the contribution of the tendon vibration in terms of an increase in baseline force when applied superimposed to NMES may not contribute to the movement of the limb in paralysed muscles, it could increase the isometric force of the muscle, which may be translated in an increased muscle endurance and thus a reduction in muscle fatigue that could have an impact in the performance of functional activities, such as standing. It may also have a positive impact in the physical health of the individual, as shown in some results of our Study 3, such as a decrease in CRP after 12-week intervention. Nonetheless, even if there is no improvement in muscle force, tendon vibration may trigger changes in the plasticity of the system (i.e. neuroplasticity) by activating muscle spindles and improving sensory stimulation (Edgerton et al., 2004). Therefore, the use of tendon vibration as a valid rehabilitation tool needs to be further investigated.

The excitatory effects found in positive responders to tendon vibration may have been the result of the vibration-induced augmented response of motor neurone produced when combined with the NMES (Burke & Schiller, 1976; Fornari & Kohn, 2008), which does not occur during high-frequency NMES (Burke & Schiller, 1976). On the other hand, the inhibitory response found in negative responders might speculatively result from the stimulation of muscle spindle secondary endings (which may provide an inhibitory signal in some conditions) and/or Ib tendon afferents leading to autogenic inhibition (Burke & Schiller, 1976; Magalhaes & Kohn, 2010).

Regardless of the mechanism, the data indicate that clinicians may need to test individuals for their response to tendon vibration and wide-pulse width before the implementation of tendon vibration and decide which individuals may benefit. Based on results from the binomial logistic regression analysis performed to predict the likelihood of having a positive or a negative response to tendon vibration (STIM+Vib), torque-time integral and total number of contractions during NMES alone (STIM) appear to be good predictors of whether an individual will be a positive or negative responder to tendon vibration (STIM+Vib); this analysis was able to predict 87.5% of positive responders and 81.3% of negative responders cases. Under the conditions of the present study a positive responder to tendon vibration would perform ≤ 16 contractions whilst a negative responder to tendon vibration would perform > 16 contractions until target fatigue in the NMES-only condition.

Nonetheless, regardless of whether an individual received a benefit to their muscle performance before fatigue or not, the use of tendon vibration superimposed onto wide-pulse width NMES appeared to minimise the voluntary fatigue experienced after the training session. In this case, tendon vibration may allow for additional rehabilitation work to be performed even after a block of NMES-based training, and thus may more effectively be used for locomotion (i.e. crutches use) and activities of daily living after the session. If the reduction in TTI is only small then the imposition of vibration might still be considered to provide an overall benefit for an individual. This is an important outcome and might be useful when considering the potential overall cost-benefit effect for an individual.

However, it is still unclear whether wide-pulse width NMES can elicit greater muscle work and delay muscle fatigue under all stimulation conditions, as the literature contains conflicting results (Neyroud et al., 2014; Wegrzyk et al., 2015a,b). Also the results of Study 1 cannot be used to determine its efficacy in the wide variety of clinical conditions where the function of afferent pathways may be affected. Thus, further studies are required to systematically test the effects of tendon and electrical stimulation methods in conditions of different pulse widths and frequencies and in clinical populations with different neurologic

alterations in order to determine whether specific protocols can be identified for use. This would provide evidence as to whether these tools might be used safely and with confidence in clinical practice. Nonetheless, the wide-pulse width NMES used in Study 1 appeared to be sufficiently safe to use in people with SCI, with or without tendon vibration, since muscle damage and soreness were not observed.

Based on results of Study 1, NMES with and without quadriceps tendon vibration were provided to people with chronic SCI (Study 2). This study was important because it was the first study to use tendon vibration superimposed onto NMES in people with SCI. The results of Study 2 also identified positive and negative responders to tendon vibration, however the responses in people with SCI were less clear and a defined effect of tendon vibration superimposed onto NMES was not discerned. Results from this study showed no difference in TTI between the conditions with and without superimposed tendon vibration (STIM and STIM+Vib). In particular, and contrary to the hypothesis, completeness of the lesion did not explain the positive and negative response to tendon vibration.

