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The role of neuromuscular electrical stimulation in reversing age-related and pathological muscle atrophy

By Leanne Griffiths

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Abstract

An ageing population increases the number of frail, elderly individuals. Physiotherapists are increasingly treating frail individuals due to the associated rate of rapid muscle atrophy. A rapid loss of muscle strength can result in difficulty performing activities of daily living, making individuals more susceptible to other age-related pathologies. Some frail individuals are unable to contract their muscle sufficiently to complete a rehabilitation programme. This can be exacerbated in a neurological population such as stroke. The aim of this thesis was to prevent muscle atrophy associated with age to allow rehabilitation to commence with a quicker onset.

Neuromuscular electrical stimulation (NMES) is a treatment modality capable of producing muscle contraction. Its use is poorly understood, with little guidance surrounding optimal parameters or muscular response to treatment. This thesis has identified optimal stimulation parameters for strength training with NMES, and tested them on a healthy population of varying ages, and in a stroke population. A muscle measurement device was designed and tested to allow accurate measurements of moments about joints.

Results indicate that the protocol is effective in inducing hypertrophy, as indicated by advances in pennation angle and maximal isometric force production. The protocol was effective at producing a small decline in force associated with a hypertrophic stimulus. Results indicate that treatment should be administered with the highest available stimulation amplitude to achieve optimal results. NMES appears to be able to advance internal muscle architecture, despite lack of volitional muscle control post stroke. Variability of response was investigated through blood biomarkers (Creatine Kinase) which was demonstrated to increase in line with volitional strength training literature. The exercise status of the individual appears to be correlated with muscle response.

It is recommended that NMES could be administered in the acute period of a physiotherapy protocol to prevent muscle atrophy associated with ageing. Further work should focus on developing the strength measurement device used throughout this thesis, and investigating a protocol suitable for other applications to allow a smooth transition into clinical settings.

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Lastly I want to dedicate this thesis to my Granddad, Brian Yallop. My Granddad is an extraordinary man and has been a true inspiration throughout my childhood. He has recently been diagnosed with Lewy Body Dementia, and I am incredible proud that he will know that I have completed this thesis.

Chapter 1

Introduction

An ageing population

The world's demographics are changing as the population is living longer (Kowal, Chatterji et al. 2012). Older people are of upmost importance in health care as they are at greater risk of acquiring a chronic long term condition (for example diabetes, stroke or cardiovascular disease). As a result of this the older population is at a greater risk of acquiring a disability. A condition such as stroke results in a high percentage of suffers having a chronic disability (Teixeira-Salmela, Olney et al. 1999). This results in an increased demand on health care services, posing a major challenge in terms of cost and provision. Improving quality of life in the older population is an important concept, however becomes particularly significant in individuals with a long term disabling condition. The following model of independence has been designed by the research team to highlight the process of normal independence throughout life.

A conceptual model of Independence

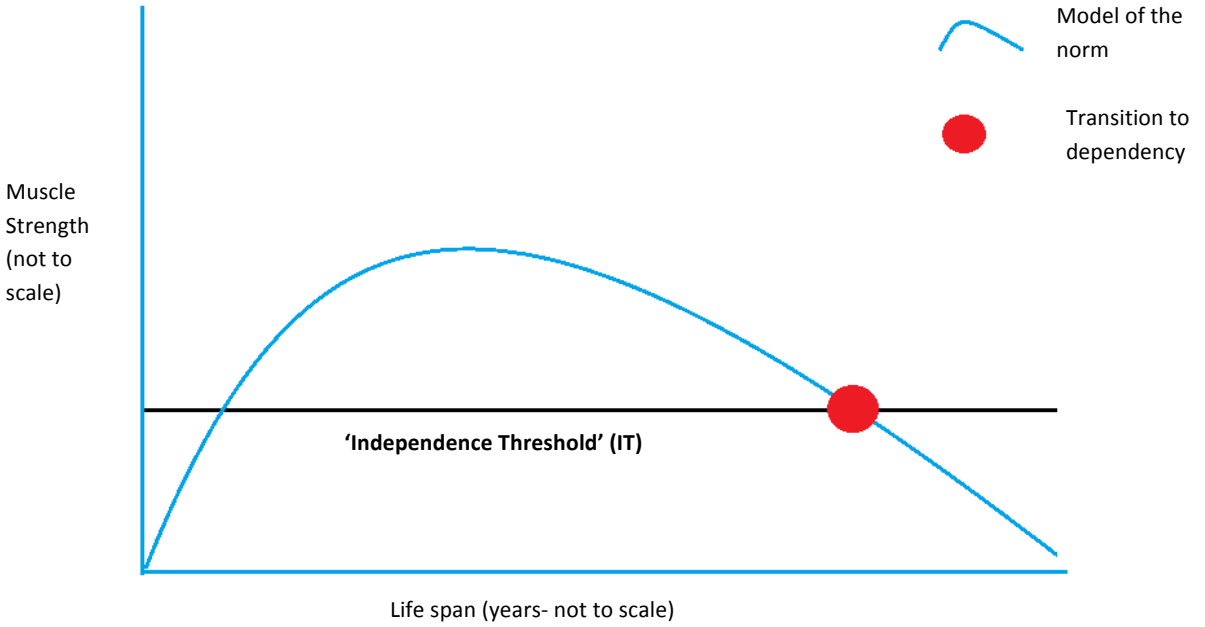


Figure 1.1 a diagrammatic representation of proposed model of independence

Figure 1.1 reflects how an individual changes their dependency status throughout life. In normal development, it can be difficult to predict when an individual will cross the Independence Threshold. An ideal trajectory will see an individual crossing the threshold at least two times in their life: as a child when they begin to walk and in the later stages of life when they are unable to fulfil the physical demands required of them on a day to day basis. However ill health or disability from birth may result in the individual not crossing the threshold at the expected time. A child with a disability affecting motor control may never gain the ability to walk, and thus have a high dependence on another individual to help them with daily activities.

When relating this model to the elderly population an individual may cross the independence threshold for the second time earlier than anticipated. Crossing of the independence threshold indicates that the individual does not have a sufficient amount of muscle strength to perform activities of daily living. This indicates that their physical activity has declined to a level that they require assistance during daily activities. It is at this point that these individuals become reliant on family members and/or carers for assistance in daily function. For the purpose of this thesis, dependency on others for daily living is regarded as crossing under the Independence Threshold. In some instances muscle weakness prevents individuals performing rehabilitation programs to a level sufficient to induce strength changing properties in the muscle. Early crossing of the Independence Threshold is pictured as an area of 'transition to dependency' in figure 1.1. Identification of an intervention to prevent frail individuals crossing the Independence Threshold prematurely would assist in maximising independent function. Medical literature is often concerned with preventing the onset of disease (Hayat, Luben et al. 2013); however this thesis is concerned with reducing the progression of disease to functional dependence. A thorough understanding of interventions to improve exercise capacity will help to maintain or improve quality of life in a rapidly ageing population.

Frailty and the natural ageing process

Frailty is a growing problem that is common in an ageing population. Confusion has arisen over the definition of frailty, and the difference between frailty and disability (Fried, Ferrucci et al. 2004). Frailty is associated with a reduced capacity of the body to withstand deterioration from multiple sources, and a reduced ability to maintain homeostasis. As frailty develops the number of co-morbidities that contributes to general health decline increases, and the individual's capacity to fight them decreases at a progressive rate. This deterioration in health often results in a decline in physical activity, which is associated with a concomitant reduction of muscle mass (Hughes, Frontera et al. 2001). In the elderly population, a loss of muscle strength due to disuse atrophy is increasingly hard to restore, which is confounded by long periods of forced rest. This can result in individuals being unable to perform exercise. When an individual is unable to perform exercise, the decline in muscle strength advances, and the individual can become dependent on others for activities of daily living. This would result in early crossing of the Independence Threshold. This process is demonstrated below in figure 1.2. Figure 1.2 was developed from clinical observations of frail individuals by the research Physiotherapist. Although there are exceptions to this model, it appears common practice in this population.

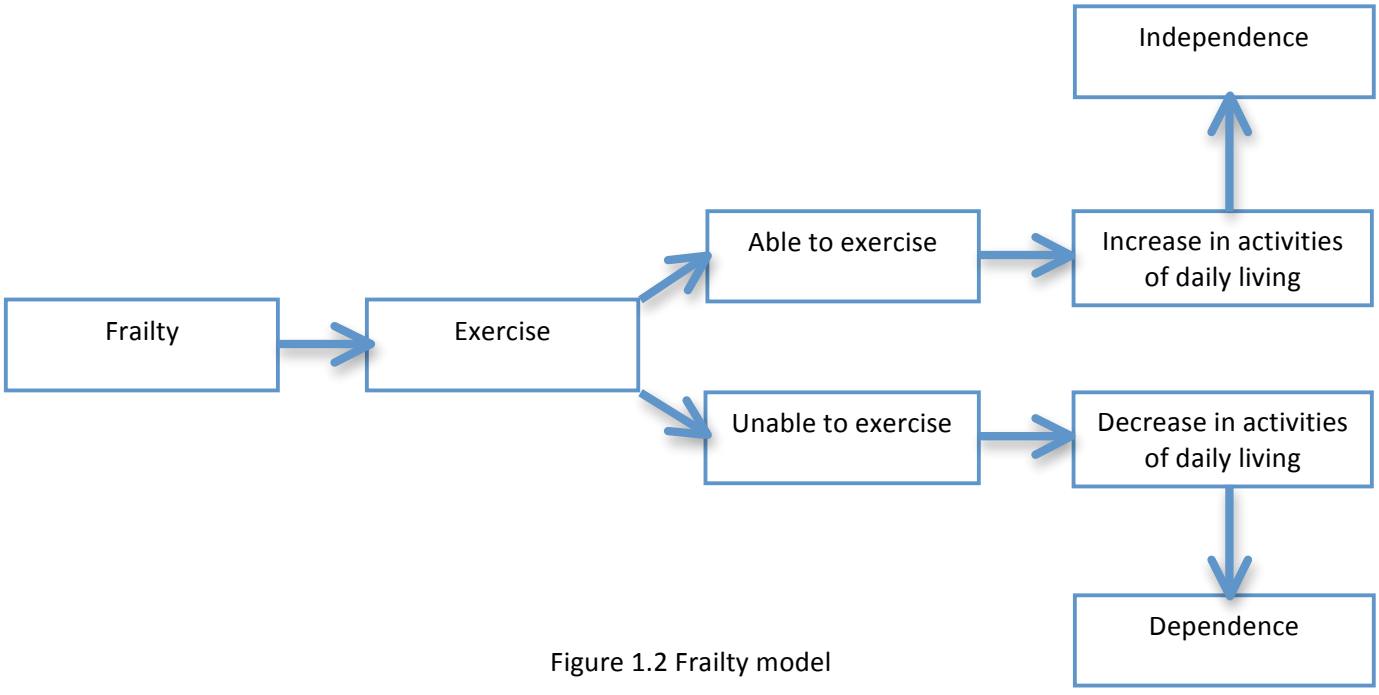


Figure 1.2 Frailty model

Muscle weakness in the frail individual

Muscle weakness has been attributed as a key determinant of frailty in elderly individuals (Fried, Tangen et al. 2001), where it contributes to a decline in mobility. The decline in architectural muscle structure associated with age is termed Sarcopenia and has been correlated to a deteriorating level of functional activity (Starling, Ades et al. 1999). Sarcopenia is a reversible condition in the elderly, with a growing body of evidence to state that exercise is able to restore muscle function (Evans 1999). The level of muscle weakness associated with old age varies, and there is no known relationship with age. Lifestyle choices such as level of exercise and nutritional status are major influence in one's physical condition. Advanced levels of Sarcopenia can often result in the inability to perform exercise protocols aimed at increasing function. Ability to exercise can be confounded by older age, number of co-morbidities and nutritional status (Breen, Phillips 2011).

Exercise rehabilitation, or resistance training is the gold standard in reversing muscle weakness. Despite this levels of exercise participation in the older individual remain low (Mayer, Scharhag-Rosenberger et al. 2011). This form of training aims to override the effects of muscle weakness (exercise dependant or age related) by initiating hypertrophy, and therefore increasing muscle strength. Hypertrophy occurs when the threshold of training is sufficient to cause changes in muscle properties. Resistance training can be administered in various forms to induce strength. These include hydrotherapy, group exercise classes, and muscle re-education (Newton, Hakkinen et al. 2002).

Problems in exercise participation in the elderly may arise from lack of resources (equipment or staff), or simply being unable to contract the muscle to a threshold sufficient to induce hypertrophy. Older adults could be excluded from participating in exercise programmes, despite a good level of muscle integrity for numerous reasons. Older adults often have orthopaedic conditions (such as a

joint replacement) contraindicating them from completing a resistance training program with the joint affected by pathology (Nelson, Rejeski et al. 2007). This population are also at a high risk of heart and cardiovascular disease; hence exercise should be preceded with caution and under supervision. The individual could be suffering from arthrogenic muscle inhibition (AMI), which is a homeostatic response designed to protect the joint from damage, commonly retarding rehabilitation protocols post-surgery (Rice, McNair 2010). This along with the heightened effects of muscle atrophy with prolonged rest in the elderly will ultimately lead to prolonged rehabilitation times. In this time frame, especially given the age of these individuals, the muscle will undergo further atrophy resulting in the exercise becoming more difficult to perform. This is reflected in figure 1.2.

The threshold to which the muscle has to work to improve function in the elderly has received little attention in the literature to date (Fujita, Kanehisa et al. 2011). If an individual is unable to activate a muscle, repeated exercise is performed in the hope of re-educating the muscle to contract in order to induce advances in strength. Improvements in muscle strength can only be achieved by tipping the balance between gains achieved through resistance training and advancing muscle atrophy caused by prolonged inactivity. This thesis proposes that the individual has to cross an Independence Threshold in order to carry out activities of daily living, as highlighted in figure 1.1. The independence threshold is the level of muscle strength required to perform everyday tasks. If underneath this threshold, the individual's physical ability to perform functional daily tasks reduces and they will ultimately become dependent on others for daily care.

Muscle weakness in a pathological population

Stroke is a condition that commonly leaves an individual with a chronic long term disability. Stroke is defined as an acute focal injury which is caused by vascular origin, and results in neurological deficit (Sacco, Kasner et al. 2013). Incidence of stroke is high, with somebody having a stroke every forty

seconds in the United States of America (Roger, Go et al. 2012). Stroke is a strong predictor of disability, which stems from the acute impact on mobility, and reductions in muscle strength. This thesis uses Stroke as a representative pathology in the elderly population that can result in prolonged bed-rest whilst the brain reorganises. This enforced bed-rest results in muscle disuse atrophy and prolonged rehabilitation times. This is regarded as an element of frailty.

Physiotherapy aims to restore independent function, which is difficult to achieve in this neurological population and highlights the continued demand for improved rehabilitation interventions to be identified (Munyombwe, Hill et al. 2014). A stroke can have numerous levels of impact on an individual's functional independence, depending on the severity of the bleed and location within the brain. In severe presentations a rapid loss of muscle strength will occur due to the disruption to innervation of the muscle. Loss of muscle strength limits the functional ability of an individual due to the negative effect on balance and proprioception. This often results in dependence for personal care. The resulting impact would be that the individuals would fall below the Independence Threshold prematurely (figure 1.1). Depending on how the stroke has affected the individual, rehabilitation can improve mobility and functional capability to a level where they re-cross the independence threshold, and are not as dependant on others.

After an adverse event, such as a stroke, one of four options can occur.

1. The individual recovers well, regaining their normal course of independence.
2. The individual shows some recovery, however still crosses the independence threshold early.
3. The individual does not show any form of functional recovery.
4. The individual dies.

This thesis will attempt to help individuals who fall into categories 2 and 3, and help them adhere to rehabilitation programs with more consistency (acutely when re-gaining neural control of muscles) and in a shorter time scale. A reduction in rehabilitation time results in less time for the muscle to under-go age related adaptations, such as sarcopenia. This would assist in reducing the effects of frailty to allow independent function in daily living upon discharge. Implementation of an intervention to prevent muscle atrophy in the acute stages of a stroke, whilst physiotherapy restores the ability of the muscle to contract is something that is not commonly used. However, it is something that may enable rehabilitation to progress faster, and promote neuroplasticity to occur in the short window of opportunity for natural recovery after neurological insult (Dimyan, Cohen 2011).

An alternative treatment

The intervention that this thesis explores to maximise muscle function is neuromuscular electrical stimulation (NMES). NMES uses small electrical currents to induce nerve impulses. Application with an intensity sufficient to depolarise the nerve results in a muscle contraction (Baker, Wederich et al. 2000). The force generated by a muscle is dependent on the intensity of stimulation, the pulse width and the firing frequency. However the interplay of stimulation parameters on muscle function is poorly understood, making its application in a clinical setting difficult to justify (de Kroon, Ijzerman et al. 2005). Application requires the presence of an intact lower motor neurone (Sheffler, Chae 2007). The mechanism thought to be associated with this is the presence of antidromic firing of motor neurones, and the ability to initiate an afferent response despite lack of nervous system control.

NMES has been investigated within varying populations, often with an aim of increasing muscle strength. Strength improvements have been observed in healthy athletic populations (Maffiuletti, Cometti et al. 2000), in a population of stroke patients (Yan, Hui-Chan et al. 2005) and in patients with spinal cord injury (Shields, Dudley-Javoroski 2006), however this list is not exhaustive. Despite a growing body of research into the ability to strength train in various populations, little understanding of the application or the response obtained from the muscle has been reported. Lack of understanding also indicates that the full potential of NMES as a treatment modality is not being utilised. It is assumed that the muscle response to stimulation with NMES is similar to that of volitional strength training. However the ability of both the muscle and the neuromuscular response to stimulation, or the response time to electrical impulse has not been investigated to date.

Justification for the use of NMES in clinical practice will not be achieved if current literature is indecisive as to its use. In recent years (2009) NMES for the correction of drop foot has been approved by the National Institute for Clinical Excellence (NICE), and is now an integral part of many clinical practices and National Health Service settings (Salisbury, Shiels et al. 2013). This recent advancement in the understanding of NMES indicates that other uses may be of clinical importance, and that their use should be investigated further.

Quantification of treatment effect

In order to establish the effects of NMES on muscle function in this thesis, a measurement device was designed. Current literature uses subjective measurement or hand held dynamometry, which document repeatability (Johansson, Cools et al. 2014), but do not provide an accurate description of muscle function. This is a limitation in current NMES literature, and further understanding will not be gained with these outcome measures despite conduction on larger samples. In order to fully explore the effect of muscle adaptability to an NMES protocol a more accurate measurement tool is

required. The ability to measure moments with high sensitivity will allow both investigation of individual muscle response to NMES and muscle changes over time. The ability to transfer the accuracy of this research focused device into a clinical setting was paramount in its development. The ability to easily, but accurately measure muscle strength of the lower limb on a hospital ward setting is a tool which is not currently available. Current devices with the ability to perform this task are not portable and expensive to purchase, making them difficult to implement in an acute setting (Stark, Walker et al. 2011). The ability to measure muscle function required primary consideration in the design of research methodology throughout this thesis, and enabled clinical studies to address the primary aims outlined below.

Summary

The overriding aim of this thesis is to investigate whether treatment with NMES can reverse, or prevent deterioration in muscle structure and function. However before identifying study specific objectives there was a need to conduct a comprehensive literature review in this subject area. The chapter that follows is the literature review, which details the project specific aims.

Chapter 2

Literature review

The population this thesis aims to address is the frail elderly. These individuals are being referred to physiotherapists more commonly as a consequence of increased life expectancy and advancements in medical knowledge (Fielding, Vellas et al. 2011). These people are often unable to complete a rehabilitation programme due to muscle weakness. An understanding of frailty as a concept will enable an insight as to how these individuals will tolerate a rehabilitation programme (Ikezoe, Mori et al. 2011). The advanced rate of muscle atrophy often supersedes advances in muscle strength resulting in a net decline in force generating capacity of the muscle. However evidence that a frail population is able to adapt in response to resistance training is emerging (as detailed below). The ability to respond to high intensity exercise opens novel avenues for new treatment modalities, such as neuromuscular electrical stimulation (NMES). This review will explore the research that has been conducted to date in this field and highlight areas where further research is required.

2.1 Introduction to frailty

As an individual advances in age the likelihood of health deterioration increases. An ageing population in today's society (Lagergren, Fratiglioni et al. 2004, Pahor, Gurainik et al. 2014) has concomitantly led to an increasing number of frail, elderly individuals (Rockwood, Song et al. 2011). In 2007, 18% of the United Kingdom's population was aged 65 years and over and this is believed to increase to 25% by 2031 (Aw, Silva et al. 2007). Many factors are contributing to this, including improved medical care, preventative education and an improved quality of life. **An ageing population is subsequently producing a demanding workload on geriatricians, with a concomitant increase in demand on health services** (Reinhardt 2003). The effect on services is also impacted by

the increased number of retired workers and demand on elderly care homes. The American Medical Association highlighted the significance of the ageing population in a white paper in 1990, stating that "one of the most important tasks that the medical community faces today is to prepare for the problems in caring for the elderly in the 1990s and the early 21st century" (Fried, Ferrucci et al. 2004). The frail elderly has been described as a challenging population, which requires input from a wide range of the multidisciplinary team. **The ageing process is not as aggressive in elderly individuals who undertake regular exercise and the consequent impact on health care services ultimately reduces in this instance** (Elward, Larson 1992, Ryan, Grant et al. 2006). A vast majority of elderly people grow old independently and do not show signs of frailty. However some people show a loss of independence and consequently become reliant or dependent on others for activities essential for daily living.

2.1.1 Frailty Definition

Frailty is difficult to define and confusion exists within clinical and academic contexts (Bortz 2002). Clinical agreement in the definition is lacking and synonyms with disability and advancing age are common. **Frailty can be referred to as a medical syndrome, with a reduced capacity of multiple systems to withstand external pressure** (Fried, Tangen et al. 2001). The inter-play of systems is evident in common conditions such as diabetes; which can consequently render an individual susceptible to other medical difficulties such as neuropathies and cardiovascular disease. An individual with diabetes is at higher risk of contracting cardiovascular disease, which may be heightened by their age, lifestyle or nutritional preferences (Barrett-Connor 2003). Both aerobic exercise and resistance training has been associated with improved glycaemic control and muscle strength in patients with diabetes (Dunstan, Daly et al. 2002) and have subsequently been included in treatment recommendations. **Advancing age is influential in health deterioration, however the**

increased number of co-morbidities and cumulative decline in multiple systems reduces the reserve to combat symptoms of frailty.

A medical syndrome indicates that multiple symptoms or clinical presentations are linked together to form a medical diagnosis of frailty. The body's capacity to react to a manifestation or condition is decreased when there are contributing factors affecting health. Co-morbidities have been documented to be a factor associated with mortality in common pathologies found in elderly care (Hu, Jiang et al. 2012). Knowledge of how common health conditions affect one another would help clinicians prepare and treat frail patients. Optimising health status in the elderly is under analysis and it is acknowledged that better screening of frail patients is required to enhance treatment outcome (Roche, Wenn et al. 2005). Cardiovascular disease and lung disease are reported to be key determinants in mortality rates, however their effect on other systems is not fully understood. Advanced knowledge of the frail individual would allow the clinician to gain an insight as to how co-morbidities will impact on each other. A confounding factor such as poor nutrition could leave the frail individual with a reduced physiological reserve, increasing the vulnerability of this age group. It is estimated that 85% of elderly individuals could demonstrate improvement in chronic conditions with a better nutritional intake (Posner, Jette et al. 1993). Nutritional advice or availability in this population, could therefore act as a treatment to improve medical status. Research into frailty is continuing and further understanding would aid both clinical and social implications. The ability of the clinician to understand how pathology links to age and the patient's ability to alter expectations in accordance with this allows healthcare to be advanced with increased outcomes (Gillick 2001).

2.1.2 Disability and frailty

Disability refers to the inability to perform activities of daily living (ADL) and the majority of researchers and clinicians are in agreement that frailty and disability are two different medical

entities (Gale, Cooper et al. 2015). Fried et al circulated a questionnaire to American and British geriatricians (n=62) to explore the relationship between the two definitions, with 97.5% agreeing that there is a distinct difference between the two (Fried, Ferrucci et al. 2004). There was also agreement that frailty is an accumulation of manifestations and cannot be diagnosed by a single pathology. The questionnaire required the clinicians to rank clinical observations, with respect to the likelihood of frailty. Three of the example observations produced a subjective score of over 70% likelihood of frailty:

- Dementia resulting in dependence in ADLs coupled with unintended weight loss of 20lbs.
- Osteoporosis with a history of single compression fracture coupled with unintended weight loss of 20lbs.
- Occasional urinary incontinence coupled with difficulty with ADLs due to weakness.

This indicates that a combination of multiple clinical symptoms are required to severely reduce the capacity of the individual to withstand external stresses (Gale, Cooper et al. 2015, Fried, Ferrucci et al. 2004). Unintended weight loss appears in the two most likely frail combinations. This suggests that weight loss is a clear contributor to frailty and an indication that the body is unable to function to its full capacity. This may be either a contributor to multiple system disruption, or weight loss may occur as a consequence of failure of other systems. Research into this factor is warranted to establish the cause of physiological decline. Other medical conditions such as diabetes, hypotension and cardiovascular disease all decrease the bodies fighting reserve, rendering the individual more susceptible to signs of frailty.

2.1.3 Contributors to frailty

It has been suggested core elements contribute to a person becoming frail and two or more of these elements must be present for a diagnosis of 'pre-frailty', or three or more for frailty to be diagnosed (Rockwood, Song et al. 2005). These elements include: age associated reduction of body weight, muscle strength, endurance, balance, walking ability and performance and physical activity (Fried, Tangen et al. 2001). The combination of these elements and other age related pathologies results in the body being unable to control homeostatic mechanisms required for a continued level of life quality. The predetermined physiological reserve of each condition for frailty to be present is unspecified, however vulnerability is assumed regardless. **The most common elements of frailty have been defined as generalised weakness, slow walking speed, exhaustion, low physical activity levels and unintentional weight loss** (Woods, LaCroix et al. 2005).

There are however exceptions to this rule, such as obesity. An obese individual is likely to have a reduced physical activity level, will generally hit a level of exhaustion with activity and subsequently have a reduced walking speed. According to Woods, this person would be categorised as frail. However, with a gradual change in diet and exercise level, this person could potentially lose some excess weight, strength their muscles and thus their exercise tolerance would improve. This indicates that some caution is required when categorising individuals and that a full medical assessment is vital in this process. The exhausted functional reserve associated with frailty continues to be investigated and screening tools developed to aid diagnosis of frailty. There is unanimous agreement that multiple physiological systems are involved in the frail individual and caution is required throughout treating due to the high risk of mortality (Ferrucci, Guralnik et al. 2004).

2.1.4 Muscle weakness vs muscle power in frailty

Many factors have been identified to make elderly individuals more prone to frailty. The muscles ability to generate power has been attributed as an influential factor affecting the performance of muscle. Research has begun to investigate a functional power activation threshold that would slow down the onset of frailty (Van Roie, Verschueren et al. 2011). Daily functional tasks require an acceleration component which argues that power is a vital component of muscle function. Power is defined as the amount of work done over a given period of time (Macaluso, De Vito 2004). As individuals age, the speed of which the muscle is able to produce a force decreases, affecting reaction time and overall strength. This reduction is suggested to be greater than that of muscle strength, making power generation a contributor to frailty (Reid, Fielding 2012). Power reduction in the elderly is due to a natural reduction in conduction time of the central nervous system with senescence (Casas-Herrero, Cadore et al. 2013). Muscle power has widely been shown to correlate with function, with a decrease in power associated with a decrease in the ability to perform everyday activities (Clemencon, Hautier et al. 2008). It has been concluded that rehabilitation should incorporate power training exercises to help minimise functional deterioration (Van Roie, Verschueren et al. 2011).

2.1.5 Frailty and physical activity

As co-morbidities develop, one of the first aspects of daily living to decline is physical activity (Mutrie, Doolin et al. 2012). Physical activity is important for independent function (Pahor, Gurainik, et al. 2014). Physical inactivity has been shown to be one of the top four factors contributing to early preventable death in the United States (Danaei, Ding et al. 2009). Guidelines published in the UK recommend a minimum of 150 minutes of physical activity per week at a moderate intensity to help improve strength and balance (Mutrie, Doolin et al. 2012), however this is commonly not achieved. Rest is a commonly prescribed treatment during illness; prolonged rest can reduce the reserve

capacity within the musculoskeletal and cardiovascular systems. The increased cerebral blood flow associated with physical activity has been linked to a reduction in the incidence of cognitive decline (Yaffe, Barnes et al. 2001). This perfusion increase is thought to be related to an increase in neuronal growth with repeated exercise. Physical activity increases bone density, which has a positive impact on the rate of fractures in this population (Forstein, Bernardini et al. 2013). An eighteen-month physical activity programme has also been shown to significantly impact body fat percentage (Beavers, Beavers et al 2014), which will have a long term influence on bone health. **These factors highlight the importance of exercise as a preventative treatment modality; potentially reducing the number of co-morbidities in this population**. Preventing decline of cognitive performance will impact other co-morbidities and allow the individual to continue with an active lifestyle. An American study found that although physical activity was beneficial to independent function, it resulted in more hospitalizations (Pahor, Gurainik et al. 2014). This could be due to the unmasking of otherwise underlying medical conditions. Although hospitalisation increases the risk of further inactivity, earlier medical intervention has positive implications.

The decline in physical activity associated with frail elderly individuals makes them more susceptible to pathologies that influence their vulnerability. In an elderly age group physical activity would be defined as activities of daily living, such as washing and dressing and walking to the local shop. **As physical activity declines muscle mass concomitantly reduces** (Hughes, Frontera et al. 2001). Although the mechanism is not fully understood, a reduction of muscle mass due to disuse atrophy is increasingly hard to restore in an elderly population (Rennie, Selby et al. 2010). Disuse atrophy results in a reduction of muscle protein synthesis, partially accounting for the reduction in force capability. The timescale of atrophy is also very rapid in the elderly, resulting in a reduced capacity to perform daily activities (Hofer, Marzetti et al. 2008). Oxidative damage and increases in iron concentration after disuse atrophy have been associated with reduced protein synthesis, which could be an influential factor in rapid muscle deterioration with reduced physical activity. Muscle

atrophy as associated with age is also confounded by long periods of rest and increased risk of other confounding factors such a stroke, respiratory or cardiovascular disease (Dempsey, Owen et al. 2014). The combination of reduced physical activity and increasing co-morbidities renders the individual susceptible to a further decline of health, on a progressively deteriorating scale. Monitoring physical activity in elderly individuals has demonstrated some positive compliance; however participants do not always adhere to recommended time scales or intensity levels (Chastin, Dall et al. 2009, Mutrie, Doolin et al. 2012, Tigbe, Lean et al. 2011). A recent systematic review also suggests that self-efficacy techniques that work for younger adults are not effective at increasing participation in physical activity in the elderly (French, Olander et al 2014). This is a key factor that requires investigation. Improving levels of physical activity will help to improve associated health decline.

2.2 Muscle Physiology

In order to understand the mechanism associated with muscle atrophy in the elderly, the basic physiology must first be understood. A muscle adapts in response to a change in physical activity level. In order to ascertain how skeletal muscle regenerates as a consequence of resistance training, it is important to understand the basic composition of a muscle fibre. Strength can be defined as the ability of a muscle to produce a force (Lieber 2009). Skeletal muscle is vital in order to hold the human skeleton in an upright position: a constant level of low muscle tone allows the body to be erect (Pereira, Silva et al. 2011). Muscles are also required to move bones about their axis of rotation. The sequential shortening and lengthening of skeletal muscle in the excitation-contraction coupling process produces movement at joints. Emerging evidence is highlighting the role of the dihydropyridine receptor and the calcium release receptor ryanodine. Although not fully understood, it is suggested that the two receptors do not interact as previously indicated; however research continues in this field (Rebbeck, Karunasekara et al. 2014). The ability of the muscle to

produce a force determines the activities that are conducted. Restoration, maintenance or improvement in this ability is a vast contributing factor in achieving a high quality of life and is a key process in the role of a physiotherapist.

The contractile region of a muscle fibre is comprised of two main proteins, actin and myosin. Both proteins are controlled by numerous regulatory proteins, with myosin being the key protein in driving the contractile process. The activity of myosin appears to be correlated with the orientation of actin filaments, indicating that the preservation and training of the filaments is vital to optimise function (Reymann, Boujemaa-Paterski et al. 2012). Titin is a molecule under investigation in the muscle contraction process and theories suggest that the tension in this connective molecule increases in the presence of calcium and plays a contributory role in the rotation of actin during a cross bridge formation (Nishikawa, Monroy et al. 2012).

The positional interplay of proteins are collectively known as a sarcomere, which is the basic element that produces a muscle contraction. Muscle contraction is initiated by the arrival of an action potential at the neuromuscular junction. Calcium release invites vesicles containing the neurotransmitter acetylcholine to cross the neuromuscular junction and initiates an action potential in the muscle cell. The subsequent activation of calcium channels allows calcium to bind to the troponin component of the actin molecule. This initiates a cross-bridge, or the interplay between actin and myosin, resulting in a muscle contraction. Muscle contraction is highly efficient and reliable due to the fact that both the impulse from the nerve and the amount of neurotransmitter released are both beyond the level that is required to reach the respective threshold for transmission. This over-estimated margin allows for as much consistency and availability of neuromuscular transmission as possible (Schiaffino, Reggiani 2011). This description of the excitation-contraction

coupling process was first described as the sliding filament theory by Andrew Huxley in 1954 (Huxley 2004).

As muscles are repeatedly trained they can increase in size which is medically referred to as hypertrophy. Hypertrophy refers to an increase in the size of existing cells and hyperplasia the addition of cells. Resistance training is the stimulus required to increase force production of the muscle and results in the addition of sarcomeres in series allowing a greater active excursion of the muscle. However the addition of sarcomeres in parallel results in an improvement of force output (Lieber 2009). If a muscle is over trained or stimulated beyond the threshold of the cells the muscle fibre can become damaged. Some muscle damage is required to stimulate hypertrophy (Schoenfeld 2012), however too much muscle damage results in a complete tear which is detrimental to movement or performance. Eccentric exercise is commonly associated with muscle damage and research indicates that there is a vast range of resulting damage (Paulsen, Mikkelsen et al. 2012). Damage can occur to the sarcolemma or individual contractile fibres, but has been found to mainly occur in type two fibres. Damage to the muscle fibre initiates an inflammatory response, with evidence that reactive oxygen species may be involved in this process. Activation of satellite cells is also suggested to result in the reversal of damage and adaptation to training. Satellite cells are progenitor cells found between the basal lamina and sarcolemma. They proliferate after muscle fibre trauma and differentiate into myoblasts before fusing to existing muscle fibres to repair the damaged tissue and stimulate growth of new tissue (Hawke, Garry 2001). Proliferation has been shown to occur 2-3 days post exercise, with the injured muscle restored to a functional level within 10 days. The adaptability of skeletal muscle is extensive, although impacted by various factors such as ageing, training the muscle to increase in size or strength is a fundamental process in everyday living.

Muscle can weaken with inactivity, pathology or age. It is well established skeletal muscle requires a repeated stimulus to maintain or regenerate muscle strength. Atrophy of skeletal muscle can occur in response to pathology, or simple disuse. Ubiquitin ligases have been identified and are thought to be responsible for the atrophy process in skeletal muscle. The up-regulation of protein degeneration during atrophy is thought to be assisted by these molecules (Bonaldo, Sandri 2013). Understanding of the mechanisms of atrophy is developing, however research is still being undertaken to advance this. Age is a process that advances atrophy and has been termed sarcopenia (discussed in full in section 2.3). The rate of atrophy related to age is unknown, however emerging evidence suggests that the process can be prevented with training (Doherty 2003). Strength is also confounded by a decrease of skeletal muscle mass, which has been shown to decrease 40% between the ages of 20 and 60. Males have been postulated to show greater declines in muscle mass than women, although being attributed to hormonal factors, the exact cause of this is unknown. Sarcopenia is therefore a factor that contributes to reduction of mobility, inability to perform functional daily tasks and general frailty in the elderly population.

2.3 Sarcopenia

2.3.1 Presentation of sarcopenia

Age related reduction in muscle mass is medically referred to as sarcopenia (Borst 2004). The reduced number of muscle fibres associated with a decrease in muscle mass consequently results in reduced muscle strength (Brooks, Myburgh 2012). Sarcopenia is highly influenced by chronic diseases (Pereira, Silva et al. 2011). A decrease in exercise level or reduction in the ability to perform functional daily activities contributes to sarcopenia. **Restoration of physical activity in the elderly appears to be sufficient to prevent age associated reduction in muscle mass** (Starling, Ades et al. 1999), although has not been shown to demonstrate improvements. Maintenance of muscle mass suggests that the number of muscle fibres remained constant throughout testing protocols and

therefore the muscle did not decrease in size. It would be interesting to ascertain the effects on muscle power, as this has been highlighted as an important factor in daily living in the elderly. Physical activity may influence other confounding factors in an elderly population, such as obesity and diabetes, potentially impacting the overall frail state of an individual. Research has indicated that the reduction in muscle mass between the ages of 50 and 80 can be as high as 33% (Borst 2004). Sarcopenia is often seen in frail individuals due to lack of mobility and fear of falling. Frail individuals can often be hospitalised (Fried, Tangen et al. 2001) and prolonged bed rest as a consequence of this results in increasing loss of muscle mass. Muscle imaging has confirmed the loss of muscle mass due to senescence in several larger muscle groups is most predominant in the lower limb (Welle, Brooks et al. 2004). This loss of muscle mass is a core element of frailty, rendering the individual more susceptible to further problems as a consequence of a reduction in physical activity.

Some researchers have suggested that sarcopenia is not selective in regards to specific muscles, with loss of strength occurring throughout the body (Jones, Stephenson et al. 2009). The same review also indicates that sarcopenia predominates in type one, postural muscles. Type one muscle fibres are slow energy release fibres and used throughout general walking and during activities of daily living. The changes in muscle fibre number and type indicate a neurogenic origin (Edstrom, Altun et al. 2007). The differences between acute muscle atrophy and sarcopenia have been discussed at length (Borst 2004). Acute muscle atrophy maintains muscle fibre number and specific force despite a reduction in muscle mass, also displaying a predominance of type two muscle fibres. Whereas, sarcopenia demonstrates a reduction in the number of muscle fibres associated with the decrease in muscle mass. This highlights how sarcopenia is a more permanent reversal of muscle structure, indicating the difficulty in restoring strength in the elderly. Research suggests that physical activity should include both aerobic and anaerobic training to maintain a level of functioning in both (Starling, Ades et al. 1999). Research is investigating the effects of strength training in the elderly and at present the mechanism and effects are not fully understood.

2.3.2 Contributors to sarcopenia

There are many contributors to the development of sarcopenia, including age related degeneration of the nervous system and the growth hormone pathway (Borst 2004). The alpha motor neurone is responsible for the innervation of the skeletal muscle extrafusal fibres, causing their contraction (Schiaffino, Reggiani 2011). Natural senescence indicates that the number of alpha motor neurones reduces with age (Roubenoff 2000). However, it has also been postulated that the number of motor neurones is unaffected, but the dendritic capacity and innervation is reduced with age. This process of neuronal atrophy hinders the transmission of nerve signals, ultimately affecting the daily function of the individual (Edstrom, Altun et al. 2007). Reduction in neurone innervation will result in less neurones innervating the muscle in a motor unit. Rate of force generation, force production and motor dexterity will subsequently be compromised. The body naturally releases neural cell adhesion molecule (NCAM) at the neuromuscular junction to compensate for this reduction. The role of the NCAM is to attract the remaining, or developing alpha motor neurones to the denervated muscle cell (Jones, Stephenson et al. 2009). This suggests that regeneration is able to occur within the ageing individual. The motor unit, defined as a single alpha motor neurone and the muscle fibres in which it innervates (Lieber 2009) then increases in size. Consequently, one alpha motor neurone has to activate more muscle fibres in the same contraction, partially explaining the decreased dexterity shown in elderly individuals. Skeletal muscle is highly adaptable to environmental changes via proliferation of satellite cells. Activation of satellite cells occurs in response to trauma, resulting in myoblasts fusing to the muscle fibre and regenerating the damaged tissue (Hawke, Garry 2001). The number of satellite cells decreases with age, resulting in the loss of force production witnessed in elderly populations (Kadi, Charifi et al. 2004). However, it is suggested that sarcopenia is preventable and that skeletal muscle mass can be regenerated in an elderly population.

2.3.3 Age Vs Disease

Although sarcopenia has been described in detail, discrepancy still remains as to where age related changes end and where disease begins. This is discussed well by Rosenberg (Rosenberg 1997), which also discusses whether muscle weakness in the elderly can be reversed. Emerging evidence suggests that resistance training is capable of increasing strength in the elderly (see review below). Evidence indicates that improvements in lower limb strength can be demonstrated in people with a mean age of sixty-eight (Pyka, Lindenberger et al. 1994). Interestingly, predominant increases in strength were shown in the first three months of a twelve month training programme, with increases plateauing for the remaining duration. This has been attributed to familiarisation with the testing procedure, with the authors highlighting that a change of protocol may be necessary to see this increase continue. This is a similar phenomenon to studies on young, healthy individuals; the muscle will become accustomed to the training intensity, with demand requiring constant alteration. Numerous studies indicate that increases in strength demonstrated in the elderly are a response of neuromuscular enhancement (Hakkinen, Alen et al. 2000). This suggests that the frequency of motor unit firing increases and is associated with the short terms improvements in force output (Hurley, Roth 2000). Although the mechanism is not fully understood, evidence suggests the role of insulin growth factors may be associated with short term improvement, although this evidence appears to be conflicting and unsubstantiated. These changes are reported to precede hypertrophy in both young and older adults. Hypertrophy of skeletal muscle has been demonstrated after a progressive and intense training program (Fiatarone, Marks et al. 1990). The effects of this programme were attributed to the progressive overload principle adopted during programme design. It is acknowledged that pre-training fat-free mass is a key indicator of muscle response; the lower the fat free mass the larger the muscle response. No association has been documented on the method of obtaining fat free mass; exercise or nutritional.

2.4 Treatment of muscle weakness in the elderly

2.4.1 Resistance Training

A growing body of research is emerging with regard to resistance training in the elderly. Resistance training has been postulated to result in significant strength gains and have a resulting effect on quality of life (Evans 1999). Exercise is conducted with the aim of reducing co-morbidities in the elderly and thus resistance training has the capacity to impact frailty. Various outcome measures have been utilised in assessing the benefits of resistance training on quality of life in this population; including muscle strength (Pyka, Lindenberger et al. 1994), functional activities (Fahlman, McNevin et al. 2011) and qualitative measures (Park, Kim et al. 2012). Despite these recommendations, statistics from the National Health Interview Survey states that over 33% of adults report that they do not participate in physical activity on a regular basis (Go, Mozaffarian et al. 2013). This indicates a need to engage more adults in physical exercise.

2.4.2 Overload principle

Studies highlight the need to utilise the over load training principle in a gradual manner in order for older participants to benefit from resistance training (Chiung-Ju, Latham 2011). The increased incidence of pathology in the elderly results in many trials evaluating resistance training in participants with a specific pathology, making comparison more challenging (Liu, Latham 2009). Liu highlighted the beneficial effect of resistance training on knee extensor strength, which subsequently had a positive effect on gait speed. Resistance training in the upper limb has been documented to demonstrate greater strength improvements than the lower limb (Sousa, Mendes et al. 2011). This has been attributed to the relative disuse of upper limb muscles in comparison to the lower limb. Lower limb muscles are utilised more frequently in daily function in comparison to upper limb muscles and thus do not suffer from the rapid atrophy of type two fibres as promptly as upper limb muscles (Gauchard, Tessier et al. 2003). Initial advances in skeletal muscle strength have often

been attributed to neuromuscular adaptation (Hakkinen, Hakkinen 1995) and it may be that the upper limb muscles are more susceptible to this influence than their lower limb counterparts. The mechanism of increased gene transcription and protein synthesis has been demonstrated to be similar in younger and older adults. However the rate of change is slower in elderly individuals (Pereira, Silva et al. 2011). It is suggested that 20% of a muscles adaptation capability is determined by genetic influences (Thomis, Beunen et al. 1998). This suggests that some variation in improvement may be expected throughout a studied population.

2.4.3 Strength Vs Power

Research indicates that both strength and power are required to achieve advances in optimum function in the elderly. Muscle power is reported to decline at a rate higher than strength, with a 3-4% reduction in power per year between the ages of 65 and 89 (Macaluso, De Vito 2004). Muscle power has been correlated to functional ability in the elderly (Suzuki, Bean et al. 2001) and is considered one of the most important physiological considerations for functional decline prevention (Foldvari, Clark et al. 2000). More specifically leg power has been identified as a main predictor. Due to the reduction in muscle strength with senescence power is utilised with everyday functional activities, such as standing from a chair. The influence of power on quality of life therefore becomes greater. Restoration or maintenance of lower limb power should be a vital consideration in physiotherapeutic programmes. The inconsistencies in application of resistance training have highlighted concern with data comparison and extrapolation of results (Liu, Latham 2009). The duration of training tends to be consistent, at 2-3 times per week, however the intensity of training and number of repetitions and sets has been shown be to variable in the literature (Chiung-Ju, Latham 2011). Adverse events associated with resistance training in the elderly are hard to define, mainly due to the variety of co-morbidities present in this population (Liu, Latham 2010). The ability

to relate research into clinical practice remains difficult, due to the uncertainty of attributing a change of status to the intervention or to an existing pathology.

2.4.4 Limitations of a Resistance Training programme

Although the benefits of resistance training in this population have been well documented, limitations have been highlighted and therefore stringent monitoring of individuals conducting this type of training is essential. Knowledge of limitations will help physiotherapists prescribe exercise based on individual needs. Resistance training has been suggested to increase blood pressure and contribute to muscle and joint pain. The American Heart Association has expressed caution when conducting resistance training in an elderly population who suffer from hypertension (Cornelissen, Fagard et al. 2011). This amalgamates from studies which report rises in systolic blood pressure and the consequent risk of haemorrhage from cardiac aneurysm. Systolic blood pressure has been identified as a key determinant in cardiovascular risk prevention and should be included in interventions related to hypertension (Chobanian, Bakris et al. 2003). Reductions in blood pressure can influence the risk of pathology in hypertensive individuals, including an 8% reduction in the risk of stroke. The addition of aerobic exercise into a programme will help to reduce hypertension (Collier, Kanaley et al. 2008), but also the increased risk of cardiovascular disease that results from obesity. Aerobic exercise is suggested to decrease arterial stiffness and improve vasodilatory capacity after four weeks of training, demonstrating improvements to general cardiovascular health. Muscle and joint pain has been highlighted as a limitation (Chiung-Ju, Latham 2011), however resistance training has been demonstrated to improve pain as measured on a validated questionnaire in patients with osteoarthritis (Jan, Lin et al. 2008). The muscle pain associated with resistance training may arise from delayed onset of muscle soreness, as a result on the increased training load demanded from the muscle. It is recommended to avoid resistance training 3-4 week post complex surgery in older individuals to enable full medical recovery to take place. However,

there appears to be limited restriction in terms of intensity utilised thereafter, with participants being assessed for one repetition maximum (Pollock, Franklin et al. 2000), with no precautions recommended. The combination of resistance training with aerobic elements conducted under supervision appears to be sufficient to help reduced the risk of associated pathologies in an elderly population.

2.5 Measuring muscle strength

2.5.1 Current measurement methods

Muscle weakness is a common problem presented to physiotherapists and rehabilitation specialists, with resistance training being the primary method of restoring strength. This is in both a young and elderly population. Accurate measurement of muscle strength is challenging to conduct in an acute rehabilitation setting, primarily as a response to impracticality of required equipment in an acute environment. Adaptation in strength witnessed in clinical populations may be achieved in small increments, however could result in profound functional implications (Umphred, Lazaro et al. 2013). Neurological pathology requires a more sensitive approach to measurement and often requires specialist adaptation of patient position, making measurement in this population difficult (Ayalon, Ben-Sira et al. 2000). The rate of strength gain can be confounded by pathology in older age, resulting in a slower rate of hypertrophy (Evans 1999). Alternatively, rehabilitation may aim to prevent muscle atrophy from disuse; something that requires a measurement tool that is sensitive to minor changes in strength. Measurement tools can be considered highly reliable, however not subtle enough to detect responsiveness of the muscle to an intervention (Flansbjer, Drake et al. 2011). **Translation of research tools into clinical practice is often lost and therefore a device aimed to fill this gap is essential**.

There are many measurement tools available in the current literature, including those which have been designed specifically for research purposes (Wietrzynski, Mazur-Rozycka et al. 2013). **The necessity to design a measurement tool to enable accurate measurement highlights an area of concern requiring development in the literature**. Isokinetic devices provide accurate measurement for research purposes on healthy mobile participants, however a pathology limiting mobility or the ability to position within a device limit use in a clinical population (O'Sullivan, Sainsbury et al. 2009, McGirr, Kennedy et al. 2014).

Strength is clinically measured by the Medical Research Council (MRC) ordinal scale, as this is both time and cost effective in a clinical setting. The subjectivity required when implementing this scale questions the validity of its clinical use; however studies disprove this finding and indicate a high level of validity (Paternostro-Sluga, Grim-Stieger et al. 2008). The MRC manual muscle test has been proven to be a reliable measurement tool in critically ill patients, once examiners have received standardised training in its assessment (Ciesla, Dinglas et al. 2011). However categorising patients exhibiting strength towards the upper spectrum of grades requires subjectivity, which is notoriously difficult to standardise in both young and old individuals. Manual methods of assessing muscle strength have been justified by the comparability with clinical practice, although highlight the method as a limitation (Pradon, Roche et al. 2013).

Hand held dynamometry is considered a useful alternative to manual testing. It is often utilised in a clinical setting due to ease of use, adaptability to patient need and a strong level of associated reliability (McGirr, Kennedy et al. 2014). It is also convenient to use in clinical settings, as compared to the larger isokinetic devices which are forced to reside in a single location. Hand held dynamometry has recently been shown to demonstrate high reliability and validity in the shoulder (Johansson, Cools et al. 2014) and the hip (Thorborg, Petersen et al. 2010). However the minimal

detectable change required to induce statistically significant findings is often higher than a result considered to be clinically relevant in a pathological population (Bohannon, Andrews et al. 2013). Examiner strength required to counter-balance force generated by the muscle has often been highlighted as a limitation, especially in the powerful lower limb muscles in healthy individuals. Intra examiner strength and testing position has been shown to have a detrimental effect on measurement with hand held dynamometry, indicating difficulty in comparing strength measured by differing examiners (Krause, Neuger et al. 2014, Stark, Walker et al. 2011). Co-treating patients is common in clinical practice and thus compromises the ability to monitor outcomes in rehabilitation. **When inferring the effect of moments for precise measurement of muscle force capacity, no commonly applied method of measurement has been identified in the current literature**. This makes accurate measurement of scientific research difficult and the subtle but clinically relevant effects of strength intervention difficult to reliably relate to physiotherapeutic practice.

2.5.2 Measurement of hypertrophy

Hypertrophy of skeletal muscle has been demonstrated to correlate with an increase in muscle size (Lieber 2009). More advanced measurement methods have been explored to measure this relationship, such as magnetic resonance imaging (MRI), computerised tomography (CT) and ultrasound imaging (Masuda, Kikuhara et al. 2003). Despite detailed analysis with accurate results, MRI and CT imaging are not extensively used within clinical settings due to the high associated cost and impracticality of repeat measurements (Bemben 2002, Kwah, Pinto et al. 2013).

Diagnostic ultrasound can be used to monitor changes in muscle architectural properties. Muscle architecture relates to the direction of muscle fibres in relation to the axis of strength generation, which has been shown to demonstrate an insight to contractile properties (Kawakami 2005). Physiological cross sectional area of a muscle relates to the cross sectional area perpendicular to its

muscle fibres and results in more muscle fibres being stored in parallel, having a direct impact on force (Lieber 2009). Muscle fibres can be identified on ultrasound imaging, with the angle at which they insert into the muscle aponeurosis (pennation angle) indicative of their strength producing ability (Strasser, Draskovits et al. 2013). Pennation angle has been shown to increase in line with strength advances (Aagaard, Andersen et al. 2001); however some authors dispute this (O'Sullivan, Sainsbury et al. 2009). Measurement can pose some limitations, with probe compression on the skin surface distorting the interplay of tissue underneath (Reeves, Maganaris et al. 2004). It has also been postulated that measurement is easier and more reliable in individuals with less adipose tissue, due to the reduced image quality at increased tissue depth (Seymour, Ward et al. 2009). Limitations are avoided with complete and thorough training, with ultrasound imaging as a muscle strength measurement tool producing a high level of reliability (E Lima, Kelly M. M. et al. 2012).

2.6 Stroke

2.6.1 Background to Stroke

Muscle weakness can be heightened by pathology in older age. The effects of sarcopenia and frailty will present more quickly with bed rest, which is inevitable in pathology such as stroke. Other contributors to frailty also act to advance these processes in this population, such as a compromised nutritional status (Scherbakov, Doehner 2011).

Stroke is a common neurological disorder affecting both middle aged and elderly individuals (Towfighi, Zheng et al. 2010, Ferri, Schoenborn et al. 2011). Over 5 million people worldwide are left with a disability as a consequence of stroke each year (Sabut, Sikdar et al. 2011). Physiotherapists aim to maximise function, however neurological impairment often precludes volitional activation in the acute phase after injury (Ferri, Schoenborn et al. 2011). An understanding of the pathology will ascertain how an intervention in this thesis could intervene in rehabilitation. A stroke is traditionally

defined as an acute cerebral injury which results from vascular origin and results in neurological deficit. However the requirement of an updated definition has been acknowledged in line with advances in clinical practice and research (Sacco, Kasner et al. 2013). A stroke most commonly occurs from ischaemic causes, resulting in a blood clot occluding the passage of blood to the brain. Hypertension can often result in weakening of the blood vessels causing lipohyalinosis and bleeding into the surrounding tissue (Haemorrhagic stroke). The area to which the blood supply has been reduced or occluded is otherwise known as the penumbra. This area may have a blood supply, however does not reach the concentration required to permit electrical activity in order for the nervous system to fire (Obrenovitch 1995). Stroke is often characterised by hemiparesis (Dragert, Zehr 2013) which is presented as a loss of function on one half of the body and occurs in 88% of stroke survivors (Ambrosini, Ferrante et al. 2011). This consequently renders stroke survivors participants of an intense rehabilitation programme. This is often difficult to partake in due to the advancing effects of age related muscle wasting diseases and a profound lack of motivation whilst coming to terms with the psychological effects of the stroke (Watkins, Wathan et al. 2011). Research suggests that motivational interviewing is effective in increasing quality of life outcomes; however this needs to be further linked to rehabilitation to assess the effect on independence.

2.6.2 Incidence of Stroke

Incidence of stroke has a strong relation to lifestyle and recent years have seen a rise in stroke incidence in Eastern Europe and Central Asia due to the unhealthy lifestyle associated with industrialisation (Kinlay 2011). The rate of stroke occurrence has reduced in the western world, which has been attributed to improved lifestyle choices (nutrition and exercise) and advanced public education, resulting in quick identification of symptoms (Jauch, Saver et al. 2013). Severity of stroke has also been shown to reduce, partly due to thrombolytic therapy administered acutely after the insult (Kinlay 2011). Thrombolysis with alteplase has been shown to break down blood clots within

the circulation, by having a direct effect on the tissue plasminogen activator (Ahmed, Kellert et al. 2013). Although this has had an effect on reducing severity of symptoms, stroke is still considered the fourth leading cause of death in the United States (Jauch, Saver et al. 2013). Stroke continues to present a problem to physiotherapists and methods to improve rehabilitation are continually being sought.

2.6.3 Recovery after stroke

A vast majority of stroke patients are left with disability post stroke. Research remains vital to establish novel therapies aimed at improving function in stroke patients, especially considering the ageing population (Dimyan, Cohen 2011). Neuroplasticity is concerned with the ability of the brain to re-establish connections in order to achieve recovery of motor function. It has been proposed that the majority of functional recovery occurs within the first four months of a stroke (Dobkin 2005), however some research indicates that the first four weeks demonstrates optimal plasticity of the brain and thus exercise should be concentrated within this time period (Verheyden, Vereeck et al. 2008). Other articles indicate that motor recovery reaches a plateau after six months (LeBlanc, Paquin et al. 2013). This implies that rehabilitation should be intensive and progressive in the acute phase post infarct. However it is also apparent that there is a wide amount of variability within motor recovery. Although repetitive practice is essential, research suggests that neuroplastic changes can be limited if exercises are not progressive (Bowden, Woodbury et al. 2013). Neuroplasticity is a result of rehabilitation and spontaneous recovery, which has been demonstrated to occur in varying degrees. Many factors such as age, lifestyle and motivation will influence this time scale. Recovery of brain function in neurological disease has been correlated with that of normal learning and thus neuroplasticity or the restoration of functional process post stroke can be attributed to a process of re-learning. This would indicate that recovery can occur over prolonged periods of time, which has been demonstrated in chronic patients (Bowden, Woodbury et al. 2013).

The mechanism of recovery post stroke is not fully understood. Initial advances in recovery have been associated with absorption of oedema in an attempt to create homeostasis within the body. Thereafter neuroplasticity is thought to occur, resulting in modification and adaptation in neural connections (Carmichael 2003). Neurones within the motor cortex adjacent to an infarct have been shown to demonstrate an increased firing frequency that lasts for several months after initial injury. This suggests that the role of damaged neurones is absorbed by surrounding intact neurones. The mechanism associated with a change in firing frequency has been associated with an increased concentration of glutamate and gamma-aminobutyric acid (GABA); neurotransmitters primarily concerned learning and inhibition respectively in the central nervous system. Axons of motor neurones have been suggested to formulate new connections through a process of sprouting that is thought to arise from an increase in growth cone markers post infarct (Caroni 2001). Growth cone markers are suggested to develop into synapse markers, highlighting the plastic change occurring in the cortex. Development of new connections within the brain will assist the body with recovery of motor function, depending on their location. The development of new neural connections has been shown to occur after a stroke (Thomas Carmichael, Wei et al. 2001), indicating a mechanism for motor relearning post stroke.

2.6.4 Restoring strength in a stroke population

Literature is beginning to highlight how individuals recover from a stroke, but the specific nature of each stroke, depending on the type and severity results in every stroke patient presenting with different symptoms. This makes understanding the level of recovery difficult, but also the response to a physiotherapeutic intervention. Some individuals will respond well to an intervention of rehabilitation and show signs of motor recovery; however the response fluctuates depending on

various factors. This could be due to physiological properties of the neuromuscular system, lifestyle or personality traits (Shaughnessy, Michael et al. 2012). As an individual recovers from a stroke (at varying rates), some muscle function can return. However, this is not a global process and selective muscles tend to compensate and take over the role of muscles that are unable to function. Compensatory behaviours to complete functional tasks are a mechanism often utilised by stroke patients to achieve goals (Carmichael 2003). Rehabilitation post stroke is currently seeking novel therapies to enhance functional outcome. Muscle weakness is a common focus of rehabilitation, which has been shown to be a high predictor of functional ability post stroke. Resistance training has been shown to be a useful intervention to induce hypertrophy in chronic patients (Ouellette, LeBrasseur et al. 2004), however the ability to justify an intensive resistance training programme in an acute setting (from 2 days) remains elusive and dependant on individual presentation.

Muscle activation is a key concept required in the initial stages of rehabilitation, which demands volitional control of the neuromuscular system and appropriate muscle integrity (Umphred, Lazaro et al. 2013). Acute rehabilitation seeks to induce neuroplasticity and thus is concerned with neural control mechanisms and muscle activation. Interventions such as mirror therapy have been demonstrated to be a beneficial adjunct to a traditional physiotherapy programme. Mirror therapy induces changes in cortex activity as demonstrated by brain imaging (Michielsen, Smits et al. 2011), which suggests that movement of the non-affected limb can affect the paretic limb (Dragert, Zehr 2013). A lack of proprioceptive input to the paretic limb is thought to be substituted by the mirror illusion, helping to recruit the pre-motor cortex. (Yavuzer, Selles et al. 2008). Constraint induced movement therapy is another treatment modality aimed at improving neural circuitry post stroke. Restriction of the non-paretic limb forces activities to be conducted with the paretic limb, encouraging repetitive activation and enhancement of neural pathways that are damaged post stroke (Grotta, Noser et al. 2004). Research is supportive in its use and indicates that

implementation in chronic patient's results in significant activation around the lesion site on brain imaging (Levy, Nichols et al. 2001).

In recent years rehabilitation is investigating the use of virtual reality and robotic therapy to induce changes in the functional ability post stroke. Research into robotic therapy in stroke rehabilitation was initiated in response to a development in technology, restrictions in resources able to conduct rehabilitation and a push to achieve early discharge from hospital (Lum, Burgar et al. 2002). The ability to apply a robotic assistive device to a severe stroke patient allows improved quality of rehabilitation, ultimately achieving enhanced outcomes. Video gaming has been extensively researched in stroke rehabilitation, which results in an improved motivational drive to perform rehabilitation and a subsequent improvement in function (Saposnik, Teasell et al. 2010).

Robotic and virtual devices tend to focus on upper limb rehabilitation (Saposnik, Mamdani et al. 2010), which falls outside the scope of this thesis. Devices that assist gait help to rehabilitate the 50% of stroke survivors that are unable to walk. Body weight support has been used to reduce the amount of physical effort required whilst rehabilitating walking ability, whilst inflicting changes in motor recovery (Barbeau, Visintin 2003). Partial body weight supported treadmill training (PBWSTT) has been extensively proposed to fulfil a gap in gait re-education, which is identified as a primary goal in many patients rehabilitation. PBWSTT is thought to activate supraspinal gait pattern generators resulting in improved ability to walk (Hesse 2008). However some limitations prevent this modality from wide use in the clinical community. The requirement for multiple therapists to assist and the high cost implication results in impracticality for use in an acute health care setting (LeBlanc, Paquin et al. 2013). **Despite the emerging supporting evidence, rehabilitation settings continue to strive for a low cost, low resource but high impact treatment modality to enhance functional independence post stroke.**

2.7 Neuromuscular electrical stimulation (NMES)

Neuromuscular electrical stimulation (NMES) delivers small electrical impulses to a nerve in order to initiate propagation of action potentials (Baker, Wederich et al. 2000). It activates intramuscular nerve bundles in superficial muscles to cause muscle contraction (Maffiuletti 2010). The electrical current is required to meet the threshold of the unmyelinated muscular nerve branches which results in propagation along the motor axon. Muscle contraction is initiated once the action potential has resulted in cross bridge formation (Baker, Wederich et al. 2000). Surface electrodes are placed directly over the skin of the muscle to be activated, creating an electrical field between the anode and cathode (Gobbo, Gaffurini et al. 2011). Positioning of electrodes is important to achieve the required contraction or movement; re-positioning of electrodes can be conducted to alter associated movement patterns, or participant comfort during application (Gobbo, Gaffurini et al. 2011).

2.7.1 Stimulation parameters

Application of NMES has been conducted with various stimulation parameters making comparison between studies difficult (Glinsky, Harvey et al. 2007, Schuhfried, Crevenna et al. 2012, Brocherie, Babault et al. 2005).

Setting of stimulation parameters is paramount to achieving the desired muscular response with NMES. The frequency of stimulation refers to the number of pulses delivered per second and has been shown to influence muscle outcome with NMES (Miyamoto, Fukutani et al. 2012). NMES is often applied with low stimulation frequencies (Peckham, Knutson 2005), however frequencies less than 15 Hz have been shown to initiate a twitch response and are not recommended in achieving

advances in muscular output (Peckham, Knutson 2005). High frequency stimulation has been recommended in the literature to maximise motor output of the muscle (Collins, Burke et al. 2001). The most common frequencies utilised in the literature appear to be 20-50 Hz (Baldwin, Klakowicz et al. 2006). **The incomparable parameter base has been identified as a problem in systematic reviews**, (Glinsky, Harvey et al. 2007), making the effect of treatment difficult to conclude and transfer to a clinical setting. Frequency is strongly associated with impacting the effects of fatigue associated with NMES application (Maffiuletti, Vivodtzev et al. 2014, Schuhfried, Crevenna et al. 2012). As a result a high range of frequencies are used in clinical applications. This is often linked to the apparent non-selective recruitment of nerves associated with NMES - please see below for full review (Bickel, Gregory et al. 2011). New paradigms are being explored to minimise discomfort associated with high frequency application, to improve tolerate and compliance in a clinical setting (Maffiuletti, Vivodtzev et al. 2014). Higher frequencies have also been recommended to maximise the volley sent to supra-spinal centres (Maffiuletti, Minetto et al. 2011, Mang, Lagerquist et al. 2010). The associated increase in the activation of motor neurones in the corticospinal pool results in enhanced plasticity of function; hence higher stimulation intensities have been recommended for neurological rehabilitation. **Despite this recommendation, the guidance in terms of frequency setting remains bleak** (Schuhfried, Crevenna et al. 2012). NMES is often administered with a gradual rise of the frequency and intensity to improve comfort. This helps patients tolerate higher frequencies to achieve desired outcomes. This is achieved by alterting the ramp times (increasing them), which is more commonly associated with neurological rehabilitation, and are often utilised in performance settings (Doucet, Lam et al. 2012). Ramp times are however utilised within the given stimulation period, which reduces the amount of time the muscle receives full intensity stimulation. **The effect of frequency on stimulation outcome requires deeper investigation, and shall be a focus within this thesis.**

The pulse width and stimulation intensity used to administer NMES are inversely related (Baker, Wederich et al. 2000) and care must be taken to ensure participant comfort during application. Pulse width relates to the time span of a single pulse, with longer pulses indicative of performance enhancement due to larger recruitment of muscle fascicles (Doucet, Lam et al. 2012). Longer pulses have also been shown to penetrate deeper into sub-cutaneous tissue, which may be imperative to fulfil specific treatment aims. A shorter pulse width requires a larger stimulation intensity (amplitude) to produce the same muscular response. Treatment response and comfort are often influenced by altering stimulation intensity in clinical practice; however pulse width adjustment has been shown to improve both comfort and maximal muscle recruitment. This suggests that NMES should be administered with the highest available pulse width and maximal tolerated stimulation intensity (Brull, Silverman 1995). **The effects of pulse width appear consistent and reliable in the literature. The linear relationship indicates that the highest available pulse width should be utilised throughout this thesis.**

2.7.2 NMES and muscle fatigue

Fatigue associated with NMES will be defined as the reduction of muscle force beyond that what is desired to produce hypertrophy. The effects of fatigue as a consequence of stimulation with NMES have been reported to deter clinicians from regular use in clinical practice (Maffiuletti, Minetto et al. 2011). Fatigue has been associated with inconsistent and inappropriate stimulation parameters (Schuhfried, Crevenna et al. 2012) and the effects of altered recruitment patterns (Baker, Wederich et al. 2000). NMES is proposed to activate larger diameter axons before small diameter axons, a pattern which is opposite to that of volitional muscle contraction as documented by the Henneman size principle. The larger axons have a lower resistance to current and are able to conduct impulses in a shorter time scale in comparison to their smaller counterparts (Gregory, Bickel 2005). The resultant impact is that NMES activates fast fatigable muscle fibres, in a synchronous fashion (Baker,

Wederich et al. 2000). However, this evidence is not conclusive with data suggesting that NMES activates muscles in a random pattern of recruitment (Kim, Bangsbo et al. 1995). The method of stimulation (direct or cutaneous) results in differing physiological responses of the muscle fibre. This along with differences noted with regard to adherence of electrode to the skin and subcutaneous adipose tissue makes understanding of recruitment patterns challenging. **Non-selective recruitment patterns are considered advantageous to NMES application, based on the ability to produce supra-maximal muscle contraction.** Despite current research little is understood as to what reduction of muscle force is considered beneficial for a hypertrophic stimulus. It has been indicated that a decline in muscle force output is required to initiate hypertrophy in traditional resistance training (Mitchell, Churchward-Venne et al. 2012)**. The magnitude of this decline has not been indicated, hence there is confusion as to what is 'positive fatigue' and what is 'negative fatigue' when applying NMES clinically. This is a concept that will be discussed further within this thesis.**

2.7.3 NMES and muscle adaptation

Literature has been investigating how NMES influences muscle tissue. NMES has been postulated to induce plasticity within muscle, resulting in a carry-over effect of treatment aims (Chipchase, Schabrun et al. 2011). This has been demonstrated in healthy and pathological populations in the form of peripheral adaptation in response to strength training (Gondin, Guette et al. 2005). The mechanism of how this occurs has been part of considerable debate, which is heightened by the varying outcomes emitted from treatment with NMES (Trimble, Enoka 1991).

As well as the peripheral effect on muscle properties, NMES has been documented to induce a central, supraspinal effect, which is thought to contribute to the observed carry-over (Mang, Lagerquist et al. 2010). NMES induces a strength response in a quicker time scale than would be associated with muscle hypertrophy; indicating a change in the neural response and thus further

evidence of a central component to training (Trimble, Enoka 1991). **The considerable variation in reported treatment effect, over a varying amount of time and with differing applications makes a clear understanding of the mechanisms involved difficult** (Gondin, Brocca et al. 2011). NMES produces both an orthodromic and antidromic response from the motor nerve, which results in repeated stimulation of the anterior horn cell at segmental spinal level (Rushton 2003). This unique firing mechanism is thought to contribute to plasticity caused via NMES, which has been witnessed after neurological insult. The strengthened synapses associated with both forms of activation are thought to encourage long term potentiation, a form of long term memory. An upper motor neurone insult, such as a stroke damages the pyramidal tract (Thomalla, Glauche et al. 2004), effectively reducing input to the anterior horn cell which results in a reduction of function with little recovery. NMES could therefore be a treatment modality to influence this mechanism of deterioration (Rushton 2003). The importance of volitional input associated with this finding has been noted, indicating the use of NMES as an adjunct to a physiotherapy programme.

NMES is able to induce an afferent volley to the motor cortex, resulting in increased excitability of the corticospinal tract which persists for a few days post treatment. The corticospinal tract is the communication of the motor cortex with the spinal cord to produce movement and thus it's function is paramount in rehabilitation after neurological injury (Gondin, Brocca et al. 2011). The increase in excitability has been documented in response to changes in neuroelectrical signals known as motor evoked potentials. The inhibitory centres in the corticospinal tract have been shown to decline as a result of NMES; evidence of neurotransmitter involvement is emerging (GABA), although their role is not fully understood to date (Mang, Bergquist et al. 2012). This indicates that inhibitory action is activated as a result of NMES to compensate for the reduced excitatory response after neurological insult, resulting in cortical plasticity. The cortical effect of NMES is thought to be related to the intensity of the administered stimulus, which is required to meet the threshold for a strong muscle contraction. This relationship appears significant for improvements in muscle function to be

produced (Chipchase, Schabrun et al. 2011), highlighting the importance of parameter setting. **The effect of frequency setting during application therefore requires further investigation in order for long-term effects of NMES to be addressed.**

2.7.4 Clinical uses of NMES

There is a broad range of literature investigating the use of NMES in physiotherapeutic settings. There are both studies which have advocated the use of NMES in neurological rehabilitation (Yan, Hui-Chan et al. 2005, Ambrosini, Ferrante et al. 2011) and who have achieved varying results and proceed to recommend findings with caution (Elboim-Gabyzon, Rozen et al. 2013). NMES has also been under scrutiny in the field of orthopaedic rehabilitation (Palmieri-Smith, Thomas et al. 2010, Stevens-Lapsley, Balter et al. 2012) and athletic performance (Maffiuletti, Cometti et al. 2000, Babault, Cometti et al. 2007). Studies are also emerging in specialised groups assessing NMES with clinical treatments such as Botulinum Toxin (Galen, Wiggins et al. 2012, Wilkenfeld 2013). Despite this literature, there is little guidance on application (Schuhfried, Crevenna et al. 2012) or complete understanding of the mechanism associated with training effects (Gondin, Guette et al. 2005, Chipchase, Schabrun et al. 2011).

Incomplete understanding results in multiple proof of concept studies, which do not employ high quality research designs. This causes limitation in the ability to drive basic science into clinical practice (Chipchase, Schabrun et al. 2011). Proof of concept trials also tend to investigate different pathologies or populations, resulting in a lack of understanding of underlying mechanisms or treatment effects. Studies have employed a small sample to test the effects of NMES, resulting in a lack of high quality randomised trials. Sample size is frequently less than 20 participants (Brocherie, Babault et al. 2005, Piva, Goodnite et al. 2007), which aim to demonstrate effects of treatment in specific populations. Case control studies have been conducted (Deley, Babault 2014) as preliminary

reports in an attempt to further the NMES science base. Recent studies are attempting to overcome this method flaw and recruit a higher number of participants; 50 participants (Elboim-Gabyzon, Rozen et al. 2013), 41 participants (Bruce-Brand, Walls et al. 2012), however sample size remains limited. Limited knowledge of parameter setting in the application of NMES as detailed above in section 2.7.1 also heightens the inability to perform high quality studies.

This thesis will focus on the application of NMES with the primary aim to induce advances in muscle strength in the lower limb; corresponding with the aims outlined in the introduction (Chapter one). Application of NMES in the following settings will be considered: orthopaedic rehabilitation, athletic performance and stroke rehabilitation. The following search terms were used between the periods of 1990 and 2015:

- Neuromuscular electrical stimulation [OR] Electrical Stimulation [OR] Functional Electrical Stimulation
- Resistance training [OR] strength training [OR] muscle strength
- Elderly [OR] Frail [OR] old
- Orthopaedic [OR] Rehabilitation [OR] Performance [OR] Stroke [OR] Cerebrovascular Accident

AMED, Medline, PsycINFO, SPORT DISCUS, AgeLine, CINAHL and Academic Search Complete were search via EBSCO host. Articles with full text access were screened from title and abstract and were included for review if primary aims and outcomes measures met strength training criteria. Reference lists of included studies were also screened for additional articles.

2.7.4.1 NMES for use in orthopaedic conditions affecting the frail elderly

Frail elderly individuals are often unable to contract a muscle with sufficient intensity to cause hypertrophy (Evans 1999). This can preclude them from taking part in a rehabilitation protocol. **A treatment modality aimed at restoring this ability would allow patients to begin rehabilitation quicker, and therefore reduce the impact of sarcopenia** (Park, Kim et al. 2012).

NMES has been explored to improve muscle strength in participants who demonstrate difficulty in volitional contraction (Bax, Staes et al. 2005). Muscle weakness is considered a major problem in patients after total knee arthroplasty, affecting over 60% of patients one month post-surgery (Mizner, Petterson et al. 2005). Voluntary activation reduces after surgical procedures; swelling and pain are thought to alter afferent input from the joint, resulting in a compromised efferent signal to the quadriceps which limits their ability to contract. Recovery of voluntary activation via NMES is possible in patients with osteoarthritis (Elboim-Gabyzon, Rozen et al. 2013), a population which is at high risk of knee arthroplasty. Effects on strength were not documented after receiving 12 treatment sessions with NMES (75 Hz, 200 μs). It is postulated that a longer during of treatment is required to induce strength gains. However, a 6-week intervention with NMES (50 Hz, 250 μs) alongside standard rehabilitation acutely after total knee arthroplasty has been shown to prevent loss of skeletal atrophy and improve functional ability (Stevens-Lapsley, Balter et al. 2012). NMES was found to be effective 3.5 weeks into the treatment programme. The reversed motor unit recruitment has been attributed to these gains above traditional rehabilitation. Similar findings have been documented in patients after an 8 week NMES intervention, with additional changes in muscle architecture (Vaz, Baroni et al. 2013) in patients who have undergone hip replacement surgery (Suetta, Aagaard et al. 2004) and who have rheumatoid arthritis (Piva, Goodnite et al. 2007). Lack of muscle adaptation after 4 weeks of NMES training (50 Hz) has been attributed to a time period that was too short to induce muscle adaptation (Palmieri-Smith, Thomas et al. 2010), with a review of

methodology recommended. **Literature concludes that NMES is able to influence voluntary activation of the quadriceps muscle force production post-surgery and has the ability to improve muscle strength** (Lewek, Stevens et al. 2001, Bruce-Brand, Walls et al. 2012). The method of testing muscle strength may be responsible for some of the variation witnessed within this recommendation.

The use of physiotherapy as a means of increasing pre-operative strength is increasing in clinical settings. An 8-week home based NMES programme has been shown to have excellent compliance and result in an earlier restoration of strength post-surgically (Walls, McHugh et al. 2010). Research into NMES as a pre-operative intervention is limited highlighting the need for further research to be conducted in this area. This would allow the patient to increase muscle strength to act as a buffer to post operative muscle atrophy. **Additional research into the effects of NMES on an orthopaedic population is required to ascertain optimal time scales for application.**

2.7.4.2 NMES for improving athletic performance

NMES is beginning to emerge as an adjunct to volitional resistance training to optimise performance in athletes. This allows the muscle to contract beyond volitional capabilities, hence train at a greater intensity (Regina Dias Da Silva, Neyroud et al. 2015).

Much of the literature surrounding NMES in athletic populations focuses on outcome measures to improve performance; jump height (Martinez-Lopez, Benito-Martinez et al. 2012), sprint time (Brocherie, Babault et al. 2005) and squat jump (Maffiuletti, Cometti et al. 2000). The goal of NMES in a performance setting is to elicit a supra-maximal muscle contraction to improve output (Seyri, Maffiuletti 2011). Research in athletic populations has focused on the quadriceps due to its

important role in functional movement and superficiality for ease of stimulation (Brocherie, Babault et al. 2005). A wide variety of strength gains have been documented with the use of NMES, ranging from 0-44% (Maffiuletti, Cometti et al. 2000). **The inability to justify parameter selection and variability of treatment duration has been associated with this wide range of treatment effects**.

NMES administered over a 3-week period (85 Hz, 200 μs) is capable of improving maximal strength in corporation with ice-hockey training. The short duration of treatment is likely to induce neural adaptation in the muscle (Maffiuletti, Pensini et al. 2002), with the assumption that fast twitch muscle fibres have been preferentially recruited based on the eccentric gains. Fast twitch muscle fibres have also been shown to have a greater hypertrophic potential (Brandenburg, Docherty 2002). Interestingly isometric application of NMES is able to translate into dynamic strength when paired with appropriate volitional training (Maffiuletti, Pensini et al. 2002, Seyri, Maffiuletti 2011, Brocherie, Babault et al. 2005, Babault, Cometti et al. 2007). Dynamic strength refers to the ability to produce repeatable, functional muscle contractions (Lieber 2009), however the ability of NMES to demonstrate advances in dynamic strength without associated volitional training remains to be concluded (Gondin, Cozzone et al. 2011). **This assumes that isometric NMES can be administered in conjunction with a physiotherapy protocol in a pathological population to induce functional improvements**. Some argue that explosive movements with a horizontally moving centre of mass use the muscle in quasi-isometric conditions. That is, the muscle fibres contract in an isometric fashion, with the explosive nature coming from the recoil of stored energy in the tendon (Spurway 2007). Application and training of the muscle via isometric application of NMES could therefore be an attribute to tendon properties in a functional setting, however further research into this theory is required.

Over training syndrome can be a condition many athletes are at risk of contracting, especially sports which require high intensity training loads. NMES can be used as a means of developing muscle strength during rest periods as it allows the remaining body to rest and is less time consuming than conventional resistance training (Malone, Coughlan et al. 2012). Research into this process is required to continue and could provide a useful means of maximising performance training in a short period of time.

Maffiuletti has suggested that training with NMES in an athletic population should be followed by a short period of sport specific training (Maffiuletti, Pensini et al. 2002). NMES continually stimulates the nerve pathways used to produce a movement and continued stimulation heightens the sensitivity of these pathways, allowing for better 'tuning of control'. This repetitive stimulation falls under the reflex based neurofacilitation approach to motor relearning (Shumway-Cook, Woollacott 2007). This theory suggests that normal movement occurs from higher cortical centres initiating a chain of reflexes that allows the lower centres within the nervous system to produce movement. If these higher cortical centres are damaged then abnormal movement patterns occur. This research group states that restoration of function can be returned if abnormal movement is inhibited. This could occur due to the higher cortical area being restored or a repetitive stimulus overriding the abnormal input, leading to change in function. The repetitive nature and stimulation of the nervous system during NMES mimics a normal movement pattern, which is assumed to transfer to functional tasks.

2.7.4.3 NMES for strength training in a stroke population

New technologies are constantly emerging to maximise functional independence in patients who have suffered stroke (Lum, Burgar et al. 2002). Many technologies are impractical to use in an acute environment, as a result of high cost and large specialised pieces of equipment. NMES has the

potential to be used in an acute hospital setting, as it is inexpensive and simple to apply. Research into its use in developing into upper (Price, Pandyan 2001) and lower limb functional impairments (Liu, Wang et al. 2014), however this review will only focus on the lower limb application to fulfil the aims of this thesis.

Functional improvement is a common goal in neurological rehabilitation (Ambrosini, Ferrante et al. 2011); however the strength generating ability of muscle is paramount to this. Strength of the ankle dorsiflexors has been assessed in 46 acute stroke patients (one week post). Improvements in strength as a result of 3-weeks of NMES (30 Hz, 300 μs) was applied to multiple lower limb muscles (Yan, Hui-Chan et al. 2005). Antidromic activation of motor neurone pools has been postulated as a potential mechanism for improvements in strength and transferability to functional advancements (Rushton 2003). Quadriceps were stimulated however their maximal voluntary contraction was not reported, which is unfortunate given this is a key muscle treated within this thesis.

Contralateral controlled NMES uses the unaffected limb to stimulate movement of the paretic limb with the aim of putting patients at the centre of their rehabilitation and improving central processes of neuroplasticity (Knutson, Harley et al. 2007). Ankle dorsiflexor strength of chronic stroke patients (more than 6 months) was improved with 6-weeks of NMES (35 Hz, 250 μs), with concomitant advances in walking ability. However the case report of three participants limits the reliability of extrapolating these findings. A meta-analysis (Glanz, Klawansky et al. 1996) conducted to ascertain effects of NMES in improving muscle strength identified four studies dating from 1978-1992, with which only three were conducted on the lower limb. Despite this, muscle strength was improved indicating a treatment effect from NMES. The importance of a control group was highlighted to determine the effects of natural recovery.

Functional improvements witnessed through gait are commonly employed as primary outcome measures, for example; gait speed (Robbins, Houghton et al. 2006), gait variables (Lindquist, Prado et al. 2007) and orthotic effects (Chae, Sheffler et al. 2008). Functional muscle activation or strength is a pre-requisite of mobility but appears over looked in NMES research studies. The effect of non-functional isometric strength training on functional recovery in stroke therefore requires investigation.

Many studies adopt a control group instead of a placebo treatment when administering NMES due to the difficulty in reliably blinding participants to allocation arm. This is due to the sensation produced from NMES and the erythemic response of the skin as a consequence (Brunoni, Nitsche et al. 2012). The data from Yan demonstrates how strong a placebo treatment can be compared to a control group (Yan, Hui-Chan et al. 2005). The placebo group received the same stimulation protocol (30 minutes, 5 days a week for 3 weeks) with a doubled treatment time and disconnected NMES circuit. Nine subjects re-gained the ability to walk in the treatment group (N=13) and eight subjects re-gained the ability to walk in the placebo group (N=15). Both groups continued to partake in the standard rehabilitation. This finding could be influenced by a learning effect of subjects due to the perceived thought that they are receiving an 'extra' treatment. Many clinicians underestimate the effect of a placebo treatment in rehabilitation (Margo 1999) and the power it can have in a clinical setting. When investigating pathology such as stroke, natural recovery is occurring as a result of oedema reabsorption as the central nervous system attempts to restore function and re-learn how to use existing and build new motor pathways (Carmichael 2003). The most powerful study design would adopt a Randomised Controlled Design (RCT), to monitor this process and allow this factor to be accounted for.

A systematic review of the use of electrical stimulation to improve muscle strength in neurological conditions has recently been conducted (Glinsky, Harvey et al. 2007). The review analysed eighteen studies with a neurologic basis, eleven of which focused on stroke; the condition in which most research has been conducted. However, of the eleven articles included there was a mix between upper and lower limb application, with varying muscles being treated in the lower limb (gastrocnemius, quadriceps femoris and tibialis anterior). NMES also differed in mode of application, including lower limb articles with EMG-triggered NMES (Gabr, Levine et al. 2005), NMES plus standard rehabilitation (Newsam, Baker 2004, Winchester, Montgomery et al. 1983, Yan, Hui-Chan et al. 2005) and NMES plus a functional task (Peurala, Tarkka et al. 2005). The number of weeks that treatment was delivered varies from three to eight, with frequencies varying from 20-80 Hz and pulse widths from 100-300 μs. Strength of the dorsiflexors was shown to improve by 220% after treatment, a result that displays narrow confidence intervals and a moderate methodological quality (Maher, Sherrington et al. 2003). The effect was less substantial in the quadriceps femoris (Winchester, Montgomery et al. 1983). The review recommends the use of NMES for strength training in stroke in combination with conventional physiotherapy, however highlights methodological flaws as a limitation within this. Limited guidance exists as to the application of NMES and limits justification for a high powered study to be conducted at present. **Although Glinsky has provided a comprehensive review of the literature, the variation described makes it increasingly difficult to draw any conclusions to take forward to future studies.**

The ability to walk is commonly affected by lack of ankle control, which is often a consequence of weak dorsiflexor muscles as a result of stroke (Yavuzer, Geler-kulcu et al. 2006). This can lead to an increased number of falls and a reduction in the amount of physical activity performed ultimately leading to depression (Allan, Rowan et al. 2013). NMES has been shown to increase strength of the anterior tibial muscles responsible for dorsiflexion in 51 sub-acute stroke patients (3 months post). Functional ability was improved as demonstrated via functional questionnaires, indicating a positive

response of NMES above traditional physiotherapy alone (Sabut, Sikdar et al. 2011). However, dorsiflexor strength was measured via manual muscle testing, limiting the elucidation of these results based on questionable accuracy. A systematic review of NMES used as an orthotic measure in the rehabilitation of stroke (8 articles) supports its use in functional improvements with the primary aim of improving walking speed (Kottink, Oostendorp et al. 2004). Research to contradict this finding exists after 3-weeks of training (Yavuzer, Geler-kulcu et al. 2006) and highlights the need to study optimal time lengths of applications to optimise effects. **Although NMES used in a functional pattern such as walking is considered beneficial, isometric application is able to apply a stronger level of stimulation highlighting a positive attribute of this non-functional application** (Knutson, Chae 2010). Walking is often difficult to reliably measure post stroke. Patients often adopt compensatory strategies to achieve tasks (Carmichael 2003). A lack of knee flexion in swing is often a result of quadriceps weakness or spasticity, or an increase in knee flexion can compensate for a drop foot. However compensations tend to be specific to individuals and measurement of various parameters should be employed to ascertain functional improvements (Kerrigan, Karvosky et al. 2001).

Although neuroplastic changes are believed to occur within the first few weeks post stroke, many studies do not apply treatment within such an acute period (Yan, Hui-Chan et al. 2005). The ability to infer on the effects of NMES aiding neuroplasticity are disadvantaged by testing on a chronic population although the concept is still of functional importance. A wide spectrum of time post stroke has been utilised within studies with one particular study including patients who ranged from 2-227 days post stroke (Bogataj, Gros et al. 1995). Length of time post stroke has been shown to have strong implications with regard to neurological recovery (Kwakkel, Wagenaar et al. 1997). Method of application also varies including studies that apply surface electrodes (Yan, Hui-Chan et al. 2005), intramuscular electrodes (Daly, Roenigk et al. 2006) and functional electrical stimulation

(FES) assisted cycling (Ambrosini, Ferrante et al. 2011, Ambrosini, Ferrante et al. 2012, Ferrante, Pedrocchi et al. 2008).

FES assisted cycling has been utilised as a safer alternative to FES applied during gait with the aim of transferring muscular adaptation to improvements in functional mobility. Advances in strength and functional mobility were reported with discussion surrounding how FES cycling can be used as a means of restoring trunk control in the acute phase post stroke. Trunk muscles were not directly stimulated and FES was thought to induce improved motor co-ordination and symmetry between both legs in functional tasks (Ambrosini, Ferrante et al. 2012). Trunk control has been highlighted as a vital early task in rehabilitation with lack of control highlighting increased risk of prolonged rehabilitation (Franchignoni, Tesio et al. 1997). FES assisted cycling appears sufficient to induced functional gains in mobility; however availability of specialised equipment is required to administer this treatment. The need for a portable and cost effective alternative modality to administer NMES is required to commonly see this gain achieved in acute rehabilitation settings.

Odds ratios were calculated from comparing data from three studies assessing the effect of NMES on lower limb muscle strength and walking ability (Ambrosini, Ferrante et al. 2011, Ferrante, Pedrocchi et al. 2008, Yan, Hui-Chan et al. 2005). Odds ratios allow interpretation of the probability of walking post stroke. Analysis of odds ratio allows comparison of current statistics used in physiotherapy literature and analysis dependant on small sample size, as experienced in this field of study (Schechtman 2002). **Combination of methodologies results in an individual being 11.3 times more likely to walk with NMES than without NMES compared to either placebo treatment or standard rehabilitation**. Although sample sizes are small in the aforementioned studies and we have combined studies that utilise various methodologies, this result is a good indicator that NMES is a useful treatment to strength train in stroke rehabilitation. **The ability to walk is often the main**

objective of stroke patients and restoring the ability to walk will increase an individual's quality of life. This suggests that a larger randomised trial should be conducted to investigate the true potential of this treatment.

Emerging evidence suggests that NMES has the ability to induce strength-training properties after neurological insult (Glinsky and Harvey 2007). This is based on a small cohort of studies with relatively small changes in strength displayed. **The ability to transfer this knowledge of strength increase to a clinically functional improvement is also lacking**. Although the current literature into NMES in neurological impairment has some methodological flaws at present, the current understanding has given an insight into the mechanisms involved. **The current research base has also gone some way in beginning to establish proof of concept in this population. Further clinical trials are required to confirm the effects of NMES in Stroke and establish a parameter base that could be used for protocol purposes in the field of rehabilitation**. This thesis will aim to address some of these issues.

2.8 Investigating variability in literature

The current literature surrounding the effects of neuromuscular electrical stimulation (NMES) on muscle function is variable (as highlighted above). This is both within orthopaedic, athletic and neurological populations (Elboim-Gabyzon, Rozen et al. 2013, Gondin, Brocca et al. 2011, Glinsky, Harvey et al. 2007). **At present it is unknown whether this variability is due to methodological differences throughout research or whether different muscles react differently to stimulation.**

2.8.1 Monitoring of blood biomarkers in sports medicine

The effects of blood biomarkers during high intensity exercise have been well documented in the sports literature (Brancaccio, Maffulli et al. 2007). To our understanding knowledge is sparse regarding the physiological effects of NMES on blood biomarkers in either animal or human studies. A study has investigated the use of biomarkers in animals which implies that effects are similar to that of volitional strength training (Pette, Vrbova 1992). However, the low frequency chronic stimulation that is applied throughout this review shows considerable difference in terms of primary aims and application to NMES strength training literature. **A biomarker is a molecule whose concentration changes in response to tissue damage** (Tsimikas, Willerson et al. 2006). This is an important concept to understand as changes in enzymatic activity (biomarkers) will determine the muscles response to treatment with NMES, perhaps highlighting why response is varied.

Blood biomarkers have been monitored in sport for various reasons, the primary reason being able to monitor the behaviour of muscle to different external variables (Brancaccio, Maffulli et al. 2007). Monitoring may aim to investigate different durations of exercise or whether concentric or eccentric activity has a greater impact on muscle function. This allows researchers to understand muscle function and allows exercise and rehabilitation protocols to be administered with scientific justification. Biomarker research also allows us to understand the events of how a muscle hypertrophies and the micro-trauma associated with this hypertrophy under various stresses (Armstrong 1990). Investigation into biomarker changes during application of NMES may give an insight as to the mechanisms associated with muscle adaptation, and why variability occurs.

In the sports literature scientists have been able to establish key enzymes and proteins that react to exercise and contribute to physiological muscle changes. These give an idea about how the muscle makes adaptations to training, and may be able to establish whether a muscle is reacting to NMES in

the same that that it acts to volitional muscle training. The following biomarkers are identified to have an important role in muscle strength training:

- Creatine Kinase
- Lactate Dehydrogenase
- Alkaline phosphatase
- Oxidative stress biomarkers
- Inflammatory biomarkers
- Cardiac biomarkers

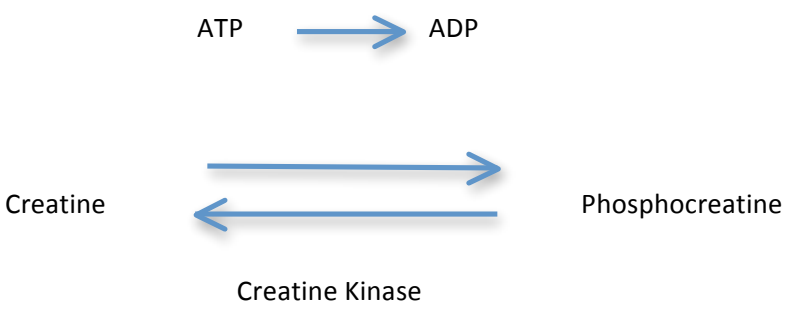
Creatine kinase (CK) is a well researched enzyme that is vital for the muscle to produce energy. Many authors have investigated the use of CK in sports and medical literature (Brancaccio, Limongelli et al. 2006, Clarkson, Kearns et al. 2006); however its effects in an NMES strength training protocol have not been investigated to date. Lactate dehydrogenase (LDH) is an enzyme which catalysis the conversion of pyruvate to lactic acid in anaerobic respiration and is frequently monitored in medical conditions such as cancer (Armstrong, George et al. 2012). The anaerobic effects of its role indicate that LDH is an enzyme that would need to up regulate in response the increase in demand from high intensity exercise. Other biomarkers have been grouped together in the literature as there are many markers which have the same goal. Broader groups (such as oxidative stress, inflammatory and cardiac) allow trends to be identified and highlight the similar role of various molecules. Oxidative stress biomarkers measure the level of free radicals and reactive oxygen species (ROS) in the cell, the levels of which are increased by muscular exercise (Alessio, Hagerman et al. 2000). Many scientists believe that oxidative stress can have a negative influence on performance and work is currently being conducted to investigate this (Radovanovic, Bratic et al. 2009). Inflammatory biomarkers have been investigated during exercise in people with obesity with the aim of linking the level of exercise

to level of cardiovascular disease (Olson, Dengel et al. 2007). In terms of physiotherapeutic management of patients with obesity, this work plays a role in understanding exercise prescription within safe limits. Finally, cardiac biomarkers such as troponin(s) can be monitored in both endurance athletes and pathology to monitor cardiac function with the increase in pressure placed on the cardiovascular system (Scharhag, Herrmann et al. 2005). Just within this short (but not extensive) list of biomarker research it is clear to see that the effect of exercise can be monitored on many systems of the body.

The final clinical study in this thesis will explore the change in concentration of Creatine Kinase to a treatment protocol with NMES. This has been employed in attempt to justify some of the variability portrayed when strength training with NMES. Creatine Kinase was chosen as the biomarker to monitor due to the advanced understanding of its interplay with lifestyle factors (Brancaccio, Maffulli et al. 2007), its acceptance as a marker of muscle work done (Vincent, Vincent 1997) and the readiness of assay preparation methods.

2.8.2 Creatine Kinase

Creatine Kinase (CK) in an enzyme located within skeletal muscle, which is utilised during muscle contraction. **CK acts to catalyse the conversion of creatine into phosphocreatine using Adenosine triphosphate (ATP) as an energy source** (Schlattner, Tokarska-Schlattner et al. 2006). The chemical reaction is shown below:



Phosphocreatine is required by skeletal muscle during contraction for buffering and regeneration of ATP. This is mainly required during high intensity exercise when consumption exceeds synthesis (Gabr, El-Sharkawy et al. 2011). ATP is a vital source of energy during muscle contraction and if not regenerated muscle contraction would cease to continue (Lieber 2009). **CK is therefore a vital enzyme in regulating the process of muscle contraction and aims to keep ATP at a constant level to allow exercise to be fulfilled**. **Elevation of CK indicates that it has played an active role in the breakdown of creatine.**

The phosphocreatine energy system (PCr) breaks down phosphocreatine to add the third phosphate molecule to adenosine diphosphate (ADP) to make adenosine triphosphate (ATP). This gives the muscle a source of energy which does not require any oxygen or produce any by products, such as lactic acid (Lieber 2009). Phosphocreatine is a molecule that is produced from the resting creatine within the muscle. Creatine is a compound made from arginine and glycine which is produced in the liver. Creatine is transported from the cytoplasm of cells to the mitochondria where creatine Kinase (CK) adds a phosphate molecule to it to make phosphocreatine. Phosphocreatine is stored in the mitochondria for when it is required for muscular contraction.

The PCr system is a short lived energy system lasting approximately 10 seconds. It is utilised on high intensity energy demand and produces short bursts of energy before aerobic forms of energy supply are utilised. Exercise produced by the PCr system includes maximal strength efforts, short sprints and some eccentric exercises.

CK functions within the phosphocreatine system (PCr) in regulating energy homeostasis (Greenhaff 2001). The CK/PCr system has been reported to have four main functions:

1. Temporal energy buffer

2. Energy transport system

3. Prevent a rise in intracellular ADP

4. Proton buffering

CK acts as a temporal energy buffer as described above by maintaining supply of ATP to muscle tissue. Short term energy is produced by anaerobic respiration and utilised in muscle contraction before aerobic forms of energy are utilised. The role of CK in energy transport was first described by Bessman and Geiger in 1981 and has had numerous other authors contribute to its understand in more recent years (Wallimann, Tokarska-Schlattner et al. 2011). This role relates to phosphocreatine linking the sites of energy production with those of energy utilisation. This allows smooth transfer of energy and use of the appropriate energy system. Creatine and phosphocreatine can then be considered plausible 'energy carriers' (Greenhaff 2001). The third function describes how the PCr system prevents a build-up of intracellular ADP which would result in accumulation of unwanted molecules in the cell. Lastly, by buffering protons the PCr system prevents unwanted acidification which is vitally important in early energy production before glycogenolysis is activated (Wallimann, Tokarska-Schlattner et al. 2011). The PCr system also releases an inorganic phosphate molecule which is required for glycogenolysis. It is therefore suggested that this release has an indirect effect on further cell functioning.