An additional, clinically-relevant finding was that the use of tendon vibration superimposed onto the wide-pulse width NMES did not reduce the fatigue elicited by the training session when compared to NMES alone, at least when re-assessed using evoked tetanic contractions (since voluntary contractions could not be produced by most subjects). Therefore, there was no evidence to support the use of tendon vibration in people with SCI, and the use of NMES alone was considered to provide similar benefits for most people. These results differed qualitatively from Study 1, and may be due to the different response to mechanical stimuli (tendon vibration) in healthy and paralysed (or paretic) muscles. In paralysed muscles, the deformation of the soft tissues provoked by tendon vibration may not be sufficient to provoke Ia afferent activation. This could be explained by the reflex pathways and intrinsic neuronal properties being negatively affected after spinal cord injury, with changes in both descending drive and sensory inputs being likely (Thompson, Lewek et al., 2011) and resulting in the variable response in muscle force production observed in Study 2. Speculatively, the negative response to tendon vibration could also be attributed to the selective activation of Ib afferent fibres (Golgi organ tendons) (Fallon & Macefield, 2007). On the other hand, the positive responses in some individuals may have resulted from stimulation of an already excitable neuromuscular system in people who developed greater levels of spasticity, and thus the Ia afferent response may have been exacerbated in those cases (Fallon & Macefield, 2007; Magalhaes & Kohn, 2010). However, these theories need further evidence to be confirmed. It is also worth mentioning that all subjects participating in this study did not report excessive spasticity, but it does not preclude a small response impacting the results.

Based on the results of Studies 1 and 2 it was considered that the use of tendon vibration was only beneficial for some individuals (positive responders to tendon vibration) and could not be applied generally to people with chronic SCI of different levels and presenting different symptoms. In Study 3, therefore, a 12-week high-intensity NMES-strength training intervention was implemented in people with chronic SCI, but tendon vibration was not simultaneously used; a wide-pulse width was still used because previous research indicated that this may allow for the recruitment of a greater total muscle mass (Gorgey & Dudley, 2008; Gorgey et al., 2010). The results of Study 3 provided evidence that high-intensity NMES strength training could be an effective exercise training method for improving muscle strength and mass, positively affecting physical health, ameliorating symptoms of spasticity and improving QoL.

The results of Study 3 have significant clinical implications in the physical rehabilitation field because the evidence for the use of different NMES protocols is inconsistent (Ho et al., 2014) and has many limitations to its introduction on a regular basis into clinical practice (Bersch et al., 2015). Findings from Study 3 support the use of high-intensity NMES strength training for increasing muscle force and mass and ameliorating symptoms of spasticity. The significant improvements in quadriceps femoris cross-sectional area (CSA) and muscle strength revealed that the use of high-intensity NMES strength training may be an important method for the reduction of the high levels of atrophy found in people with SCI. This increases in muscle size and force capacity and reductions in levels of spasticity are relevant to promote a healthier and strong musculoskeletal system that can maintain the capacity to benefit from future technology-based interventions, such as exoskeletons, epidural stimulation and stem-cells therapies. Therefore, using this type of intervention would theoretically allow people with SCI to have a real chance in the future to recover some functional abilities, such as standing and walking.

An improvement in some physical health markers, such as blood lipid profile, anti-inflammatory protein concentrations (CRP) and bone health markers were observed. The fact that the very high CRP basal levels in two people were reduced to normal levels was an interesting finding and suggested that prolonged higher-intensity NMES-based training might be of great benefit, even in people (3 of 5 subjects) who were already using NMES-based training at low intensities as part of their current rehabilitation program (i.e. FES). Although the (statistically significant) changes in bone strength and architecture (assessed using pQCT) were not deemed to be of sufficient magnitude to be clinically meaningful, they indicated that longer periods of training might possibly evoke meaningful responses. Thus, is worth pursuing changes in these parameters in further studies using high-intensity NMES strength training in

clinical populations, with a larger sample size possibly in combination with other strategies (i.e. diet, supplements, longer duration).