There are three isoforms of Creatine Kinase (CK) found within muscle and the brain (CK-MM, CK-BB, CK-BM). In human skeletal muscle CK is expressed as 98% of the CK-MM isoform and 2% CK-MB. Cardiac tissue is the only tissue in which the isoform CK-MB exceeds 5% with the exception of muscle diseases and athletes who display higher values. In skeletal muscle a large proportion of CK is located in the myofibrillar M-line of the sarcomere (Baird, Graham et al. 2012). This accounts for 5-10% of the total muscle CK (Brancaccio, Maffulli et al. 2007). This particular review suggests that the M-line therefore has an important structural and enzymatic role in regenerating ATP and providing the myosin with enough energy to work under high intensity exercise.

Normal CK levels vary with gender with males displaying an average of 24-195 units per litre of blood and women 24-170 units per litre of blood (Mougios 2007). Although normal values have been established, there are factors that can influence these resting levels which are dependent on numerous factors. These factors can include age, gender and race. Males have a higher resting serum level of CK compared to females which decline slightly in later years. Females display reductions in resting serum CK during pregnancy; however this increases back to normal during late gestation. After high intensity exercises these gender differences remain unchanged resulting in the interpretation of serum CK becoming more complex. Race also influences resting serum CK with black men having a higher CK concentration than Caucasians; however reviews have debated this considerably (Eliakim, Nemet et al. 1995). It is also important to note that CK is related to body mass and exercise levels, with resting levels remaining elevated in athletes as an adaptation to training (Brancaccio, Maffulli et al. 2007). The variations described above make it vital to ascertain personal and physical activity levels to enable interpretation of how serum CK changes with exercise.

2.8.3 Role of Creatine kinase in muscle contraction

Physical exercise that damages the muscle sarcomere has been shown to produce elevations in CK levels (Baird, Graham et al. 2012). It is suggested that high intensity exercises damages the membrane permeability resulting in the release of enzymes. The level of CK increase is usually lower in trained subjects compared to untrained subjects with trained subjects who partake in regular physical activity displaying continued elevation of CK (Vincent, Vincent 1997). CK activity has been shown to demonstrate the greatest post-exercise increase after prolonged exercise such as marathon running (Kyrolainen, Pullinen et al. 2000), however high intensity especially eccentric exercise has been proven to show high serum CK elevation (Malm, Sjodin et al. 2004). Serum CK elevation can peak up to 8 hours after exercise and has been shown to remain elevated for 24 hours with elevations continuing depending on the amount of subsequent exercise (Baird, Graham et al. 2012). Some studies suggest that activity has been at its highest after 5 minutes of exercise demonstrating a broad range of data present in the literature (Koutedakis, Raafat et al. 1993). This particular article suggests that exercise intensity and duration are the main contributing factor to elevations rather than the individual fitness level.

In clinical practice Creatine Kinase (CK) is tested to help diagnose and identify acute myocardial infarction (MI), severe muscle break down (rhabdomyolysis), muscular dystrophy and acute renal failure (Brancaccio, Maffulli et al. 2007). In these instances CK is a marker that is well understood and easily tested via blood extraction. In research settings CK can be used as a marker to identify when a muscle has been working to assess both the types of muscle contraction on muscle function and also the intensity of exercise on muscle function. **It is this area of CK testing that we plan to manipulate using the well documented sporting literature and means of preparing assay samples to apply this to our specific population and means of exercise.**

Various confounding factors associated with application of NMES have often been postulated in the literature such as reduced impedance of electrodes and an increased sub-cutaneous adipose layer (Maffiuletti, Morelli et al. 2011). **Establishing the physiological impact NMES has at muscular level will determine whether these variables are viable or not**. Although research into the physiological changes during NMES is not established, there is a good understanding of volitional strength training to compare results too. Being able to understand how the muscle responds to NMES will be able to guide its place in clinical practice, determining whether it is an effective treatment to include in rehabilitation protocols or not. **If we can establish whether people will respond to treatment with NMES based on a simple blood test, we may be able to guide treatment with more scientific justification.**

2.9 Conclusion

The frail elderly are a vulnerable population who are at high risk of contracting further co-morbidities. Pathology (such as a stroke) results in a spiralled decline of function which is confounded by a rapid loss of muscle strength witnessed in older age. The continued decline in health status results in a reduced amount of physical activity and renders these individuals increasingly hard to rehabilitate. Resistance training is a method that has been shown to increase muscle strength in elderly individuals and is safe and effective to administer contrary to historic beliefs. The problem faced in this population is that they are often unable to contract their muscles with a sufficient intensity resulting in prolonged rehabilitation and risk of further health decline due to inactivity. This is heightened in neurological pathology such as stroke, with individuals struggling to restore strength whilst attempting to remain in a medically stable position after the stroke.

Emerging evidence suggests that NMES is able to induce strength changing properties in skeletal muscle both in young athletic populations and in patients who have had a stroke. However,

methodological flaws and lack of understanding of the optimal parameter settings makes it difficult to apply this information to a wider population base. There is also uncertainty as to the mechanism of strength gain and why strength gains have been produced without functional, goal-orientated application of NMES. NMES may be a treatment that could be used to prevent unwanted muscle atrophy in the initial stages of a physiotherapy programme. However, NMES has demonstrated variability in the literature which contributes to a lack of understanding around its use. Creatine Kinase has been shown to be a strong indicator of muscle function in sporting and medical conditions. Monitoring of creatine Kinase through a treatment programme with NMES may help to understand its mechanism of function with more certainty.

This thesis aimed to investigate the role of NMES in reversing age-related and pathological muscle atrophy. The main objectives of this thesis are outlined below:

1. To identify a suitable NMES stimulation frequency (maximum possible contraction with minimum discomfort) with the aim of increasing muscle strength. The remaining parameters and treatment dose will be designed based on current strength literature (both NMES and volitional). The tested stimulation protocol will be trialled over a full treatment programme. This will determine if it is a tolerable treatment dose that has potential to increase force-generating capacity.
2. To investigate the use of the NMES protocol on different populations, namely different aged muscle and stroke patients. Associated morphological changes will be investigated (e.g. hypertrophy). These studies will form proof of concept to guide larger clinical trials.
3. To investigate observed variation in treatment response to NMES protocols by monitoring biomarkers.

In order to achieve the aims outlined above, four clinical studies have been conducted:

1. To identify a stimulation frequency with the potential to increase muscle strength over a full training programme with NMES.
2. To test the effects of the NMES strength training protocol on age; the relationship between muscle response and adaptation to NMES on muscle in young and older adults.
3. To test the strength training protocol on a clinical population (stroke) to ascertain the effects on muscle affected by pathology.
4. To test the physiological response of muscle to NMES by monitoring biomarkers during treatment, in the hope of establishing why variation occurs when using NMES.

Chapter 3

Device development: Leg Measurement Device (LMD)

The development of a measurement tool was paramount for achieving the outcome measures of this thesis. A commercial device was not available that allowed adjustment for patient size and have the ability to be used on an acute hospital ward. The ability to measure muscle strength, whilst allowing adaptability of movement at the knee and ankle joints was paramount. The development of this tool would also fill an existing gap in clinical practice. Practitioners are dependent on unreliable and often subjective measures of measuring muscle strength (Bohannon, Andrews et al. 2013, McGirr, Kennedy et al. 2014). This results in inadequate recording of patient progress, potentially limiting the design of an appropriate rehabilitation programme. A device which is easy to set up in an acute environment, and accurate in measurement would enable greater precision when treating patients. The Leg Measurement Device (LMD) was designed and developed in accordance with the goals set within this thesis.

3.1 Leg Measurement Device (LMD) design

Accuracy of measurement required a device that was adjustable to participant size and shape, whilst maintaining comfort throughout testing periods. The following criteria were used as guidance throughout the design process. These criteria were essential for the device to be used in a clinical setting:

- Cross knee and ankle joint with hinges aligned as closely as possible to the anatomical axis.

- Capable of both immobilising the knee and ankle joint in any given available range of motion, and passively moving the joints through their available range.
- Able to resist maximal isometric contraction of the quadriceps femoris (knee) and gastrocnemius (ankle) in any locked position.
- Adjustable to participant size.
- Able to fit either leg (i.e. right or left).
- Suitable for use in sitting or supine.
- Comfortable, easily cleaned and suitable for use in a ward environment.

The design of the device was outside the scope of the author, and thus external help was necessary to produce a device suitable to achieve the thesis aims. A design team was responsible for developing the LMD based on the specification above. Materials were sourced, and clinical engineers constructed the device. The author of this thesis was responsible for ensuring practicality and ease of use in a clinical setting. The LMD design was developed and constructed as illustrated in image 3.1.

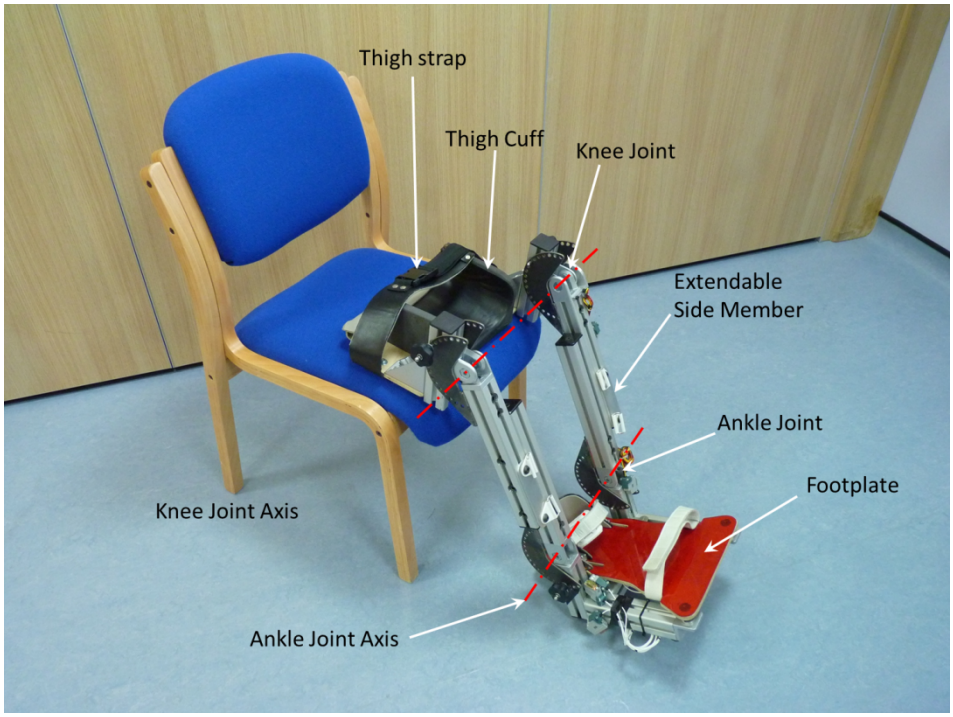


Image 3.1 The LMD

The LMD had to be small, and light weight to transport around a hospital ward. Initially the thigh cuff section was extended so that the patient sat on a padded area during testing; however modifications were made to this during implementation of study 3, which was conducted on an acute stroke ward. Positioning of severe stroke patients into the LMD resulted in difficulty, resulting in staff having to hoist participants into position. The help of extra staff was not always available on a busy hospital ward, hence modifications to the design. The base of the thigh cuff was reduced by approximately 50%, in order for the researcher to slide the LMD under the leg of the participant whilst they were sitting. This was successful at improving the ease of using the measuring device on an acute stroke ward.

Potentiometers were inserted in the knee and ankle joint, which were able to measure joint range of movement. This was adjustable via the bracket on the side of the joint, allowing the joint to be in a fixed or freely movable position. The knee joint was able to be moved between 0° extension and

100° of flexion. The ankle joint was able to be moved through 10° dorsiflexion, to 20° plantarflexion. Both the side members of the main frame and both the knee and ankle joints were adjustable with regard to length via an Allen key. This was designed in accordance with standardised measurements for adult men. The Allen key was able to unlock the side members and joints in order to slide them in relation to each other, to determine the required position. The Allen key was then used again to tighten the lock when aligned with the joint centre of rotation, and prevent the side member or joint from moving. This allowed accurate measurement of joint moments around the knee and ankle joint. In order for this to be accurate the position of the patient in the LMD required some standardisation, and subsequent padding and strapping to maintain this position was employed. Straps were placed across the femur and anterior ankle joint to prevent unwanted movement. Extra padding was required during study 4, to account for flaccid limbs post stroke. Foam blocks and pillows were utilised to standardise position.

Force transducers were positioned in the footplate to record plantarflexion moment, and in a thigh strap that attached onto the side members (Image 3.2) to measure knee extension moment. Three transducers were used in the foot plate, and two in the thigh strap. The thigh strap was appropriately padded with foam to reflect optimum position for tibia contact; added support enabled variations in shank size to be accounted for when testing knee extensor moment.

Image 3.2: Shin strap- two force transducers (left) and straps to attach onto LMD (right)

The force transducers on the footplate and knee strap were calibrated (as described below) to distances that were fixed. The information recorded during testing was collected via a laptop, and moment was calculated via the calculations presented below.

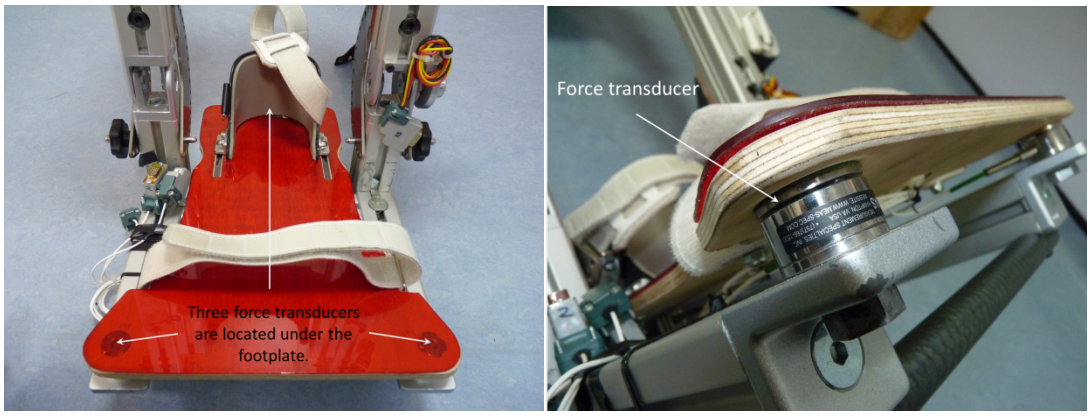


Image 3.3: Three force transducers in footplate

3.2 Foot plate

The foot plate was required to measure plantarflexion moment. A wipe clean material was required to allow barefoot testing of multiple participants. As well as measuring moments, the foot plate was required to adjust to various ranges of movement, and thus required two ankle potentiometers. The potentiometers became the reference point for joint centre positioning within the LMD. The potentiometers were positioned on each side of the ankle joint, which is encompassed by an adjustable cuff. The cuff is adjustable in the sagittal plane to allow accurate positioning of the ankle joint centre (malleoli) with the potentiometers. This allows measurement of ankle range of movement, within the limits of the ankle joint. The foot is held in place by two Velcro straps; one over the forefoot, and one across the anterior ankle joint.

Force measurement was conducted via three force transducers built into the foot plate. One was placed centrally at the back of the plate, with the remaining two in the opposite corners (Image 3.4).

Generated force was calibrated in accordance to the distance away from the central rotation of the joint at three known places. These were the central point between the ankle potentiometers, and at both 100 mm and 200 mm forwards from this point. The device was loaded with 25 kg, in increments of 5 kg via a vertical loading apparatus. The LMD was connected to a software package (Biometrics DataLink system) for data collection. Data was then transferred to a calibration spreadsheet for conversion to newton metres, and subsequent analysis.

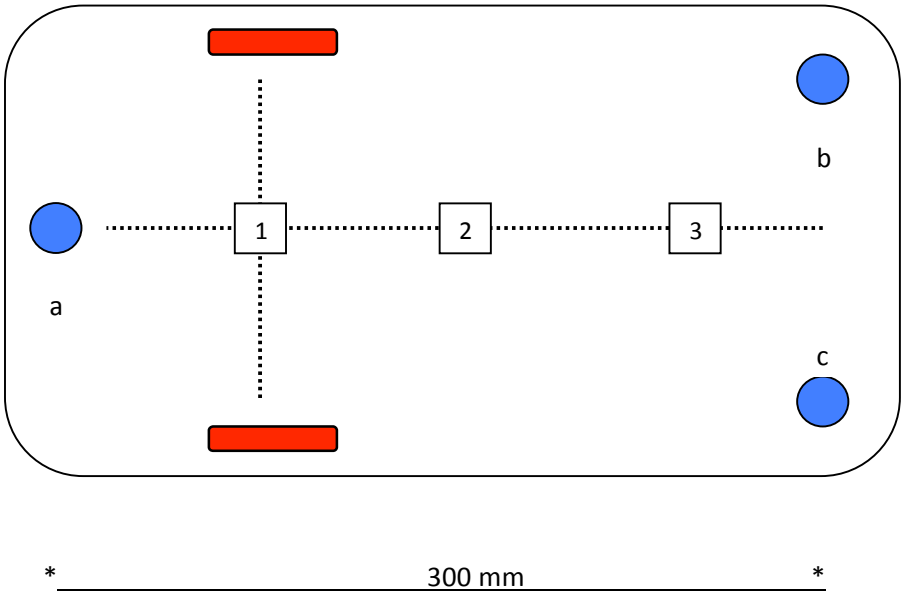


Image 3.4 diagrammatic representation of the foot plate. The blue circles represent the force transducers, and the red rectangles represent the ankle potentiometers (which are lined up with the ankle joint centre and held in place by an ankle cuff). The points labelled 1, 2, and 3 represent the three separate positions for calibration loading.
Distance 1 - a = 90 mm
Distance 1 – 2 = 100 mm
Distance 2 – 3 = 100 mm
Distance c – a = 300 mm

Calculation for working out moment:

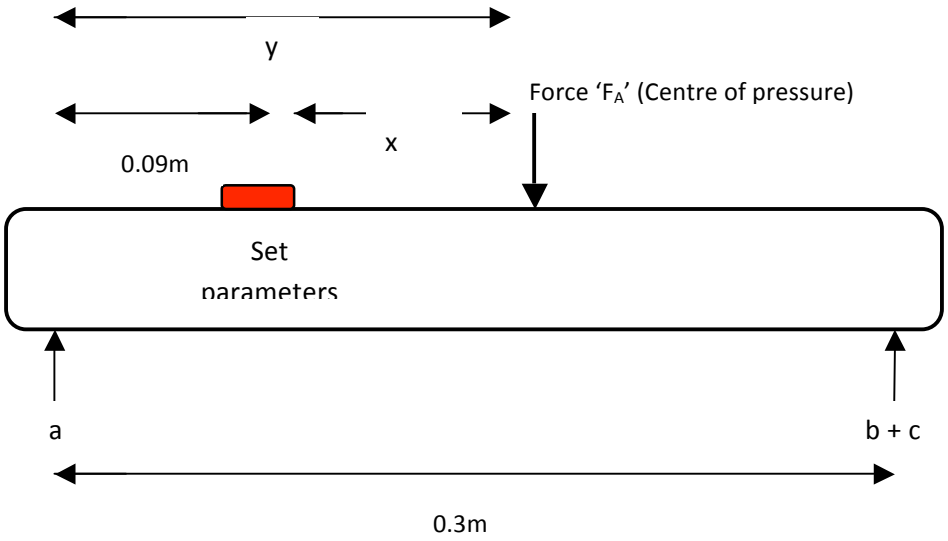


Image 3.5 Diagrammatic representation of foot plate for moment calculation

$$F_A \times y = 0.3(b + c)$$

$$y = \frac{0.3(b + c)}{F_A}$$

$$y = \frac{0.3(b + c)}{(a + b + c)}$$

$$0.09 + x = y$$

$$x = y - 0.09 = \frac{0.3(b + c)}{(a + b + c)} - 0.09$$

Image 3.6 mathematical calculation of foot plate moment

The moment about the ankle is determined by multiplying the force by the distance you are measuring (i.e. F_A multiplied by x). However, 'x' is an unknown value, and requires calculation before moment is determined. It is accepted that turning moments of opposite directions are equal to each other, as illustrated by Newton's third law of motion. This means that the turning moment

from the combined effort of the force and transducer 'a' are equal to the turning moment of transducer 'b' and 'c' (because the foot plate is in equilibrium). We are able to determine 'y' by dividing this equation by the total force (i.e. a, b and c). Therefore we are now able to calculate 'x' as shown above, allowing a final figure for moment to be calculated. This was inputted into a calibration spreadsheet, and the moment values used for subsequent analysis.

3.3 Shin Strap

The shin strap was developed to measure knee extensor moment. Original plans aimed to combine the knee strap onto the side members of the LMD with an opening and lock in mechanism to allow passage of the leg into the device. However variability in participant size excluded this plan at the development stage and the knee strap was developed as a separate entity. Velcro straps over the force transducers enabled secure attachment of the strap to known distances away from the knee joint potentiometer (to which knee joint centre was aligned). The Velcro was tested and proved to be strong enough to withstand the force of maximal quadriceps contraction.

The knee strap consisted of two compression force transducers, positioned 100 mm apart on a cuff, lined with foam to encompass the shank. Precision was required in positioning the knee strap in the centre of the tibia, to ensure extensor torque was accurately measured. Alteration of position was made if required. The first force transducer was positioned 200 mm from the knee joint centre and the second 300 mm.

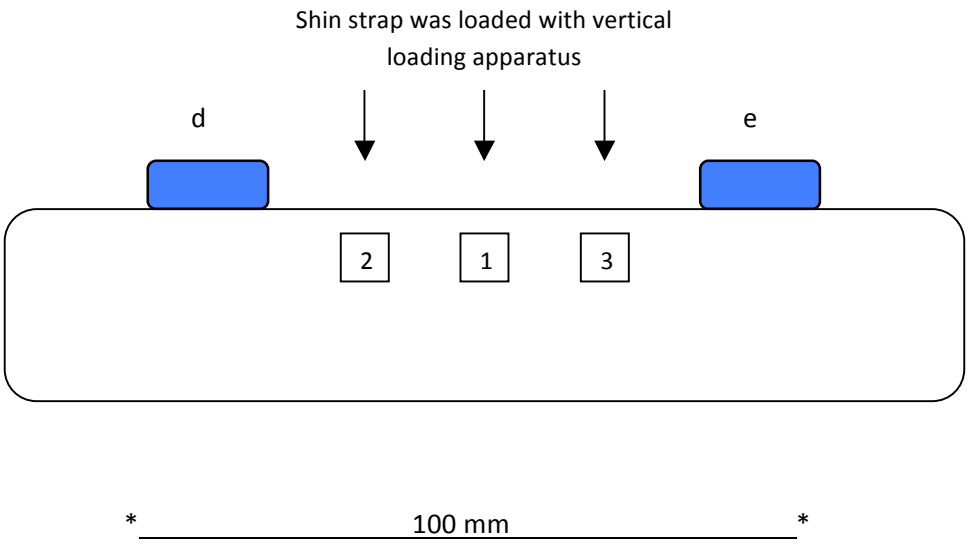


Image 3.7 Diagrammatic representation of the shin strap. ‘d’ and ‘e’ (blue rectangles) represent force transducers. Positions 1, 2 and 3 represent where the shin strap was loaded. Distance from d to e = 100 mm. Distance from d to knee joint centre = 200 mm. Distance from e to knee joint centre = 300 mm. 1 = central point between d and e. 2 = d – 25mm. 3 = e – 25mm.

When taking these measurements into consideration, the force for knee extension can be worked out with the following equation:

Moment = force x distance

Moment = (scaled force of T1 x 0.2) + (scaled force of T2 x 0.3)

Image 3.8 Calculation to determine moment for the shin strap

For calibration purposes the shin strap was mounted on a wooden block, to ensure it was horizontal to the floor. Another wooden block was mounted on top of the force transducers, to accommodate

the difference in transducer heights, and a tubular spirit level used to ensure it was level. The device was loaded with 25 kg (245 N), in 5 kg stages via a vertical loading apparatus (please see Image 3.9).

3.4 Calibration

The recordings were collected via external software (Biometrics DataLink system), and transferred into a calibration spreadsheet for analysis. Data was collected at a sampling rate of 20 Hz, which was used for data collection throughout all studies in this thesis. Data for Force, Moment and Centre of Pressure was calculated. To ensure that the shin strap was being loaded vertically, the whole process was conducted over a force plate, and video vector analysis was monitored in a Gait Laboratory via a Vicon Nexus 1.5 tracking system. The shin strap was tracked via Vicon markers, as shown by the grey spheres in Image 3.10 in order to show its plane. A vector was generated by the pressure on the force plate, as shown by the red arrow, and a superimposed blue arrow has been inserted to indicate true vertical. A graph highlighting the recorded force was produced by the Vicon system, which was comparable to the DataLink system (Image 3.11). The shin strap was loaded at the central point between the two force transducers, and at 25mm either side of this point. The transducers were not calibrated directly over them due to the fact that the shin strap would not remain horizontal under this pressure.

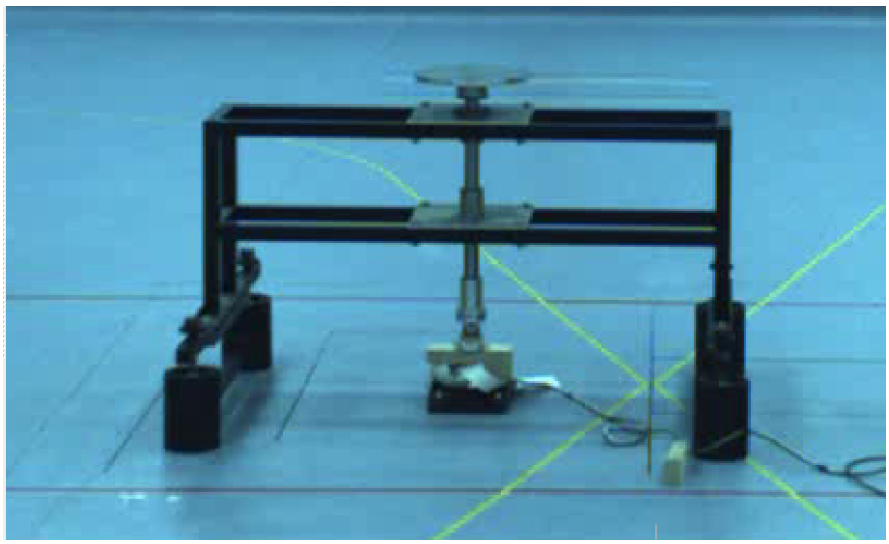


Image 3.9 Loading of shin strap via vertical loading apparatus on a force plate

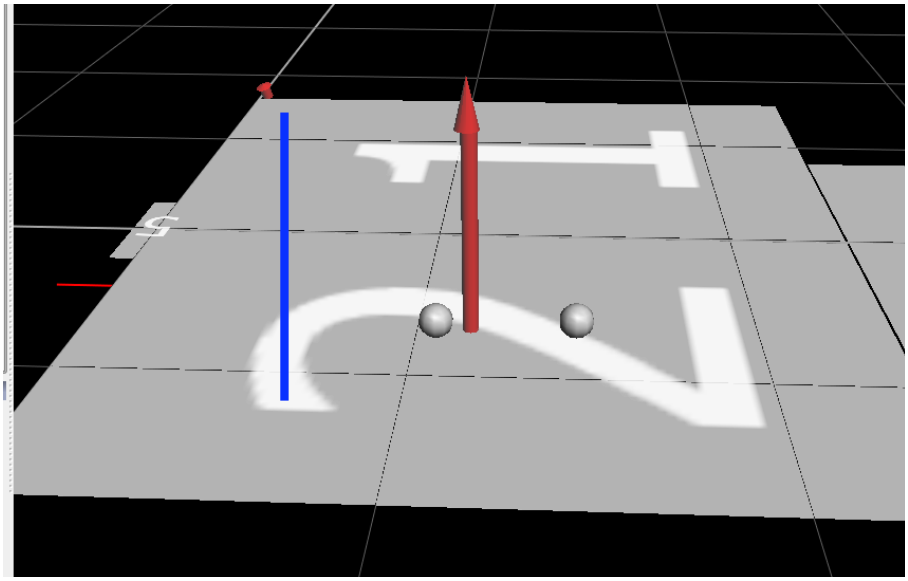


Image 3.10 Vicon Nexus 1.5 tracking system with the shin strap being loaded 25 mm to the left of the central point of the transducers.

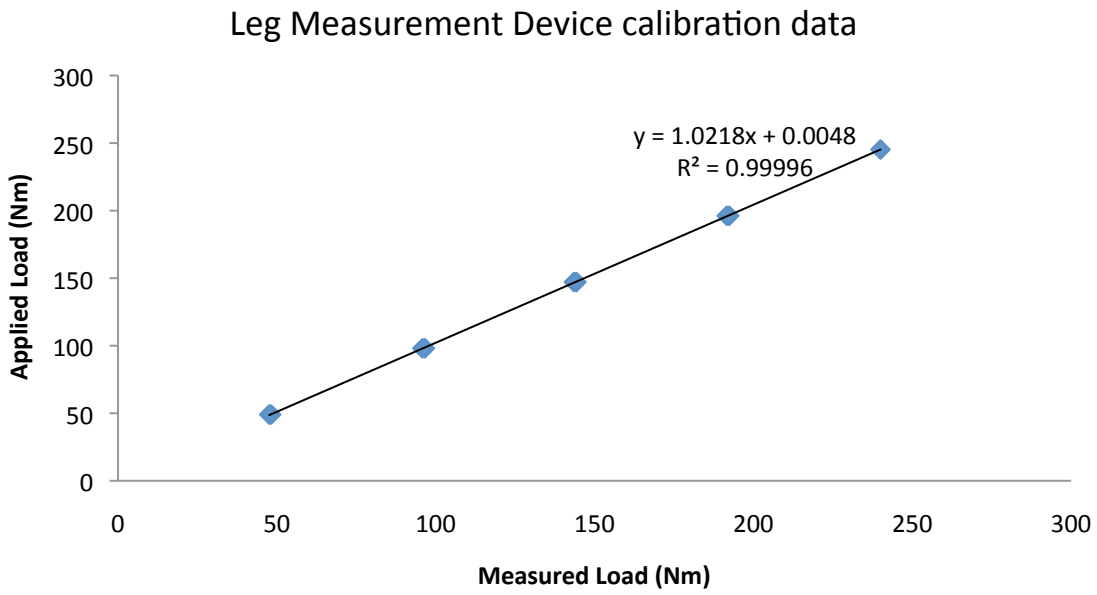


Image 3.11 Calibration force data showing linear correlation.

A calibration spreadsheet was required to convert the raw data into total force, moment, and centre of pressure of all the transducers; a separate spreadsheet was produced for the foot plate and knee strap respectively. The transducer sensitivity was accounted for as per original transducer specification, and then the total force of all the transducers was collated (three transducers for the foot plate and two for the knee strap). Once an appropriate scaling factor was derived, based on the theoretical assumption that 25 Kg of force would produce 245.25 N (force= mass X gravity), the total force was calculated, and used for all subsequent testing measurements.

3.5 Testing the LMD

Calibration indicated that the LMD was able to provide measurement comparable to literature. It was then necessary to test the device in a healthy population, before advancing onto pathological populations. The LMD required testing to ensure adequate sensitivity, accuracy of measurement and ease of use. Neuromuscular electrical stimulation (NMES) was administered to a participant within the device, allowing muscle response to stimulation to be measured.

Chapter 4

Study one: Establishing treatment parameters and dose for remaining clinical trials.

The first clinical study within this thesis aims to establish stimulation parameters and treatment dose for strength training with NMES. Guidance will be sought from literature regarding pulse width and stimulation intensity, as their interaction has been recognised (Brull, Silverman 1995). Frequency will be the focus of this study, as literature surroundings its application remains elusive (Schuhfried, Crevenna et al. 2012). The selected frequency will then be tested in one full training session, which has been proposed based on volitional strength training literature as indicated below. If aims are achieved, this protocol will be utilised throughout the remaining studies in this thesis. The secondary objective is to test the Leg Measurement Device (LMD) over a training session, to establish participant tolerance and comfort.

4.1 Introduction

Stimulation with Neuromuscular Electrical stimulation (NMES) will only produce a muscle contraction if it is strong enough to depolarise the axon (Gobbo, Gaffurini et al. 2011). The resultant muscle contraction must also be of sufficient intensity to elicit changes in muscle properties for alterations in strength to occur (Maffiuletti 2010). Parameter setting must comply with patient comfort; NMES stimulates sensory nerves as well as motor, and careful application can prevent unwanted noxious stimuli (Piva, Lasinki et al. 2013). Parameter setting should be guided by the aim of the intervention (for example, strength training), and guidance from literature is compulsory for efficient treatment with NMES to be administered.

There is currently no guidance for setting stimulation parameters when using NMES for strength training (Schuhfried, Crevenna et al. 2012, Mang, Lagerquist et al. 2010). This has resulted in existing

studies using a variety of frequencies to administer NMES with limited or no scientific reasoning behind choices (Chipchase, Schabrun et al. 2011). Comparability with current literature appears to be the most common choice for parameter selection when using NMES for strength training (Palmieri-Smith, Thomas et al. 2010, Bruce-Brand, Walls et al. 2012). Frequency selection varies according to the pathology being treated making comparison difficult. A recent review indicates that NMES for treatment of osteoarthritis uses a range from 25-50 Hz. The reasoning behind this variability is unexplained (Giggins, Fullen et al. 2012). Frequencies higher than 50 Hz have been highlighted to cause unnecessary muscle fatigue (Schuhfried, Crevenna et al. 2012), however application in research continues to rise beyond this level (Maffiuletti 2010, Maffiuletti, Pensini et al. 2002, Seyri, Maffiuletti 2011). Limited research has been conducted to reason why parameters have been selected, although understanding of the influence of each parameter on response has been documented (Baker, Wederich et al. 2000). It appears that electrode placement is a key variable for muscle response, and the positioning will determine motor output (Bickel, Gregory et al. 2011).

Once a stimulation frequency has been identified it must be tested on a full strength training protocol. Guidance on protocol development is currently not available from the NMES literature (Glinsky, Harvey et al. 2007, Chipchase, Schabrun et al. 2011). In the absence of any guidelines recommendation must be sought from volitional strength training literature. Current guidance for volitional strength training indicates that exercise should be performed two to three times per week. Intensity values vary within the literature. Guidance states that 40-50% of the subjects one repetition maximum (1RM) should be used for untrained individuals looking to improve dynamic strength, and 80-85% 1RM for more advanced strength training and neural adaptation (Kraemer, Ratamess 2004). The concluding repetitions of a strength-training program have been shown to produce muscular fatigue along with an increase in motor unit activity. This supports the idea that neural adaptation precedes early signs of muscular hypertrophy (Brandenburg, Docherty 2002).

Although a decline in maximal force output after repeated stimuli indicates that the muscle is undergoing an element of fatigue (Campbell, Neil et al. 2012), Enoka 2012), research indicates that fatigue can be an important stimulus for muscle development (Stevens-Lapsley, Balter et al. 2012, Schoenfeld 2012). This indicates that a drop in maximal force development during the NMES protocol may be beneficial to stimulate hypertrophy. Fatigue of this nature is considered beneficial, acting as a stimulus to induce hypertrophic changes. Fatigue is negative when the muscle has been worked to an excessive level; rendering the muscle unable to contract with sufficient intensity. It is therefore imperative that the stimulation parameters are set to allow a low level of positive fatigue to occur.

Although using the same parameters in order to compare results to clinical practice has some justification for helping NMES to be accepted into the clinical world, practitioners will not incorporate NMES into clinical protocols unless there is evidence as to the benefits and guidance to its application. Some practitioners will use NMES as a treatment for acute muscle wasting, however this is not a treatment that is widely used. NMES has been extensively researched as a means of preventing drop foot, and has recently been recommended by NICE for use in clinical practice (Shiels, Wilkie et al. 2011). This level of certainty must be readily available for practitioners to confidently and safely use NMES for strength training within physiotherapeutic programs.

4.2 Aim

The aim of this study was to investigate the effect of varying frequencies on force-generating capacity in skeletal muscle. A frequency appropriate to increasing force will then be tested over a pre-set treatment dose to establish effects from prolonged stimulation. This study acts as proof of concept to inform future studies within this thesis.

This study refers to 'optimal' frequency. Optimal will be considered as the frequency that produces a strong tetanic muscle contraction, and a low level of fatigue over the treatment session. Based on literature, these effects from treatment are considered optimal for increasing muscle strength.

4.3 Methodology

Design: An experimental design was utilised. Ethical approval was obtained from The South Staffordshire Local Research Ethics Committee to conduct this study (reference number: 09/H1203/92, see appendix 1).

Sample size: Twenty healthy participants aged between 18-80 were recruited from the staffing population of the Robert Jones and Agnes Hunt Orthopaedic NHS Foundation Trust (RJAH).

Method of recruitment: Participants were approached via an email, with the contact details of the researcher included. If interest was expressed, a participant information sheet was provided and any additional questions were subsequently answered. The participants were given 24 hours to decide whether they wished to participate in this trial; this time period was followed up with a telephone call.

Inclusion criteria: Participants were able to participate in this study if they fulfilled the following criteria:

- Aged between 18 and 80
- In a good state of health
- Able to comfortably tolerate NMES protocols

Exclusion criteria: Participants were screened for contraindications that included:

- Active pacemakers
- Uncontrolled epilepsy
- Active exercise in the previous 48 hours to testing
- Musculoskeletal injuries of the lower leg in the past 8 weeks
- Cuts or grazes over electrode site
- Skin or allergy conditions preventing electrode placement
- Any contraindication to using NMES

Informed consent: Informed consent was obtained once the participant had had chance to ask the researcher any questions that arose, and had been screened for contraindications.

Method of randomisation: All participants received treatment; no randomisation was required.

Testing position: Testing took place on the non-dominant Gastrocnemius, as determined by the participant. The non-dominant leg was regarded as the stabilising leg (the leg which was most commonly grounded) during an activity. This leg was utilised as it was anticipated that a low level of muscle tone would be required for stabilisation instead of powerful eccentric actions, which would be utilised through functional or sporting activities (Young, Rath 2011). Throughout all testing procedures the participant was positioned in the leg measurement device (LMD), with their knee joint in 60° flexion, and their ankle in a plantargrade position (Image 4.1). Velcro straps were placed over the femur and anterior ankle to maintain this position during stimulation with NMES. This

position was chosen as the lower limb muscles have been demonstrated to perform optimally in this position (Lieber 2009).

Electrodes (50 X 90 mm) were placed horizontally across the two heads of Gastrocnemius, as per current recommended guidelines (Baker, Wederich et al. 2000). Experience lead the placement of electrodes over the muscle belly, as this is often more comfortable for the participant. The proximal electrode was placed 3cm below the posterior knee fold, and the distal electrode 2cm below the proximal (Image 4.2). Immediately prior to testing, an acclimatisation session was conducted with the participant to ensure that they were comfortable with the sensation of NMES and the intensity of a single contraction. Stimulation intensity was increased gradually whilst receiving constant feedback from the participant. Once the participant indicated that they had reached their maximal level of intensity, the muscle was stimulated on two more occasions; to allow another increase to be performed if the muscle further acclimatised to the sensation. This session allowed the stimulation intensity for the testing protocol to be set before testing begun. Once the participant was comfortable and happy to proceed the researcher explained what the remaining session entailed (approximately 15 minutes). This rest period eliminated any possible effects of fatigue from the muscle that may have resulted from the acclimatisation session.

The one off training session (part two) was conducted one week after the initial identification of optimal frequency (part one). The same limb position and electrode placement procedure was followed (as above). The participant was encouraged to stimulate with the same or higher stimulation intensity than the first part of testing to maximise force output from the muscle.

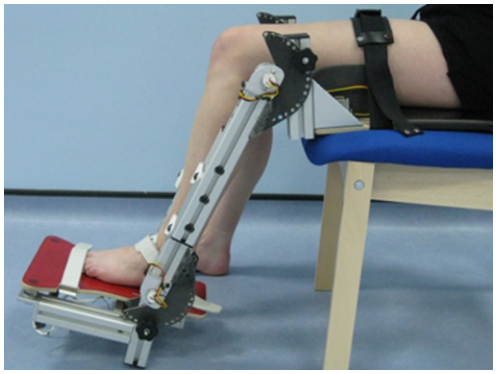


Image 4.1 testing position

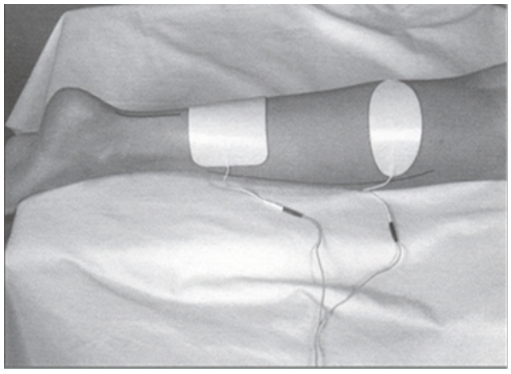


Image 4.2 Electrode position (Baker, Wederich et al. 2000)

4.3.1 Testing protocol

This study has been split into two parts:

1. Identification of optimal frequency
2. Testing optimal frequency over a full training session

In part one the participant was stimulated with eight different frequencies, whilst all remaining parameters remained constant. Research suggests that pulse width has a proportional relationship with maximal force, indicating that the higher the pulse width, the higher the resultant force produced from the muscle (Alon, Allin et al. 1983, Baker, Wederich et al. 2000). This study utilised a pulse width of 450 µs as this was the highest available pulse width on the stimulator used throughout this thesis. There is also evidence to suggest that the higher the pulse width, the lower the intensity that will be needed to produce equal force production with the same frequency. This holds advantage as higher intensities are likely to cause noxious stimuli (Baker, Wederich et al. 2000). The stimulation time was set to 3 seconds, and the rest period between contractions 90 seconds. This was set to achieve a strong muscle contraction, with enough rest to minimise any negative fatigue. Ramp up and down were both fixed at 0.5 seconds in order to improve participant comfort, however maximise the stimulation time. Stimulation intensity was set to maximal tolerated

by the participant (established in acclimatisation session) and remained constant throughout each testing session. This allowed a maximal muscle contraction to be produced. Frequency was varied with the following values (in order of administration): 20, 30, 40, 50, 60, 70, 80, 100 Hz. This was the range available on the NeuroTrac 2 stimulator (Verity Medical LTD). The frequencies were administered to the Gastrocnemius twice, with 20 minutes between testing sessions. Participants were encouraged to use the same or a higher current intensity on the second application. They were asked to relax throughout testing procedures, and not assist the muscle contraction that was produced. They were not aware of the order that frequencies were being administered. A flow diagram of the methodology for this part of the study is provided below (figure 4.1).

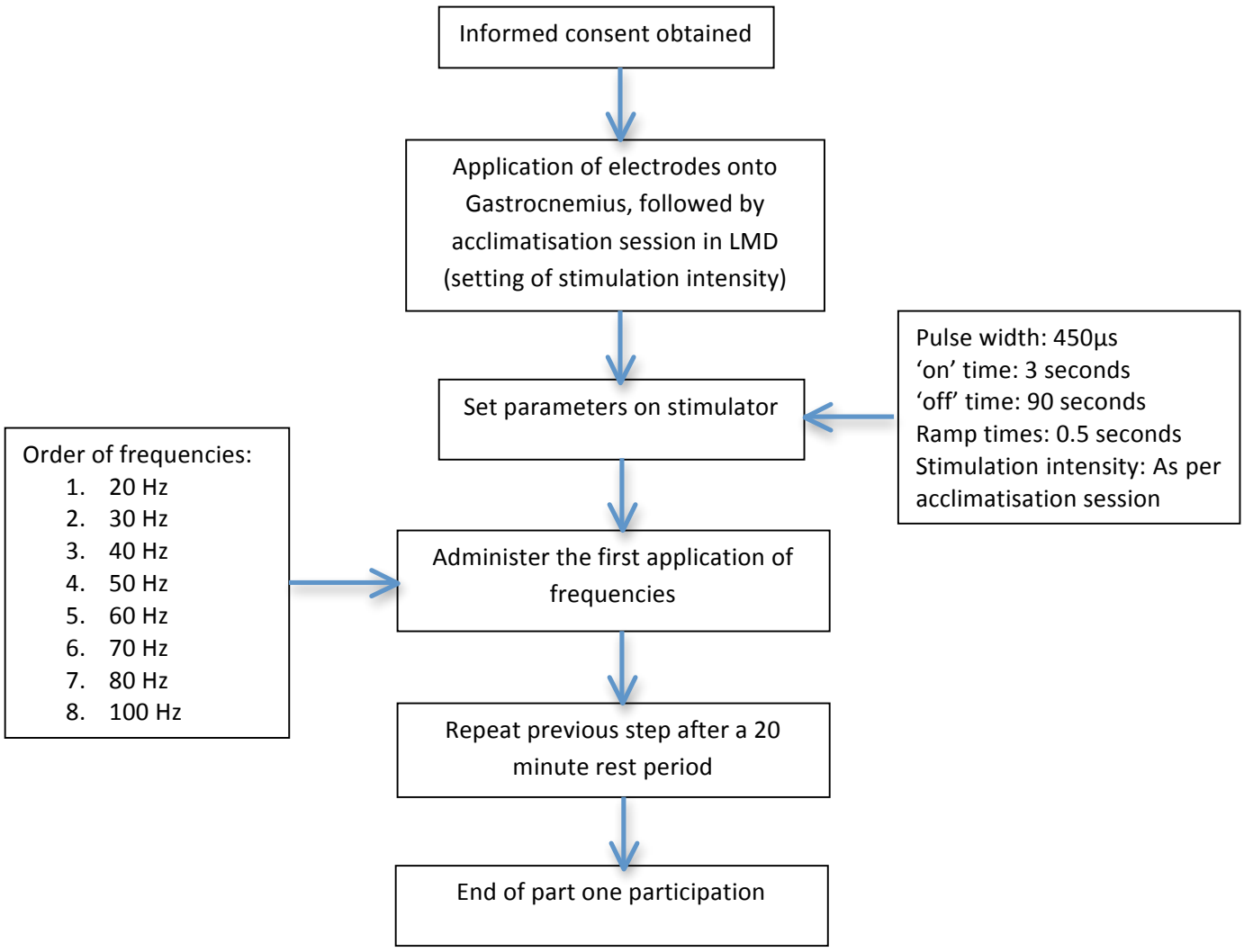


Figure 4.1 Part one methodology flow chart

The second administration of frequencies was considered for analysis, as customisation to the protocol was considered to be an influential factor from the first set of data. The participant may have been more relaxed in the second attempt, which would allow the muscle to respond more freely to stimulation with NMES.

During part two a stimulation frequency of 50 Hz was identified as 'optimal' (see results below for full analysis). Stimulation parameters were set as detailed in table 4.1. The stimulator was set to administer 45 contractions to the Gastrocnemius.

Stimulation parameter	***Setting***
Frequency	50 Hz
Pulse Width	450 μs
'On' time	3 seconds
'Off' time	10 seconds
Ramp times	0.5 seconds
Intensity	Maximal tolerated (ma)

Table 4.1 Part two stimulation parameters

During both parts of this study, a muscle contraction of the Gastrocnemius was produced resulting in a plantarflexion moment (measured in Newton metres: Nm). The force of this was recorded on the footplate of the LMD for analysis. Data was collected at a sampling rate of 20 Hz in real time via a Data Link Biometrics system (Biometrics LTD), which was connected to the LMD. Participants were

not shown real time data, as we did not want this to influence remaining results. Results were later transferred to a Mathcad spreadsheet for identification of outcome measures; process of measurement is detailed in the outcome measure section below.

4.4 Outcome Measure

Maximal Electrically Stimulated Force Production (MFP-ES)

MFP-ES allowed us to assess the peak moment produced from the plantarflexors during each muscle contraction produced via the NMES protocol. This outcome measure will also allow us to comment on the participant's compliance with the treatment protocol and whether they were able to relax throughout the session.

Raw data was extracted from the Data Link system. Data was converted to Newton metres (Nm) and transferred into mathematical analysis software (Mathcad). Each of the 45 contractions was identified when the force output reached 20% over the baseline reading. This figure was chosen due to the high level of baseline noise produced from the footplate. The greatest Nm value for each contraction was identified. This was considered to be peak moment, and regarded as MFP-ES during analysis.

4.5 Data analysis

4.5.1 Initial data analysis (part one)

The outcome measure used throughout this study was Maximal Electrically Stimulated Force Production (MFP-ES). This is a ratio level measure. A parametric approach to data analysis was taken in order to study both the patterns in behaviour and also the magnitude of the variability.

Initially, descriptive statistics (mean, standard deviation, coefficient variation and graphs) were used to identify if any trends existed within the data either at the level of an individual or at the group level.

4.5.2 Updated data analysis (part one)

Our initial analysis suggested that the optimum frequency to take forward to future studies was 50Hz. However, at a later stage when exploring the data in depth we realised we may have made an error in our initial assumption as there was a significant outlier. We have since reanalysed the data and this process is described below.

We used three paired sample t-test (p value adjusted for multiple comparison < 0.02) to test if there was a difference between the

- lowest frequency (20Hz) and the maximum frequency (100 Hz),
- The originally selected frequency (50Hz) and the maximum frequency (100 Hz)
- The frequency identified in the updated analysis (60Hz) and the maximum frequency (100Hz)

(NB: A repeated measure ANOVA was not carried out as our sample was small and we had eight different frequencies, i.e. 8 repeated measures for each subject)

Initial analysis showed large variability. It was possible that the variability in responses could have resulted from local factors (e.g. skin impedance, intensity variability, etc.) hence we also checked the variability in response by normalising to the maximal response for each subject.

An individual's muscle response to changes in frequency was modelled using a method of linear regression (Matthews, Altman et al. 1990). We split the data set into three sets of frequencies (a) 20 to 40Hz, (b) 40 to 60 Hz and (c) 60 to 100Hz. The range of frequencies used in literature varies, but a common frequency is 40Hz. Our initial analysis suggested the optimal frequency was 50 and the latter analysis showed 60 to be the optimum frequency. However, initial graphs suggest that the response kept increasing until 100 Hz for the group so the response beyond 60 Hz was investigated.

The final analysis was to explore the relationship between the response and the maximum tolerable intensity.

4.5.3 Data Analysis (part two)

Similar parametric approaches to analysis were taken to part one, which identified trends in behaviour and magnitude in change of force.

Initial descriptive statistics (mean, standard deviation, coefficient of variation and graphs) were used to identify any difference in individual or group response. Coefficient of variation represents a normalised measure of dispersion, and allowed the variation to be compared within the sample.

Individual participants were grouped into categories, with 8 participants demonstrating similar mean MFP-ES values (low), and 3 exceeding this (high). When testing for differences with a one-way ANOVA, no statistically significant finding was noted. Patterns in muscle response to the 45 contractions were also identified on an individual basis. This was initially conducted by identifying the difference between first and last contraction. This allowed a descriptive approach to the analysis. Rate of change was also taken into consideration by using linear regression analysis. This

allowed any change throughout the treatment protocol to be taken into consideration, allowing a sense of the overall reaction of the muscle to stimulation with NMES. Three trends were identified:

1. Increasing MFP-ES
2. Stable MFP-ES
3. Decreasing MFP-ES

These patterns represent different muscle responses to treatment with the NMES protocol.

There was one week between part one and part two of testing. This allowed comparison between the stimulation intensity used for both parts of the study. Both internal and external factors can influence tolerance to NMES and therefore may alter muscle response. Comparison was made on a descriptive level based on the value of stimulation intensity used. Stimulation intensities were categorised in a similar way to part one, which indicates that participants were able to increase the intensity that stimulation was delivered with on the second day of testing.

A linear relationship was identified between stimulation intensity and MFP-ES. This indicates that the higher the stimulation intensity the larger the muscle response to NMES. When testing the response of these two variables with a one-way ANOVA a significant difference was noted.

4.6 Results

4.6.1 Part one analysis

Data was collected from 20 participants. Analysis was conducted on 15 due to the foot plate of the leg measurement device (LMD) not recording all data points in 5 participants. For these five participants stimulation peaks were not able to be identified with mathematical analysis, and manual analysis was not possible. All participants were able to tolerate stimulation, with no-one

reporting negative effects or adverse reactions to stimulation. All participants also reported comfort, and ease of use with the LMD. Raw data is presented in appendix 2. Participant demographics are summarised in table 4.2 below. There were a higher number of males than females participate in this study, with a low mean age of 39 years.

	Gender	Age	Height (cm)	Weight (kg)
Mean	Male n= 9 Female n=6	39	181	78
Standard deviation (SD)	n/a	6.2	7.4	10.6

Table 4.2 Participant demographics

Maximal electrically stimulated force production (MFP-ES)

Results are reported from attempt two of the frequency application. Attempt two was anticipated to be more reflective of frequency response as the participant would be acclimatising to the protocol in attempt one. The peak MFP-ES tended to be higher during attempt two. Analysis was initially performed on an individual level to account for a small sample size and high levels of variability. Table 4.3 shows the descriptive statistics for maximal electrically stimulated force production (MFP-ES).

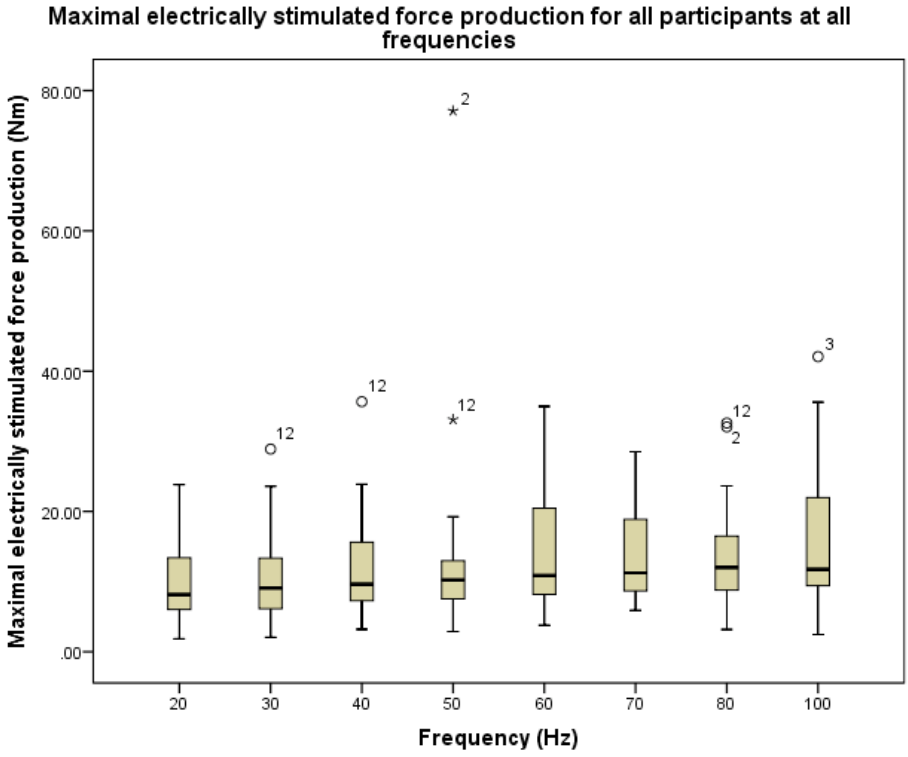
	Frequency (Hz)							
	20	30	40	50	60	70	80	100
Mean	10.1	10.9	12.1	15.5	14.5	14.2	14.3	16.3
SD	5.8	7.4	8.5	18.6	9.2	8.1	8.8	11.3
COV	0.6	0.7	0.7	1.2	0.6	0.6	0.6	0.7

Table 4.3 Descriptive statistics for each frequency

SD: standard deviation, COV: coefficient of variation

When comparing mean values, 100 Hz produced the greatest gastrocnemius mean force (16.3 Nm), followed by 50 Hz (15.5 Nm). 20 Hz produced the smallest force of 10.1 Nm. There is a decline of

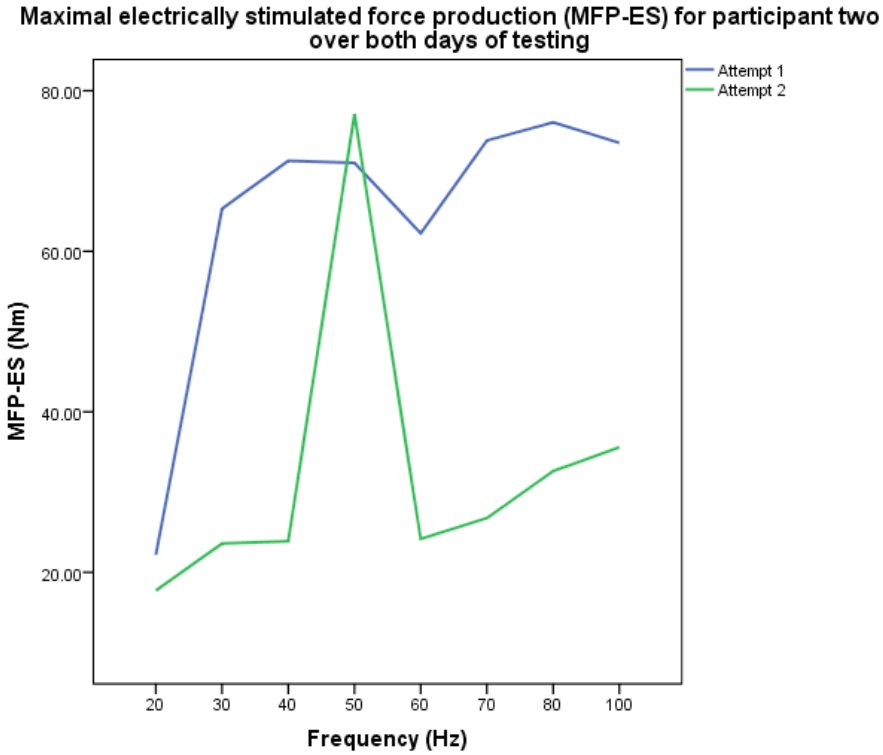
moment evident between 50 Hz and 60 Hz (-1 Nm), which is the greatest drop in moment throughout the dataset. When considering individual data, results are more varied, showing rise and fall of maximal moment in varying patterns. This is reflected by the high standard deviation (mean 9.7), which peaks at 50 Hz (18.6). 100 Hz also has a high SD. Remaining frequency values demonstrate smaller standard deviations, indicating less variability in data within participants. The Coefficient of Variation remains similar in all frequencies except 50 Hz, indicating the highest level of variability at this point. Mean maximal electrically stimulated force production (MFP-ES) and standard deviation values are depicted in graph 4.1 below.



Graph 4.1 a graphical representation of mean MFP-ES (Nm) over each frequency

Graph 4.1 indicates that maximal moment peaks at 50Hz, and then plateaux's before rising again at 100 Hz. The greatest increase in peak moment is found between 40 Hz and 50 Hz, a rise of 3.4 Nm. The high standard deviation in the 50 Hz category compared to other frequencies indicates that there is an outlier, which has been identified as participant 2, and lies beyond the upper quartile on

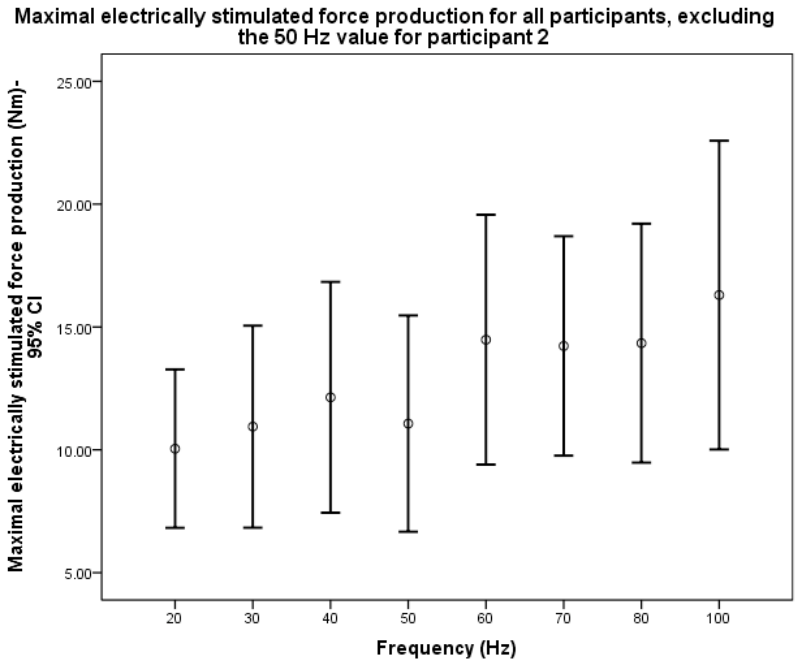
box plot analysis. Participant 2 is a 31 year old male who is a regular runner. Participant 2 produced a moment of 77.1 Nm at 50 Hz, which is 54% higher than the next highest value, which was seen at 100 Hz (35.58 Nm). When considering the MFP-ES data of the two separate attempts for participant 2, it is interesting to note that attempt one demonstrated a higher and more stable MFP-ES than attempt two (graph 4.2): a pattern which is individualistic to this participant. Participant 2 also used a higher stimulation intensity on day two of testing (4 mA higher).



Graph 4.2 MFP-ES for attempt one and attempt two (participant two)

Subsequent studies in this thesis have been designed based on assumptions made up until this point in analysis. Analysis was conducted in a short turn around time to allow the same participants to be used in both parts of this study. Data was further explored upon writing of this thesis and is presented below. On reflection the updated analysis may have been more reflective of optimal frequency, and gives indication for further exploration of the interplay between frequency and stimulation intensity.

Participant two is excluded from Graph 4.3, and it is interesting to see that a level of variability remains. The mean value for 50 Hz has reduced to 11.1 Nm, a decline of 4.4 Nm. The frequency causing the highest maximal moment before decline has risen to 60 Hz instead of 50 Hz as seen previously with the inclusion of all participants. The presentation of data beyond this maximal remains similar: a steady state of decline followed by a rise at 100 Hz.



Graph 4.3 A graphical representation of MFP-ES (Nm) over all frequencies, without data from participant 2. Error bars represent 95% CI

A paired t-test was used to ascertain whether there was a difference of MFP-ES production at different frequencies. Three pairs were tested as detailed in table 4.4 below. The significance value was adjusted for multiple comparisons using the Bonferroni method of correction (Bland, Altman 1995). A difference was found between 20 Hz and 100 Hz (P= 0.063), however was not significant. This indicates that frequency has a linear effect on MFP-ES. However no statistical difference was found between either 50 Hz or 60 Hz (60Hz demonstrated a greater treatment effect). Clinical indications must therefore be considered in interpretation.

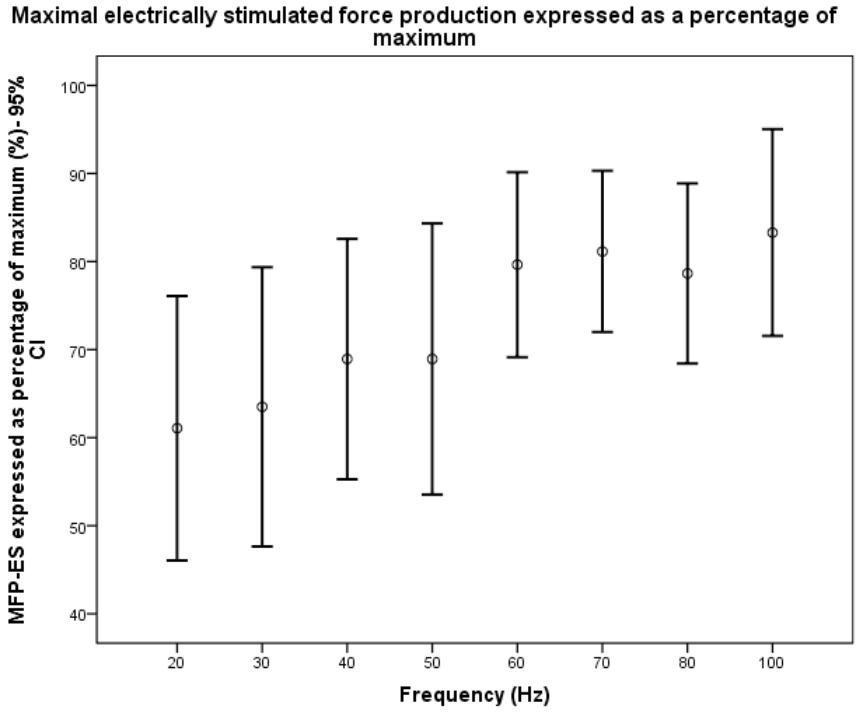
Pair	Frequencies (Hz)	95% confidence interval		P value (< 0.02)
		Lower	Upper	
1	20-100	-11.192	0.343	0.063
2	50-100	-9.683	1.974	0.177
3	60-100	-3.791	1.526	0.374

Table 4.4 Results of t-test for MFP-ES based on frequency

Literature often presents data as a percentage of maximum. Percentage maximum allows the differing stimulation intensities to be accounted for, without impacting coefficient of variation. The results are presented as a percentage of maximum in table 4.5 and graph 4.4. When reviewing frequency data as a percentage of maximum the greatest rise in mean moment is again between 50 Hz and 60 Hz, with an increase of 10.7%. The increase in percentage maximum increases by 7.8% from 20-50 Hz, and 3.7% from 60-100 Hz. This indicates that the greatest amount of increase in peak moment is found between 20 and 50 Hz frequencies. 40 Hz and 50 Hz produced the same percentage maximum value (68.9%). When looking at the co-efficient of variation, the variation remains stable throughout, indicating some variability throughout the data set. The frequency which causes maximal peak moment (MFP-ES) tends to flutuate, again indicating variability in our results.

	Frequency (Hz)							
	20	30	40	50	60	70	80	100
Mean	61.1	63.5	68.9	68.9	79.6	81.1	78.6	83.3
SD	26.0	27.5	23.6	26.7	18.2	15.9	17.7	20.3
COV	0.4	0.4	0.3	0.4	0.2	0.2	0.2	0.2

Table 4.5 Descriptive statistics for MFP-ES expressed as a percentage of maximum (%)
SD: standard deviation, COV: coefficient of variation.



Graph 4.4 Maximal electrically stimulated force production (MFP-ES) expressed as a percentage of maximum (%). Participant 2 was excluded from analysis. Error bars represent 95% CI

In order to ascertain whether there is a frequency that would be considered optimal for strength training with NMES, it is important to understand the variability of the results. Linear regression analysis indicates that a high level of variability was found within the dataset of each participant. Table 4.6 indicates the rate of change found at each frequency. Frequencies were sub-categorised into smaller groups to enable identification of trends. The group with the highest number of participants showing the greatest improvement is the 40-60 Hz group (8 participants showing the highest level of change from each group of frequencies.

Participant	Rate of change (%)		
	20-40 Hz	40-60 Hz	60-100 Hz
1	0.166	**0.426**	0.001
3	0.034	**1.38**	0.186
4	**0.308**	-0.242	0.113
5	0.093	**0.17**	0.026
6	-0.097	**0.08**	0.032
7	0.142	**0.24**	-0.083
8	0.002	**0.062**	0.039
9	0.068	**0.105**	0.018
10	-0.174	-0.036	**0.049**
11	**0.031**	-0.05	-0.054
12	**0.591**	-0.487	-0.032
13	0.123	-0.054	**0.311**
14	-0.099	**0.192**	-0.06
15	**0.075**	-0.04	-0.114

Table 4.6 Rate of change of MFP-ES expressed as a percentage of maximum for all participants. Values highlighted in red indicates greatest improvement in that sub-category

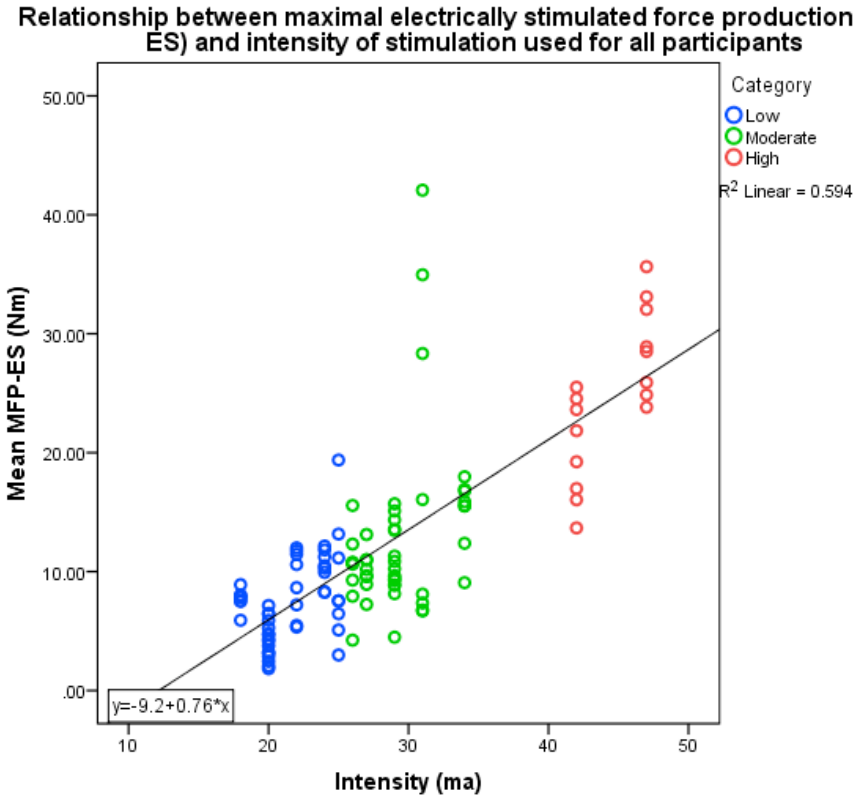
Stimulation intensity

As well as comparing MFP-ES the stimulation intensity used during stimulation with NMES was also taken into consideration. For analysis purposes stimulation intensity has been grouped into three categories, as identified in table 4.7. This enabled any trends in both level of gastrocnemius force production, and response to frequency to be identified. The majority of participants stimulated with a moderate stimulation intensity (n=7), and only two participants increased stimulation intensity to a high value.

Stimulation Intensity (mA)	Category	Number of participants
15-25	Low	6
26-36	Moderate	7
37-47	High	2

Table 4.7 Stimulation Intensity of NMES split into categories for analysis

Graph 4.5 indicates that there is a linear relationship between MFP-ES and stimulation intensity: the higher the intensity the greater the MFP-ES (R^2= 0.594). This indicates that the participants who used a higher intensity whilst stimulating produced a higher output from the gastrocnemius. The moderate category of intensity produced two MFP-ES values that were higher or equal to the MFP-ES produced by the high intensity category. These both relate to participant three, who used a stimulation intensity of 31 mA, but who produced the highest MFP-ES of all participants of 42.07 Nm at 100 Hz, and the second highest of 34.96 Nm at 60 Hz.



Graph 4.5 the relationship between MFP-ES (Nm) and stimulation intensity (mA) for all participants.

4.6.2 Part Two Results

In the second part of the study the fifteen participants from part one returned one week after initial testing to complete part two. Analysis was performed on 11 of the participants, due to the foot plate

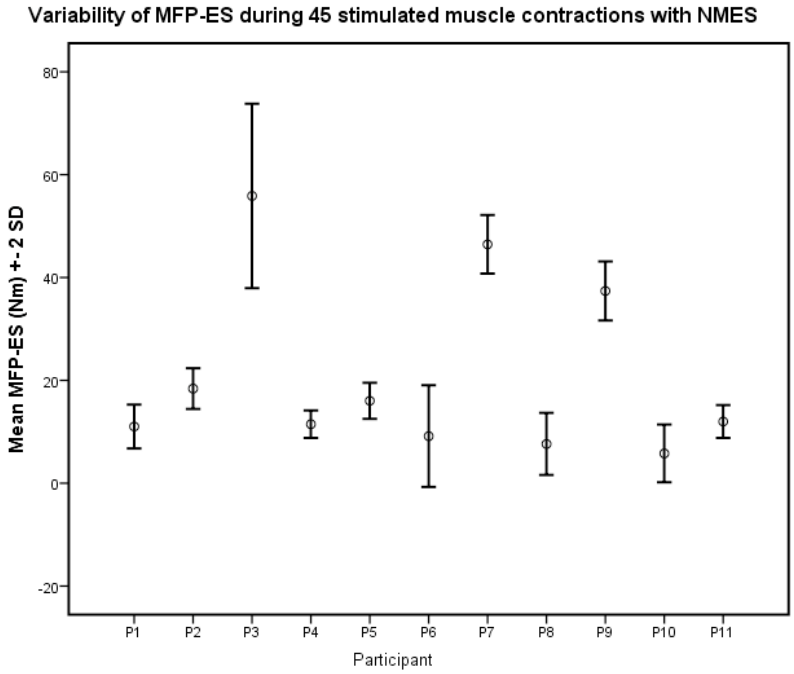
of the dynamometer not recording all data points in 4 participants. Again, no negative effects from stimulation were noted. Raw data is provided in appendix 3. From part one to part two 1 male and 3 female participants were lost. Participants complied with protocols; the participants were withdrawn during the analysis process where peak force (MFP-ES) was unable to be identified during analysis.

Maximal electrically stimulated force production (MFP-ES)

Table 4.8 shows the descriptive statistics for MFP-ES for all 45 muscle contractions produced during stimulation with NMES. There were a wide range of MFP-ES values between participants, ranging from 5.8 Nm to 55.9 Nm. Standard deviations tend to be quite low (average 3.1), despite the range of MFP-ES. There are two participants, 3 and 6 who demonstrate standard deviations higher than expected compared to the remaining data. Participant 3 produced the highest MFP-ES of 55.9 Nm, and a standard deviation of 9, whilst participant 6 had a MFP-ES of 9.2 Nm and a standard deviation of 4.9. This is depicted in graph 4.6. Coefficient of variation was higher in participants who demonstrated a lower force output from NMES (participants 6, 8, 10).

	Participant										
	1	2	3	4	5	6	7	8	9	10	11
Mean	11.0	18.4	55.9	11.5	16.0	9.2	46.4	7.6	37.4	5.8	12.0
SD	2.1	2.0	9.0	1.3	1.8	4.9	2.8	3.0	2.9	2.8	1.6
COV	19.1	10.9	16.1	11.3	11.3	53.3	6	39.5	7.8	48.3	13.3

Table 4.8 descriptive statistics (mean, standard deviation (SD) and coefficient of variation (COV)) for MFP-ES for all participants over 45 stimulated muscle contractions (Nm)



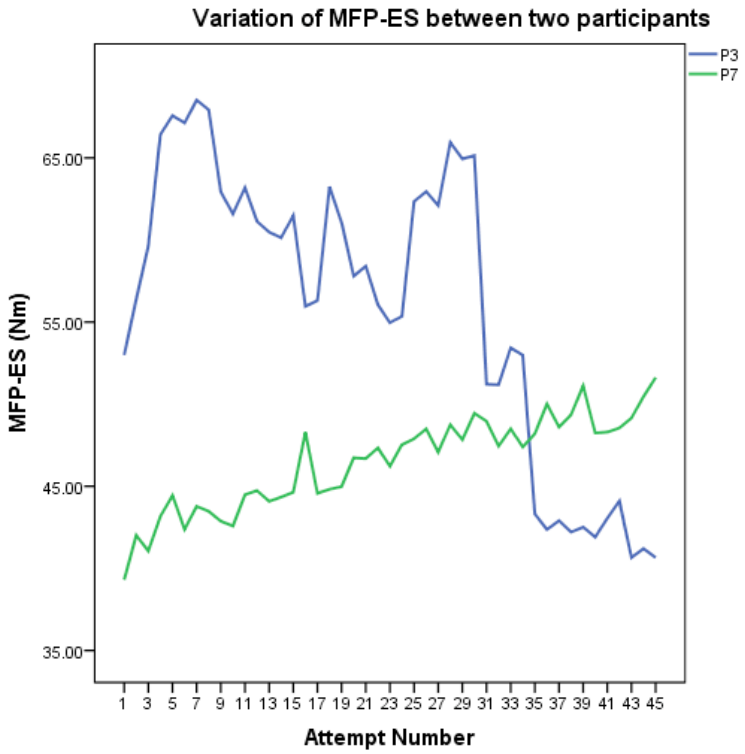
Graph 4.6 variability in MFP-ES in all participants (Nm) indicating ± 2 SD

Given the variability witnessed, it is important to look at the results based on individual presentation. Graph 4.6 indicates that 8 participants had similar MFP-ES values, and 3 participants exceeded this. Table 4.9 groups MFP-ES into categories. Participants 3, 7 and 9 produced a MFP-ES in the high category, which correlates with the presentation in graph 4.6. When comparing all participants with a one-way ANOVA to assess for relationship, no significant finding was found (P= 0.850, F= 0.038). The 3 participants in the high MFP-ES category were not the participants with the large variance witnessed.

MFP-ES (Nm)	Category	Number of participants
5-20	Low	8
21-35	Moderate	0
36 +	High	3

Table 4.9 MFP-ES category descriptions

When exploring within participant data, multiple trends were identified. Some participant's demonstrated positive linearity in their MFP-ES, indicating that MFP-ES increased over the 45 stimulated muscle contractions (participant 7 in graph 4.7). Some decline was also witnessed as indicated by participant 3 below. Participant 3 was identified as having a wide standard deviation of MFP-ES, with a range of MFP-ES from 68.5 Nm to 40.7 Nm. Both these participants produced a low coefficient of variation (16.1% and 6% respectively).



Graph 4.7 MFP-ES of participant 3 and 7 during the stimulation programme.

Some participants produced a stable response of MFP-ES over the stimulation protocol, for example participant 4 whose MFP-ES values ranged from 9.3 Nm to 14.9 Nm. This participant indicates that a stable muscle response can be produced from a constant stimulation intensity. Table 4.10 highlights participants who demonstrated increases or decreases in MSP-ES over the 45 stimulated muscle contractions. A stable response has been identified as a change in MFP-ES of 0-1 Nm. The participants who produced a high mean MFP-ES value (3, 7 and 9) did not demonstrate similar

trends in table 4.10. Participant 3 demonstrated the largest decline in MFP-ES over the 45 stimulated muscle contractions, however participant 7 demonstrated the largest increase. This indicates that response to NMES is varied, and reasons for this variability should be investigated.

	Participant number										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Difference from contraction 1- 45 (Nm)	**-3.7**	**2.9**	**-12.3**	**2.3**	**6.1**	**-10.3**	**12.3**	**0.4**	**1.0**	**-7.1**	**-2.4**

Table 4.10: A table to show the increase (indicated in **green**) decline (indicated in **red**) or stable (indicated in **black**) response of MFP-ES between contraction 1 and contraction 45. Units are displayed in Nm.

Linear regression analysis was conducted to establish the variance of force output during the stimulation protocol (instead of pre-post). Analysis grouped responses into three presentations: stable, fatiguing and increasing. Four participants demonstrated increases in their MFP-ES over the 45 stimulated muscle contractions (participant 2, 4, 5 and 7). This indicates that five participants witnessed decline as a result of the NMES programme. Two participants demonstrated overall stability of response. The average decline of participants over the treatment protocol was -0.13 Nm per contraction. Participant 7 demonstrated one of the top three MFP-ES forces as a result of treatment however demonstrated a positive rate change; the lifestyle of this participant should be taken into consideration.

Stimulation intensity

During part two of this study participants were encouraged to use a stimulation intensity equal or higher than the value they used in part one. The stimulation intensity used was therefore compared to part one. Table 4.11 indicates that all participants except four increased their stimulation intensity

from part one to part two. Participant 2 was excluded from analysis in part one, because of a wide variance of data, indicating voluntary involvement to assist NMES. The reduction of the intensity during the second part of participation could be a consideration for the decreased variability witnessed in participant 2 data (standard deviation of 2). Participant 12 demonstrated the highest decline in intensity (25 ma), and produced a low mean MFP-ES of 5.8 Nm in this part. Participant 3 produced the highest increase in stimulation over the two parts of 21 ma. It is interesting to note that participant 3 also produced the greatest MFP-ES, with the greatest amount of variability between the 45 muscle contractions. The three participants (3, 7 and 9) who produced the highest MFP-ES also used the highest stimulation intensities, 52 mA, 39 mA and 39 mA respectively. A mean increase of stimulation intensity of 2.4 mA was observed between the two days.

Participant	Part one	Part two	Difference
1	42	34	8
2	39	35	4
3	31	52	21
4	29	23	6
5	22	27	5
6	18	32	14
7	26		
8	34	39	5
9	20	30	10
10	27	39	12
11	20		
12	47	22	25
13	25	28	3
14	24		
15	29		

Table 4.11 Stimulation intensity values used throughout the stimulation protocol (mA). Blank entries indicate that data was unavailable. Highlighted entries indicate decline.

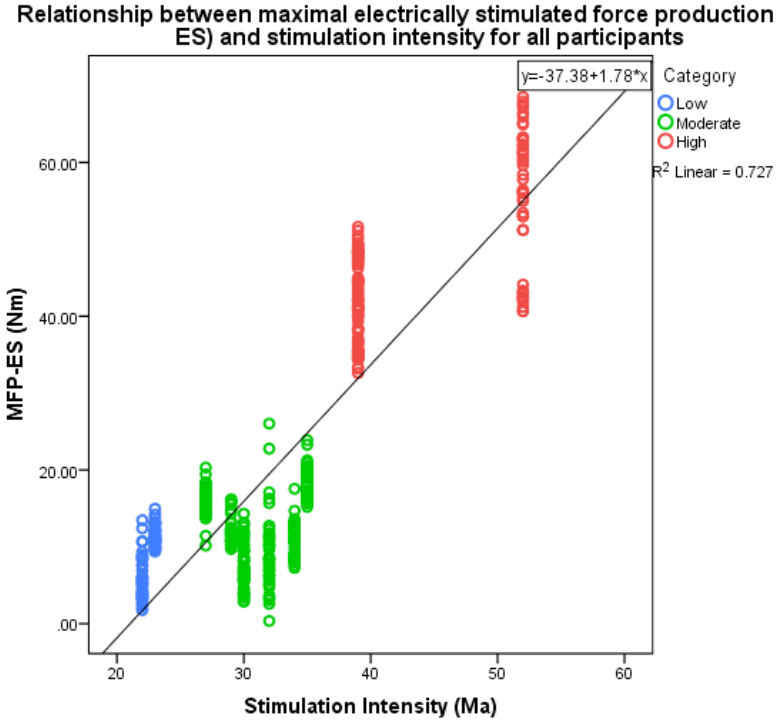
Stimulation intensities have been split into categories, as used in part one. Table 4.12 indicates the categorisation of stimulation intensities. From part one there are 4 less participants in the low

category, and 1 more participant in the high category. This demonstrates that participants were able to increase the intensity that stimulation was delivered with on the second day of testing.

Intensity (mA)	Category	Number of participants
15-25	Low	2
26-36	Moderate	6
37 +	High	3

Table 4.12 Stimulation intensity categories for analysis

From graph 4.8 below it is evident that a linear relationship exists between stimulation intensity and MFP-ES over the 45 stimulated muscle contractions (R^2= 0.727). This is similar to the relationship identified during part one. There appears to be a relationship between the intensity category (low, moderate or high) and variability of MFP-ES during the 45 muscle contractions. When conducting a one-way ANOVA a statistical significance was identified between MFP-ES and stimulation intensity (F= 1580.9, P= 0.000). This indicates that those who administered NMES with a high category of stimulation intensity resulted in a higher MFP-ES during the stimulation protocol.



Graph 4.8 Relationship between MFP-ES and stimulation intensity for all participants during the stimulation programme.

4.7 Overview of Study One results:

Part one

The aim of this study was to ascertain whether an optimal frequency existed, and if it was effective over a training session with NMES to improve muscle strength. The frequency should be high enough to produce a strong tetanic contraction, whilst initiating some fatigue in the muscle to stimulate morphological changes. Results were analysed by taking four areas into consideration:

1. The maximal response of MFP-ES- was a plateaux identified
2. Trends within individual data
3. Trends with individual data expressed as a percentage of maximum
4. The highest level of rate change on linear regression analysis

Results were initially analysed using data from all participants, which was carried forward for subsequent study design. **This resulted in 50 Hz being regarded as 'optimal frequency'**. Retrospective analysis indicates that removal of an anomalous data point alters the maximal response. On reflection, 60 Hz may have been best suited for future studies as it produced a mean maximal response. There was however a wide range of variability within participants. Interestingly linear regression analysis indicated that the highest change in MFP-ES was witnessed between 40 Hz and 60 Hz. Using the middle value of these three frequencies supports our initial assumption that 50 Hz should be carried forward for future studies. This was supported by the greatest increase in MFP-ES being identified between 20 Hz and 50 Hz; the response of MFP-ES increases beyond 60 Hz, but at a smaller rate. This indicates that 50 Hz did not produce the greatest maximal moment from NMES; however it demonstrated the greatest rate of change throughout the data set.

Stimulation intensity analysis indicates that the participant should increase the intensity as high as they can tolerate, considering the linear relationship identified with MFP-ES in graph 4.5. Further analysis may be required to ascertain the impact of stimulation intensity on force response to NMES, as this was not the main focus of this study.

Part two

Part two aimed to test the proposed 'optimal frequency' in one session of a strength training protocol. There was a degree of variability witnessed within the results, with multiple trends being identified when assessing individual data. When comparing the full cohort of participants there was a greater decline of MFP-ES over 45 muscle contractions than there was incline, as evidenced through linear regression analysis. **This indicates that stimulating at 50 Hz requires the muscle to work at sufficient level to cause changes in muscle structure.**

A positive relationship was identified between stimulation intensity and MFP-ES: the greater intensity used, the greater the MFP-ES. Part two has identified that 50 Hz produces a variety of muscular responses when administering a NMES strength training protocol; increasing, decreasing and stabilising. **50 Hz has therefore been accepted as a stimulation frequency that is able to produce an adequate muscle response to a strength training protocol with NMES in combination with the other stimulation parameters used throughout this thesis.**

4.8 Discussion

The aim of this study was to investigate whether an optimal frequency exists, and if it is effective over a treatment session when stimulating the gastrocnemius with neuromuscular electrical stimulation (NMES). An optimal frequency was decided upon after initial analysis (50 Hz). Part two aimed to ascertain whether a 50 Hz frequency used in combination with other set parameters was tolerable and effective when administering 45 stimulated muscle contractions with NMES with the intention of increasing muscular strength. The information gained from this study was gathered to inform further studies in this thesis, where the protocol will be administered on different populations.

Study one was the first study that utilised the leg measurement device (LMD). This study was conducted on the gastrocnemius, resulting in the foot plate being our sole means of measurement. The LMD proved to be simple and effective to set up. Alteration to the side members in response to participant limb length was easy to conduct whilst the participant was seated, and provided a standardised measurement of knee and ankle position during stimulation. The Velcro straps were efficient at maintaining limb position. Standardisation of limb position was vital as the gastrocnemius is a bi-articular muscle. The electrodes were easiest to apply before the participant

was positioned in the LMD; a factor that was retrospectively reflected upon for future testing. Subjective feedback was positive; the device was comfortable, and all participants reported that they would accept this position during a treatment session for a longer period of time if required. Due to the young and healthy nature of our sample, no problems were encountered with positioning within the LMD. However, participants were required to balance on one leg in order to lower into the device. Although a demonstration and a chair with hand rails were provided to ease this process, the design of the device may have to be re-considered for future studies on pathological patients.

The LMD was connected to Data Link Software for data collection, which proved easy and effective to use. No problems were encountered in the extraction of data to the software. Extracted data was converted to Newton meters, and transferred to a mathematical software programme (Mathcad). The data produced for some of the participants was unable to be read by the Mathcad program written for analysis. Manual analysis would not have been reliable due to inconsistencies in reading peak force. It is anticipated that this noise was due to the foot moving or pressing on the foot plate, causing oscillations in the recording. There is a possibility that the participant was contributing to the stimulation, whether assisting or resisting. Either contribution would affect our results, preventing us from comparing the data to both within participant frequency trials and between participants. An example of both a noisy and a clean dataset is presented in image 4.3 below. Noisy data was therefore excluded from analysis. A time measurement device could have been utilised and activated on application of the stimulation in order for a contraction to be manually identified with confidence. This would have prevented data being excluded from analysis.

The transducers on the footplate were adjusted for sensitivity as per manufacturer guidelines. Peak moment was extracted from the Mathcad programme, which was considered to be the highest data point from the force data (Nm). This is highlighted in red in image 4.3 below. The start of a muscle

contraction was identified by reading data after 20% of maximal jerk (measured in seconds and shown in blue on image 4.3). This value was more consistent in identifying a true effort when compared to a 10% or 30% cut-off. It was considered to be the value that gave the most detail from the data considering its variation. In future studies we propose to continue to use the method of analysis developed for this study, as any change would run the risk of not being able to identify small changes in force, which could have a clinical impact on a patient population. When testing pathological populations, such a stroke, small changes in strength are anticipated given the strong neurological influence of the condition (Umphred, Lazaro et al. 2013). Small changes in muscle strength, along with changes in neuromuscular control are able to impact functional activity, which is a primary goal of rehabilitation (Pomeroy, Ward et al. 2014). If the sensitivity of the footplate was altered, these changes may not be witnessed. Therefore participants who do not comply with the NMES protocols, and who are not able to tolerate NMES to a sufficient level will be excluded from future studies.

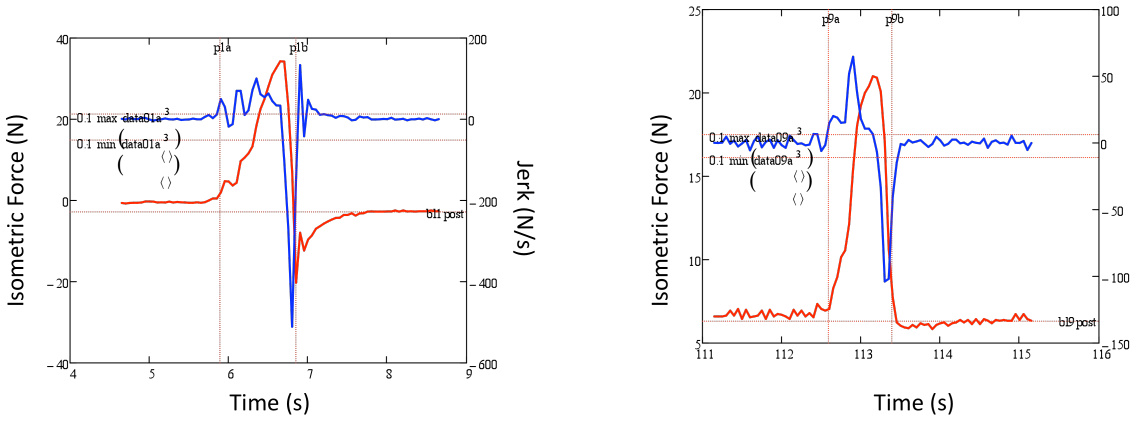


Image 4.3 Force data from mathcad spreadsheet. The image on the left shows a noisy contraction, and the image of the right a clean contraction. Force is represented in red and Jerk is represented in blue.

When looking at the participants who were excluded on this basis, there were three females and one male. It could be inferred that a female population have less tolerance to the sensation of NMES that males. This is supported by recent research, which indicates that sensory thresholds are lower in females compared to males, and that this should be taken into consideration when designing stimulation protocols (Maffiuletti, Morelli et al. 2011). This maybe a result of a higher peripheral adipose layer, which has been reported in females, compared to males (Karastergiou, Fried et al. 2012). The relationship between muscular response to NMES for strengthening and tolerance of stimulation intensity in genders is something that should be investigated further, as this may create a barrier to therapeutic findings.

This study recruited 20 participants; with analysis being conducted on 15 in part one and 11 in part two. A convenience sample was recruited from staff at the hospital where the research was conducted. This sample was used for ease of participants volunteering to take part in the research. The benefits included having limited travel and time out of a working environment. The research team also had the benefit of internal email communication, which given the time constraint to collect data (based on funding implications) was vital. The convenience sample used was beneficial for recruitment, but limits the ability to translate the results to a wider population (Marshall 1996). A convenience sample is not necessarily reflective of the wider population and could have similar demographics. This factor could be reduced by using a judgement sample, however by selecting a population deemed to be appropriate a level of bias is entered into the methodology.

This study recruited a limited sample based on the proof of concept design. Sample size is associated with expected outcomes and as this study was expected to produce a linear response, a smaller sample was justified (Marshall 1996). Given that this study aimed to inform protocol development, and was not administering a treatment to ascertain effect, we decided to recruit 20 participants. This

was under the understanding that this sample size would be able to identify trends to inform further protocol development in part two. Although a larger sample size may have given this study more strength to reliably infer from the results, the data was going to be further tested in a full strength training protocol. Given the limited time available to collect data, a smaller sample was deemed sufficient. In order to fully achieve the aim of this study a power calculation could have been performed to ascertain how many participants would be required to generalise results to a wider population. There are a variety of methods available to calculate sample size (Dupont, Plummer Jr. 1998). A power calculation was not conducted as the logistical issues outweighed the need given the linearity of expected result and further development of result in subsequent studies: It was not within the scope of this study.

There are some methodological factors that should be taken into consideration when inferring from the results of this study. During part one testing each frequency was administered twice, and the second attempt considered as MFP-ES. The two attempts were split by a 20-minute rest period. Comparison between attempt one and attempt two data was not conducted. Attempt two was used for analysis as it was assumed that the participant would be able to relax and allow the stimulation to produce a muscle contraction once they were accustomed to the methodology. To develop and increase the reliability of our results each frequency could have been administered more times, with the variability between the contractions compared.

Both administrations of frequency values in part one were administered in a linear order, which may have induced a learning effect during the protocol. Participants were not informed which order frequencies were administered, in the thought that they would not be affected by the ordering of frequencies. On reflection, a random application of frequencies would have improved confidence when deducing from results, especially given our small sample size. During part two the perceived

increase in strength could have initiated a learning effect whereby the perceived increase in volitional strength could be transferred into actual strength gains. Learning effects can be a powerful placebo when referring to treatment response (Flatten, Blumenthal 1999). In order reliably say if this were true the study design should ensure that a control group is employed for comparison, and a larger sample size used for testing. Sham treatments with NMES are difficult to implement. Sensory outputs from NMES result in tingling, muscle twitching or contractions, and even noxious stimuli (Baker, Wederich et al. 2000). Delivery of a sham treatment would result in the participant being aware of the lack of sensation from NMES; effectively breaking the blinding. Blinding is also difficult within the research team as participants who have received stimulation with NMES are commonly left with an erythema under the electrode site as a result of heat from the electrodes (Brunoni, Nitsche et al. 2012). The use of another person for analysis was not available within the scope of this PhD research. When considering the results obtained from this methodology there is no significant indication that a learning effect occurred. It is therefore presumed that this has not occurred, and these results are deemed reliable to take forward for further analysis in a full 6-week program.

Part one of this study was conducted with a 90 second rest between electrically induced muscle contractions. This rest period was incorporated into the protocol based on recent literature highlighting the impact of fatigue whilst using NMES (Gobbo, Maffiuletti et al. 2014). Although only producing 16 muscle contractions (both sessions), a protocol that induced direct fatigue was not appropriate in achieving study aims. Fatigue is often highlighted as a negative training influence in NMES literature (Schuhfried, Crevenna et al. 2012, Gregory, Bickel 2005). It must be noted that volitional strength training literature uses the overload training principle to induce muscle fatigue. This induces eccentric muscle contractions and a stimulus for hypertrophic changes (Vogt, Hoppeler 2014). For this reason a 10 second off time was utilised in part two of this study, which reflects a volitional strength-training program.

Much of the NMES literature discusses the concept of fatigue as an influential factor during its administration. Fatigue is often thought to be due to a build-up of metabolites such as lactic acid in the muscle tissue (Westerblad, Allen et al. 2002), which is regarded as peripheral fatigue. Peripheral fatigue often occurs from anaerobic exercise, and is a result of a build-up of hydrogen ions in the muscle cell, causing acidosis. Westerblad (2002) also discusses the idea of increased inorganic phosphate from the break-down of creatine phosphate as another contributor to the mechanism of peripheral fatigue. There is debate as to whether fatigue contributes to the processing at Central Nervous System (CNS) level. The impact on CNS functioning is regarded as central fatigue and has been extensively researched during muscle training (Gandevia 2001). This results in a reduction in the efferent input to the muscle. Inactivation of the muscle can result from a lack of drive in activating motor neurons from the CNS, or a reduction or inability of the neuromuscular system to activate the muscle to produce a force.

Short-term training with NMES is thought to induce changes in the central components of muscle activation (Maffiuletti, Pensini et al. 2002). This indicates a development in the muscles neuromuscular response rate. It is assumed that some central fatigue as a result of training is required for this change to occur, although research to support this is limited. Literature proposes that the synchronous firing of muscle fibres during NMES develops fatigue with a quicker onset than volitional muscle contraction (Baker, Wederich et al. 2000). Rest between contractions has been shown to be the optimal way to eliminate fatigue during stimulation (Gandevia 2001). This means that we are able to reliably assume that the frequency methodology (part one) has not induced fatigue, however the contraction as a direct result of the stimulation was at an intensity sufficient to induced fatigue. Fatigue has been highlighted in the NMES literature as a vital stimulus in the development of muscle hypertrophy (Stevens-Lapsley, Balter et al. 2012). The level of fatigue

required to induce hypertrophic changes and the threshold when fatigue results in a reduction of muscle force production requires further investigation. The average decline in force in part two of this study indicates that a low level of fatigue has been produced, which we can assume is beneficial to induce an increase in muscle strength.