Of note, it should be remembered that there are many barriers when performing research in clinical populations that need to be considered. For instance, the recruitment of participants who could commit twice-weekly for a period of 16 weeks to undertake the intervention study (Study 3) was challenging and was one of the main reasons for the moderate sample size in Study 3. Thus, aiming for longer intervention periods may not be practical in people with SCI unless substantial transport, financial and other support is offered. This would help the urgent need to close the gap between research and clinical practice. If results from scientific research can be implemented in clinical practice, then people with a neurological condition will benefit from obtaining the best physical rehabilitation methods to improve their function and QoL.

Finally, there are some research processes that may need to be implemented in the future to perform efficient and good quality research in clinical populations. Firstly, it would be worth considering the use of surveys or interviews to clinicians and patients to find out what the main aims and problems encountered during the physical rehabilitation process are, so the research questions and the methodology for future research studies can then be developed. Secondly, clinicians and patients should be actively involved in the design of methodologies and implementation of the research as they can provide invaluable information in terms of feasibility and practicability of the research, which can increase the quality of the research. Thirdly, if research studies find positive results from any physical intervention and this finding is rigorously validated then the intervention should be first tested in clinical practice, under the supervision of an experienced researcher, before being adopted more widely. Subsequently, records of the outcomes obtained from the specific rehabilitation intervention during clinical practice should be reported and then systematically used in different clinical populations with the supervision of both experienced clinicians and researchers. It is my strong belief that if these recommendations are systematically implemented during any research study involving clinical populations, then the quality of the research and the practical implementations will improve dramatically. It only takes one step in the right direction to make the changes needed to take that step if we aim to improve the function, physical health and QoL of people living with a profoundly debilitating neurological condition.

In conclusion, when superimposed onto moderate-frequency wide-pulse width NMES, patella tendon vibration of the quadriceps elicited variable (positive and negative) effects in both healthy subjects and people with SCI. The positive response was clearly distinguished in healthy populations, but not in people with SCI. Although further testing is required to substantiate this finding, clinicians should test individual responses to tendon vibration to

determine whether it might allow for a greater total contractile impulse to be achieved before muscular fatigue in clinical practice. Nonetheless, the results indicate that high-intensity NMES strength training may be an effective exercise training method in people with SCI, based on the substantive increases in muscular strength and mass (cross-sectional area), improvements in some physical health outcomes, symptoms of spasticity and QoL observed in the current subjects.

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APPENDICES

Appendix A The Spinal Cord Injury Spasticity Evaluation Tool (SCI-Set) Questionnaire



The University of Sydney
Faculty of Health Sciences

THE SPINAL CORD INJURY SPASTICITY EVALUATION TOOL (SCI-SET)

Client: _____

Date: _____

Instructions: For each of the following choose the answer that best describes how your spasticity symptoms have affected that area of your life **during the past 7 days**. When I talk about "spasticity symptoms, I mean:

- a) Uncontrolled, involuntary muscle contraction or movement (slow or rapid; short or prolonged).
- b) Involuntary, repetitive, quick muscle movement (up and down; side to side)
- c) Muscle tightness
- d) What you might describe as "spasm"

Please, let me know when a question is not applicable to you.

DURING THE **PAST 7 DAYS**, HOW HAVE YOUR SPASTICITY SYMPTOMS AFFECTED:

	Extremely Problematic	Moderately Problematic	Somewhat Problematic	No effect	Somewhat Helpful	Moderately Helpful	Extremely Helpful
1. Your showering?	-3	-2	-1	0	+1	+2	+3
2. Your dressing/undressing?	-3	-2	-1	0	+1	+2	+3
3. Your transfers (to and from bed, chair, vehicle, etc)?	-3	-2	-1	0	+1	+2	+3
4. Your sitting positioning (in your chair, etc)?	-3	-2	-1	0	+1	+2	+3
5. The preparation of your meals?	-3	-2	-1	0	+1	+2	+3
6. Eating?	-3	-2	-1	0	+1	+2	+3
7. Drinking?	-3	-2	-1	0	+1	+2	+3
8. Your small hand movements (writing, use of computer, etc)?	-3	-2	-1	0	+1	+2	+3
9. Your ability to perform household chores?	-3	-2	-1	0	+1	+2	+3
10. Your hobbies/recreational activities?	-3	-2	-1	0	+1	+2	+3
11. Your enjoyment of social outings?	-3	-2	-1	0	+1	+2	+3
12. Your ability to stand/ weight bear?	-3	-2	-1	0	+1	+2	+3
13. Your walking ability?	-3	-2	-1	0	+1	+2	+3
14. Your stability/balance?	-3	-2	-1	0	+1	+2	+3
15. Your muscle fatigue?	-3	-2	-1	0	+1	+2	+3
16. The flexibility of your joints?	-3	-2	-1	0	+1	+2	+3
17. Your therapy/exercise routine?	-3	-2	-1	0	+1	+2	+3
18. Your manual wheelchair use?	-3	-2	-1	0	+1	+2	+3
19. Your power wheelchair use?	-3	-2	-1	0	+1	+2	+3
20. Your lying positioning (in bed, etc)?	-3	-2	-1	0	+1	+2	+3
21. Your ability to change position in bed?	-3	-2	-1	0	+1	+2	+3
22. Your ability to get to sleep?	-3	-2	-1	0	+1	+2	+3
23. The quality of your sleep?	-3	-2	-1	0	+1	+2	+3
24. Your sex life?	-3	-2	-1	0	+1	+2	+3
25. The feeling of being annoyed?	-3	-2	-1	0	+1	+2	+3
26. The feeling of being embarrassed?	-3	-2	-1	0	+1	+2	+3
27. Your feeling of comfortable socially?	-3	-2	-1	0	+1	+2	+3
28. Your feeling of comfort physically?	-3	-2	-1	0	+1	+2	+3
29. Your pain?	-3	-2	-1	0	+1	+2	+3
30. Your concern with falling?	-3	-2	-1	0	+1	+2	+3
31. Your concern with getting injured?	-3	-2	-1	0	+1	+2	+3
32. Your concern of accidentally injuring someone else?	-3	-2	-1	0	+1	+2	+3
33. Your ability to concentrate?	-3	-2	-1	0	+1	+2	+3
34. Your feelings of control over your body?	-3	-2	-1	0	+1	+2	+3
35. Your need to ask for help?	-3	-2	-1	0	+1	+2	+3

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SCORE:

- Number of (+) items: ____
- Number of (-) items: ____
- Number of (0) items: ____
- Negative Score: ____
- Positive Score: ____
- **TOTAL SCORE:** ____
- Applicable items (#): ____
- **Average Score:** ____

Appendix B Quality of life Index. Spinal cord Injury Version-III



The University of Sydney
Faculty of Health Sciences

Quality of Life Index Spinal Cord Injury Version - III

Client: _____

Date: _____

PART 1. For each of the following, please choose the answer that best describes how *satisfied* you are with that area of your life. Please mark your answer by circling the number. There are no right or wrong answers.

HOW SATISFIED ARE YOU WITH:

	Very Dissatisfied	Moderately Dissatisfied	Slightly Dissatisfied	Slightly Satisfied	Moderately Satisfied	Very Satisfied
1. Your health?	1	2	3	4	5	6
2. Your health care?	1	2	3	4	5	6
3. The amount of pain that you have	1	2	3	4	5	6
4. The amount of energy you have for everyday activities?	1	2	3	4	5	6
5. Your ability to take care of yourself without help?	1	2	3	4	5	6
6. Your ability to go places outside your home?	1	2	3	4	5	6
7. Your ability to clear your lungs?	1	2	3	4	5	6
8. The amount of control you have over your life?	1	2	3	4	5	6
9. Your chances of living as long as you would like?	1	2	3	4	5	6
10. Your family's health?	1	2	3	4	5	6
11. Your children?	1	2	3	4	5	6
12. Your ability to have children?	1	2	3	4	5	6
13. Your family's happiness?	1	2	3	4	5	6
14. Your sex life?	1	2	3	4	5	6
15. Your spouse, lover, or partner (if you have one)?	1	2	3	4	5	6
16. Not having a spouse, lover or partner (if you do not have one)?	1	2	3	4	5	6
17. Your friends?	1	2	3	4	5	6



HOW *SATISFIED* ARE YOU WITH:

	Very Dissatisfied	Moderately Dissatisfied	Slightly Dissatisfied	Slightly Satisfied	Moderately Satisfied	Very Satisfied
18. The emotional support you get from your family?	1	2	3	4	5	6
19. The emotional support you get from people other than your family?	1	2	3	4	5	6
20. Your ability to take care of family responsibilities?	1	2	3	4	5	6
21. How useful you are to others?	1	2	3	4	5	6
22. The amount of worries in your life?	1	2	3	4	5	6
23. Your neighborhood?	1	2	3	4	5	6
24. Your home, apartment, or place where you live?	1	2	3	4	5	6
25. Your job (if employed)?	1	2	3	4	5	6
26. Not having a job (if unemployed, retired, or disabled)?	1	2	3	4	5	6
27. Your education?	1	2	3	4	5	6
28. How well you can take care of your financial needs?	1	2	3	4	5	6
29. The things you do for fun?	1	2	3	4	5	6
30. Your chances for a happy future?	1	2	3	4	5	6
31. Your peace of mind?	1	2	3	4	5	6
32. Your faith in God?	1	2	3	4	5	6
33. Your achievement of personal goals?	1	2	3	4	5	6
34. Your happiness in general?	1	2	3	4	5	6
35. Your life in general?	1	2	3	4	5	6
36. Your personal appearance?	1	2	3	4	5	6
37. Yourself in general?	1	2	3	4	5	6



PART 2. For each of the following, please choose the answer that best describes how *important* that area of your life is to you. Please mark your answer by circling the number. There are no right or wrong answers.

HOW IMPORTANT TO YOU IS:

	Very Unimportant	Moderately Unimportant	Slightly Unimportant	Slightly Important	Moderately Important	Very Important
1. Your health?	1	2	3	4	5	6
2. Your health care?	1	2	3	4	5	6
3. Having no pain?	1	2	3	4	5	6
4. Having enough energy for everyday activities?	1	2	3	4	5	6
5. Taking care of yourself without help?	1	2	3	4	5	6
6. Being able to go places outside your home	1	2	3	4	5	6
7. Your ability to clear your lungs?	1	2	3	4	5	6
8. Having control over your life?	1	2	3	4	5	6
9. Living as long as you would like?	1	2	3	4	5	6
10. Your family's health?	1	2	3	4	5	6
11. Your children?	1	2	3	4	5	6
12. Being able to have children?	1	2	3	4	5	6
13. Your family's happiness?	1	2	3	4	5	6
14. Your sex life?	1	2	3	4	5	6
15. Your spouse, lover, or partner (if you have one)?	1	2	3	4	5	6
16. Having a spouse, lover or partner (if you do not have one)?	1	2	3	4	5	6
17. Your friends?	1	2	3	4	5	6



HOW IMPORTANT TO YOU IS:

	Very Unimportant	Moderately Unimportant	Slightly Unimportant	Slightly Important	Moderately Important	Very Important
18. The emotional support you get from your family?	1	2	3	4	5	6
19. The emotional support you get from people other than your family?	1	2	3	4	5	6
20. Taking care of family responsibilities?	1	2	3	4	5	6
21. Being useful to others?	1	2	3	4	5	6
22. Having no worries?	1	2	3	4	5	6
23. Your neighborhood?	1	2	3	4	5	6
24. Your home, apartment, or place where you live?	1	2	3	4	5	6
25. Your job (if employed)?	1	2	3	4	5	6
26. Having a job (if unemployed, retired, or disabled)?	1	2	3	4	5	6
27. Your education?	1	2	3	4	5	6
28. Being able to take care of your financial needs?	1	2	3	4	5	6
29. Doing things for fun?	1	2	3	4	5	6
30. Having a happy future?	1	2	3	4	5	6
31. Peace of mind?	1	2	3	4	5	6
32. Your faith in God?	1	2	3	4	5	6
33. Achieving your personal goals?	1	2	3	4	5	6
34. Your happiness in general?	1	2	3	4	5	6
35. Being satisfied with life?	1	2	3	4	5	6
36. Your personal appearance?	1	2	3	4	5	6
37. Are you to yourself	1	2	3	4	5	6