Although 100 Hz produced the highest maximal moment when stimulating for one contraction, the impact over 45 muscle contractions requires consideration. Literature indicates that stimulating at higher frequencies for a long period of time will produce undue fatigue (Neyroud, Dodd et al. 2014). Interestingly, the level of detrimental fatigue has not been verified. NMES induced contractions of the plantarflexors at 75Hz resulted in both peripheral and central fatigue, which resulted in a motor function decline (Boerio, Jubeau et al. 2005). Functional decline was associated with reduced axon propagation, resulting in a decreased firing capacity of the neuromuscular system. NMES does not initially favour type one muscle fibres as seen during voluntary muscle contraction (Lieber 2009). Synchronicity of stimulation activates type two muscle fibres, resulting in a strong contraction due to their fast properties. This can be beneficial as a strong muscle contraction is produced which is often stronger than volitional capacity in an impaired population (Bax, Staes et al. 2005). However the impact of fatigue as a stimulus for change or a detrimental influence has not been tested here. Until this relationship is investigated this study will follow guidance from the literature and consider higher frequencies to have the potential to be detrimental over a longer period of training.

Variability has been identified at all frequencies, indicated by the high number participant outside the interquartile range on box plot analysis (graph 4.1). This indicates that there are differences in how a muscle responds to NMES. On consideration of individual participants, participant two was highlighted as causing the exceptionally high standard deviation found at 50 Hz (18.6). Participant two was a 31 year old runner, who ran 6 miles three days previous to study participation. He used a

moderate stimulation intensity of 35 mA. Although producing a higher than average MFP-ES at 50 Hz, the remaining frequencies produced moments in line with other participants. This implies that the participant assisted the contraction at 50 Hz, rather than detrimental fatigue from the previous exercise. It may be that he altered position in the leg measurement device (LMD), or pushed his foot onto the foot plate which would have resulted in a higher peak moment being recorded. When considering the participants attempt one and attempt two data separately it appears that there is a decline between the two sessions. The attempt one session resulted in a higher mean MFP-ES than the second attempt. This decline over time indicates that some residual fatigue was present, and his gastrocnemius was unable to hold the level of muscle contraction when all muscle fibres were stimulated synchronously. This may have considerable implications when administering a strength protocol with NMES, as the muscle may not have time to replenish its metabolites in the time it would do with a volitional contraction. Although this did not appear to be an issue during one training session with NMES, it will be interesting to see the pattern over a 6-week training period as is planned for subsequent studies. Participant two was removed from analysis in this study, allowing more reliable interpretation of the data to be performed.

Variation in individual response was expected, however ranged more than anticipated. As far as we are aware limited research has been conducted in artificially stimulated muscle, however factors that influence voluntary muscle contraction are assumed to apply to artificially contracted muscle. Aspects such as nutrition, hydration, previous level of exercise and sleep all have an effect on volitional exercise (McArdle, Katch et al. 2010), and although afferent signals are artificially produced, the efferent response is comparable. Minimal qualitative data was extracted from participants in this study; however deeper understanding of the condition of a muscle would allow more accurate assumptions on the relationship between force and frequency to be made. This would also indicate people who are more likely to respond to treatment with NMES, and allow certainty in clinical outcomes to be used if incorporated into a treatment programme. It must be

highlighted that when using NMES in a clinical setting, this data would not be available to the clinician, so the information gathered in this study is reflective of a clinical scenario.

Identification of peaks resulted in too much variability to reliably assume an optimal frequency. The variability also hindered the use of plateaux identification as an indicator of optimal frequency. When considering mean values, the greatest rise in MFP-ES was between 40 and 60 Hz. This prompted linear regression analysis. The rate of change identified the frequency producing the greatest response to stimulation with NMES. This has profound clinical implications as an increase in frequency is only warranted if the muscle is still producing an increased response to the stimulus. The muscle may produce a higher force as a result of stimulation, but given the variability in this aspect of analysis, the higher force must be justified by an increased muscle response. The optimal frequency carried forward to subsequent studies is supported by this analysis. Parameter setting should be focused on the aim of the stimulation. The aim throughout this thesis is to improve muscle strength: a larger response from the muscle is crucial to achieve this.

During part two there were three participants who produced a maximal electrically stimulated force production (MFP-ES) that was surprisingly higher than the other participants (although non-significant on testing). These participants also used the highest stimulation intensities. This indicates that the higher the stimulation intensity used to administer NMES the higher the force output of the muscle being stimulated. This is supported in literature (Stevens-Lapsley, Balter et al. 2012). Interestingly these participants did not produce the highest variability within their data set. Participant 3 demonstrated wide variability, and will be discussed separately, but participants 7 and 9 both produced a standard deviation of less than 3. This indicates that higher stimulation intensity does not have a detrimental influence on the muscle response to NMES.

It is interesting to note that the three participants who utilised a high stimulation intensity were all male. This indicates that the males in our sample were able to tolerate higher stimulation intensities than females. NMES literature supports this finding (Maffiuletti, Morelli et al. 2011). This could also be attributed to the level of testosterone in males influencing their competitiveness (Zilioli, Watson 2012). The 'competition effect' is a widely researched concept, and indicates that male testosterone levels remain elevated in those that succeed, with a constant drive to improve performance. This attribute would see males taking their stimulation intensity as high as they could, whereas females may more be more cautious with their application. This infers that males would see a greater benefit from NMES compared to females. Research in NMES needs to investigate this issue.

There was one participant who responded to NMES differently to other participants. Participant three was a 27 year old male. This particular participant increased their stimulation intensity by 21 mA from part one to part two. The results from this participant indicate a varied response of the gastrocnemius to stimulation, as indicated by graph 4.7. The decline of MFP-ES witnessed over the training programme suggests that this muscle produced a response to the stimulation. This may be a result of muscle fatigue. Muscle fatigue results in a reduced force output (Enoka 2012), as was witnessed in this participant. Damage to the muscle sarcolemma has been suggested to initiate satellite cell proliferation, and thus generation of new muscle cells (Schoenfeld 2010). The point where fatigue stops acting as a positive stimulus and begins to negatively affect the muscle cell has not been investigated to date. Comment on this is outside the scope of this study, however requires investigation to successfully apply NMES as a strength training modality. It may be that the muscle became accustomed to the intensity of the contraction causing force decline. Increasing the stimulation intensity throughout the testing protocol may have resulted in either a smaller amount of force decline, or an increase in force output. Other studies report much higher stimulation intensities ranging between 60 and 100 mA with little detriment to the muscle, and an increase in force output (Maffiuletti, Cometti et al. 2000). The subject may be able to tolerate higher stimulation

intensities if the neuromuscular system was well conditioned as a result of regular volitional training. We are unable to comment on this factor, as information regarding lifestyle was not collected during this study. As participant three was alone in this rapid pattern of decline, it is assumed that this is a response specific to this individual. An increase in sample size and expansion of outcome measures would help to reliably draw conclusions.

The stimulation intensity that participants used during the testing protocol was monitored and a linear relationship identified (graph 4.5 and 4.7). This indicates that the participants who stimulated with a higher intensity produced a greater maximal electrically stimulated force production (MFP-ES) of the gastrocnemius. This implies that the participant should be encouraged to use a high stimulation intensity to gain the greatest force from the muscle. Further investigation as to the effects of stimulation intensity is warranted based on this analysis. The relationship between stimulation intensity and pulse width has been identified (Brull, Silverman 1995). The linear response could also be present for stimulation intensity and frequency.

Participants did not report discomfort during stimulation, and were comfortable with testing procedures. Research has recently been conducted into the use of NMES in a hospital setting with similar subjective results reported (Broderick, Kennedy et al. 2011). Reported sensation of NMES tends to generate reluctance to its use in an acute clinical setting from clinicians (Gobbo, Maffiuletti et al. 2014, Schuhfried, Crevenna et al. 2012). A clear understanding of the effect of stimulation parameters on patient comfort and guidance of their use would help to address this issue. Participant comfort assumes that the acclimatisation session was effective in ascertaining maximal intensity, and allowed the participant time to adjust to stimulation sensation. More time could be spent on this aspect of the method, allowing a greater compliance with intensity to maximise force output.

4.9 Conclusion

The aim of this study was to highlight whether an optimal frequency exists, and is effective when stimulating to develop strength with NMES. **An optimal frequency of 50 Hz was identified with initial analysis and will be carried forward to future studies within this thesis**. The greatest rate of change in force production was produced between 40 and 60 Hz. The middle value of these three frequencies was considered to be optimal, as it would result in the largest force change during stimulation.

Subsequent analysis indicates that variability influenced our results and the optimal frequency carried forward may not be reflective of the overall picture. A higher frequency may be able to produce a similar, if not higher force response from muscle. On reflection, 60 Hz may be best placed to take forward as optimal frequency. This produces an area for further development along with the relationship between frequency and stimulation intensity.

Muscle fatigue is a factor that cannot be avoided with artificially induced muscle contraction (due to fibre recruitment pattern). Using fatigue as a stimulus for hypertrophy should be considered when using NMES for strength training. **Further investigation into the impact of fatigue for a NMES strength training protocol should be considered**. The variation of both stimulation protocols and muscle response in the literature makes it difficult to apply recommendations to protocol development within this thesis.

During one training session we anticipated that the muscle would demonstrate a slight decline in maximal force output over the training session, as a result of work done by the muscle: this would

indicate a small level of fatigue to stimulate hypertrophic changes. **The response was more varied than this with trends indicating an increase, decrease and stable response of MFP-ES to the training protocol.** The majority of participants demonstrated a small average decline of MFP-ES, which was confirmed via linear regression analysis. **This indicates that the protocol utilised was effective in producing a strength response from the muscle**. We will therefore carry this stimulation protocol forward for use in the remaining studies in this thesis.

This study indicates that force output as a result of stimulation with NMES increases with higher stimulation intensities. Further investigation into participant tolerance to stimulation would therefore positively affect the output from the muscle. Further use of the acclimatisation session and incremental increases in stimulation intensity during protocol application may be useful to maximise treatment response.

Chapter 5

Study 2: Is the response to NMES similar in younger and older adults?

The protocol used in study one (Chapter 4) produced a response that would allow the muscle to hypertrophy if continued. The protocol will therefore be carried forward into this study. A key outcome was the variation in response from the muscle. This will be monitored throughout this study to ascertain whether it continues and highlight any trends within this.

The protocol will now be tested in a full 6-week training protocol on different aged participants. It was decided to test two age groups as literature to date is often conducted on young healthy participants. This will help establish whether the effects of the proposed protocol are similar to that of current literature, and of a frail elderly population.

5.1 Introduction

Much of the NMES literature has focused on improving sporting performance, and has therefore used a young population to conduct their testing (Maffiuletti, Cometti et al. 2000). Research that has been conducted on an older population is often inconclusive, or highlights the need to conduct further testing (Elboim-Gabyzon, Rozen et al. 2013). When used in a clinical setting the aim of NMES is primarily restoration of muscle strength or prevention of disuse atrophy. This indicates that the fundamental population requiring intervention is the frail elderly, and the neurologically impaired. Research has begun to investigate the effects of NMES primarily for strength training in various pathological populations, including osteoarthritis (De Oliveira Melo, Aragao et al. 2013) and total knee arthroplasty (Stevens-Lapsley, Balter et al. 2012). However, inconsistencies in application of NMES and varying outcome measures have limited the ability of NMES to be integrated into a

clinical setting (Giggins, Fullen et al. 2012, Schuhfried, Crevenna et al. 2012). Understanding the fundamental principles of NMES application would allow intervention to be applied with more certainty. This understanding is required for NMES to be accepted as an intervention in a clinical environment.

The understanding of strength training using NMES in an athletic population is developing (Sillen, Franssen et al. 2013). However, there is limited evidence to suggest that we can transfer knowledge of NMES in young muscle, to that of older muscle. We can therefore not be certain of the effects of NMES in a frail population at this stage.

The population of frail elderly individuals is increasing given advances in medical technology and education (Mackenbach, Slobbe et al. 2012). Advancing age is concurrent with a decrease in muscle strength, and subsequent increased risk of falls (Evans 2010). Muscle strength has been shown to increase with a volitional strength training protocol delivered to the elderly, which has positively impacted on daily functional activities (such as sit to stand endurance) and a decreased risk of falling (Fahlman 2011). This change of thought in treating the frail elderly is beginning to embed into clinical settings. However it does not take into account that some individuals are unable to perform exercise to the intensity required to see improvements in strength and function.

Muscle strength is increasingly hard to restore with age, given the rapid onset of sarcopenia. In order to achieve this an externally stimulated intervention to initiate the strength training process may be useful. It is important to focus on increasing this population's level of mobility, which will have a direct consequence on their quality of life (Li, Fisher et al. 2003). A greater understanding of different aged muscle's response to strength training with NMES will also help to prepare

appropriate rehabilitation programs which are capable of achieving their aims: to induce muscle strengthening.

5.2 Aim

This study aimed to test the proposed protocol on healthy participants of differing ages. The comparison of a younger population compared to that of an older population will help to establish treatment effect and address some of the gaps in the current literature. The effects on an older population will help this thesis to achieve its overriding aim: to establish the effects of NMES in reversing age-related muscle atrophy.

5.3 Methodology

Design: Unblinded RCT with stratification based on age. Ethical approval was obtained from the South Staffordshire Local Research Ethics Committee to conduct this study (reference 09/H1203/92, appendix 1).

Sample size: Twenty healthy participants were recruited into this trial: 10 in the younger category (5 treatment 5 control), and 10 in the older category (5 treatment, 5 control).

Method of recruitment: Participants were recruited from the Robert Jones and Agnes Hunt Orthopaedic NHS Foundation Trust staff, and a local University of the third age group (U3A). Both hospital staff and external participants were approached via email, providing contact details of the researcher. Participants emailed, or telephoned the researcher for more information about the study: which was responded to by telephone call, and a participant information sheet. Participants

were given 24 hours to decide whether they would like to participate, and this time period was followed up with a telephone call to discuss any questions that arose.

Inclusion criteria: Participants were invited to partake in this study if they fulfilled the following criteria:

- Aged within either age bracket: 18-30 or 60-80
- In a good state of health
- Able to comfortably tolerate NMES protocols

Exclusion criteria: Participants were screened for contraindications which included:

- Active pacemakers
- Uncontrolled epilepsy
- Musculoskeletal injuries of the lower leg in the past 8 weeks
- Any contraindications to using NMES

Informed consent: Written informed consent to participate in the study was obtained once the participant was screened for contraindications, and had been provided with all relevant details of study involvement.

Method of randomisation: Participants were randomly allocated to the treatment or control group. 10 of the places were allocated to the treatment arm of the study, and labelled 'treatment old' (n = 5) or 'treatment young' (n = 5), and the remaining 10 allocated to the control arm of the study, and labelled 'control old' (n = 5) or 'control young' (n = 5). Randomisation cards were placed in blank envelopes, which were shuffled and offered to the participant after informed consent had been

obtained. Randomisation envelopes were compiled by an individual who was independent of study testing or participation.

Testing position: Testing was conducted on the non-dominant Gastrocnemius, and the participant was positioned in an identical position to study one (detailed in section 4.3). Throughout all treatment applications and outcome measurement testing the participant was positioned in the Leg Measurement Device (LMD). Their knee joint was positioned in 60° flexion, and their ankle joint in a plantargrade position. This is the same as study one (see image 4.1).

Electrodes (50 X 90 mm) were placed horizontally across the two heads of gastrocnemius, as detailed in study one (Baker, Wederich et al. 2000). See image 4.2. Modifications to electrode position were made if the participant reported noxious stimuli associated with stimulation. NMES depolarises cutaneous sensory nerve endings, which have a lower propagation threshold than alpha motor neurones, due to their superficiality (Sheffler, Chae 2007). Alteration of electrode position and application of some water on the under surface of the electrode are recommended in the literature to make stimulation more comfortable.

Before the first treatment session an acclimatisation session was performed where the participant was introduced to the sensation of NMES, and the intensity of a single contraction that would be administered during the protocol. This also allowed an understanding of participant tolerance to stimulation. Stimulation intensity was gradually increased over a number of muscle contractions to a level that the participant was comfortable with, but considered their maximal. This intensity was used to stimulate the muscle on two more occasions, allowing a further increase if the participant became accustomed to the setting. Once the stimulation intensity was set for the initial treatment session, the researcher explained how the session would progress (15 minutes). This time span would eliminate any possible effects of fatigue before the session begun.

5.3.1 Treatment group

Participants in the treatment group underwent a 6 week strength training protocol of NMES on their non-dominant gastrocnemius. Testing was restricted to one muscle due to time constraints; testing of two muscles would result in double time required for each participant for full outcome measure assessment to be implemented. We chose to test the Gastrocnemius as it has been shown to produce training responses that are representative of all muscles (Lieber 2009).

Treatment sessions involved 45 individual muscle contractions on their non-dominant Gastrocnemius, on 3 different days a week, over a 6 week period. The participant was positioned in the LMD during each treatment session. Stimulation parameters are detailed in table 5.1. Stimulation parameters were utilised due to recommendations from the scientific literature (see literature review, chapter 2), and from the results of previous studies (Chapter 4). Chapter 5 details the development of this protocol, which will be used throughout this thesis to allow for comparability.

Stimulation parameter	***Setting***
Frequency	50 Hz
Pulse Width	450 µs
'On' time	3 seconds
'Off' time	10 seconds
Ramp times	0.5 seconds
Intensity	Maximal tolerated (ma)

Table 5.1 Stimulation parameters used throughout testing

Stimulation intensity was set to maximal at the beginning of each testing session. This allowed increases to be made from acclimatisation to the sensation of NMES, or any muscle response throughout the treatment programme. Stimulation intensity was monitored throughout the 6-week protocol.

Stimulation from the NMES protocol produced a muscle contraction of the Gastrocnemius. This plantarflexion moment was recorded via the footplate of the LMD. Data was collected in real time via a Data Link Biometrics system (Biometrics LTD), which was connected to the LMD via a laptop. Participants were not shown real time data, as we did not want this to influence remaining results. Results were later transferred to a Mathcad spreadsheet for outcome measure analysis (details provided in outcome measure section below).

5.3.2 Control group

The control group participants underwent outcome measure testing, but did not participate in any other intervention.

5.4 Outcome measures

Outcome measures were collected at baseline, at 3-weeks and completion of the 6-week study period. Outcome measures are described below:

Volitional Maximal Isometric Force Production (MIFP)

Isometric volition force production was assessed, to establish any carry over effects of treatment with NMES. Volitional Maximal Isometric force production (MIFP) was assessed via the LMD which was designed to measure moments about the knee and ankle joints (further details provided in

chapter 3). The participant was positioned in the LMD, as described previously. Volitional gastrocnemius force production was measured with the participant's ankle in a plantargrade position, and the knee joint at 60° flexion. Mid-range of the gastrocnemius fibres will enable the optimal force to be produced, and allows a standardised position that is comparable to literature (Reese, Bandy 2009).

Gastrocnemius volitional force production was assessed by asking the participant to push their foot down onto the footplate as quickly, and as hard as possible. Verbal encouragement was given. Maximal force was regarded as the highest of 3 attempts: which will be referred to as MIFP for the remainder of this chapter.

Pennation angle

Pennation angle was considered to be the angle that which the muscle fibre inserted into the superficial muscle aponeurosis, as measured by 2D ultrasonography. Pennation angle has been shown to relate to muscle strength (Aagaard, Andersen et al. 2001), therefore this would give an indication of muscle adaptation to the NMES protocol.

Pennation was measured both at baseline and after the 6-week intervention period. The measurements were conducted with the participant lying in a prone position. The participant was asked to hang their feet off the edge of a treatment plinth, in order to maintain a plantargrade position. The lateral head of the gastrocnemius used throughout testing was measured.

The probe position for measurement was identified by finding the central point between the proximal border of the muscle and the myo-tendinal junction. The probe was placed longitudinally

at the mid-way point between the medial and lateral border of the muscle belly, or as close to the point as possible to obtain a clear image of the muscle fibres. Multiple images were taken for analysis.

Images were extracted from the ultrasound machine via USB connection, and transferred to a computer for analysis with ImageJ software (ImageJ LTD). Pennation angle was manually identified (image 5.3), and the mean of three images considered pennation angle (Koppenhaver, Parent et al. 2009).

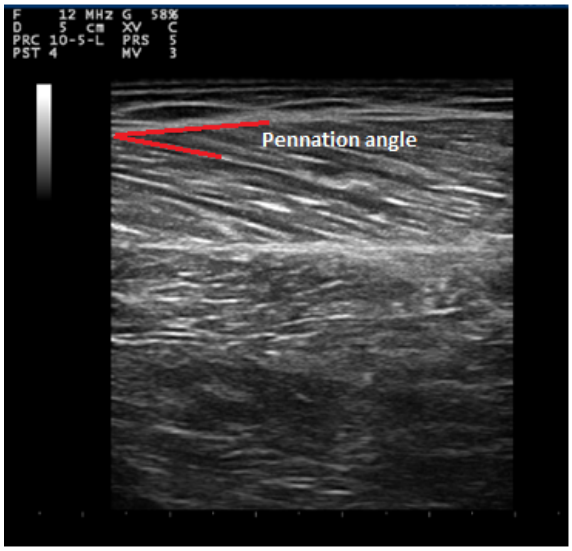


Image 5.3 example ultrasound image

Maximal Electrically Stimulated Force Production (MFP-ES)

MFP-ES was considered to be the maximal plantarflexion moment (Nm) achieved during each contraction of the NMES protocol. Previous literature has suggested that NMES predominantly recruits type 2, fatigable muscle fibres in a synchronous fashion (Maffiuletti 2010). For this reason NMES is often thought to cause detrimental fatigue, which has been implied as a reason for its lack of clinical use (Chou, Binder-Macleod 2007). Measuring MFP-ES during the NMES application will

establish if the level of force produced during the stimulation protocol reduced over time, and also if the peak force of contraction alters over the 6-week testing period.

MFP-ES was calculated once the data from the NMES protocol was extracted and transferred to a mathematical software spreadsheet (Mathcad). Data was extracted from each of the 45 muscle contractions. Maximal moment from each contraction was identified and regarded as peak moment; this value will be referred to as MFP-ES. This outcome measure also provides a measure of treatment compliance; was the participant able to relax throughout the electrically induced contractions, without assisting the contraction.

Duration of NMES contraction (DUR)

DUR was calculated for each muscle contraction produced from the NMES protocol. Data was extracted from the LMD, and calculated in the Mathcad spreadsheet. DUR was considered to be the period of time (seconds) that the muscle was in a contracted state as a response of the NMES protocol. A contracted state was considered to be when force exceeded 20% of the baseline jerk (measured in seconds from when the muscle began to contact). DUR was considered until the force fell below 20% of jerk at the end of the contraction. Figure 4.3 presents a muscle contraction as used for analysis. The blue line indicates jerk, and the red force. DUR allowed the real 'on' time as a result of stimulation to be established, and also indicated the time taken for the muscle to respond to the electrical stimulus (Time on minus DUR). This would give indication to adaptations in the neuromuscular system in response to the NMES protocol.

5.5 Data analysis

This study utilised four ratio level outcome measures. Two of the outcome measures relate to the volitional capacity of the muscle, and the other two the muscle response to treatment with NMES. A parametric approach to analysis was conducted to identify both trends within groups and within individuals. Descriptive statistics were used (mean, standard deviation, effect size and graphs) as a means of studying magnitude of trends given the small sample recruited in each sub-group.

When considering Maximal Isometric Force Production (MIFP) three measurement attempts were reduced to one for analysis. The maximum of three attempts was considered for analysis, as opposed to calculation of the mean. Maximal consideration of MIFP both encouraged the participant to achieve their best force output and allowed analysis of the muscles maximal force generating ability. Literature suggests that the second attempt of three tends to hold the highest value, and that similar reliability has been shown with one attempt, mean values or maximal consideration (Roberts, Denison et al. 2011).

MIFP was analysed by comparing descriptive statistics before and after treatment. This allowed for variation in baseline to be accounted for. Both treatment groups improved MIFP from baseline, however variation has been highlighted within this. Paired sample T-tests indicate that the effect is significant for both groups. Effect size was calculated to ascertain the magnitude of treatment effect, which was greater in the under-30 treatment group.

A proportional relationship was observed when comparing differences in pennation angle before and after treatment. This indicates that pennation angle increased post NMES, an effect that was greater in the under-30 treatment group. A relationship was also investigated between MIFP and

pennation angle, however this was not clearly distributed. Paired T-tests indicate a significant difference during the six-week intervention period for both treatment groups.

When looking at Maximal Electrically Stimulated Force Production (MFP-ES) we firstly compared descriptive values over time, comparing among age groups. It appeared that variation within groups was dependant on initial tolerance to stimulation. Error bars highlighted trends within age groups, and variation within this. The variation was greater in the over-60 treatment group. Curve estimation analysis indicates a rise in mean rate of improvement over time, indicating that the muscle was able to increase its artificial force generating capacity. Within session data suggests decline over the 45 stimulated contractions. The relationship with stimulation intensity was also investigated, indicating that a rise in stimulation intensity resulted in an increase in MFP-ES.

Duration of NMES contraction (DUR) was compared via descriptive statistics over time. DUR was a small measurement of change within the 3-second stimulation period; percentage increases were able to demonstrate this as a clinically relevant change. Error bars indicated the changes within age categories, which were described on an individual basis. The under-30 treatment group demonstrated a relatively steadily increase, with the over-60 treatment group highlighting some fluctuation in response. Despite this variation, linear regression analysis indicates a positive rate of change. This indicates that both groups increased DUR as the treatment period progressed.

5.6 Results

Nineteen participants completed the six week strength training intervention with NMES to their gastrocnemius. One treatment participant withdrew in the early stages of the study due to time constraints preventing attendance of treatment sessions. Raw data is presented in appendix 4 a-c.

Table 5.2 identifies the sample size within each group. All participants were able to tolerate treatment protocols, and no adverse effects were reported as a result of stimulation. Participant demographics are displayed in table 5.3.

Under 30	T (n=4) C (n=5)
Over 60	T (n=5) C (n=5)

Table 5.2 Sample size for each age category T: treatment, C: control

	Age (years)	SD	Gender
Under-30	24.8	2.5	Male n = 5 Female n = 5
Over-60	69.8	4.8	Male n = 7 Female n = 3

Table 5.3 Participant demographics

The age of participants recruited into this study had a low standard deviation, which was slightly higher in the over-60 category (table 5.3). This is reflective of the convenience sample recruited. The gender split between allocated treatment arms differs between age group. There were 2 males and 3 females in the under-30 treatment group, compared to 4 males and 1 female in the over-60 treatment group. The under-30 treatment participant who withdrew was a female.

Maximal Voluntary Isometric Force Production (MIFP)

MIFP has been defined as the maximal of three attempts of gastrocnemius voluntary force production. MIFP was assessed both before and after the 6 week intervention period. Table 5.4 shows the descriptive statistics for MIFP displayed for treatment group and age.

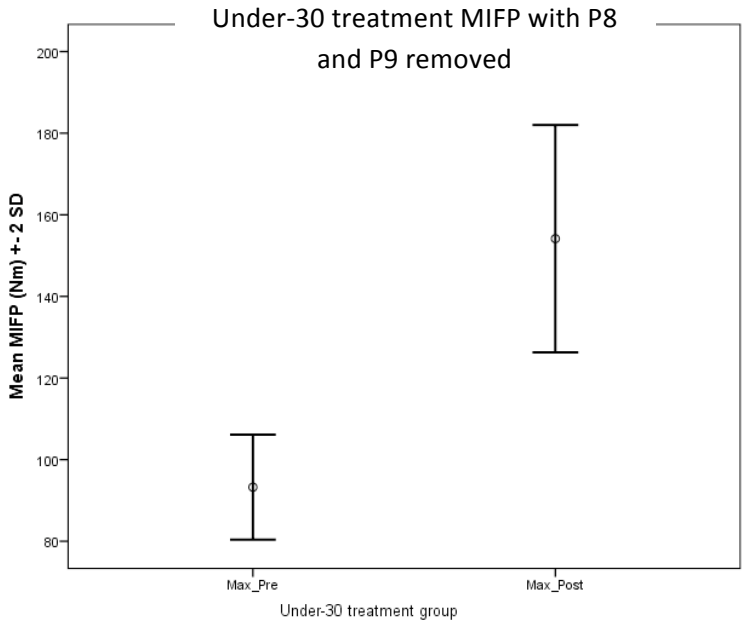
	Mean (SD) of MIFP (Nm)	
	Pre	**Post**
U30 Control	**63.08 (17.2)**	**64.02 (15.9)**
U30 Treatment	**76.5 (20.6)**	**109.7 (51.9)**
O60 Control	**56.6 (15)**	**61.6 (20.1)**
O60 Treatment	**45.4 (20.3)**	**64.9 (21.6)**

Table 5.4 Descriptive statistics (Mean and standard deviation- SD) of MIFP pre and post treatment intervention (measured in Newton Metres- Nm).

U30: Under 30, O60: Over 60

The mean of the maximal MIFP is displayed in table 5.4, which has been categorised by age and treatment group. Results indicate that the intervention with NMES had an effect on MIFP in both the under-30 and over-60 age groups. When looking at the under-30 group, maximal MIFP increased by 0.94Nm in the control group, and by 33.2Nm in the treatment group. This compares to an increase of 5Nm in the over-60 control group, and 19.5Nm in the over-60 treatment group. Both treatment groups displayed an increase in mean maximal MIFP compared to their respective control group, and when comparing both treatment groups the under-30 treatment group showed an increase of 13.7Nm more than the over-60 treatment group.

When looking at table 5.4 it is clear to see that the MIFP varies significantly from each group at baseline. Baseline considerations were not taken into account during analysis, due to the small sample size in each group. Measurements were considered in relation to participant individual baseline attempt, helping to make analysis of strength changes as accurate as possible. A small sample size also warrants individual analysis of results. As expected the under-30 groups baseline mean maximal MIFP is higher than the over-60 groups, however there is also a difference between groups. The under-30 treatment group were on average 13.4 Nm stronger than their respective control group at baseline, and the over-60 control group was 11.2 Nm stronger than their respective treatment group. The under-30 treatment group demonstrate a high level of variation within their sub-group, with a standard deviation of 51.9 post-treatment (table 5.4). P8 demonstrated a decline in MIFP over the 6-weeks (1.3 Nm), which was the only decline witnessed in this sub-group. P9 demonstrated a small increase of MIFP in comparison to other participants (20.69 Nm smaller). Removal of P8 and P9 from the under-30 treatment group demonstrated smaller standard deviations in post MIFP (13.9), as highlighted in graph 5.1. Removal of these two participants results in a sample size of two, which will be discussed later in the chapter.



Graph 5.1 Under-30 treatment MIFP with P8 and P9 removed

Differences between age categories and within age categories have been investigated via paired t-tests (table 5.5). Both treatment groups demonstrated a significant increase in MIFP over the 6-week intervention with NMES, indicating that NMES has an influence on voluntary muscle strength.

Group	Pair	95% confidence interval		P (<0.05)
		Lower	Upper	
T30	Pre-Post	-49.495	-11.222	.005
C30	Pre-Post	-8.147	6.013	.751
T60	Pre-Post	-32.074	-9.766	.001
C60	Pre-Post	-13.224	6.374	.458

Table 5.5 Paired samples T test to test for significance of MIFP for each age category (T= treatment, C= control)

The effect size of MIFP change is greater in the under-30 participants (1.5) compared to the over-60 participants (0.9), as shown in table 5.6. The indication is that the magnitude of effect shown in MIFP is greater in the under-30 participants. Wider confidence intervals exist in the under-30 participants which is reflective of the small sample used.

Group	Effect size	95% confidence interval	
		Lower	Upper
U30	1.5	-13.4	16.4
O60	0.9	-5.9	7.7

Table 5.6 Effect size for MIFP in both the Under-30 (U30) and over-60 (O60) age groups

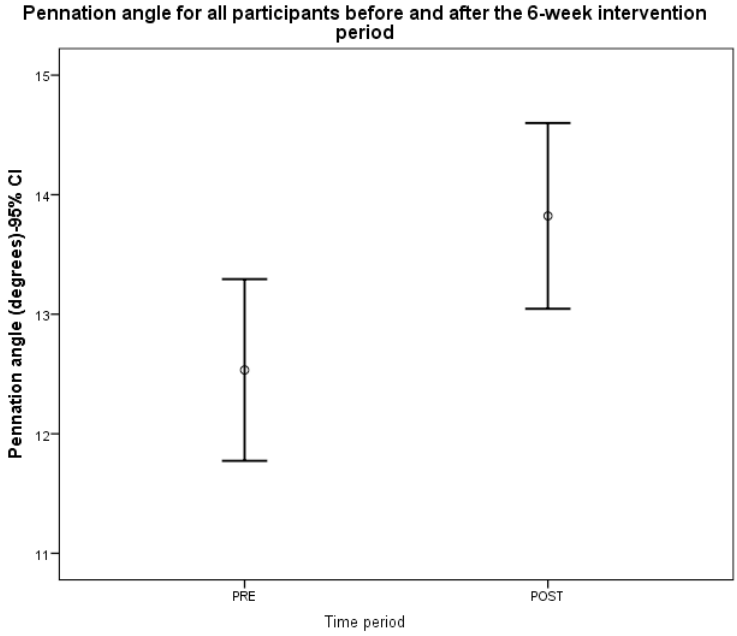
Pennation angle

Pennation angle of the lateral head of gastrocnemius was measured via 2D ultrasonography. Measurements were taken at baseline (PRE) and after the 6-week intervention period (POST). The researchers measurement error was not recorded. Pennation angles are summarised in table 5.7 based on treatment group and age.

Group	**Pennation angle (degrees)**		**Difference (degrees)**
	PRE	**POST**	
C30	**14.8**	**15.3**	**0.4**
T30	**13.2**	**15.6**	**2.4**
C60	**11.2**	**11.3**	**0.1**
T60	**11.3**	**13.5**	**2.2**

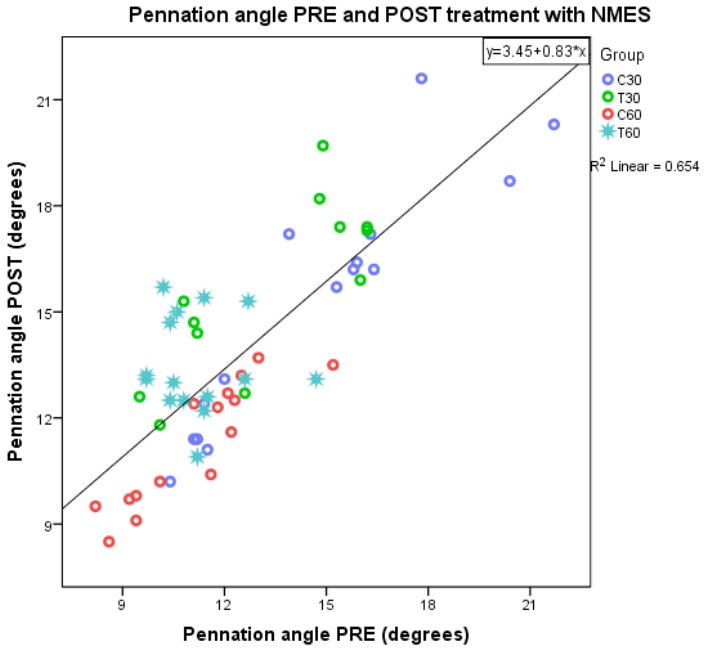
Table 5.7 Pennation angle of treatment group from pre to post (degrees) C=control, T=treatment

Table 5.7 indicates that both treatment groups increased pennation angle over the 6-week intervention period. The under-30 treatment increased pennation angle by 0.2° more than the over-60 treatment group. There was also a rise witnessed in both control groups, by 0.3° more in the under-30 group. All participants demonstrated an overall rise in pennation angle; expect one control participant in the over-60 age category who demonstrated a decline of 0.1°. The greatest increase of pennation angle was made by P4, a female participant in the over-60 age category (3.7°). Variation at baseline is noted; however individual differences from PRE to POST have been used for analysis. The highest baseline is the control group of the under-30 age category, which is 1.6° higher than their respective treatment group. Pennation angle for all participants is depicted in graph 5.2.



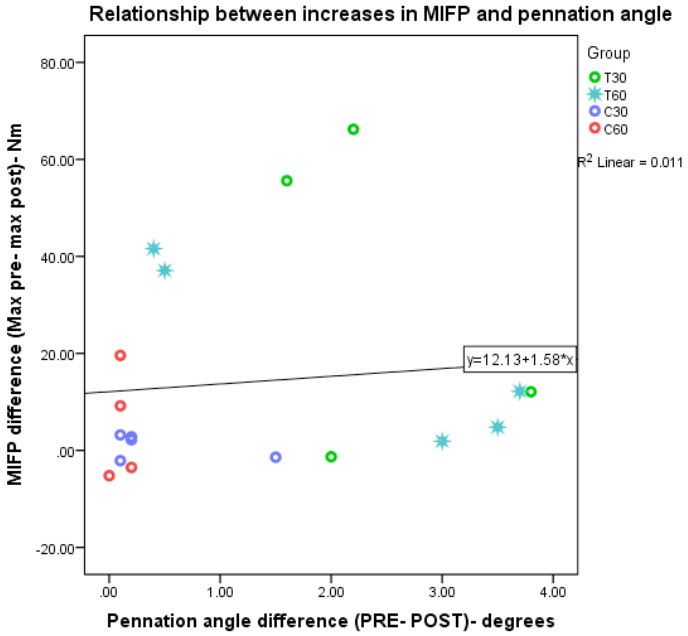
Graph 5.2 pennation angle for all participants before and after the 6-week intervention period

The scatter graph below shows pennation angle PRE and POST treatment with NMES (graph 5.3). A proportional relationship exists (R^2= 0.654) between the two measurements, with POST treatment values increasing alongside PRE treatment. Markers have been set by group: the highest pennation angles were witnessed from the under-30 age category, followed by the over-60 age category. More variability appears to come from the under-30 age category.



Graph 5.3 Pennation angle PRE and POST treatment with NMES (degrees)

Pennation angle was also analysed in relation to maximal isometric force production (MIFP), to ascertain relationship with increases in voluntary strength (graph 5.4). Both control groups (red and blue circles) demonstrate a small increase in MIFP and a small increase in pennation angle. The relationship within the treatment groups is more varied. When looking at the under-30 treatment group (green circles) the increases in pennation angle were higher than the respective control group, however a wide range of increases in MIFP is demonstrated (-1.3 Nm to 66.20 Nm). The over-60 treatment group (turquoise stars) again demonstrated some variability. The increase in pennation angle from PRE to POST was more varied in this group (0.4° to 3.7°), as was the MIFP. Two participants increased by a mean of 39.3 Nm (SD: 3.2), and the other three by a mean of 6.3 Nm (SD: 5.3).



Graph 5.4 Relationship between increases in maximal isometric force production (MIFP, measured in Newton metres, Nm) and pennation angle (degrees)

Comparison of pennation angle differences between age and treatment group was conducted through a paired t-test. Table 5.8 indicates that a significant finding was observed for both treatment groups (P=<0.05). This implies that the response of the muscle to NMES can be attributed to the treatment. The wide confidence intervals must be noted. Both control groups did not display a significant finding, however the under-30 control group demonstrated more of a difference (P=0.179).

Group	Pair	95% confidence interval		P (<0.05)
		Lower	Upper	
C30	PRE-POST	-1.342	0.276	.179
T30	PRE-POST	-3.416	-1.351	.000
C60	PRE-POST	-0.624	0.304	.472
T60	PRE-POST	-3.384	-1.216	.000

Table 5.8 Pennation angle paired T-test results

C=control, T=treatment

Maximal Electrically Stimulated Force Production (MFP-ES)

MFP-ES is a measurement of the strength of gastrocnemius contraction elicited artificially by each contraction of the NMES protocol. Control group participants did not take part in the NMES protocol, hence are not included in this analysis. The NMES protocol delivered 45 muscle contractions during each treatment session, which were measured by the leg measurement device (LMD). These have been converted into Newton Metres (Nm), and the average per week displayed in table 5.8 (total number of data points for all treatment participants N=5085). Data for MFP-ES for the final under-30 treatment participant was unavailable (P9), due to noise in the data set. MFP-ES for the under-30 treatment group has been analysed with n=3 as a consequence of this (total data points N=2055).

Participant	Week1	Week2	Week3	Week4	Week5	Week6
P1 (O60)	**15.14**	**12.02**	**15.97**	**30.76**	**20.08**	**28.94**
P2 (O60)	**21.62**	**25.06**	**26.69**	**29.72**	**25.67**	**23.66**
P3 (O60)	**16.92**	**18.79**	**21.80**	**26.71**	**28.13**	**22.09**
P4 (O60)	**40.66**	**49.49**	**76.31**	**50.71**	**67.47**	**76.39**
P5 (O60)	**35.71**	**62.66**	**76.07**	**62.38**	**70.61**	**75.52**
P6 (U30)	**29.23**	**79.05**	**74.59**	**117.75**	**138.35**	**185.44**
P7 (U30)	**63.01**	**88.08**	**104.52**	**128.24**	**141.02**	**152.44**
P8 (U30)	**177.97**	**147.36**	**162.66**	**190.27**	**181.96**	**208.04**

Table 5.9 Mean MFP-ES per week for all treatment participants (Nm)

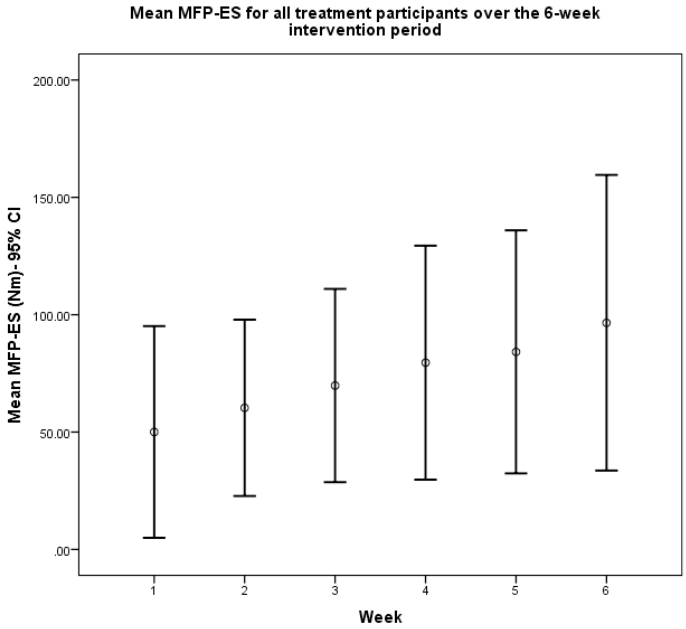
O60: over-60, U30: under-30

Table 5.9 indicates that mean MFP-ES increased for all participants over the six week intervention period. Table 5.10 demonstrates the increase in MFP-ES from week one to week six. The under-30 sub group produced a higher mean force production as a result of the NMES contraction (72.6 Nm higher), but also a mean increase of 91.9 Nm between week 1 and 6. The over-60 participants increased by a mean total of 19.31 Nm. P6 from the treatment category displayed the greatest increase in MFP-ES (156.21 Nm), which is 66.79 Nm greater than P7 and 126.14 Nm greater than P8. When looking at the raw data P8 has the greatest MFP-ES value of 208.04 Nm in week 6. This participant produced a MFP-ES of 177.97 Nm in week 1, indicating a good initial tolerance of the treatment protocol. P6 had a low MFP-ES (29.23 Nm) in week 1, which increased by 49.82 Nm by week 2. A steady rise in MFP-ES was then demonstrated over the following weeks. This indicates that initially P6 had low tolerance of NMES, and potentially not utilising maximal stimulation intensity in week 1.

Participant	Increase (Nm)
P1 (O60)	13.80
P2 (O60)	2.04
P3 (O60)	5.17
P4 (O60)	35.74
P5 (O60)	39.81
P6 (U30)	156.21
P7 (U30)	89.42
P8 (U30)	30.07

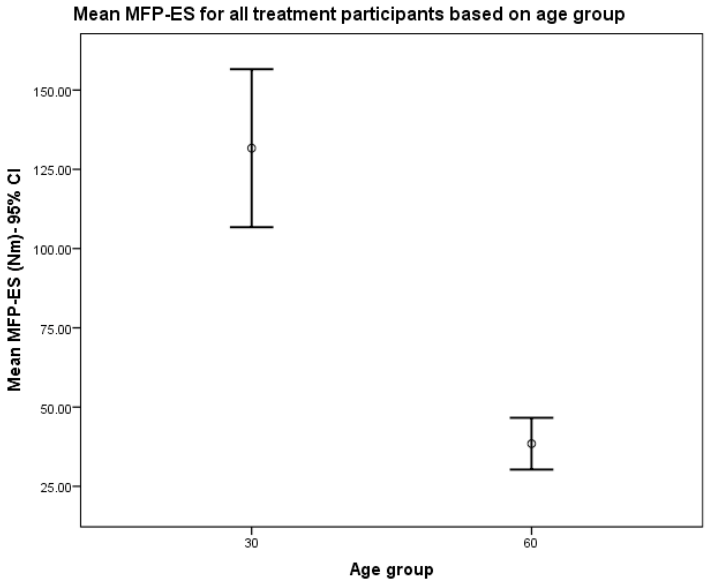
Table 5.10 Mean increase in MFP-ES from week one to week six (Nm) O60: over-60, U30: under-30

Graph 5.5 shows how all participants display a steady increase in MFP-ES over the 6 week intervention period, despite age category. This indicates that participants either tolerated a higher stimulation intensity as the protocol progressed, or the muscle was able to produce more force as a consequence of the NMES. The error bars display a 95% confidence interval for the mean, showing that our data has some variation, which increases towards the later stages of the study.



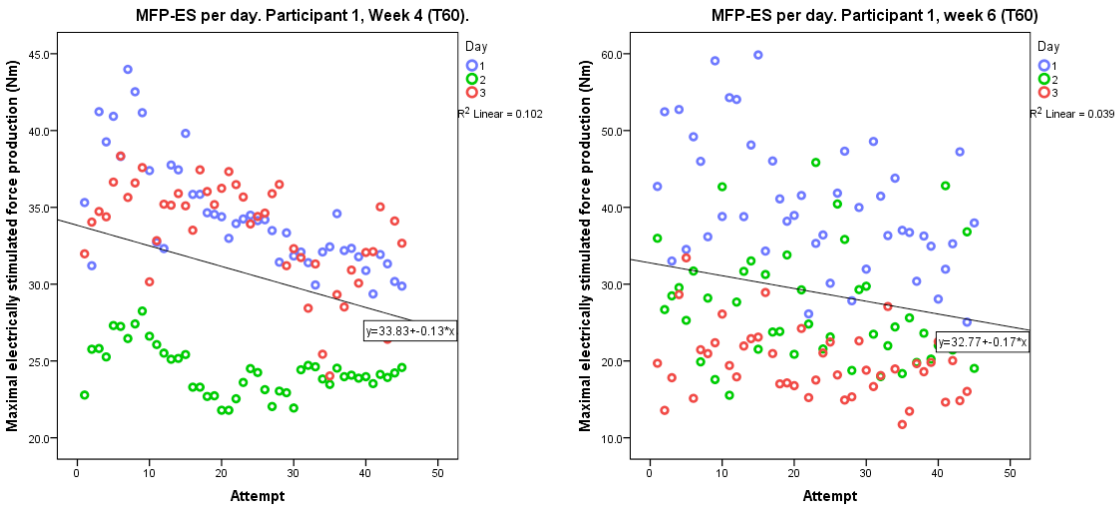
Graph 5.5 Mean MFP-ES of all participants during the six week NMES intervention period, displayed in Nm

When looking at graph 5.6 the under-30 participants produced a greater MFP-ES as a result of NMES than the over-60 participants. (P= <0.05, 95% CI 97.11-101.88) However, the error bars indicate a much greater variation of force in the under-30 participants than the over-60. The MFP-ES of the over-60 category was 64.37 Nm, whereas in the under-30 category it was 178.81 Nm.



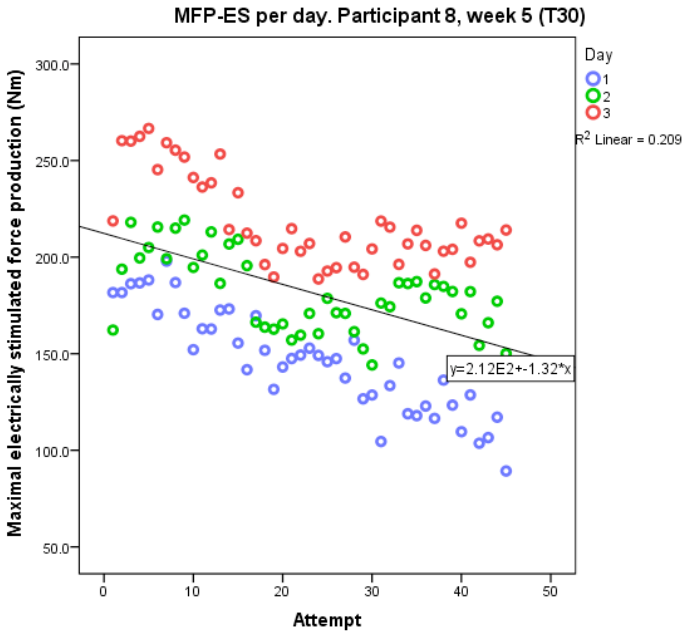
Graph 5.6 Mean MFP-ES of the six week NMES intervention period in both groups, displayed in Nm. '30': under-30, '60': over-60.

The over-60 participants demonstrate a steady rise of MFP-ES over the first 3 weeks of treatment. Between week 3 and 4 the contraction level fell by 3.31 Nm, and then increased by another 5.26 Nm in the final two weeks. The under-30 participants also display a linear increase in MFP-ES over the 6 week treatment period. The increase in this age category is greater, with a rise of 91.9 Nm from week 1 to week 6. There are no declines evident between weeks, and the greatest increase in MFP-ES occurs between week 3 and 4, with an increase of 31.5 Nm.



Graph 5.7 Variation within participant. Both graphs relate to participant 1 (treatment, over-60). The left hand graph is week 4, the right hand graph is week 6.

Analysis has been conducted using means of maximal electrically stimulated force production (MFP-ES) per week. However, it is important to take the variation within participant data into consideration. Graph 5.7 demonstrates the variability within participant 1. The response to NMES in week 4 (left hand graph) is proportional; the response of the muscle to NMES declines in a steady fashion as the attempts increase. However at week-6 (right hand graph), the response is more varied, with little pattern emerging between MFP-ES and attempt. When considering the under-30 participants, more linearity is displayed (graph 5.8), indicating that the muscle response to NMES is less variable in younger participants.



Graph 5.8 Relationship between MFP-ES and attempt. Participant 8, week 5 (treatment, under-30)

Table 5.11 displays results from a curve estimation analysis. This indicates that the under-30 age group had a mean rate of improvement of 18.22 Nm (P=0.004). The over-60 group had a smaller rate of improvement, with a value of 3.42 Nm.

	Curve estimation analysis		
	R square	**P (95% confidence interval)**	**Rate of improvement (Nm)**
Over-60	**.073**	**.148**	**3.42**
Under-30	**.409**	**.004**	**18.22**

Table 5.11 Curve estimation analysis of MFP-ES for both age groups.

Table 5.12 indicates the current intensity used when administering NMES, and its variability over the six week NMES intervention period. The mean values in the final column indicate that the distribution is quite varied, with no distinct pattern noted. It is interesting to note that P7 displayed

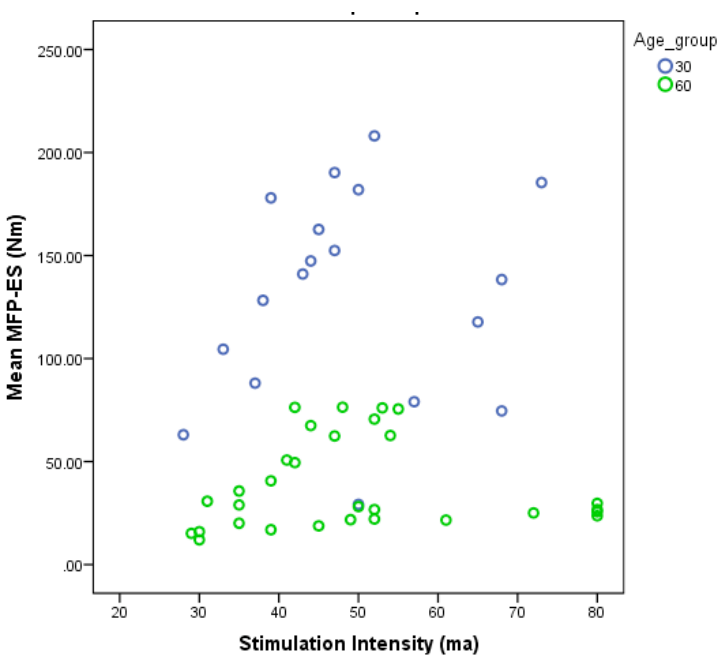
the second lowest mean current intensity, however produced the second highest mean increase in MFP-ES over the 6 week intervention period. When looking at the raw data of Maximal Isometric Force Production (MIFP) P7 increased by 66.2 Nm, which is the highest of the under 30 treatment participants. The highest current intensity available on the NeuroTrac device used in this study was 80 mA, and P2 (an over-60 participant) was the only participant to reach this threshold.

	Stimulation Intensity (ma)						
Participant	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Mean
P1 (O60)	29	30	30	31	35	35	32
P2 (O60)	61	72	80	80	80	80	76
P3 (O60)	39	45	49	52	50	52	48
P4 (O60)	39	42	42	41	44	48	43
P5 (O60)	35	54	53	47	52	55	49
P6 (U30)	50	57	68	65	68	73	64
P7 (U30)	28	37	33	38	43	47	38
P8 (U30)	39	44	45	47	50	52	46

Table 5.12 Mean stimulation intensity per week for each participant (displayed in milliamps- ma)

Stimulation intensity was considered in relation to MFP-ES, in order to establish any trends within age groups (graph 5.9). As expected MFP-ES is higher in the under-30 category compared to the over-60 category. There is a wide range of stimulation intensities across both age groups, and no clear relationship is noted. This is reflected by the mean current intensity of both groups being similar; 50 mA for the over 60 group, and 49 mA for the under 30 group.

Relationship between stimulation intensity
and mean MFP-ES based on age group



Graph 5.9 Relationship between mean MFP-ES and stimulation intensity

Duration of NMES contraction (DUR)

DUR is a measurement of how long the muscle is producing force during the NMES contraction. The table below (5.13) displays the mean value of DUR during each week of contraction for each participant. Control group participants did not receive intervention with NMES and are therefore not included in this analysis. Total number of data points for all treatment participants N=5055. The table below displays a consistency of DUR over the six-week intervention period, with some participants demonstrating an increase (table 5.14). Missing data points relate peaks that were unable to be read from the Mathcad spreadsheet.

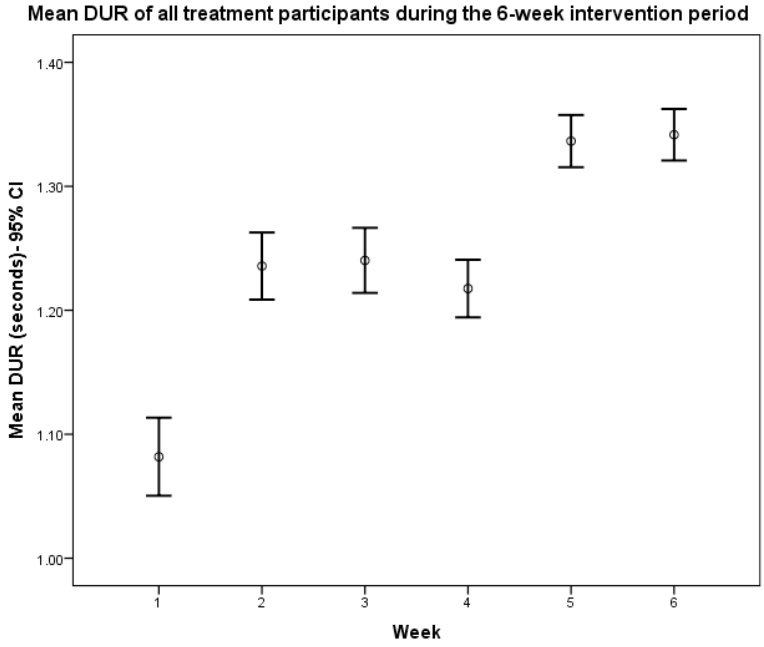
Participant	Mean DUR (seconds)					
	Week1	Week2	Week3	Week4	Week5	Week6
P1	0.78	1.06	0.68	0.84	0.78	0.90
P2		1.18	1.51	1.58	1.63	1.70
P3	1.16	1.22	1.23	1.23	1.26	1.28
P4	1.45	1.40	1.46	1.56	1.49	1.50
P5	1.33	1.42	1.43	1.44	1.48	1.53
P6	0.79	0.82	0.88	0.93	1.15	1.14
P7	1.28	1.13		0.95	1.33	1.35
P8	1.33	1.44	1.45	1.50	1.46	1.53
Mean	1.18	1.31	1.26	1.33	1.33	1.38

Table 5.13 Mean DUR over the six week intervention period for each participant (displayed in seconds)

Participant	Difference	Percentage increase (%)
P1 (O60)	0.12	15
P2 (O60)	0.52	44
P3 (O60)	0.11	10
P4 (O60)	0.05	3
P5 (O60)	0.19	15
P6 (U30)	0.35	44
P7 (U30)	0.07	5
P8 (U30)	0.20	15

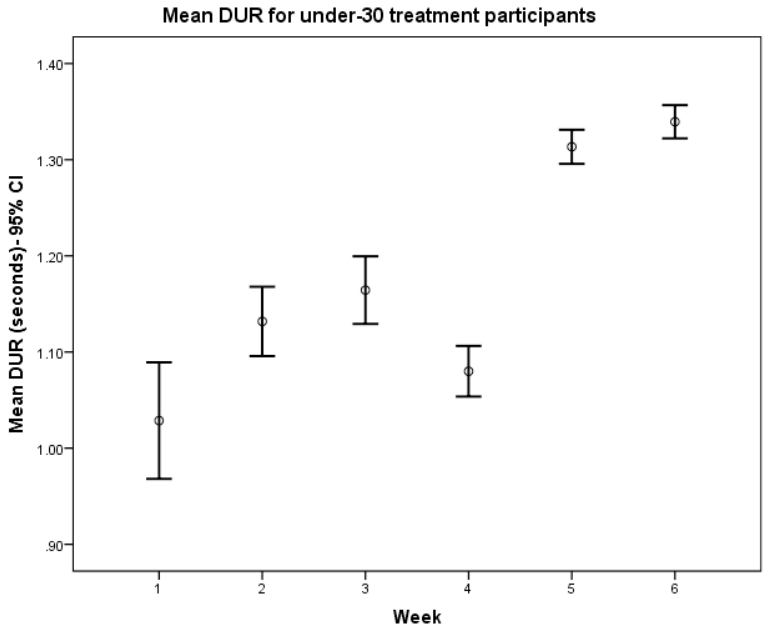
Table 5.14 Increase in mean DUR (Seconds) values over the six week intervention period for all treatment participants

Table 5.14 demonstrates that each participant demonstrated a small increase in DUR over the six week intervention period. There is some variation in the level of increase between the 8 participants over the 6 week period (1.63 seconds).When looking at the increase between groups the under-30 group displayed a smaller increase (0.21 seconds) than the over 60-group (0.44 seconds). The table also displays the results as a percentage of increase in the 3 second activation period. P6 (under-30) and P2 (over-60) demonstrate a greater increase than other participants (44%), the next highest being from an over-60 age group participant (P1), and an under-30 participant (P8) both with a 15% increase. P4 displayed the lowest percentage increase of DUR over the 6-week intervention period.



Graph 5.10 Mean DUR during the NMES treatment protocol for all participants, error bars indicate 95% CI of the mean

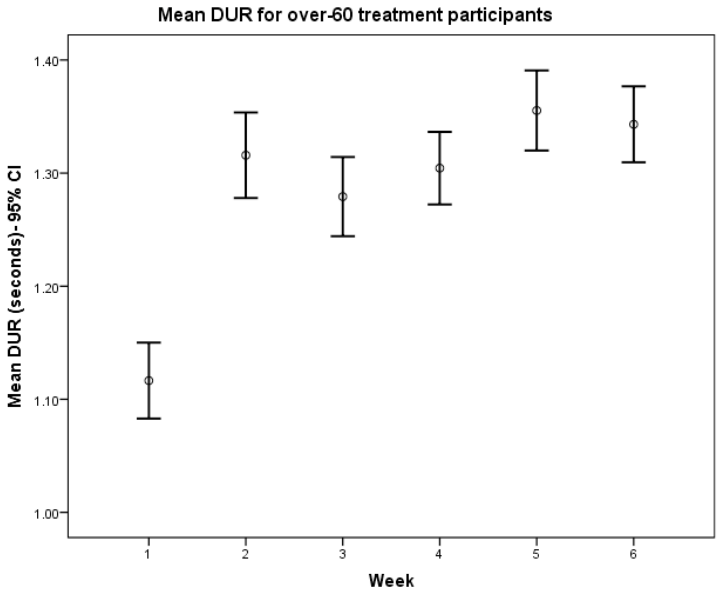
Graph 5.10 demonstrates how mean DUR for all participants varies over the six-week intervention period. Although changes are small, there are differences demonstrated over the time frame which could relate to clinical implications. DUR shows an overall increase from week one to week six, with rises generally accompanied by a week of stable response. The initial rise from week 1 to week 2 is the greatest, with a rise of 0.15 seconds. The response to stimulation stabilised between weeks 2 and three, which was followed by the only decline throughout the intervention programme between week 3 and 4: 0.02 seconds. Another rise was produced between week 4 and 5 (0.12 seconds), again which stabilised leading into week 6. There was a total of 0.26 second increase in the time the muscle was switched on during a NMES contraction from week 1 to week 6. It is interesting to note the overall increase in this graph, however to gain a better understand this has further been broken down into age group changes, as shown in graph 5.11 and 5.12.



Graph 5.11 Mean DUR for under-30 participants

Although the under-30 participants demonstrate an overall increase of 0.31 seconds between week 1 and week 6, there is some variability in the data set. Week 4 demonstrates a decline in mean DUR,

which is the only decline within this age group. When looking at the raw data participant 7 demonstrated a decline in mean DUR of 0.18 seconds between week 2 and 4. Unfortunately data is not available for week 3. The remaining two under-30 treatment participants show values that demonstrate a relative rise in mean DUR from previous weeks. P6 displayed mean DUR values that are lower than the other two under-30 participants (table 5.13), however is consistent throughout. P6 utilised the highest stimulation intensity (mean 64 ma) of the under-30 participants.



Graph 5.12 Mean DUR for over 60 participants

The mean DUR for over-60 participants increases by 0.2 seconds from week 1 to week 6 (graph 5.12). This is 0.11 seconds less than the under-30 category. This group demonstrates a sharp rise from week 1 to week 2, with an increase of 0.13 seconds. The mean DUR demonstrates two small declines, however fluctuates mildly between weeks 2 and 6. The biggest decline is witnessed between week 2 and 3 with a decline of 0.05 seconds.

Linear regression indicates a positive rate of change of DUR over the 6 week intervention period (0.42 seconds/week). When considering age group DUR increased by 0.003 seconds/week more in the over-60 age group than the under-30 age group.

Subjective feedback

Although there was no formal qualitative measurement to report treatment feedback, some participants verbalised their perceptions towards NMES. General feedback was positive, and all participants expressed that they would agree to NMES being administered as part of a rehabilitation intervention. Participants subjectively felt that NMES would be beneficial as an adjunct to another strength training modality.

P2 (over-60) reported that he felt subjectively 'stronger' from the intervention with NMES, and felt more confident walking longer distances after the 6-week intervention. A change in sensation was noted from this participant (spinal stenosis has resulted in a reduction in sensation to the leg prior to study involvement). The participant reported that although his sensation was not fully restored, the subjective change in sensation was notable, and appeared to influence his daily function. P4 also reported a specific change that should be taken into account. Although no reference to strength was reported, this participant indicated a feeling of increased muscle bulk comparatively to the non-tested gastrocnemius.

The under 30 participants did not report such specific differences post-treatment, but commented on areas of performance, such as a subjectively improved reaction time and time to peak contraction.

5.6.1 Overview of study three results

The aim of this study was to ascertain the effects of the NMES protocol on muscle function over a six week period. Two age groups were analysed (under-30 and over-60) to identify correlations between treatment effect on participant age. Four outcome measures were utilised, two related to voluntary capacity of the muscle, and two related to the muscles response during the stimulation.

Maximal isometric force production (MIFP) was assessed both before and after treatment with NMES. The treatment group of both age groups demonstrated a rise in MFIP, the under-30 group more than the over-60 group. All participants (excluding one- under-30) increased MIFP (treatment and control), with the under-30 age category demonstrating more variability within this outcome measure. Similar results were seen with pennation angle, both treatment groups improved after treatment with NMES, with the under-30 age category showing a greater improvement. The greatest improvement was from an over-60 treatment participant. A small correlation was demonstrated with MIFP; however this was not as strong as expected from literature.

Voluntary muscle force production is a product of the function of the neuromuscular system, hence it was important to investigate changes related to this. Maximal electrically stimulated force production (MFP-ES) was a measure of how the muscle responded to the NMES stimulus. MFP-ES increased in all participants, with a greater increase in the under-30 age category. This indicates that the muscle responded to treatment over time and was able to increase its force generating capacity. When looking at within treatment session data the MFP-ES demonstrated decline in some participants. This is reflective of the fatiguing stimulus discussed in chapter 5. What was interesting was the high level of within participant variation, despite constant NMES parameters and participant position. This variation was less in the under-30 age category. There was a small correlation between MFP-ES and the stimulation intensity used to administer NMES. Although this implies that higher

stimulation intensities will produce a greater muscle response, there were anomalies to this finding, and given the small sample size caution should be used until further testing is complete.

Duration of NMES contraction (DUR) demonstrated that the time the muscle was activated by the NMES increased after treatment in all participants. This increase is small, but potentially relevant clinically given the 3-second stimulus with NMES. The under-30 age category demonstrated a greater improvement than the over-60 age group, with the over-60 age group demonstrating a steep rise from week 1 to week 2, followed by a fluctuating stable response thereafter.

The results of this study have identified differences of muscle response after treatment with NMES, but also how both age groups respond to NMES differently. These results are discussed below.

5.7 Discussion

This study aimed to investigate whether age influences the response of the Gastrocnemius to a six-week strength training protocol with NMES. This study was the first in this thesis to administer the NMES protocol over a full training period. Identifying differences between age groups on protocol acceptance was vital in transferring results from this study into a clinical setting where both age groups would be targeted. Although the Leg Measurement Device (LMD) has previously been used in study one, this was the first time it was subjected to a full training protocol making this an opportunity to assess for ease of use, adjustability and comfort. This study achieved its aim; all participants in the treatment arm received six weeks of NMES training, and the LMD was effective in extracting the outcome measures that were planned.

When designing this study a sample size of twenty was targeted. Of this sample, one participant withdrew their consent in the second week of the NMES protocol. This reduced our sample of the under-30 treatment group to four participants. P10 withdrew consent due to work restrictions, not allowing sufficient time to attend the training sessions. The participant reported that neither the study design, sensation of NMES nor the testing methods used in this study influenced this decision. The reduction of this sub-group to four participants limited our analysis to descriptive means and restricts our ability to infer our results to a wider population. The data produced for each outcome measure was maximised (considering the small sample) due to the high number of treatment repeats. This allowed analysis to be as reliable as possible. Adopting a randomised controlled trial (RCT) study design allowed us to compare the treatment group to a non-intervention group, which gives justification for a larger RCT to be conducted. When planning this study, we were limited by both funding and time constraints, which limited the sample size we could recruit. One person collecting data along with the high number of sessions (3 sessions every week for 6 weeks) was time consuming. Inclusion of specific assessment from each NMES contraction (MFP-ES, DUR) as part of our outcome measurements resulted in the inability to issue a home based intervention program. We felt it important to maintain the current methodology, as this acts as a proof of concept study, and gives a good basis for a larger RCT to be conducted.

The inability to read NMES protocol related data resulted in a treatment participant being excluded from the under-30 age category (reducing the sample in this group to three). The outcome measures related to volitional capacity of the muscle have been used throughout analysis. P9 was a female (aged 22) who used an average stimulation intensity of 42 ma. The inability to read the data may have resulted from the participant not tolerating the stimulation to produce a strong enough contraction. This may be due to electrode placement or adherence to the skin, which can affect the transition of the electrical impulse through the sub-cutaneous tissue. Noxious sensations may result in the reduction of stimulation intensity, limiting the output of the muscle force (Gobbo, Gaffurini et

al. 2011). Further time could have been spent positioning electrodes, to maximise participant comfort. Increased time spent altering position, and setting stimulation intensity may have resulted in further acclimatisation to sensation, thus allowing data to be read.

Despite a low muscle output from the NMES protocol, the data exported into mathematical analysis software had a wide range of data points in this participant (P9). The variation at baseline did not allow identification of moment peaks during analysis. The moment peak may not have reached the threshold required to be identified as a peak, or the participant could have moved their foot on the footplate. Data available is unable to identify the reason for this. An example graph of a noisy dataset is provided in image 4.3. Participants were encouraged to relax and remain in a fixed position throughout the protocol (not assisting the NMES). This is difficult to identify visually during data collection due to the high level of sensitivity of the force transducers. Again further time could have been spent explaining procedures to participants and providing reminders during data collection. This would have allowed us to maximise our participant numbers, which is important considering our low sample size.

Literature surrounding the use of NMES for strength training in an elderly population is developing in clinical populations (Stevens-Lapsley, Balter et al. 2012), although its mechanism is not fully understood. The application of NMES in a clinical setting is therefore not something that is regularly used. By categorising our cohort of participants into two age groups we were able to compare the effects of an elderly population to their younger counterpart, in whom the effects of NMES for strength training are slightly better understood (Maffiuletti 2010). Comparison of the effects of NMES on age ranges resulted in careful consideration of the age boundaries in each group. An age of 60 was considered as the lower limit in the old category. Muscle mass has been shown to decline by 2% per year over the age of 50 (Buford, Anton et al. 2010). Sarcopenia has been shown to increase in

prevalence over the age of 60, which continues into older ages. However, the variability in diagnosis and assessment makes it difficult to highlight an exact age of onset (Bijlsma, Meskers et al. 2013). Recruiting participants who were over-60 into this study also had some practical benefits. Participants were still able to work at 60, hence could be recruited from the host hospital without impeding on their lifestyle. Establishing links with a local over-60 club allowed all members to be eligible for study participation. When considering the younger age group, an age of 30 was carried forward as the upper limit of this group. Muscle force has been shown to peak between the ages of 20 and 30 (Evans, Hurley 1995), and thus encapsulation of muscle function before this upper limit is vital for this study design. Practical advantages in terms of recruitment were also considered; this age range was readily available within the host hospital for participation.

A wide array of outcome measures were utilised when assessing both volitional and artificial responses of the muscle to NMES. The LMD recorded all outcome measurements and allowed accurate, repeatable measurements: something that is difficult to produce in a clinical rehabilitation setting. Outcome measures relating to muscle function or performance tend to be subjective (often based on a numerical scale), and therefore can commonly be seen as a limitation in a research design. Clinicians are required to assess muscle weakness on a numerical scale, with boundaries for each point on the scale notoriously subjective (Vanhoutte, Faber et al. 2011, Harbo, Brincks et al. 2012). Production of a muscle force against resistance is considered to be a level 5 of 5 on the Medical Research Council Scale. There is little definition of how a muscle force improves within this level, highlighting the need for an accurate device to record muscle strength in a research setting.

Research indicates that advances in muscle strength can be caused as a result of improvements in the neuromuscular capacity of the muscle; the nervous system is able to respond with a quicker firing capacity (Hortobagyi, Maffiuletti 2011). This factor makes it important to look at the

electrically induced response of the muscle to NMES, as well as the volitional capacity. Differences in neuromuscular capacity of the muscle can be addressed via the LMD during the protocol, as this monitors moments about the ankle with a high level of sensitivity. Advances in neuromuscular function will be clinically portrayed as improvements in volitional strength capacity. Neural adaptation has been likened to improvements in motor learning, whereby the neuromuscular system has adapted to the training stimuli resulting in functional improvements (Carroll, Riek et al. 2001). It is the voluntary ability of the muscle to improve with NMES that would make a clinical difference to the lifestyle of an individual. For this reason we investigated the ability of the muscle to produce an isometric contraction. Although isometric contractions are not functional movements, we felt it important to make our results as accurate as possible, given our small sample size. If we had investigated a functional task such as walking distance or sit to stand ability, the results would become more subjective. If an elderly participant is unable to perform a movement, they tend to compensate with another muscle or movement pattern; allowing the task to be fulfilled. This would not give a true indication as to the effects of the treatment protocol. By sitting the participant in the LMD, we eliminated this variable, allowing us to draw more from our results.

Volitional capacity was also assessed by monitoring any changes in muscle architecture; measurement of pennation angle via ultrasonography. Pennation angle has been shown to correlate with muscle strength (Aagaard, Andersen et al. 2001), indicating that improvements in strength are proportional to increases in muscle pennation angle. This allows comparison of the measurements obtained via the LMD with internal muscle structure. These outcome measures give a wide understanding of muscle function, allowing us to gain a large depth of information from the data collected. The mean of three measurements was taken as this has been shown to produce the lowest measurement (REF1). Researcher measurement reliability was not conducted due to time constraints and the potential of bias with only one examiner. This method of analysis reduced the

likelihood of intra-examiner measurement error as much as possible. This method of analysis was used throughout this thesis.

When looking at Maximal Isometric Force Production (MIFP) both the under-30 and over-60 treatment groups improved their gastrocnemius force generating capability compared to their control group. This indicates that a six week strength training protocol with NMES is capable of producing adaptation of the gastrocnemius in both age ranges. Both control groups produced marginal strength gains, highlighting a possible learning effect from the assessment process in the LMD. However these gains were not sufficient to infer that this would affect our results.

There was a high level of baseline variability demonstrated in all participants when considering maximal isometric force production (MIFP). This was anticipated, due to the presence of sarcopenia, and degeneration of the neuromuscular system in the older participants (Lang, Streeper et al. 2010). The primary aim of this study was to investigate the effects of the treatment protocol on muscle strength, where differences in strength were monitored. The baseline variation is therefore a factor that did not influence analysis. The under-30 treatment group showed a greater volitional strength increase than the over-60 treatment group over the 6 week intervention period, however both groups improved. Muscle of young individuals is more plastic than older individuals, which has been attributed to an abundance of myoblast activity, and an enhanced neuromuscular system (Raue, Trappe et al. 2012). The increased differentiation of myoblasts into myotubes demonstrated after mechanical stress to the muscle as a result of resistance training has been shown to contribute to strength development in both trained and untrained young individuals. Although gene transcription alteration has been demonstrated in old muscle (80 years +) the number of genes affected is reduced compared to their young counterpart (30-40 years), and occurs after an extended time period compared to young muscle (Raue, Trappe et al. 2012). Satellite cell function in response to

muscle damage has been demonstrated to be delayed with advanced age, along with a wide variance of gene alterations between young and old muscle, despite similar Creatine Kinase serum increases. This demonstrates that old muscle has the ability to regenerate with exercise, but the mechanism behind the delay witnessed in older adults is not fully understood. Research does however suggest that the stimulus required for strength advances in old muscle is smaller than that required of young muscle (Thalacker-Mercer, Dell'Italia et al. 2010). The delayed response in older muscle to exercise stimulus in the literature may indicate that the training programme in this study requires extending. The 6-week training period may not have been sufficient to elicit the full strength increase capacity of the older muscle, and clinical use may warrant a longer period of training to maximise functional gains.

Despite the higher overall improvement, the under-30 treatment participants demonstrated a larger amount of variability in MIFP than the over-60 participants. P8 was a 24 year old male who demonstrated a decline in MIFP (1.3 Nm), despite producing a MIFP in line with other participants. P8 regularly conducted resistance training at a gym, where he conducted both lower and upper limb exercises. It may be that this participant was suffering from muscular fatigue and causing the strength of his contraction to be reduced. Peripheral fatigue is often associated with lactic acid. However the build-up of hydrogen ions in the cell has been shown to demonstrate acidosis, which hinders the strength of contraction post exercise (Westerblad, Allen et al. 2002). If this participant were to be suffering with a build-up of metabolites in his muscle, it may have hindered his performance when performing his maximal MIFP.

This participant (P8) produced the highest force output during the NMES protocol (Maximal electrically stimulated force production- MFP-ES), which was higher than the volitional force produced. This indicates that his muscle had the ability to contract (with NMES) beyond what it was

able to do volitionally. The synchronicity of muscle fibre activation with NMES may contribute to this supra-maximal contraction that has been produced via NMES (Bergquist, Clair et al. 2011). NMES also produces a sensory volley as a result of contraction, resulting in an afferent signal being produced from the muscle receptors (e.g. muscle spindle), which is sent to the central nervous system, in a synchronous fashion. This level of contraction with NMES could have also resulted in fatigue within the muscle, which with the intensity of the six-week program, plus his additional gym work could have begun to accumulate and affect the results of this study. It is also possible that this subject did not remember how to conduct the assessment process properly, and more time should have been spent ensuring he was confident with testing protocols. The small sample size utilised limits our ability to rule this factor out.

Improvements in force generating capacity were shown in the over-60 participants in the 6-week NMES intervention period. Volitional resistance training research is gradually accepting the fact that elderly individuals are able to undergo muscle hypertrophy (Cadore, Moneo et al. 2014). However evidence is still conflicting as to the effects of NMES on elderly populations (De Oliveira Melo, Aragao et al. 2013). Variation in MIFP improvements were witnessed in the over-60 category in this study, and testing a larger proportion of individuals to assess whether this improvement is consistent would be beneficial. Improvements in strength ranged from 1.9 Nm to 41.6 Nm in the older treatment category. When looking back at participant activity levels there are some differences. P3 (1.9 Nm increase) is a sedentary male, in his early sixties, whereas P5 (41.6 Nm increase) is an active 73 year old lady. Logic would dictate that the older individual should demonstrate less improvement in strength, as the neuromuscular system has had longer to decline with age (Stenholm, Tiainen et al. 2012). It could be inferred that the older participant in this instance was more active, and thus her muscle had maintained a greater ability to regenerate. An increased functionality of an individual has been shown to reduce the decline of muscle mass associated with senescence, hence maintain the ability of the muscle to perform work (Fahlman,

McNevin et al. 2011). However, the improvements in voluntary strength could also be attributed to the older participant experiencing more of a learning effect from the LMD. From a functional point of view, this study has not investigated the association between participants who have responded to treatment, and participants who did not. This variability is highlighted in all outcome measures. It would be interesting to assess the metabolic demands of an NMES protocol, and whether there is a reason behind this variability witnessed.

Current literature suggests that initial increases in strength with NMES are attributable to modifications in the neuromuscular system as opposed to muscle hypertrophy (Maffiuletti 2010). The time scale from which neuromuscular adaptation results in muscle hypertrophy is poorly understood which is heightened by the diversity of protocol application in the literature. Ability to transfer knowledge from voluntary strength training literature is also difficult due to the difference in physiological principles of muscle recruitment in NMES (Gondin, Brocca et al. 2011). Application of this protocol over 6 weeks indicates that neuromuscular adaptation may have been produced as opposed to pure muscle hypertrophy. Regardless of neuromuscular adaptation or muscle hypertrophy, the changes in muscle response as a result of the NMES program indicates that some modification had occurred, and it is suggested that this modification would continue if our protocol was conducted over a longer period of time.

Measurement of pennation angle allows assumptions based on voluntary contractibility of the muscle to be made. Pennation angle has shown to have a proportional relationship with muscle force, indicating that increases in pennation angle occur alongside improvements in force generating capacity (Aagaard, Andersen et al. 2001). Pennate muscle allows more muscle fibres to be arranged in parallel, which increases the physiological cross sectional area of the muscle. Measurement of pennation angle allows correlation between internal muscle architecture and voluntary force

production in this study. Both treatment groups increased pennation angle, the under-30 treatment group by more than the over-60 treatment group. This correlates with the under-30 treatment group showing a greater improvement of voluntary strength. The changes in pennation angle indicate that adaptation to the muscle fibre occurred as a result of the NMES protocol: improvements in pennation angle suggest that the muscle had increased the number of fibres in parallel (Strasser, Draskovits et al. 2013). Monitoring changes in pennation angle over shorter periods within the 6-week programme would allow indication as to when muscle changes begun to occur within the programme. The relationship between pennation angle and maximal isometric force production (MIFP) in this study is small (r^2=0.011). The small sample size may be a limiting factor in this analysis, and testing over a larger sample may reflect knowledge of the current literature.

We monitored the ability of the gastrocnemius to produce a force as a result of the NMES protocol; without voluntary control (MFP-ES). We decided to monitor this as a variable as this gives an indication of how the muscle responds to NMES over time, and whether the muscle is able to increase its peak force generating capacity; i.e. train to induce a higher level of strength. It also indicates whether the participants' muscle is resisting the NMES, which would prevent the muscle producing a peak force, affecting the training effect of the protocol (participant treatment compliance). By monitoring this during each session we were able to instruct participants to relax through the stimulation, or adjust the stimulation intensity level to a setting sufficient for the resistance to reduce. This then builds up the participant's confidence level, enabling a gradual increase in the stimulation intensity gradually throughout the protocol.

When looking at the MFP-ES results, both treatment arms were able to improve in the level of gastrocnemius contraction produced by NMES. The under-30 treatment group demonstrated a

greater improvement in MFP-ES than the over-60 treatment group. The under-30 participants may have decreased the time taken for the muscle to produce a force, through repetition of the protocol. As young muscle has the ability to accommodate to new stresses placed upon it in a short period of time (Marshall, McEwen et al. 2011), the participants' neuromuscular system may have been able to alter the speed in which it responds to a stimulus, or increased the motor area that the neurone innervates. This would effectively allow an increased response of the muscle, due to both a greater 'on' time, but also as a result of more efficient neuronal firing. The assumption would be made that the under-30 participants were able to tolerate NMES better than the over-60 participants.

The results from this study suggest that the under-30 participants spent less time adjusting to the sensation of NMES; however they were not able to tolerate NMES to a greater level than the over-60 age group. This result is interesting and not one which was expected. Younger muscle was expected to be of a better internal integrity than older muscle; more muscle fibres, larger muscle thickness and well-structured alignment of fibres (Lieber 2009). The muscle properties along with a reduced sub-cutaneous adipose layer associated with a larger muscle would indicate that under-30 participants would be able to tolerate a higher current intensity, with a corresponding increase in the level of muscle contraction produced. However the fact that the average current intensity in both groups was very similar, and the second lowest intensity was from an under-30 participant suggests otherwise. This suggests that the greater muscle integrity and neuromuscular function under 30 participants indicates that the under-30 participants do not require a high level of stimulation intensity to produce a strong muscle contraction. The threshold of nerve propagation in this age group may be reduced as a result of the enhanced neuromuscular system. It must be considered that the small sample size in this study contained participants who were not reflective of the general population, and thus studies on a larger population are required to further extrapolate from this finding. The over-60 participants' neuromuscular system would be slower to react due to age, but appears able to react to the same stimulus from NMES to reach the threshold for muscle

contraction (Thalacker-Mercer, Dell'Italia et al. 2010). Although requiring similar stimulation intensity, this study suggests that the over-60 participants were not producing the same level of contractile ability. This links back to the changes in responsiveness of the neuromuscular system with age, showing how it is more difficult for frail, elderly individuals to contract a muscle, in both an artificial and external environment. When linking this to frail individuals with pathology, performing a rehabilitation program, it is easier to understand why they find it difficult to perform activities required of them by gold standard rehabilitation programs.

It is interesting to see the variability in MFP-ES across all participants, but especially within the under-30 treatment category. P6 demonstrated an increase of 156 Nm, whereas P8 only increased by 30 Nm over the 6 week intervention period. It is interesting to note that P8 tolerated a high intensity of NMES from the outset, whereas it took P6 a week to tolerate a higher level of stimulation. In terms of physical properties, P8 is more active than P6, perhaps accounting for this factor. P8 participated in regular resistance training, and P6 regular aerobic endurance training. P8 may have tolerated a higher initial stimulation intensity due to the ability of his muscle to withstand greater loads as a result of the repetitive practice from the resistance training load. Skeletal muscle adaptation to exercise allows greater force to be placed on it through training, which will allow a greater muscle contraction to be produced via NMES. As previously discussed, P8 declined in his MIFP making this variable difficult to account for or justify, and this is also hindered by our small sample size. We are unable to ascertain whether this result is reflective of the wider population of younger individuals of an intensive resistance training regime, or whether this participant displayed a non-reflective result. A larger sample size in each age group is required to investigate this.

The aerobic endurance training of P6 would result in an abundance of type 2a (fast twitch oxidative) muscle fibres to enable periods of prolonged exercise but with a high level of contraction (Aagaard,

Andersen et al. 2011). This would account for the high level of MFP-ES increase over the 6-week period; the muscle had a solid underlying integrity, and demonstrated rapid response to the new training stimuli. The lack of maximal force training in the aerobic endurance programme would also suggest that the muscle is not accustomed to high intensity load being placed on it; resulting in a reduced initial tolerance to the stimulation with NMES. Results from MFP-ES indicate that there is a high level of variability within a NMES training programme, and that clinical applications must account for this in terms of expectations when designing a specific protocol.

Assumptions on how quickly the neuromuscular system was able to respond to the external stimulus from NMES was assessed by the outcome measure DUR; duration of which the muscle was producing a force during the NMES contraction. It was hypothesised that DUR would increase over the six weeks, as the time frame for the nervous system to detect a stimulus, the muscle to be activated and the muscle fibres to move to a contracted state should reduce with training (Cormie, McGuigan et al. 2011). This reduction in response of the neuromuscular system allows a prolonged period for the muscle to produce a force, which would benefit the muscle in terms of adaptation to the programme. The protocol was set to deliver a stimulus to the muscle for 3 seconds; however it must be taken into consideration that this time also includes the time for the neuromuscular system to respond to the stimulus.

Both treatment groups displayed an increase in the DUR over the 3 week period, with increases showing a small level of variation. The under-30 treatment group displayed a slightly higher mean increase than the over-60 participants, however this effect was small. In fact variation between all participants was small. Similar high adaptation responses to DUR were witnessed in both age categories, demonstrating that age does not influence the ability of the neuromuscular system to adjust to intervention with NMES. Despite low values, it is still thought that these values are of

significant interest to clinical practice, especially given the short 3 second stimulation time frame. For this reason we have been unable to perform substantial statistical analysis on the data, however have continued to make assumptions based on the physiological implications. The results of DUR increases have been displayed as percentage increases in tabular format, as this is more reflective of clinical relevance than the small time increment. However when graphically presenting the values of DUR in the results section, we have maintained the used of raw data, allowing a true reflection of our results given the limited sample size in this study.

The under-30 participants displayed more variation within their results than the over-60 participants, suggesting that this response was not repeatable. The increase in DUR suggests that the time of the neuromuscular system to respond to the stimulus and activate the muscle to contract has decreased. Clinically this improved efficiency of the muscle may contribute to improved function. A quicker response of the muscle to a stimulus would enable the body to better anticipate threat. The response generated in the muscle may prevent injury, or prevent the individual from falling. The initial generation of muscle power occurs quicker than peak muscle contraction, and has been shown to improve in an elderly population previously (Suetta, Aagaard et al. 2004). Further testing is required to ascertain whether the improved neural control of the muscle transfers to volitional activities, or whether further training to induce these effects in daily function is required.

The over-60 participants demonstrate a sharp increase in DUR, which is followed by a relatively stable response thereafter. This indicates that we elicited some adaptation in elderly muscle, with alteration of the neuromuscular system. Early adaptation could be reflective of background in physical activity of the over-60 participants: the more active they are the better they respond to treatment. This cohort was relatively active compared to age; activities include walking (P4, P5), and

working as a football referee (P1). Further investigation into effects on participants who do not exercise is recommended.

The greatest increase in DUR was demonstrated from an over-60 participant, who increased his DUR by 0.52 seconds. P2 tolerated the highest stimulation intensity of all participants. The increase in DUR may be a reflection of this, or an indication that substantial advances in the neuromuscular system were made. P2 has a low level of spinal stenosis (consultant permission was sought to recruit this participant), which effects his walking ability. The reduced function of his lower limb muscles as a consequence of this may have induced a rapid early response, as the neuromuscular system has the ability to contract, but it has been operating with limited stimulus. The subsequent increase in efferent motor drive after exercise displays a rapid increase in muscle function, despite little adaptation in the muscle fibre (Cadore, Izquierdo et al. 2013). Adaptation in the muscle fibre (hypertrophy) has been witnessed in an elderly population. Although variation in time scales have been reported (Valenzuela 2012), strength increases in the elderly have been demonstrated to occur after approximately 4 weeks. This particular participant increased the stimulation intensity to the maximum the stimulator would allow. This may have been a result of having a high tolerance to the sensation of NMES, coupled with a high level of self-competitiveness driving improvements. The spinal stenosis may have impacted his sensation as a result of narrowing of the nerve root (Slatis, Malmivaara et al. 2011), allowing increases in stimulation intensity to occur whilst not resulting in an increase in sensation. Outcome measures performed in this study limit our ability to infer the cause of this increase. A larger sample of participants is required, with varying levels of exercise and pre-existing muscle function to reliable draw conclusions.

Regardless of age group, the mean increase of DUR in all treatment participants is 1.25 seconds, indicating that it takes 1.75 seconds for the neuromuscular system to detect the stimulus and

produce a contraction of the muscle in response. This indicates that there is a substantial response time of both age groups to produce a response to the NMES stimulus, and that clinical guidelines should be produced to reflect this. From our understanding there is no advice on this aspect of protocol implementation in the current literature and therefore the 'on' time of stimulation that is indicated in the methodology is not what is being delivered to the participant. This also means that we are unable to make comparisons of NMES literature to that of volitional strength training, which is vital when the primary aim of NMES is to improve an individual's capacity to undertake rehabilitation as opposed to using it to improve performance.

Subjective perceptions of the NMES protocol have been taken into consideration, as these may be indicative of physiological change. An over-60 treatment participant (P2) made contact after the study had finished reporting that he could walk further, with less pain in his leg. This particular participant has a degree of spinal stenosis that can result in reduced cutaneous sensation when walking for long periods of time (over 20 minutes on entry to study). By the end of the study he was able to walk his dog for 40 minutes with no pain. Superficial stimulation directly onto a muscle belly activates a muscle tissue without the central nervous system (CNS) initiating the original signal. This results in the peripheral nerve being activated without having to travel through the stenotic area. Research indicates that an afferent response is emitted as a result of artificial stimulation to a muscle, which would travel through up through the spinal area (Bergquist, Clair et al. 2011). Repetitive neural input to this muscle from NMES could influence the ability of the neuromuscular system to restore its function, which would result in improvements in pain. A prolonged period of time with limited or no stimulation to a muscle would result in disuse atrophy (Hofer, Marzetti et al. 2008), and an elderly male who is scared of walking due to pain would walk less, heightening the atrophy (Parry, Finch et al. 2013). Fear of falling has also been correlated to adaptations in gait characteristics such as a slower gait speed and shorter step length, reducing the demand of the muscle. The impact of this vicious cycle would contribute to this male becoming frailer.

Another over-60 participant (P4) reported a subjective feeling that their muscle had 'increased in definition' compared to its counterpart. This would indicate that the muscle had undergone a degree of hypertrophy, which is reflected by this participant demonstrating the greatest increase of pennation angle in all participants. Subjective responses provide indication that effects of NMES correlate with functional improvements. This would heighten the clinical justification for using NMES in a patient population to aid physio-therapeutic intervention programmes. However, outcome measures did not address this issue in this study, and further testing is warranted in this field.

5.8 Conclusion

During this study NMES has been shown to improve volitional strength in both old and young muscle, however more so in young muscle. Both age ranges have shown signs of improvement of voluntary and artificial ability to contract, and we have been able to make conclusions about how the muscle has adapted over a 6 week period. These results have given an insight into how NMES could be used in a clinical environment. Elderly patients often find it difficult to exercise their muscle to a level sufficient to induce strength adaptations, due to various confounding reasons including pain, pathology and surgical procedures. **NMES could therefore be an adjunct to a physiotherapy program, aimed at activating the muscle to a level sufficient to produce strength adaptations**. Application of both traditional rehabilitation plus NMES could rehabilitate to a more functional level, in a shorter time scale. In terms of the younger age group, NMES has the potential to shorten recovery times post-surgery, allowing muscle contraction when post-operative swelling or pain does not volitionally allow this. NMES could also be administered during a non-weight bearing period after surgery as this does not affect joint compression. The NMES would stimulate blood flow, helping to bring nutrients and fresh blood to the area, resulting in a maximised potential for advances in muscle strength and reduction in swelling. NMES could also help to improve

performance in a young healthy population, demonstrated by the fact that NMES can produce muscle contractions beyond what the muscle can produce volitionally. From this study we feel that we can make the following assumptions:

- **NMES can improve the volitional force output of both young and old muscle after a 6 week intensive program.**
- **Although unable to fully ascertain whether the improvement in volitional force output was adaptations in the neuromuscular system or muscle hypertrophy, this is of significant clinical value. Frail elderly individuals can have limited physical capacity to undertake volitional strength training programs, and beginning a rehabilitation program with NMES could enable them to fulfil this task.**
- **A muscle (regardless of age) is able to improve the response time of a muscle to produce a contraction during stimulation with NMES.**
- **Both young and old muscle require similar stimulation intensities to elicit strong tetanic muscle contractions.**
- **A muscle (regardless of age) will not receive the full 'on' time programmed into an NMES unit**

The results from this study have highlighted areas that NMES can be considered beneficial to rehabilitation. Although suggesting a positive result from our intervention, there are still confounding factors that require addressing before we can make definitive assumptions.

The variability witnessed throughout all outcome measures suggests that some people respond better to treatment than others. Testing on a pathological population will enable us to ascertain whether this variability remains, and whether it affects the response of muscle to stimulation in a

specific population. At present physiological and lifestyle properties have not been able to given reason to this variability, and if there is a metabolic reason for it, variability may be able to be influenced by an external intervention. The sample size in this study also limits are ability to reliable infer from this data. However it does provide proof of concept to justify a larger randomised control trial to be conducted.

Chapter 6

Study three: Strength training with electrical stimulation; is this a feasible treatment to administer on an acute stroke ward?

Study two (Chapter 5) indicates that NMES can be a useful intervention to strength train in older muscle as well as young muscle. This indicates that the effects of sarcopenia associated with frailty have the ability to be reversed. In order to study this effect further and investigate the clinical impact the protocol must be tested in a pathological population. Stroke is a condition that affects many individuals and can be disabling to a frail population. A population of individuals who have suffered a stroke will often suffer from frailty and will be prone to rapid muscle atrophy as a result of forced rest.

6.1 Introduction

A stroke is a debilitating neurological disorder which affects over 110,000 people in England every year (Lee, Shafe et al. 2011). Recent research has focused on reducing the risk of vascular stress on the body, which has resulted in a reduced incidence of stroke among Caucasians. This trend has not continued among other ethnic populations, and thus further development of preventive measures should be investigated (Kleindorfer, Khoury et al. 2010). The number of people under the age of 55 who experience stroke is increasing, which has been attributed to the increased incidence and risk of diabetes and obesity in today's society (Kissela, Khoury et al. 2012). The change in population results in Physiotherapists being faced with altered primary aims of rehabilitation post stroke; rapid restoration of functional independence and an enhanced quality of life.

The neurological influence of a stroke can result in a diminished capacity of the muscle to produce a contraction, which is fundamental for independent, functional living. Stroke results in impaired motor unit recruitment, ultimately leading to a rapid incidence of muscle atrophy which is difficult to restore (Prado-Medeiros, Silva et al. 2012). Restoration of muscle strength is a problem faced by Physiotherapists, which is confounded by the initial focus of developing proximal control, such as sitting balance and mid-line awareness before distal focus can begin. Proximal control must be developed to allow independent function. The time delay in neurological recovery results in further atrophy of muscles; an intervention to prevent this rapid decline in function is paramount for maximising rehabilitation potential.

Rehabilitation research investigates modern technologies aimed at restoring function post stroke (Dipietro, Krebs et al. 2012), which can often be expensive and impractical on a day-to-day basis. Neuromuscular electrical stimulation (NMES) has been previously utilised in Stroke rehabilitation, and has been associated with the ability to increase individual muscle strength (Sabut, Sikdar et al. 2011) and gait speed (Daly, Zimbelman et al. 2011). The mechanism is thought to be attributed to advances in neuroplasticity as a result of afferent motor neurone stimulation (Schuhfried, Crevenna et al. 2012). Some controversy regarding the effects of NMES in stroke rehabilitation remains, due to some conflicting results and varying methodologies. Lack of understanding of stimulation parameters has resulted in multiple stimulation frequencies and number of repetitions within a protocol (Chipchase, Schabrun et al. 2011). Advances in muscle strength as a result of NMES have been reported, with studies using manual muscle testing or dynamometry as primary outcome measures (Sujith 2008). Although this has some benefit in terms of comparability with assessment in a clinical setting, little understanding has been developed as to how the muscle is responding to treatment. Further understand of this process is required before larger randomised trials can be developed.

It is important to establish resources required to justify development of future studies through proof of concept trials. The use of the leg measurement device (LMD) in this study is a novel approach to accurately measuring the muscle response to stimulation with NMES. Reporting moments as a measurement unit provides a precise way to accurately measure muscle function. Ultrasonic monitoring of muscle architecture remains a novel approach in stroke rehabilitation, which has received little attention to date. Research that has focused on muscle architecture relates to upper limb muscles, or does not include the use of pennation angle (Gao, Zhang 2008), which has been investigated as a marker of muscle hypertrophy in this thesis. This study aimed to identify trends that result from the stimulation protocol developed throughout this thesis, in the hope of having a clinically relevant effect on the lower limb muscles used throughout the gait cycle.

6.2 Aim

This proof of concept study aimed to establish the effects of the NMES treatment programme on a population of patients who acutely suffered a moderate to severe stroke. This includes feasibility of treatment with NMES and the use of the Leg Measurement Device (LMD) on an acute stroke ward.

6.3 Methodology

Design: This study adopted a Randomised Controlled Trial. Ethical approval was obtained through the South Manchester Local Research Ethics Committee (Reference number: 08/H1003/44).

Sample size: Ten participants who had suffered a first ischaemic stroke were recruited from the acute Stroke unit of Aintree University Hospital NHS Foundation Trust.

Method of recruitment: Participants were identified from inpatient records. Inpatient lists were screened on a daily basis, and information of patients who fulfilled the inclusion criteria were passed to the consultant associated with the research study. Participants were approached by conversation with the consultant. Interest in the study resulted in them being given an information sheet (appendix 5). Further questions from the participant or family were answered by the lead researcher after a period of 24 hours to process study involvement. Time was spent with the family and participant in an attempt to reduce withdrawal rates and to ensure that both parties understood all relevant study information.

Inclusion criteria: Participants were able to participate in this study if they fulfilled the following criteria:

- Aged 18-80
- 2-6 weeks post first acute ischaemic stroke
- Medically stable
- Capable of supported or independent sitting
- Lower limb muscle strength of 0-2 on the MRC grading scale
- Independently mobile before their stroke (including walking with a walking stick).

Exclusion criteria: Participants were excluded if they had any contraindication to NMES, which were:

- Active pacemakers
- Uncontrolled epilepsy
- Inability to comply with or understand treatment protocols.

Informed consent: The participant or their family were able to speak to the research team, or the consultant overseeing the study regarding questions that arose about participation. Written informed consent was obtained from the participant where possible; however in the case that the participant was unable to verbally communicate, or write as a consequence of their stroke, the next of kin was able to give assent on their behalf.

Method of randomisation: Participants were randomly allocated to the treatment (n=5) or control group (n=5). Randomisation was performed by placing allocations in blank envelopes, and asking the participant to choose one. Randomisation envelopes were developed using a random number generator, by an independent assessor.

Testing position: Electrodes were placed as per current recommended guidelines (Baker, Wederich et al. 2000) on the Quadriceps Femoris and Gastrocnemius of the affected leg. Quadriceps Femoris electrodes (50mm X 90mm) were placed 5cm proximal to the patella, and 5cm proximal and 3cm lateral to this. Two (50mm X 90mm) electrodes were placed longitudinally and centrally over both heads of the Gastrocnemius, 3cm below the knee joint and 2cm apart (figure 6.1 a & b). This position was subject to change if the participant reported discomfort. NMES can induce noxious sensations, due to superficial stimulation innervating cutaneous receptors; altering the position of the electrodes and placing some water on the under surface of the electrode can alleviate this (Baker, Wederich et al. 2000).

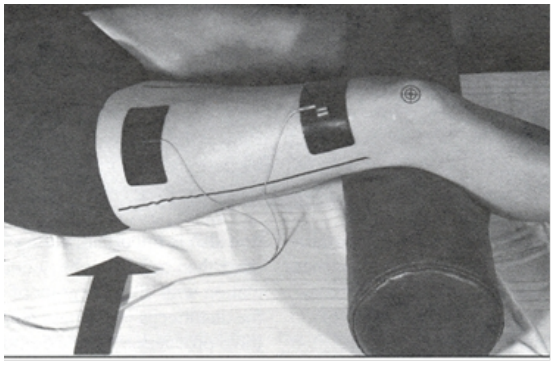


Figure 6.1a Quadriceps electrode position

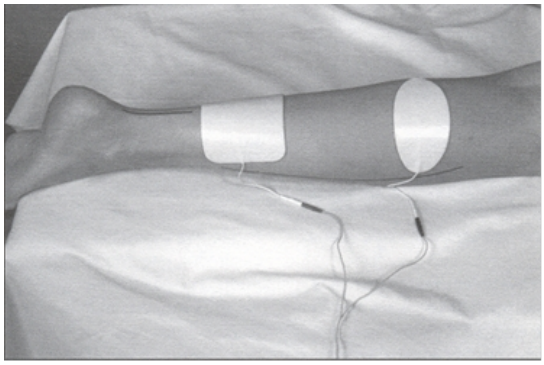


Figure 6.1b Gastrocnemius electrode position

Reference for figures (Baker et al 2000)

The participants' affected leg was positioned in the leg measurement device (LMD) that could be used to measure strength of the knee extensors and ankle plantarflexors during application of NMES (Chapter 3). The LMD was altered to allow transducers to be aligned with the joint centre of the knee and ankle joints of each participant. Limb position was fixed to allow allowed 60° knee flexion and plantargrade at the ankle (figure 6.2). Straps were placed across the thigh and anterior ankle to maintain position throughout testing. The participant was appropriately padded into the LMD to ensure the joint centre of the limb aligned with the device in the case of a flaccid limb. Data was not collected during the stimulation protocol (due to small moments achieved associated with the neurological condition) hence position was subject to modification if necessary. Measurement was also restricted by uncertainty as to whether the device would be tolerated on an acute stroke ward, and whether the limb could be positioned with accuracy. Participants often required assistance from multiple members of staff with manual handling and were prone to loose stools, both of which could prevent the participant from being positioned outside of their bed or within the LMD. If position in the LMD was not permitted, the affected limb was positioned as close to this as possible to maintain comparability. The participant was positioned either half lying (inclined to 45° hip flexion in bed), or sitting in a chair. The participant would preferably be positioned in a chair to keep the hip at 90° flexion (as per previous studies).

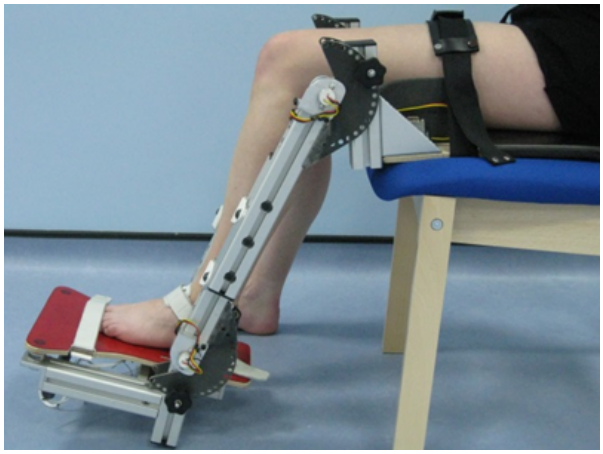


Figure 6.2 LMD position

6.3.1 Testing protocol

The aim of this study was to ascertain whether muscle strength could be improved with NMES after a moderate to severe stroke. Both treatment and control groups were monitored over a 6-week period, with outcome measures being assessed at study enrolment (baseline), at 3-weeks and at the end of the 6-week period. Both the treatment and control groups received daily standard NHS physiotherapy. Participant medical or nursing care was not affected by study involvement.

6.3.2 Treatment Group

Participants in the treatment group underwent a 6-week strength training program with NMES. Before testing began participants were introduced to the sensation of NMES in an acclimatisation session on both the Quadriceps Femoris and Gastrocnemius muscles of their affected limb. Stimulation parameters were set according to Chapter 5 (table 6.1). Verbal description of NMES sensation was communicated before application to ensure that the participant knew what to expect from stimulation. The stimulation intensity was then increased in small increments, whilst receiving constant feedback about the sensation from the participant. Once maximal tolerated has been established, three separate muscle contractions with NMES were produced at the identified

intensity, to allow further increase if acclimatisation occurred. This was conducted to ensure that the participant was comfortable with the sensation of NMES, plus to allow the first treatment session to be conducted in full and at maximal tolerated stimulation intensity. The acclimatisation session was conducted the day before treatment with NMES commenced. This allowed the participant enough time to process the demands of study involvement, and ensure they were comfortable with the stimulation.

Treatment sessions were conducted 3 times per week, for a period of 6-weeks. Each session delivered 45 muscle contractions on both the affected Gastrocnemius and Quadriceps Femoris. Stimulation to each muscle was delivered separately. During each treatment session, the stimulation intensity was set to the maximal amount that the participant could tolerate. This value was guided by the acclimatisation session; however the participant was encouraged to increase their stimulation intensity on a session by session basis. This was monitored throughout the 6-week programme. Frequency was set at 50 Hz, pulse-duration at 450 μs, time on was 3 seconds, time off 10 seconds, and ramp times 0.5 seconds (table 6.1). These settings were chosen in accordance with results from Chapter 5 to maintain comparability within this thesis.

Stimulation parameter	***Setting***
Frequency	50 Hz
Pulse Width	450 μs
'On' time	3 seconds
'Off' time	10 seconds
Ramp times	0.5 seconds
Intensity	Maximal tolerated (ma)

Table 6.1 Parameter settings throughout testing protocol with NMES

6.3.3 Control group

Control group participants undertook no additional intervention from normal standard care.

6.3.4 NHS Physiotherapy care

Treatment and control participants participated in daily standard NHS physiotherapy programs. Physiotherapy sessions were monitored in all participants on a daily basis (information was extracted from medical notes). Physiotherapy sessions were also audited by the research physiotherapist, once every two weeks, allowing functional progression to be monitored. This information was useful in attributing changes in key outcome measures in a limited sample.

Exercises conducted by physiotherapists included:

- Sitting balance
- Specific muscle activation techniques
- Practice of transfers
- Sit to stand practice
- Stepping practice
- Gait re-education

6.4 Outcome measures

All participants underwent outcome measure assessments at baseline, 3-weeks and at completion of the 6-week study. Outcome measures conducted are described below:

Volitional Maximal Isometric Force Production (MIFP)

Volitional force capacity of the muscle enabled any adaptations to muscle response as a result of the treatment protocol to be established. Volitional Maximal Isometric force production (MIFP) was assessed via the Leg Measurement Device (LMD) which was designed to measure moments about the knee and ankle joints (for full details please see chapter 3). The participant was positioned in the device, as described in section 6.3 (60° knee flexion, plantargrade ankle joint). Research indicates that hypertrophic changes occur in the position they were trained, therefore the testing position used when administering NMES was used to assess outcome measures (Kitai, Sale 1989).

Gastrocnemius volitional force production was assessed by asking the participant to push their foot down onto the footplate of the LMD as quickly, and as hard as possible. Verbal encouragement was given. MIFP was regarded as the highest of 3 attempts.

Quadriceps Femoris volitional force production was assessed by asking the participant to straighten their knee against the LMD as quickly, and as hard as possible. Verbal encouragement was given, and MIFP was regarded as the highest of 3 attempts.

The maximal attempt was considered as MIFP throughout analysis as encouragement to fulfil the aims of this study (measure maximal strength). The use of maximal data has been shown to be indicative of reliable analysis (Roberts, Denison et al. 2011). The data from both muscles was recorded in real time via the Biometrics Data Link software (Biometrics LTD), and later converted into moments. Peak moments were analysed in mathematical software (Mathcad) for comparison. Details of this can be found in chapter 4- image 4.3.

Muscle architecture

Muscle architecture can be used to infer force generating capacity of the muscle (Aagaard, Andersen et al. 2001). The architecture of the Lateral Gastrocnemius head (LG) and the Vastus Lateralis (VL) were assessed using 2D ultrasonography. Where possible the participants were seated in the standard testing position in the LMD (knee 60° flexion, ankle plantargrade). Participants often required padding in the frontal plane to maintain this position. If this were not possible due to probe orientation or specific participant needs, participants were seated as close to this position as possible in a standard chair. In this instance a goniometer would be used to measure joint angle.

In order to reliably re-measure muscle architecture probe position was monitored during assessment. LG was measured by identifying the central point between the proximal border of the muscle and the myo-tendinal junction. The probe was placed longitudinally at the mid-way point between the medial and lateral border of the muscle belly, or as close to the point as possible to obtain a clear image of the muscle fibres. The middle point of the muscle was deemed appropriate to demonstrate representation of the pennate muscle structure.

VL was measured by identifying the distal border of the muscle, and placing the probe 10cm proximal to this, mid-way between the medial and lateral borders of the muscle. The probe position could be altered slightly to obtain a clear image of the muscle fibres.

Three images were taken from each muscle. Images were extracted from the Ultrasound machine via USB connection, and later analysed via the computer software ImageJ (ImageJ LTD). Images were analysed with the intention of obtaining as many measurements on one image as possible. The mean of the measurements was considered for analysis. Three measurements were taken as a

minimum. This reduced the impact of measurement error. Muscle subcutaneous adipose layer, muscle thickness and muscle pennation angle were identified and measured for each image as shown in figure 6.3. When referring to figure 6.3 below, measurements 4, 5 and 6 refer to the distance between the lower layer of the epidermis and the top layer of the muscle aponeurosis. This was regarded as subcutaneous adipose layer. 1, 2, and 3 refer to the distance between the superior and inferior muscle aponeurosis, equating to muscle thickness. Finally, 7ang and 8ang refer to pennation angle: the angle that the muscle fibre inserts into the top layer of the muscle aponeurosis.

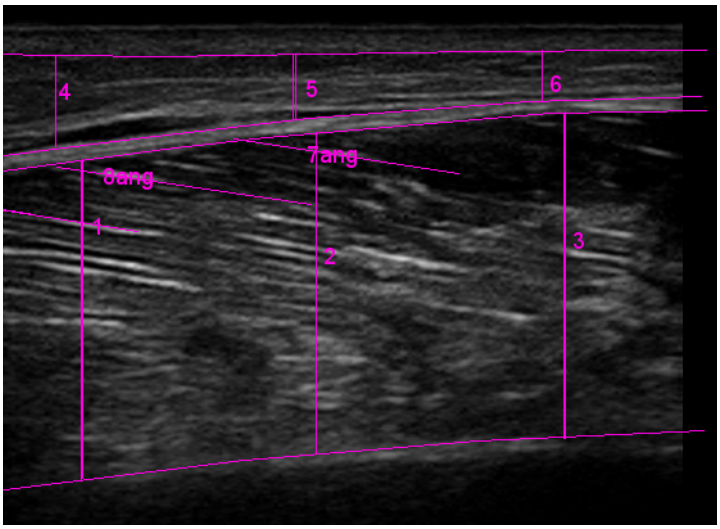


Figure 6.3: Example ultrasound image

Measurements were conducted by the lead researcher, and were therefore not blinded. This was a constraint limited by availability of personnel to conduct analysis. Measurements were analysed at baseline and completion (0 and 6 weeks), care was taken not let previous measurements influence analysis, to eliminate bias as much as possible as reliability was not tested.

Muscle Volume

Muscle volume of the affected quadriceps and gastrocnemius were measured via Magnetic Resonance Imaging (MRI). Due to funding constraints, half the participants received an MRI scan. This was determined during randomisation: the treatment and control groups were allocated with or without an MRI scan

MRI scans were taken at baseline and at 6-weeks. Analysis of MRI scans was not conducted due to poor quality of images. This is described throughout the results section of this chapter.

Functional ability

Where the participant was able, a timed up and go test was performed. This was regarded as the time (seconds) taken to raise from a standard arm-chair, walk 3metres, turn around and return to sit in the chair using any appropriate walking aids. The timed up and go test has been validated as a useful functional measure in assessing changes in walking speed in Stroke patients (Ng, Hui-Chan 2005). Correlation has also been identified with the Barthel Index, which has also been utilised in this study (see below).

Inclusion criteria prevented this measurement being conducted at study enrolment; however each participant was assessed for ability to perform this assessment at completion of study participation.

Functional Questionnaires

Functional changes in participants were monitored to ascertain any developments in activities of daily living. Two qualitative measures were used to asses a range of global and more specific functions. The Barthel index and the Nottingham Extended Activities of Daily Living questionnaire

(NEADL) were completed both at the beginning and end of study involvement. Research suggests that two scales to assess disability and function may be better than one (D'Olhaberriague, Litvan et al. 1996). Both questionnaires have been validated, and are in regular use within the Stroke literature (Quinn, Langhorne et al. 2011, Sarker, Rudd et al. 2012). Example questionnaires are provided in appendix 6)

6.5 Data analysis

A parametric approach to data analysis was conducted to ascertain trends within groups and individual participants. The small sample size recruited resulted in a limited ability to make assumptions based on group. This was also confounded by a large variation in functional ability as a result of the stroke, for example age and walking ability. Consideration of participant demographics at baseline was vital in identifying changes over time. This has resulted in descriptive analysis being the key method throughout this study.

Maximal isometric force production (MIFP) was first considered based on descriptive statistics (mean, standard deviation) and graphs. This was important to establish the effect of treatment with NMES on the ability to produce a muscle force. Differences between both muscles were compared, to establish whether differences were equal between the two muscles tested. A paired t-test was utilised to ascertain the effect of NMES on muscle force over the study time period.

Muscle architecture was measured via 2D ultrasonography. Small subject numbers resulted in descriptive and graphical presentation of data to demonstrate main findings. T-tests were used to assess the level of change, however the small sample size resulted in wide confidence intervals and caution is required when using statistical analysis. The relationship between pennation angle and

Gastrocnemius MIFP was investigated via graphs, to understand whether the results in this study reflect findings from strength training literature. A small relationship was identified which is supportive of the fact that increases in muscle strength were accompanied by increases in internal muscle structure.

Functional outcome measures (timed up and go and functional questionnaires) were also analysed based on descriptive statistics, as a wide variation in outcomes and a small sample make statistical analysis difficult. The purpose of a proof of concept trial is to identify trends in data to provide justification for larger studies.

When considering walking ability, statistical analysis was performed despite the small sample. One of the primary goals of physiotherapy post stroke is to improve the ability to walk, and thus the ability of NMES to influence walking ability is vital to consider in order to justify further studies in this field. Odds ratios were used to interpret the effect of NMES on walking ability. Odds ratio analysis was performed to describe the effect of NMES or no NMES on the ability to walk. Representing probability gives a good indication of the effect of NMES, and frequent use in medical journals allows comparison with literature (Bland, Altman 2000). It must be noted that the odds ratio is expected to produce a high confidence interval due to the small sample size, and thus interpreted with caution. This will be taken into consideration in the analysis. Description of walking ability was also useful to determine the wide variety of compensation mechanisms utilised by participants as the body strives to achieve the goal of walking.

6.6 Results

The study recruited 9 participants (4 male, 5 female, mean age 66.6 years, SD 10.5) from the acute stroke ward at Aintree University Hospital. Strict inclusion criteria within the 9-month recruitment period prevented the last participant being recruited. Six participants completed the trial: one withdrew due to constant neuralgic pain, one due to being unhappy with control arm allocation, and the third passed away before study completion (unrelated to study- pulmonary embolism). Of those that completed the study until completion, no adverse effects were reported. Participants who received treatment with NMES reported that the stimulation was comfortable, and that they would be happy to receive it within standard NHS treatment programs. Raw data is presented in appendix 7.

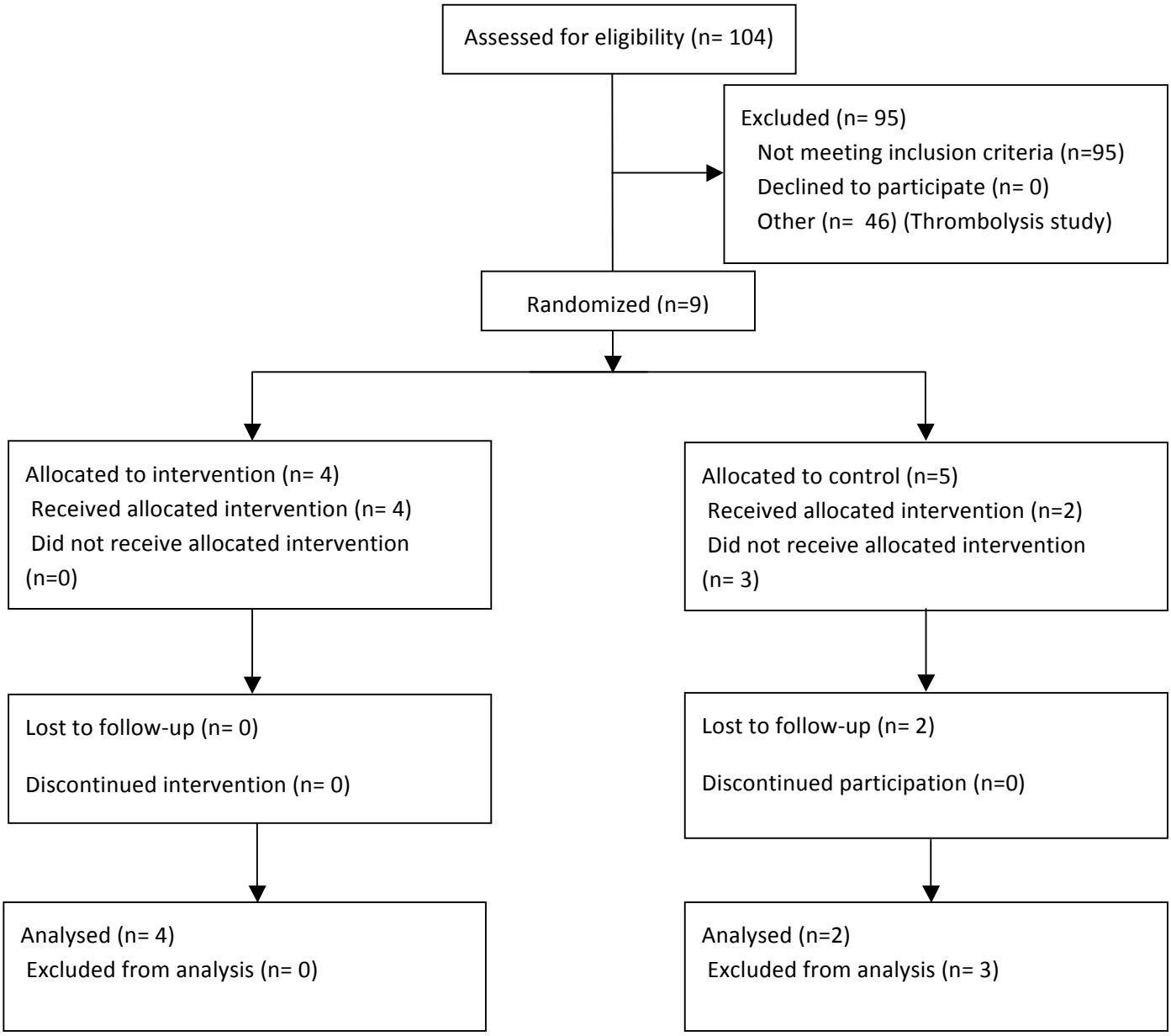


Table 6.2 A CONSORT diagram to show study recruitment process

A CONSORT diagram was produced based on guidelines from the CONSORT statement (Schulz, Altman et al. 2010) and displayed in table 6.2. Total number of participants screened for study enrolled was high in comparison to the number recruited. The participants not meeting inclusion criteria (N=95) reflects the difficulty in matching specific inclusion criteria in a stroke population. Participant demographics and type of stroke are summarised in table 6.3.

Subject number	Study group	Age/ gender	Type of stroke
1	T	64 (M)	R LAC
3	T	75 (F)	R PAC
4	T	79 (F)	L MCA
7	T	49 (M)	R MCA
2	C	54 (M)	R MCA
5	C	69 (F)	L MCA
6	C	74 (F)	R TAC
8	C	69 (M)	R PAC
9	C	74 (F)	R Cerebellar

Table 6.3 Subject demographics and type of stroke
T= treatment group, C= control group, M= male, F= female
LAC= lacunar, MCA= medial cerebral artery, PAC= partial anterior circulation, TAC= total anterior circulation

There was a wide age range of participants recruited into this study (49-79 years), with a mean of 67.4 years. The distribution between treatment and control group is relatively evenly distributed, with the two youngest participants split between groups. A variety of types of stroke were recruited, with the majority of participants suffering a medial cerebral artery stroke (MCA). The majority of strokes were right sided (N=7).

Variation in baseline functional ability was high. Participants ranged from being dependant for activities of daily living (unable to wash, dress, toilet etc.) to being able to transfer from bed to chair with the assistance of two members of staff. Assistance was needed for a variety of reasons; inability to control lower limb, flaccid presentation, spastic presentation or inability to control the core. The progression of functional ability and status on discharge is documented in table 6.4 to allow individual analysis.

Participant number	Study group	Functional status week 0	Functional status 6-weeks	Hospital discharge status
1	T	Dependant	Dependant	Discharged to NH
3	T	Dependant	Mobile with assistance 1	Discharged home
4	T	Transfer with assistance 2	Mobile (walking stick)	Discharged home
7	T	Transfer with assistance 2	Mobile (walking stick)	Discharged home
2	C	Transfer with assistance 2	Mobile (walking stick and assistance)	Discharged home
5	C	Transfer with assistance 2	Transfer with assistance 1	CW- neuralgic pain.
6	C	Transfer with assistance 2	Mobile (walking stick)	CW- unhappy with control allocation
8	C	Dependant	Dependant	Discharged to NH
9	C	Dependant	Passed away at week two	CW- Passed away at week two

Table 6.4 Participant status throughout study (T= Treatment, C= Control, NH= Nursing Home, CW= Consent Withdrawn

Maximal Isometric Force Production (MIFP)

Raw data of volitional MIFP throughout the study is presented in table 6.5. When looking at the quadriceps femoris both participant 1 (T) and 8 (C) were not able to produce any force throughout the duration of the study. The largest increase in quadriceps force (19Nm) was demonstrated by a control participant; participant 2. Participant 2 suffered from spasticity in the hip flexors throughout study participation. This was followed by an increase of 4.2Nm and 7.5Nm for participant 3 (T) and 4 (T) respectively. All participants (except participant 4, 0.4Nm) began the study with no trace of quadriceps activity. There was reasonable variation with regard to timescale of recovery; participant 2 showed most recovery of quadriceps force generating ability in the first 3 weeks (13Nm), whereas participant 3 produced more than 50% of their increase in the second half of the study (2.7Nm). The

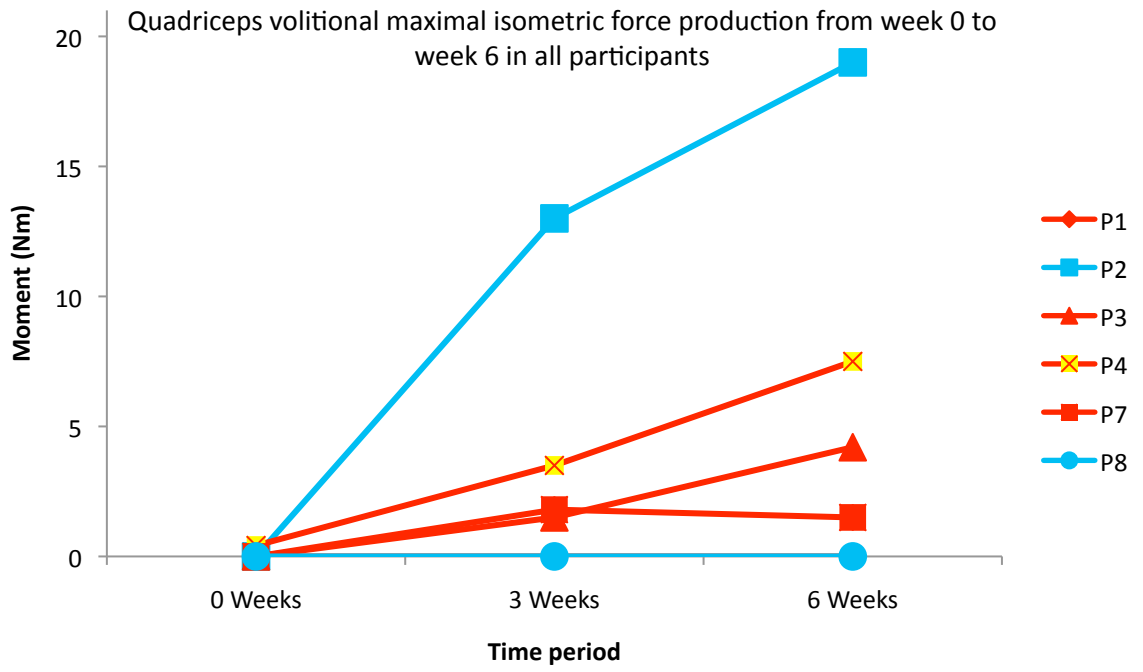
treatment participants produced a mean increase of 4.2 Nm of quadriceps force, whereas the control participants demonstrated a mean increase of 19 Nm (N=2).

When observing changes in gastrocnemius MIFP, participant 3 (T) shows the greatest increase in force (26 Nm), where the improvement was obtained reasonably equally between both half's of the study (14Nm in first 3 weeks, 12Nm in second 3 weeks). Participant 4 (T) demonstrated the next highest increase (19.7Nm) however the majority of the increase (16Nm) was obtained within the second 3 weeks of the study. Although demonstrating 19Nm of quadriceps force, participant 2 (C) was only able to produce 2.6Nm worth of gastrocnemius force within the 6 week period. Again, participant 1 and 8 did not show any signs of volitional muscle activity throughout the study. Mean increase of gastrocnemius force production in treatment participants was 17.9 Nm, whereas the control group increased by a mean of 2.6 (N=2)

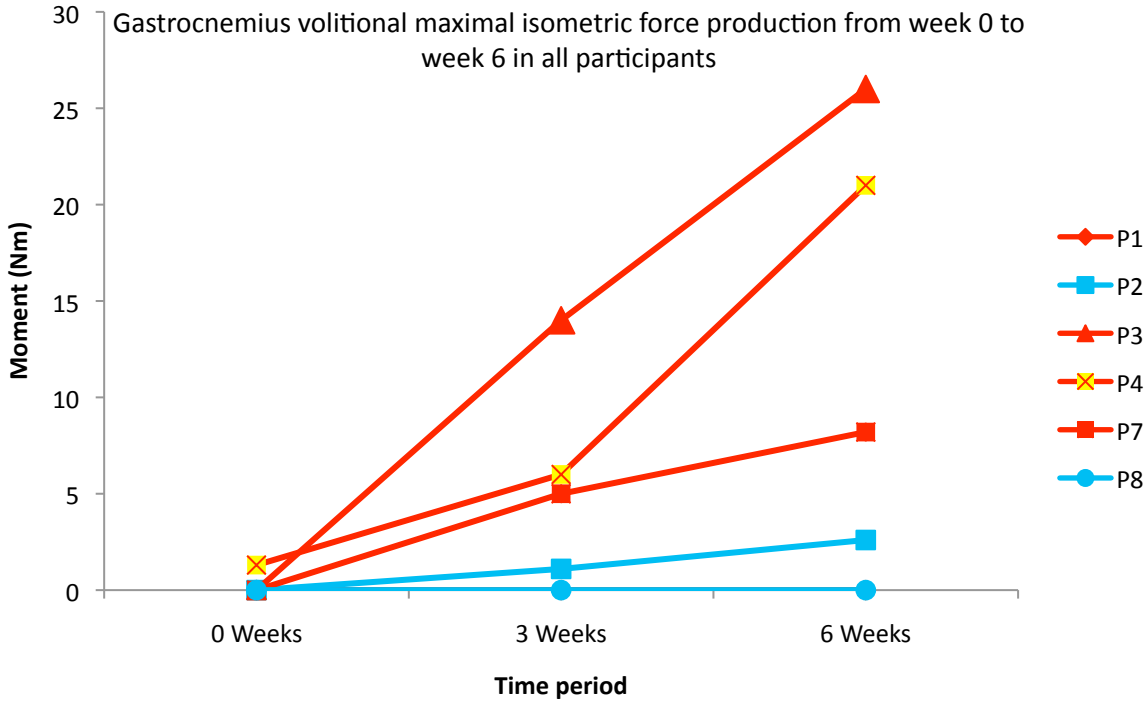
Participant Number	Quadriceps volitional MIFP (Nm)				Gastrocnemius volitional MIFP (Nm)			
	Pre	3wk	Post	Change	Pre	3wk	Post	Change
1 (T)	0	0	0	0	0	0	0	0
3 (T)	0	1.5	4.2	4.2	0	14	26	26
4 (T)	0.4	3.5	7.5	7.1	1.3	6	21	19.7
7 (T)	0	1.8	1.5	1.5	0	5	8.2	8.2
2 (C)	0	13	19	19	0	1.1	2.6	2.6
8 (C)	0	0	0	0	0	0	0	0

Table 6.5 Raw data for quadriceps femoris and gastrocnemius volitional maximal Isometric Force Production (MIFP) at baseline (pre), 3 weeks (3wk) and at the completion of 6 weeks (post), measured in Newton Metres (Nm). ('Change' indicates difference between post and pre measurement).

Changes in MIFP for both quadriceps femoris and gastrocnemius are depicted in graphs 6.1 and 6.2 respectively.



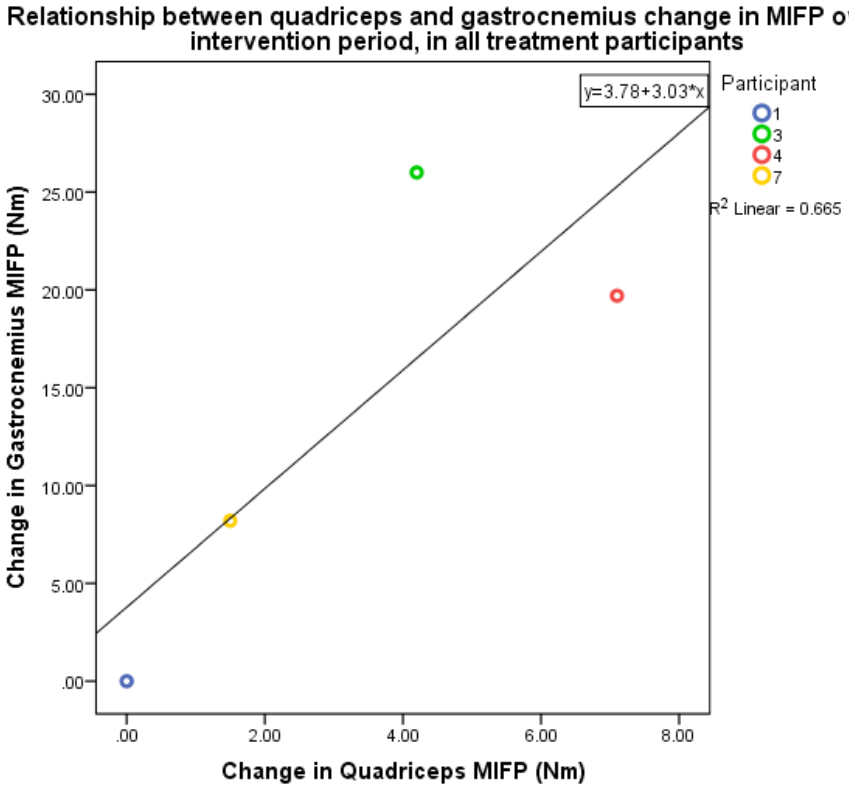
Graph 6.1 Quadriceps MIFP from week 0 to week 6 for all participants
P= Participant. **Red** indicates a participant in the treatment group, **blue** indicates control group. (Note P1 and P8 did not deviate from 0Nm throughout)



Graph 6.2 Gastrocnemius MIFP from week 0 to week 6 in all participants
P= Participant. **Red** indicates a participant in the treatment group, the **blue** a participant in the control group. (Note P1 and P8 did not deviate from 0Nm throughout)

Two treatment participants demonstrate steady progression of quadriceps MIFP over time (4 and 3), and one demonstrates a decline from 3-weeks (7), however effects are small. Treatment participants demonstrate a greater increase in Gastrocnemius force compared to Quadriceps force, with a progressive increase demonstrated over the 6-week period.

A proportional relationship is demonstrated between quadriceps and gastrocnemius restoration of strength after treatment with NMES (R^2=0.665). Although a relationship has been identified, this finding is not significant (P=0.113 95% CI -24.9-4.4) which is reflective of the low sample size being analysed (graph 6.3).



Graph 6.3 Relationship between quadriceps and gastrocnemius change in MIFP over the intervention period in treatment participants

Paired T-tests were used to ascertain effects of the gain in muscle strength over the 6-week period. Gastrocnemius demonstrated more of a correlation, however neither were significant (table 6.6). Clinical implications of this finding will be discussed later in this chapter.

Muscle	Group	P (<0.05)	95% confidence level	
			Upper	Lower
Quadriceps	Pre-Post	0.133	-8.18	1.78
Gastrocnemius	Pre-Post	0.103	-31.97	5.01

Table 6.6 Paired T-test results of MIFP over the 6 week intervention period in treatment participants

Stimulation with NMES was applied with maximal tolerated stimulation intensity. The mean stimulation intensity utilised by each treatment participant is provided in table 6.7. A small range of mean stimulation intensities were demonstrated (65-76 ma), with small associated standard deviations. This implies that participants were able to adjust to stimulation protocols well. Participant 1 stimulated with the highest intensity, however did not produce a force output with either muscle tested. He was the only participant to reach the highest intensity available on the stimulator: 80 ma. The remaining participant's demonstrated larger standard deviations and all stimulated with similar intensities.

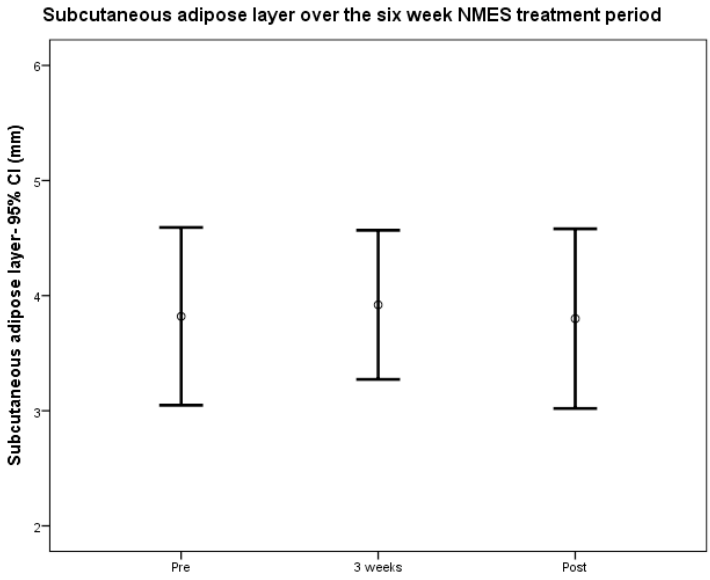
Participant	Stimulation Intensity (ma)	
	Mean	SD
1	76	1.3
3	65	4.5
4	73	5.1
7	71	7.2

Table 6.7 Stimulation intensities utilised by treatment participants

Muscle architecture

Muscle architecture was measured at baseline (0-weeks), 3-weeks and at the end of the treatment period (6-weeks) by 2D ultrasonography. No problems with measurement protocols were encountered during testing. Ease of measurement was enhanced if the participant was sitting in the leg measurement device (LMD), due to advantages in probe alignment and limb position. Measurements of subcutaneous adipose layer and pennation angle have been reported. Images reporting muscle thickness did not produce reliable measurements to include in analysis due to limitations in depth of view.

Subcutaneous adipose layer changes over time are depicted in graph 6.4. The change between pre and post measurements were not significant (P=0.847 95% CI -2.49-0.28) indicating that the treatment protocol with NMES did not influence the subcutaneous adipose layer. The largest increase (0.3 mm) was witnessed in participant 1 (T), who did not respond to changes in force as a result of treatment. The biggest decrease in subcutaneous adipose layer (0.2 mm) was produced by participant 3 (T), indicating that NMES may have influenced changes in lower limb architecture. It must be noted that changes in this measurement are however small which is confounded by the small sample. Treatment participants decreased subcutaneous adipose by a mean value of 0.05 mm, whereas control participants increased by 0.1 mm.



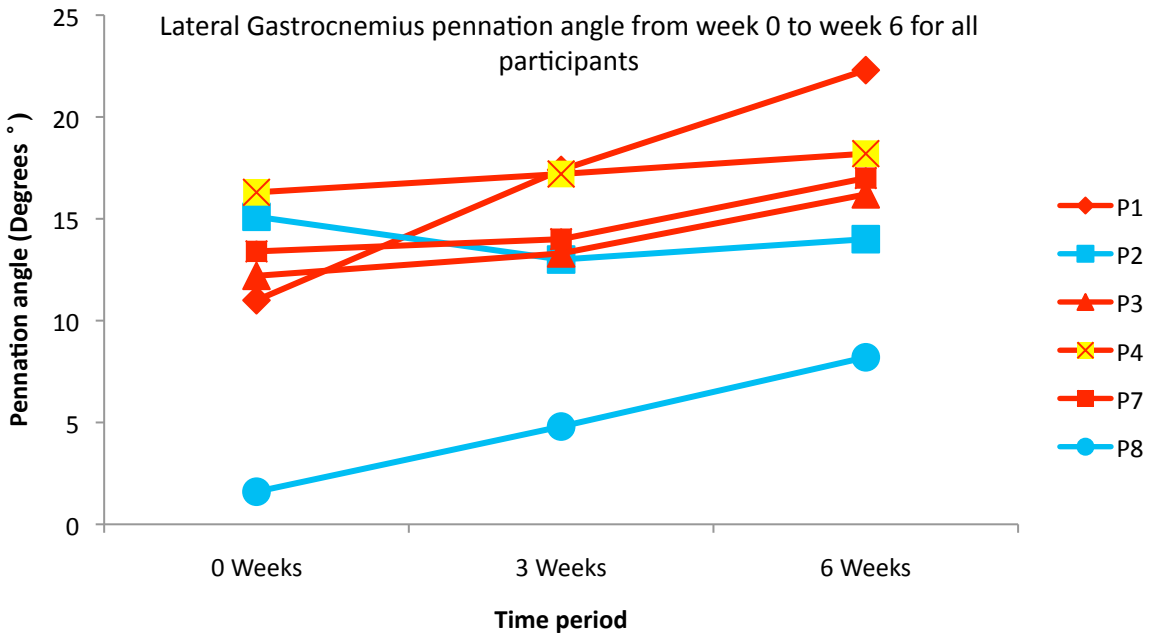
Graph 6.4 Subcutaneous adipose layer changes over the 6-week treatment period

Raw data of the lateral head of gastrocnemius pennation angle is displayed in table 6.8 for all participants. All participants bar one increased pennation angle at study completion. Participant 1 (T) displayed the greatest increase in pennation angle, which was equally distributed to 6 degrees in both halves of the testing duration. Participant 8 (C) produced the next highest increase in angle (6.6°); note that both these participants did not regain any force in either muscle group. Participant 2 (C) was the only participant to show a decline in pennation angle (1.1°). Participant 7 received an injection of botulinum toxin in the affected gastrocnemius to reduce spasticity which was administered in week 4.

Participant Number	Ultrasound pennation angle (Degrees °)			
	Pre	3wk	Post	Increase
1 (T)	11.0	17.4	22.3	11.3
3 (T)	12.2	13.3	16.2	4.0
4 (T)	16.3	17.2	18.2	1.9
7 (T)	13.4	14.0	17.0	3.6
2 (C)	15.1	13.0	14.0	-1.1
8 (C)	1.6	4.8	8.2	6.6

Table 6.8 Raw data for Lateral Gastrocnemius Pennation angle at baseline (pre), 3 weeks (3wk) and at the completion of 6 weeks (post). ('Increase' indicates difference between post and pre measurements).

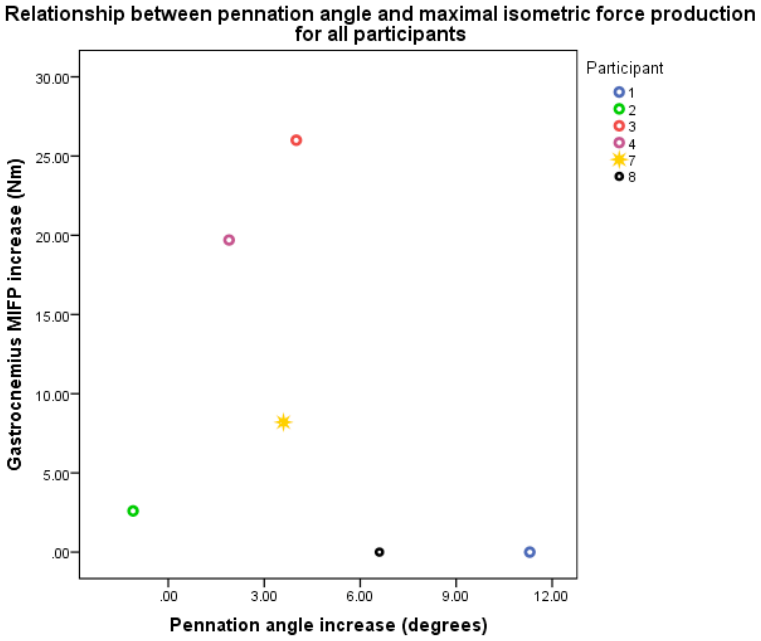
Progression of pennation angle over time is demonstrated in graph 6.5. The treatment participants gained a mean increase of 5.2°, whereas the control participants demonstrated a mean increase of 2.7°. Participant 8 had the lowest baseline pennation angle, which was 10.6° lower than the next lowest baseline; participant 3 (T). There does not appear to be a relationship between baseline pennation angle and treatment allocation.



Graph 6.5: Lateral Gastrocnemius pennation angle from week 0 to week 6 for all participants. P= Participant

Linear regression analysis in indicates that pennation angle is shown to decrease in control participants (b1= -2.45). The clinical implications of this must be reflected upon; and the effect of the botulinum toxin in a treatment participant must be taken into consideration. The difference between pennation pre-post in treatment participants is not significant (P=0.088, 95% CI -11.83-1.43). Wide confidence intervals are reflective of the sample size used in analysis.

Rate of gastrocnemius pennation angle increase was compared to that of maximal isometric force production (MIFP) increase to identify correlations. This is depicted in graph 6.6. Note participant 1 and 8 did not restore any gastrocnemius force. When considering the remaining four participants, a small correlation has been noted (R^2=0.445). This suggests that a rise in gastrocnemius MIFP results in an increase in pennation angle. The response appears to be more apparent in treatment participants (**3**, **4** and **7**), compared to the remaining control participant (**2**).



Graph 6.6 relationship between pennation angle and MIFP in all participants

The pennation angle of Vastus Lateralis (VL) proved difficult to both image and analyze. The oblique angle of the muscle fibers made measurement difficult, which was heightened by atrophy in this population. The ability to measure architectural differences from these images was therefore not able to be conducted.

Muscle Volume

Half of the participants were randomized to receive an MRI scan to measure muscle volume at recruitment. Of those who had an MRI scan at baseline (N=4), two had a 6-week week scan at completion of the study. Participant 1 was incompliant in lying still in the scanner, due to being disorientated and was therefore unable to read the first scan, or receive the second scan. Participant 9 passed away during the data collection period, and therefore did not receive the final scan. Image quality for all scans was below the standard required for reliable manual measurement. Flaccidity of the affected limb also affected muscle mass distribution and thought as to appropriate limb position is warranted to reliably measure as per current methodology protocol. Below is an example of the image quality produced by the MRI protocol used for this study (figure 6.4). Confidence in identifying muscle borders with enough accuracy to fully estimate the muscle volume of the limbs was low. MRI data was therefore not utilized in our analysis.

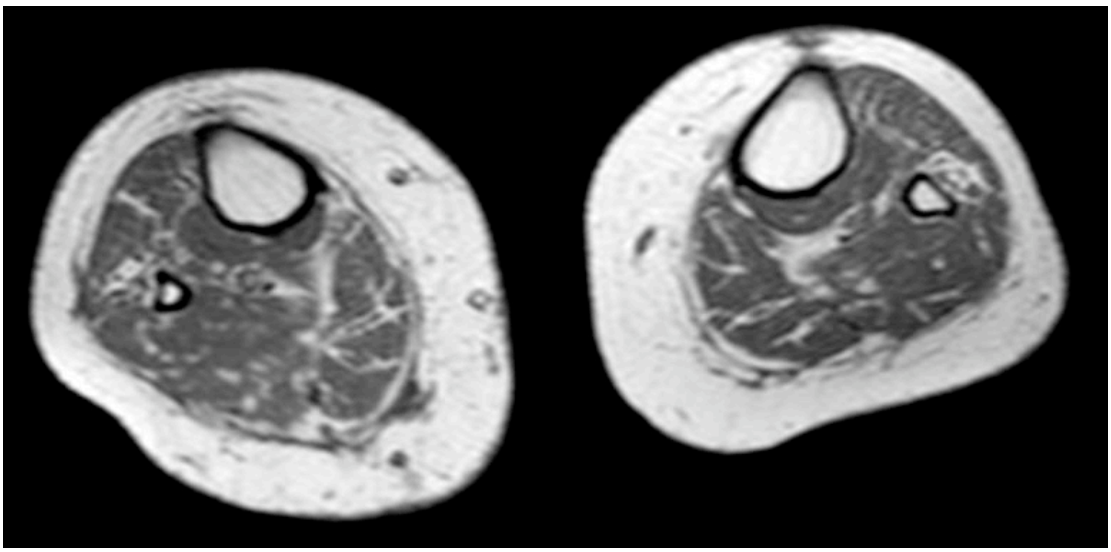


Figure 6.4 MRI scan of participant 4 gastrocnemius pre-treatment

Functional ability and questionnaires

The timed up and go test was used as a measure of functional ability. Participants were unable to perform the test at baseline, due to inclusion criteria preventing the ability to mobilize. Table 6.9

outlines participants who were able to conduct the test at the completion of study participation. Participant 1 and 8 were unable to perform the test at 6-weeks, however all other participants completed the test. The mean time of completion for the treatment participants was 15.5 seconds, 1 control participant completed the test with a time of 16.4 seconds. This indicates that gait speed improved more in the control participant than the treatment participants.

Participant	Timed up and go test (seconds)	
	Baseline	6-weeks
1	0	0
2	0	16.4
3	0	16.6
4	0	14.4
5	0	NA
6	0	NA
7	0	13.8
8	0	0
9	0	NA

Table 6.9 timed up and go results for all participants
NA= not applicable (consent withdrawn)

Due to the varying nature of stroke the functional changes experienced by each participant were considered on an individual basis. Table 6.10 demonstrates the main functional changes witnessed throughout the NHS physiotherapy program, and any significant effects on muscle function of the NMES protocol.

Participant Number	Main changes witnessed through study	Changes in functional questionnaires
1 (T)	Week 3- able to sense NMES on application (previously unable to sense stimulation) 11.3° increase in pennation angle of gastrocnemius despite no change in volitional muscle force	*NEADL* Pre-stroke: 12 Baseline: 1 6 week: 1

	No functional recovery: discharged to nursing home	*Barthel Index* Baseline: 10 6 week: 10
3 (T)	Week 2- first evidence of muscle force in quadriceps (1/5) Patient displayed difficulty activating quadriceps in non-functional patterns Week 5- begun mobilising along corridor with assistance	*NEADL* Baseline: 11 6 week: 11 *Barthel Index* Baseline: 15 6 week: 45
4 (T)	Week 1- able to stand with assistance of 3 people Week 2- begun to mobilise with assistance Week 3- reported full sensation of NMES (partial sensation previously) Week 4- experiencing reflex patterns with NMES Week 6- mobilising with tripod independently	*NEADL* Baseline: 4 6 week: 16 *Barthel Index* Baseline:25 6 week: 60
7 (T)	Week 1- Quadriceps muscle force evident (1/5). Clonus evident in gastrocnemius in standing Week 2- mobilising in parallel bars Week 5- mobilised up and down stairs (with tripod, step to pattern). Application of botulinum toxin to gastrocnemius and upper limb.	*NEADL* Baseline: 5 6 weeks: 11 *Barthel Index* Baseline: 40 6 weeks: 70
2 (C)	Week 3- muscle force in hip flexors and quadriceps increased from 0/5 to 3/5 and 2/5 respectively Week 2- spasticity of lower limb apparent; especially hip flexors, plantarflexors Week 6- mobilised in parallel bars for first time	*NEADL* Baseline: 6 6 week: 10 *Barthel Index* Baseline: 15 6 week: 55
5 (C)	Withdrew consent due to hyper-sensitivity and pain on affected side (unrelated to NMES- control participant)	N/A
6 (C)	Withdrew consent due to being unhappy with allocation to control arm. Participant saw involvement in study as 'pointless'	N/A
8 (C)	No recovery of volitional muscle function 6.6° increase in pennation angle of gastrocnemius despite no change in volitional muscle force	*NEADL* Baseline: 2 6 weeks: 2

	No functional recovery: discharged to nursing home	*Barthel Index:* Baseline: 5 6 weeks: 10
9 (C)	Passed away in week 2 due to a pulmonary embolism (unrelated to study participation)	N/A

Table 6.10 Functional changes experienced by participant and functional questionnaire results. T= treatment, C= control, NEADL= Nottingham Extended Activities of Daily Living Questionnaire

Table 6.10 demonstrates a wide variability between recovery of participants. Generally participants who were functionally mobile at the end of the 6 week intervention period improved on their functional questionnaire scores (participant 3, 4, 7 and 2). The improvement was seen in both NEDAL and the Barthel Index. Participant 3 improved on the Barthel Index (improvement of 30), but not the NEADL (improvement of 0). Participants who did not regain functional capability (participant 1 and 8) did not demonstrate change on either questionnaire. When comparing differences between changes in both questionnaires there was a small relationship identified (R^2=0.381), however variability in functional changes and a small sample size results in individual analysis being paramount.

Four participants regained the ability to walk at study completion (three treatment [95% CI 0.3-0.95] and one control [95% CI 0.06-0.70]). The odds ratio of a participant in the treatment group regaining the ability to walk is 3 (95% CI 0.2-162.5). This indicates that a participant receiving NMES as part of their physiotherapy intervention is 3 times more likely to walk than somebody receiving physiotherapy alone. The high confidence interval suggests that this method of analysis is reflective of the small sample size and will be interpreted with caution. On average treatment participants took 4.3 weeks to regain the ability to walk, where the only control participant who regained ability to walk took 6 weeks to restore this function. The variance in ability is noted by indicating the main

compensation to assist walking ability in table 6.11. Participants 3 and 4 restored a gait pattern that was reflective of normal gait, and thus there was no compensation that predominantly assisted.

Participant	Group	Week able to walk	Main compensation
2	C	6	Hip flexion
3	T	5	
4	T	6	
7	T	2	Hip flexion

Table 6.11 Participants who gained the ability to walk during study participation

6.6.1 Overview of study four results

The first significant finding of this study was the extended recruitment time to fill inclusion criteria. The number of individuals screened was 10.5 the amount recruited into this study. A research trial investigating thrombolytic treatment was running parallel to this study; often preventing participation in this trial. Unfortunately three participants were lost to follow up (reasons outlined in table 6.4).

Maximal isometric force production (MIFP) was measured in the leg measurement device (LMD). Interestingly the control group increased quadriceps force more than the treatment group, however this was confounded by the control sample size (N=1) and hip flexor spasticity in the control participant. Gastrocnemius MIFP increased more in treatment participants than control participants, and a proportional relationship was identified between the two muscles; those who increased quadriceps MIFP also increased gastrocnemius MIFP. All participants stimulated both muscles with high stimulation intensities. Two treatment participants reported a reduced sensation to NMES at study enrollment, which was restored during the six week intervention period.

When considering muscle architecture of the lateral head of gastrocnemius some interesting observations were noted. There was some indication that the NMES protocol influenced subcutaneous adipose layer, however the effect was greater when considering pennation angle. The participant to decrease subcutaneous adipose layer with the highest value was in the treatment group. There was a larger increase in pennation angle in the treatment group compared to the control group, suggesting that NMES produced adaptations in muscle architecture. Interestingly participant 1 produced the greatest increase in pennation angle, however did not demonstrate restoration of muscle strength. This suggests that NMES is able to improve internal muscle structure without volitional control of the muscle. Participant 8 was a control participant who also increased pennation angle, but to a lesser extent, indicating that physiotherapy has the ability to cause muscular adaptation. There was a relationship identified between pennation angle and MIFP, indicating that pennation angle increased in proportion to MIFP. The small sample size (N=4) must be considered in extrapolating from this information.

Functional measures varied throughout the sample and indicate that individual analysis is required. The timed up and go test was shorter in the treatment participants compared to the control participant, indicating that the control group improved gait speed more than the treatment group. The sample size in the control group (N=1) and young age must be considered here. Four participants re-gained the ability to walk after treatment with NMES; 3 treatment participants and 1 control participant. This equated to a participant in the treatment group being 6 times more likely to walk as a result of NMES than those who did not receive NMES. Table 6.12 summarizes the main findings throughout this study.

Finding	Number of participants who improved	
	Treatment (N=4)	Control (N=2)
Regained ability to walk	3	1
Improved sensation during treatment with NMES	2	0
Reported lower limb spasticity during study involvement	1	1
Improved Quadriceps Femoris MIFP	3	1
Improved Gastrocnemius MIFP	3	1
Improved subcutaneous adipose layer on 2D ultrasonography	3	0
Improved pennation angle on 2D ultrasonography	4	1
Improved timed up and go test	3	1
Improved on NEADL questionnaire	2	1
Improved on Barthel Index questionnaire	3	2

Table 6.12 Summary of main findings throughout study three

6.7 Discussion

The aim of this study was to ascertain whether a 6-week strength training protocol with NMES was able to prevent muscle disuse atrophy in stroke patients during the acute rehabilitation period. The use of ultrasonography to explore changes in internal muscle architecture is something that has had limited use to date in clinical literature (Kwah, Pinto et al. 2013). Monitoring of muscle structure as well as force producing capability provides a greater understanding of the effects of a NMES treatment protocol on skeletal muscle. Prevention of acute disuse atrophy as a result of post-stroke forced inactivity would enable rehabilitation programmes to progress in a quicker time scale. This

study was successful in working with physiotherapists to incorporate NMES into rehabilitation programmes.

Participants were approached to take part in this study once they had been screened for relevant inclusion and exclusion criteria. Consultant opinion was critical in determining whether the patient was likely to maintain medical stability throughout the study. This was one of the more difficult inclusion criteria to fulfil, as many acute stroke patients have co-existing medical problems in the acute phase of hospitalization (Diederichs, Muhlenbruch et al. 2011). Awaiting medical clearance often resulted in an extension beyond our 2-6 week post stroke timescale.

Medical status is often difficult to predict in the acute phase of hospitalization, which was highlighted by the withdrawal of participant 9 (passed away as a result of a pulmonary embolism). An experienced consultant's opinion is the most secure method of identifying appropriateness for participation, and no problems were faced with communication with medical staff throughout this study.

Another inclusion criterion that was challenging during the recruitment process was severity of stroke. All patients who entered the acute stroke ward were screened for participation, however many were too independent post stroke to fulfil criteria. As many patients as possible (those who were admitted in the relevant timescale, and those who consented) were thrombolysed on admission to the unit. This was conducted in line with a research study that was running alongside this study. Some patients demonstrated recovery of symptoms through re-absorption of oedema and haemorrhage as a result of the thrombolysis (Ahmed, Kellert et al. 2013), resulting in a functional ability that excluded them from participation in this study. The recruitment period had to be extended as a result of these issues. Extension was however limited due to funding restraints,

which limited the number of participants recruited. Recruitment resulted in 9 participants consenting to take part in the study. All participants who were approached and maintained medical stability throughout the recruitment process (approximately three days once all relevant parties had been informed of study processes) consented to participate, indicating that the methodology was well accepted in the hospital setting. Although the original proposed sample size was small (n= 10), not reaching our target had implications for statistical analysis and data interpretation.

Research in an acute medical setting runs a high risk of participant drop out. This is especially evident in a population such as stroke due to the advanced risk of acute medical conditions whilst in inpatient care. Three participants were lost throughout this study, which impacted an already small sample in terms of analysis. The withdrawal in all three participants was unrelated to the study protocol; in fact all three participants were allocated to the control arm of the study. This left a control sample of N=2, which restricts the ability to infer from these results.

Participant 5 withdrew consent due to increased neuralgic pain and hypersensitivity on the affected side as a result of the stroke. She also refused to consent to standard NHS physiotherapy during this period. Neuralgic pain is a common problem in stroke survivors, in which electrical stimulation has been cited as an encouraging therapy to assist (Pittler, Ernst 2008). However, this was not the aim of this study.

Participant 6 decided not to take part in the study, as she was disappointed in being allocated to the control arm. The potential effects of treatment with NMES highlighted in the information sheet were strong enough to encourage her to consent, but despite explanation as to the importance of control group use, she decided to withdraw. It may be that more time should be allocated to the explanation of research design in an acute medical setting, which may reduce withdrawal rates.

Participant 9 passed away during the 6-week period. The medical condition of this patient deteriorated with prolonged bed rest, a risk that is unavoidable in acute medical research. Recruitment of a larger sample would have lessened the effect of participant withdrawal, and maximised the use of the control group to ascertain the effect of treatment with NMES. If a larger sample was targeted, further time and funding would have been required, which was not available at the time of completion.

As with previous studies throughout this thesis, the leg measurement device (LMD) was utilised for testing muscle force. Both positive and negative aspects of its usage throughout the testing period were encountered. A design modification was employed early on in the recruitment process to enable easier positioning of participants into the device. The sitting plate was extracted to enable the device to be positioned underneath the participant's femur without moving them from their sitting position. This was necessary as the level of stroke severity often required hoisting the participant out of their chair to be positioned for the study. This was not always possible due to the number of staff required for manual handling purposes. The changes were effective at easing positioning of the patient, however contributed to a reduction in positional stability. The LMD remained in contact with the chair, and this sacrifice was deemed necessary for completion of outcome measure testing. In participants with a flaccid limb presentation, pillows were used to maintain the leg in a stable position. Participants reported that they found the LMD comfortable, and the majority of participants enjoyed having their limb in a stable position. The portability of the LMD meant that we were able to transfer it in a car, to the hospital and between patient beds. It was also wipe clean in order to adhere to infection control policies. Both of these issues made its use practical, effective and easy to setup in an acute ward environment. Once transferred onto a computer the results were sensitive enough to detect small changes in strength. This was a vital

aspect of our protocol, as small changes in muscle control could be the difference between clinical dependence, and clinical independence.

Participant feedback with regard to the sensation associated with NMES was positive. All participants who completed the study were comfortable with the stimulation, and reported that they liked seeing their muscles contracting, when many were unable to perform this volitionally. From a psychological perspective, this was important to maintain their motivational levels to participate in rehabilitation protocols. Many stroke patients find it difficult to accept the effect a stroke has on their lifestyle, with the possibility that their functional independence may not be equal to their pre-stroke level (Allan, Rowan et al. 2013). Upon recruitment into the study many participants were apprehensive about the sensation of the stimulation. The acclimatisation session was effective at reducing any anxiety, and allowed participants to better understand what the treatment entailed. Research also indicates that enhanced stimulation intensity maximises force output from the muscle, indicating the importance to acclimatise to the sensation (Stevens-Lapsley, Balter et al. 2012). Investigation as to the effects of the stimulation intensity used to administer NMES within neurological rehabilitation is still to be conducted. The acclimatisation session also ensured that the participant had time to gain trust in the researcher, and that they would approach the application of NMES in a progressive manner. By the end of the study all participants reported that they would be happy for NMES to be administered as part of standard physiotherapy. Along with the effects on the neuromuscular system, this is the most important outcome of this trial. If participants would not consent to having NMES as part of a standard clinical intervention, there would be no justification in continuing research in this area.

The aim of this study was to act as a proof of concept for three main issues; can NMES be effectively administered on an acute stroke unit; is the LMD practical and accurate when testing muscle

strength on a hospital ward; and finally can muscle architecture accurately be measured to infer strength gains in this population. Rationale for testing on a large population is limited and thus a small sample size was deemed sufficient to address these primary goals. The small sample is also reflective of the funding available to conduct this study, and reflects the practical ability to recruit in a limited time period. A larger sample size would have enabled more statistical rigor when inferring from results, especially given the high probability of complications that result in an acute neurological population. This was especially evident in the control group of this study (N=2), which demanded individual consideration of results. However, acknowledgement in the literature has been provided that feasibility and logistical implications often prevent appropriately sized samples from being tested (Tan, Machin et al. 2012). This study adopted a randomised controlled trial (RCT) design, despite a small targeted sample size. A control group was deemed necessary to ascertain the effects of natural recovery in a neurological population such as stroke. A respectable quality of methodology is essential to conducting research for medical purposes, and maximises the ability to accept results despite a small sample.

One of the primary aims of this study was to accurately and simply measure muscle strength in a neurologically impaired population in a hospital setting. The leg measurement device (LMD) enabled this aim to be fulfilled. Neurological rehabilitation is associated with small advances in strength and function, making high transducer sensitivity vital when designing a measurement device. Hand held dynamometers, which are commonly used during research (Bohannon, Andrews et al. 2013) are able to measure moments, however the LMD designed and implemented within this thesis is likely to be more accurate and repeatable due to its design. Participants were tolerant and compliant with all study measurements. The participant was positioned in the LMD whilst measurements were taken, to standardise joint position and the gravitational effects on flaccid limbs. The small sample size made measurements vital to obtain if medically and practically possible. Measurement of muscle architecture with ultrasonography was also conducted in the LMD to maintain a standard limb

position. The quality of ultrasound image enabled confidence to be placed in images. Reliability in measurement of the researcher could have been performed, and verified by an independent source which would have allowed greater confidence in this outcome measure. Time and resources limited this and was outside the scope of this PhD research. Muscle thickness was not included in analysis, due to lack of depth in the images. Increasing depth would have had a consequent effect on image quality, which would have hindered ability to infer from this measurement. Unfortunately the MRI scans did not provide high image quality, and the conclusion was made that manual measurement could not reliably outline the muscle belly borders in order to perform analysis. Issues were also concerned with participant comfort in the scanner, indicating that imaging was not always possible (Bigley, Griffiths et al. 2010). This was disappointing as comparison of ultrasound images with MRI scans would have been beneficial for acceptance of ultrasound as a cost effective measurement tool in clinical practice. The protocol used in implementing the MRI scan requires modification to address image quality before subsequent trials are conducted.

Neurological rehabilitation is fundamentally governed by maximising functional independence. The ability to relate the results to functional ability was therefore paramount in achieving the aims of this study. Lower limb rehabilitation has a strong associated with walking ability, as the ability to mobilise dictates functional independence. Participants 3, 4 and 7 were all treatment subjects who demonstrated an improvement of walking ability. It is difficult to ascertain the probability of walking from the treatment group compared to the control group, as the randomisations were not equal, however odds ratio calculation indicates that a participant in the treatment group was 3 times more likely to walk than a control participant. Although the sample for using odds ratio analysis is small we feel it is justified. It enables probability of one of the primary goals of rehabilitation to be highlighted, and thus provides evidence for a larger trial to be conducted (Schechtman 2002). The results have to be interpreted with caution in this proof of concept trial. If conducted on a larger sample, it is not envisaged that odds ratio analysis will produce such a high probability of walking.

However it does lend support to the argument that NMES is having an effect on muscle, which should be further investigated.

Participant 7 was the treatment participant who demonstrated greatest improvement of functional capability. He presented with a dense hemiplegia, and was discharged not long after study completion (2 weeks after) walking with a stick. Participant 7 was the youngest participant in the study (49 years), who suffered a right middle cerebral artery stroke. Neuroimaging of the brain during rehabilitation is creating a novel insight as to how the cortical brain reorganises after neurological insult. Interestingly activated areas in the motor cortex are higher in aged individuals compared to a younger counter-part, suggesting that compensatory actions commonly occur in younger individuals to account for reduced cell function (Calautti, Serrati et al. 2001). This could explain the high level of function restored in this participant. The underlying mechanism of increased activation in older individuals remains unclear. Advances in brain plasticity as a response to exercise have been associated with diet and cardiovascular health (Reuter-Lorenz, Lustig 2005). Poor diet and cardiovascular health is often thought to be risk factors for stroke (Roger, Go et al. 2011) making these patients vulnerable to a longer rehabilitation period. Participant 7 may have utilised compensation mechanisms or had a larger cognitive reserve due to his age; a larger amount of intact neurones that had not been effected by ageing pathology before the stroke (Scarmeas, Zarahn et al. 2003). This reserve of function may have contributed to the rapid advances in walking ability that were gained.

Research into mechanisms involved in human locomotion is developing, with the understanding of central pattern generators (CPG). CPG's are a collection of neurones found at spinal cord level that combine to form a reflex that can be used to produce patterns of movement, such as gait (Grillner 2003). Using indirect evidence, this information has been collated to better understand human

locomotion. Afferent information from the vestibular, visual and proprioceptive systems is combined and utilised by the CPG to produce learnt sequences of movement. After a stroke or cerebral insult, the activity of the spinal reflex is thought to be altered (Dietz 2003). This interrupts processing of afferent information and consequently results in impaired locomotion. Restoration of this mechanism through exercise repetition has been documented as a potential mechanism responsible for increased function, with an associated increase in neurotransmitter activity (Marder, Bucher 2001). This suggests that rehabilitation with the aim of developing the timing of associated movement reflex patterns will help to achieve functional improvements in performance. Motor re-learning approaches to stroke rehabilitation, aid this process (Langhammer, Stanghelle 2000). Results in this study indicate that NMES has a positive effect on walking ability. Repetitive stimulation of afferent pathways that have been damaged during the stroke may result in a quicker recovery period. Inclusion of NMES into a physiotherapy treatment protocol would therefore reduce the non-ambulatory period post stroke, effectively reducing the period of CPG impairment associated with this. Quicker restoration of gait implies that less time is available for deterioration, and therefore functional ability would be restored as a result of training.

Participant 2 was a control participant who restored the ability to ambulate during the study. This participant was also uncharacteristically young to have suffered a stroke within such a small sample (54), indicating that his cognitive reserve may have enabled him to progress faster with rehabilitation. Muscle spasticity was evident in hip flexors and plantarflexors, although not directly measured in this study (concluded by consultant opinion). Spasticity is recently thought to be a transient increase in muscle tonicity, to which the incidence declines with chronicity (Bakheit, Fheodoroff et al. 2011). Interestingly plantarflexor spasticity has been shown to be an active restraint to dorsiflexor activity, contributing to their weakness. Dorsiflexors have been identified as a primary determinant of gait improvement post stroke, and thus should remain a focus of rehabilitation (Ng, Hui-Chan 2012). Measurement of plantarflexor activity in this participant may also

have been affected. A toe initial contact was utilised during gait, and a walking stick used for balance in the unaffected arm. It may be that the dorsiflexors of this participant were weak, reducing stability during gait (de Niet, Weerdesteyn et al. 2013); however the ability to compensate for this was aided by his age. A larger sample of control participants is required ascertain the effects of NMES on recovery of walking ability. Physiotherapy is able to improve functional recovery; identification of interventions such as NMES to reduce the time scale of recovery is of importance in maximising potential from rehabilitation.

The aim of treatment with NMES in this study was to demonstrate improvements in the voluntary force capability of muscle; measured via maximal isometric force production (MIFP). All participants who regained ability to generate force demonstrated improvements in quadriceps and gastrocnemius force production. Interestingly the participant who demonstrated greatest quadriceps improvement (19 Nm) was in the control group (participant 2). Although showing the greatest increase in quadriceps muscle force, this participant demonstrated little improvement in volitional gastrocnemius muscle force (2.6 Nm over 6 weeks). Although activation of proximal muscles tends to occur before distal muscles, the vast difference indicates that the quadriceps activation was not a true representation of strength. Ability to activate the hip flexor muscles was documented by physiotherapists in week 3 of study participation, and could provide a compensation mechanism used to achieve the witnessed force. Bi-articular rectus femoris may have contributed to the quadriceps force as measured via the LMD, and thus not be a true representation of the muscles function. The ability to restrict hip flexor input to knee extensor force is limited, and therefore is a compensation that cannot be eliminated for research purposes via the LMD. The individuality of symptom presentation in stroke patients would assume variation in results making testing of a larger sample difficult, and therefore indicates that individual analysis is required. Additional testing of electromyography (EMG) would have enabled activation of specific muscles to be monitored, and therefore allow a more accurate justification to be produced. It must be noted that compensations

to allow functional independence are considered positively in motor-learning forms of rehabilitation (Arya, Pandian et al. 2011).

Participant 7 was the participant to demonstrate functional improvement within the quickest time period (2 weeks). The increase in strength of both muscles was the lowest of the treatment participants. As previously discussed, the age of this participant may be reflective of an enhanced ability to restore pre-learnt functional movement patterns. However the ability of this participant to ambulate implies that the level of force required to walk has been met. Threshold of functional muscle activation has been touched upon in a frail population, however only three studies are able to guide practice (Fujita, Kanehisa et al. 2011). The ability to transfer this finding to neurological recovery has not been investigated on completion of this thesis. The level of isometric torque required to perform functional daily tasks in an elderly population has been estimated at 3 Nm, below which function is anticipated to reduce (Ploutz-Snyder, Manini et al. 2002). Interestingly participant 7 only achieved a maximum of 1.8 Nm of quadriceps force, which is below the threshold currently indicated for functional activity. It may be that compensations allowed function to be achieved or that instruction as to the movement required for assessment could have been improved. More time spent ensuring outcome measures were assessed in an accurate fashion is important, especially given possibility of damaged cognitive function and therefore understanding as a result of the stroke. Threshold for gastrocnemius function has not been investigated. Understanding of its contraction requirements may be paramount in understanding the ability to perform functional activities in a population undergoing rehabilitation given the propelling role of the muscle during gait (Perry, Burnfield 2010). This particular participant (7) achieved 8.2 Nm of gastrocnemius force, which may be reflective of a functional threshold.

Changes in muscle architecture were monitored via 2D ultrasonography, which has been identified to correlate with improvements in muscle strength (Aagaard, Andersen et al. 2001). Although correlations in healthy human muscle have been observed, little research has been conducted in neurological impaired populations, and even less so in a stroke population (Gao, Grant et al. 2009). Research into muscle architecture in stroke has focused on chronic patients, with the aim of addressing the rate of atrophy. Investigation into pennation angle in this study therefore provides an insight as to how the muscle adapts to NMES in an acute time period. Pennation angle was demonstrated to increase more in treatment participants than control participants; which correlates with changes in maximal isometric force production (MIFP). This suggests that treatment with NMES demonstrated changes in muscle architecture, as opposed to changes in neuromuscular function. During hypertrophy of skeletal muscle, satellite cells are activated to formulate and regenerate muscle fibres (McCarthy, Mula et al. 2011). The resultant increase in muscle related proteins, and the addition of sarcomeres in parallel increases the physiological cross sectional area of muscle (Schoenfeld 2010); more sarcomeres in a given area. This process is required to see changes in hypertrophy; investigation should now focus on the long-term follow up of these changes.

Participant 1 demonstrated the greatest improvement in pennation angle of all participants (11.3°) suggesting that the internal muscle structure would be capable of producing a greater muscle force at the end of the study than when he enrolled in the study. However, participant 1 did not demonstrate any improvement in either quadriceps or gastrocnemius volitional force. A flaccid limb presentation was present throughout the testing period, with dependence on others for activities of daily living. Allocation to the treatment group resulted in this participant receiving 6 weeks of stimulation with NMES. This indicates that NMES was able to produce improvements in internal muscle structure despite no restoration of volitional force production. The change in architecture also suggests that atrophy as would normal be witnessed after prolonged rest was prevented in this participant (Armbrecht, Belavy et al. 2010). Stimulation with NMES appears to be a means of muscle

contraction to prevent decline in contractile ability despite no sign of neurological recovery. It may be that this increase in pennation angle is a vital aspect of recovery, resulting in the ability of the muscle to contract if some functional capability were to be restored. Prevention of muscle atrophy in this acute period would enable rehabilitation to focus on functional gains, thus decreasing rehabilitation time scales.

Participant 8 (control group) also demonstrated an increase in pennation angle over the 6 week testing period despite no volitional muscle capability. The increase was smaller than participant 1, and therefore measurement error cannot be eliminated. Further reliability testing of pennation angle measurement is required for confirmation of this. The assumption that changes in muscle architecture witnessed in control participants are a result of physiotherapy input cannot be disregarded. Although muscle contraction was not initiated, activation of damaged afferent nerve pathways has been shown to be initiated as a result of contralateral limb movement (Michielsen, Smits et al. 2011). However, the activation of nerve pathways in stroke survivors has not been correlated with a change in muscle hypertrophy, and would at best advance neuromuscular firing rates if function were to be restored.

Hypertonicity is a common presentation in stroke patients, and a symptom that was present within this small sample. The knowledge of how spasticity affects muscle architectural changes on ultrasonography has not been fully explored. Research has been conducted in children with cerebral palsy, indicating that fascicle length is shortened in children with spasticity (Shortland, Harris et al. 2002). Limited research has been conducted using pennation angle as an outcome measure in stroke, however it is suggested that smaller pennation angles may be indicative of fascicular tension as a result of spasticity (Gao, Grant et al. 2009). Participant 2 (control) was the only participant to decrease pennation angle, despite making functional changes over the 6-week monitoring period.

Spasticity of the gastrocnemius was documented in the medical notes of this participant, and although he did not receive treatment, the spasticity may have been responsible for the decrease in pennation witnessed. On reflection measurement or a rigorous form of monitoring of spasticity could have been employed throughout this study to ascertain the effect on outcome measures given the small sample size used. Electromyography would also be able to indicate the state of the muscle upon testing, giving an indication as to whether there is cortical input as a result of the stroke (Skold, Harms-Ringdahl et al. 1998). This would enable confidence in associating changes in adaptation of muscle to NMES, and give justification to conduct larger studies. However, as a proof of concept study, the changes in muscle structure noted highlight the demand of a larger study.

On initial application of NMES participant 1 was unable to sense the stimulation of NMES, despite it producing a strong visible tetanic contraction. The visible contraction produced by NMES appeared to have a psychological effect of increasing motivation to participate in rehabilitation (Langhorne, Bernhardt et al. 2011). Consideration was taken as to whether the NMES was ethical to administer if sensation was impaired. However, discussion with clinical staff decided that application was safe to apply under careful application and monitoring (Baker, Wederich et al. 2000). The stimulation was taken to an intensity where the muscle produced a strong tetanic contraction, but not beyond this point. Participant 1 began to respond to NMES in week 2 of testing; reporting that he could feel pins and needles in his quadriceps femoris during the stimulation. The change in sensation progressed over the remaining 4 weeks to being able to fully feel the sensation when stimulating both muscles. Diminished sensation indicates that the stroke had affected the innervation to the mechanoreceptors responsible for cutaneous sensation, resulting in them not transmitting signals to the motor cortex (Bowden, Lin et al. 2014). Application of NMES to a muscle innervates cutaneous nerve endings under electrode sites, regardless of if they have been affected by neurological insult. Repeated stimulation of this afferent pathway via NMES could induce a learning effect, resulting in the cutaneous distribution being able to feel the sensation. Studies suggest that stimulation induces

cortical reorganisation, and redistribution of afferent pathways resulting in improvements in sensory output (Peurala, Pitkanen et al. 2002). Sensory stimulation has also been linked to improvements in motor control and movement ability in chronic stroke patients (M.Dimitrijevic, Stokie et al. 1996), demonstrating the ability of NMES to influence multiple functions. The repetitive nature of treatment with NMES could result in a process known as sensitization. Sensitization is a learning response whereby a response to stimuli strengthens or amplifies (Shumway-Cook, Woollacott 2007). Changes in potassium conductance have been suggested to be responsible for this response; this affects this excitatory post synaptic potential allowing a greater amount of potassium to be released. This allows an action potential to be transmitted to the CNS, informing the individual of a sensation. This finding was only witnessed in one participant; hence it is not a result that can be transferred to a greater population until further research has been conducted to test the effect of NMES on cutaneous sensation in acute stroke. The effect of natural neurological recovery cannot be established when inferring from one participant, but this finding may give justification to deepen the research surrounding this area of NMES use.

Functional ability of participants was monitored via a timed up and go test and functional questionnaires. Statistical analysis of functional ability was difficult to perform due to individual symptom presentation affecting outcome. Functional ability was therefore assessed on an individual basis. All treatment participants (except one) were able to be measured in the timed up and go test on study completion. This indicates that NMES influenced function gain post stroke, however the small sample in the control group limits the ability to infer from this finding. Participant 2 (control) was able to mobilise, and performed the timed up and go test within one standard deviation of the treatment participants. The timed up and go test has been validated for use as an outcome measure in stroke patients (Ng, Hui-Chan 2005); however the effect of age on the test has not been established to date. The age of this participant must be taken into consideration which may have influenced the rapid ability to perform this measure in the control group. Treatment participants

demonstrated a greater improvement on qualitative outcome measures than control counterparts; indicating that NMES had a positive influence on functional gain post stroke. The transferability of treatment effects with NMES has little solid link to improvements in functional ability in stroke patients. A larger RCT with a primary goal of assessing functional ability should be conducted. NMES as administered throughout this thesis is isometric, which is a non-functional application. Future research could consider the effects of a functional (weight bearing) application of NMES to assist a movement pattern (sit to stand) in relation to a non-functional application. The ability to infer from qualitative data would therefore be greater. Functional ability has been related to primary outcome measures throughout this discussion, namely walking ability and maximisation of muscle strength, and should be considered in the transferability of results to a wider population.

6.8 Conclusions

This study highlights that NMES is able to be administered on an acute stroke ward, and was accepted by the clinical staff. Both medical and physiotherapy staff were tolerant of the research study, and took time to assist with study processes. Incorporation of NMES into an acute clinical setting requires acceptance from the multidisciplinary team in order for its use to be effectively integrated into existing protocols. This study provides a further step towards achieving this aim. **Both the LMD and the ultrasound were measurement devices that had to ability to be used in an acute clinical setting with little disruption.** Both devices were accepted by participants and staff, and confidence would be placed in their continued use. A number of clinical findings have been highlighted as a result of this study, and have been described below:

- **The probability of walking as a result of NMES was higher in participants who were treated with NMES as an adjunct to physiotherapy compared to physiotherapy alone.** Physiotherapy and natural recovery of symptoms are thought to increase the likelihood of

walking. The goal of NMES would be to decrease the rehabilitation time that it takes to mobilise, with the aim of preventing unwanted deterioration of muscle function. It appears that NMES was effective in achieving this aim, and further investigation is warranted in a larger study.

- **Maximal isometric force production (MIFP) demonstrated some variability within each group, although treatment groups demonstrated a larger increase**. The wide age range and unknown speed of natural recovery implicates this. Results have been related to a threshold of strength required to achieve function. Further research in this field is required to identify the point in rehabilitation where the individual is most likely to demonstrate improvements in function; thus allowing structure and maximisation of the acute rehabilitation period.
- Advances in pennation angle were witnessed in a treatment participant who did not restore voluntary muscle strength. **This indicates that NMES has the potential to maintain or advance internal muscle structure, or force generating capacity in a population who is unable to achieve this by volitional means**. This would enable the individual to have a greater likelihood of functional ability if natural recovery were delayed due to the stroke.
- **Cutaneous sensation was restored in a participant who received treatment with NMES**. Restoration of sensation was initially noticed during stimulation sessions, and advanced throughout study participation. **The ability to restore cutaneous sensation has clinical implications for individuals in identifying areas of pressure in prolonged bed rest, or mis-fitting orthotics before they present a problem to medical staff**.

The findings above demonstrate the potential of NMES for use in an acute environment. Acknowledgement must be made to the small sample size, which limits the ability to confidently attribute these findings with treatment. Individual description of participants is often required in this population due to the varied nature of clinical presentation. Further investigation on a larger scale is required to fully investigate the trends identified in this study. The use of electromyography to

ascertain effects on specific muscles would help to develop the methodology used and justify responses obtained. It would also be useful to utilise the LMD to establish the effects on muscle contractile properties, to ascertain whether these differ from a healthy elderly population. The time the muscle takes to respond to a stimulus may differ in a neurological impaired population. The methodology used has been tested throughout this thesis, and the stimulation parameters appear effective in enhancing muscle function in this population. Despite this, the response of muscle to NMES appears to vary both between participants and within participants. The muscle response is not constant despite a constant stimulus being applied. This indicates that other variable factors may be influencing how a muscle responds to stimulation. This is a finding that is hindering the acceptance of NMES into clinical settings, and requires further investigation.

Chapter 7

Study four: Investigation of biomarkers resulting from ex-vivo electrical stimulation of muscle

Studies one to three have highlighted optimal stimulation parameters that are able to induce strength training effects after a 6-week application of NMES. There appears to be variability within this effect, with some participants responding to treatment better than others. Advances in strength are small, indicating that variability may be influencing the interpretation of results. Investigation of this variability is required to ascertain the true muscle response to NMES. This study aims to address this issue.

7.1 Introduction

Previous trials in this thesis have investigated the use of NMES as a strength training modality in different populations. The overriding aim of the thesis is to treat people of different ages and pathologies, making the conclusions as clinically relevant as possible. When considering older and younger healthy adults, the six week protocol was effective at increasing maximal isometric force production (MIFP): 9 participants increased MIFP in both age groups (n=10). Within this increase, variation was witnessed with some participants demonstrating a greater increase than others (Mean= 6.5 Nm). This variation indicates that adaptations in the muscular, or neuromuscular system of participants may have occurred (Ozmun, Mikesky et al. 1994); however the muscle response time to stimulation varied within this. Increases in neuromuscular control have shown to be a precursor to hypertrophic changes in muscle in the initial stages of a strength programme, resulting in an improved firing rate or number of neurones within the motor unit. NMES is accepted to stimulate nerve fibres before muscle fibres, as the propagation threshold is lower in neural tissue (Peckham,

Knutson 2005). This supports a change in neural control when stimulating with NMES, which is reinforced in current literature (Hortobagyi, Maffiuletti 2011).

Changes in muscle morphology were witnessed in participants with Stroke (pennation angle measured via ultrasonography), despite 1 of the treatment participant's not demonstrating improvement in MIFP. This indicates that muscle adaptation is able to occur within the six week training period used throughout this thesis. Both studies indicate that NMES is able to influence muscle tissue, with a variety of responses witnessed from participants. This variation of strength gain restricts the understanding of the underlying mechanisms responsible when administering NMES. This mechanism requires exploration to fully understand the response of muscle to stimulation with NMES.

Literature is well established in terms of how the body responds to volitional exercise through research into blood components (Brancaccio, Maffulli et al. 2007). Research into the use of Creatine Kinase (Teixeira, Borges 2012) as a marker of muscle activity after exercise has developed over the last 70 years, and continues to be investigated. Creatine Kinase is a measurement of Creatine conversion to Phosphocreatine within the muscle. Creatine is utilised during exercise to enhance the energy supply of the muscle. The understanding of muscle cellular response to exercise provides an indication as to how the muscle will adapt over a longer period of training (refer to chapter two for a full literature review). Animal studies suggest that the effect of creatine kinase in muscle which has been subjected to NMES is similar to that of volitional exercise (Pette, Vrbova 1992), however little research has been conducted on human muscle. Understanding of biomarker adaptation during stimulation with NMES would be beneficial in understanding the variability of strength increase witnessed throughout previous studies. Investigation into human muscle's biomarker response to NMES would aid the understanding of mechanisms involved during treatment. This level of

understanding is required in order for NMES to be accepted in the clinical community. This study aims to ascertain the response of healthy human muscle tissue to NMES at a cellular level. An understanding of muscle response to treatment with NMES would highlight responders, and pharmaceutical intervention is a future possibility to increase responder rates in neurological rehabilitation. The ability to identify responders to treatment would enable a physiotherapist to incorporate NMES into a patient's rehabilitation program with an increased likelihood of success.

It is expected that levels of CK will increase over the treatment period, which would correlate to voluntary muscle literature. This would give justification for the adaptation in muscle witnessed throughout previous studies.

7.2 Aim

To investigate how Creatine Kinase (CK) levels fluctuate over a one off treatment session with NMES. The activity of CK will be compared to that of volitional muscle strength training as documented in current literature. This study forms proof of concept in establishing whether biomarkers can highlight muscle that will respond to NMES treatment.

7.3 Methodology

Design: Experimental, observational study. Ethical approval was obtained by Keele University (see appendix 8)

Sample size: Forty healthy participants were recruited from Keele University student and staff populations.

Method of recruitment: Participants were approached via an email, and posters strategically placed around the university campus. The research team was contacted by email, where a participant information sheet was sent to the potential participant (appendix 9). An appointment to attend a testing session was scheduled on expression of interest.

Inclusion criteria: Participants were invited to take part in this study if they fulfilled the following criteria:

- Aged between 18 and 80
- In a good state of health
- Able to tolerate NMES protocols
- Able to tolerate blood extraction protocols

Exclusion criteria: Participants were excluded from participation if they fulfilled any of the following:

- Contraindications to NMES (active pacemakers, uncontrolled epilepsy)
- Any reason preventing the participant from blood extraction, including but not limited to blood disorders, skin conditions and religion.

Informed consent: Informed consent to participate in the trial was taken once the participant was screened for contraindications, and all relevant information and questions had been answered.

Method of randomization: All participants received treatment, no randomization was required.

Lifestyle questionnaire: A questionnaire was completed by the participant once informed consent had been obtained and the participant had been screened for contraindications. The lifestyle questionnaire gave the research team a greater understanding of the participants nutritional and exercise status over the previous 24 hours. This enabled trends to be identified between deviations from normal blood concentration levels and the participant's lifestyle, or allow us to attribute any deviations to the NMES protocol. The lifestyle questionnaire can be found in full in appendix 10, questions included:

- Demographics: age, height, and weight
- What did you eat for dinner last night?
- How often do you exercise per week?
- Have you done any exercise in the past 48 hours?

This questionnaire was completed and signed by both the participant and the principle investigator. If required time was spent expanding on detail provided.

Testing position: The participant was not supported in the Leg Measurement Device (LMD) during this study, as this would limit testing to one participant at a time. The LMD was not required to extract outcome measures. It is not anticipated that the cellular response would differ if isometric testing were conducted. Testing was conducted in a sitting position on a standard chair, with the hip at 90° flexion, and the knee at 60° flexion. This was measured by a goniometer. The ankle was relaxed on the floor, or supported on a raised box if necessary to maintain knee position. Despite being instructed to maintain the limb position throughout testing procedures, we cannot be sure of exact position throughout the full testing time period. Participants were verbally instructed if they made any adjustments to testing position. The participant was positioned between two tables,

which were both equipped with a pillow. This allowed blood to be extracted from either arm during testing procedures, without compromising participant position.

7.3.1 Testing protocol

Once all information about study participation had been communicated to the participant, the NMES electrodes were set up on the participants right Quadriceps Femoris. Electrodes (50mm X 90mm) were placed longitudinally across the muscle belly the first 5cm proximal to the apex of the patella, and the second 5cm proximal and 3cm lateral to this. This corresponds to electrode position in previous studies, which was deemed appropriate in producing a strong tetanic contraction (chapter 6). Literature is non-specific and variable with regard to electrode placement, with some studies stimulating over the motor nerve in the femoral triangle (Bircan, Senocak et al. 2002); however this was found to cause unwanted noxious stimuli. Electrode placement is demonstrated in figure 7.1.

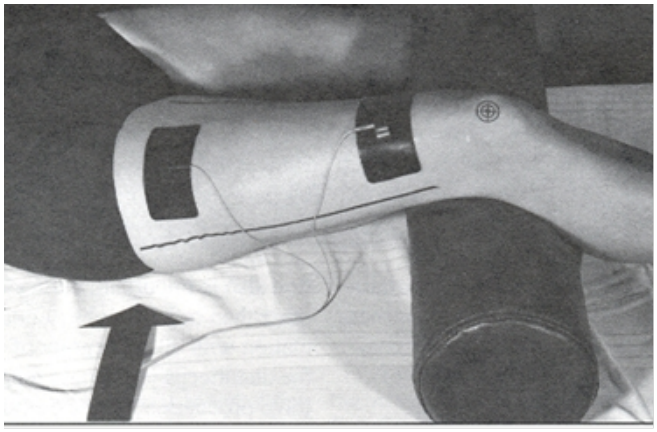


Figure 7.1 Electrode placement on the Quadriceps Femoris.

Based on evidence from CK literature, adaptations to the current NMES protocol were required. The protocol used throughout this thesis may not have demonstrated enough time to test the aims of this study. Adaptations were made under the assumption that this is a proof of concept study, and therefore deemed necessary to provide justification for a larger trial with the original protocol. The

stimulation frequency and pulse width are comparable to previous trials. The stimulation on time, and treatment time has increased to maximise any change witnessed.

Stimulation with NMES was not commenced until the baseline blood sample was obtained. NMES was delivered over a 30 minute period. The parameters used throughout the testing protocol are identified in table 7.1. The participant was verbally introduced to the sensation of NMES, and what to expect. Stimulation intensity was introduced on the first muscle contraction at an intensity that produced a visual muscle contraction. The value of this varied between participants. The stimulation intensity was increased during each contraction until the participant stated that they did not want to increase further. Constant feedback was utilised throughout this process. Rapid increases were made as the participant became accustomed to the stimulation. As the muscle begun to show evidence of force decline (a visual assessment) the stimulation intensity was increased. This allowed the level of visual muscle contraction to be maintained throughout. The main researcher controlled the increase in stimulation intensity in all participants, and therefore aggressiveness in its increase was standardised. This study aimed to elicit a supra-maximal muscle contraction, making it imperative to maintain constant stimulation intensity increases as the intervention progressed. The participant was instructed to relax throughout the stimulation, and allow the leg to respond if the muscle contraction produced movement. Stimulation with NMES continued for 30 minutes, with constant feedback to the research team regarding the participants comfort. A blood sample was collected 15 minutes into the protocol; however the stimulation was not affected by this.

Stimulation parameter	*Setting*
Frequency	50 Hz
Pulse Width	450 µs
'On' time	15 seconds
'Off' time	5 seconds
Ramp times	0.5 seconds
Intensity	Maximal tolerated (ma)

Table 7.1 Stimulation parameters used throughout testing protocol

Once the NMES protocol had finished the participant was instructed to remain seated for 30 minutes. They were asked not to walk around, or consume food or drink in this time period. This would allow us to investigate whether any effects of NMES would carry over this time frame, or whether any changes identified resumed to their resting levels immediately.

After 30 minutes rest and collection of the final blood sample, the participant was offered refreshments before they left. This ensured stability in blood sugar levels which may have been affected by the extraction of blood.

7.3.2 Blood samples

Four ~10ml samples of blood were extracted from each participant via a venepuncture needle into an untreated vacutainer. The Vacutainer contained separation gel allowing good separation of coagulated blood and serum. A medical student, who was also a trained phlebotomist extracted

blood throughout this study. The phlebotomist washed their hands and wore gloves throughout all extractions. The participant was seated comfortably in a chair with their arm resting on a pillow by their side. The phlebotomist chose which arm to draw blood from depending on the venous presentation. A tourniquet was used above the venepuncture site (cubital fossa) to enlarge the vein, and the participant was asked to make a fist to encourage blood flow. The phlebotomist tapped the vein to encourage dilation, and wiped the area of skin to be pierced with an alcohol wipe. The sample of blood was then drawn.

Blood was drawn at four time intervals:

A. Baseline (No NMES)

B. 15 minutes (after 15 minutes of NMES)

C. 30 minutes (once the 30 minute NMES protocol had finished)

D. 60 minutes (after 30 minutes rest)

Labelling of samples allowed us to ascertain which participant, and which time frame we were analysing.

Blood samples were allowed to clot for ~2 hours post testing, before being spun in a centrifuge (1300 RPM, 5 minutes) to separate the various components by density. The serum was pipetted into 0.5ml aliquots and stored at -80 °C to preserve for analysis (Ratcliffe 2006). Aliquots were stored for short periods of time (6-12 hours) at -20 °C local to the laboratory used for assay preparation, before being transferred to the main storage facility.

7.4 Outcome measures

Our sole outcome measure throughout this study was measurement of Creatine Kinase activity through blood assay preparation. All assays were purchased and used as suggested from suppliers. Observations in Creatine Kinase activity were compared to answers on the lifestyle questionnaire during analysis.

Creatine Kinase (CK) assay preparation

The blood samples were returned to room temperature immediately prior to analysis. Assay preparation was conducted using Abcam assay ab155901 (Abcam LTD), a colorimetric CK assay kit. This specific kit converts creatine into phosphocreatine and adenosine diphosphate (ADP). The phosphocreatine and ADP generated react with the CK enzyme mix of the assay kit to form an intermediate. This creates a coloured product with a high absorbency level (λ = 450 nm) which is measured by a plate reader. Serum was pipetted in 2µl aliquots into a 96 well plate format. To this 50µl of assay buffer was added and 48µl of reaction mixture, as per assay protocol. Using a Biotek Synergy 2 plate reader the optical density was measured at 450 nm for each well, with a control buffer background being taken. A CK calibration was also carried out on the same 96 well plate, using standard CK concentrations of 0, 2, 4, 6, 8 and 10 nm/well, again using a buffer background. A kinetic assessment of optical density was carried out with each sample being analysed every 5 minutes over 1 hour, at 37 °C. Data was exported to Excel directly after being collected using the Synergy 2 collection software.

7.5 Data Analysis

Data was analysed based on time points of blood extraction. This enabled trends to be identified in data, which could then be related back to the NMES protocol. Time points included:

- Baseline (0 minutes)- analysis of CK-A
- 0-15 minutes- analysis of CK-A and CK-B
- 15-30 minutes- analysis of CK-B and CK-C
- 30-60 minutes- analysis of CK-C and CK-D

Statistical analysis was limited in this study due to the relatively low sample size and high level of data produced. Statistical findings would not be able to be related to a larger sample with confidence, and therefore is outside the scope of this study. It is acknowledged that this is a limitation, and identification of positive trends would warrant a larger study of this nature to be performed.

Each time period was analysed based on descriptive statistics (mean, standard deviation, coefficient of variation and graphs), highlighting mechanisms of how the blood components could work during a stimulation programme with NMES. Further division was made based on response to the lifestyle questionnaire; allowing trends in data based on current exercise levels. Exercise levels were taken into consideration based on the wide range of influential factors reported in literature that may influence results (Brancaccio, Maffulli et al. 2007). Where possible independent T-tests and analysis of variance were performed to indicate any significant findings within the time periods. Linear regression analysis also indicated direction and rate of change. Results are discussed in relation to percentage of baseline to show comparison based on small changes, however actual values were used, as this is deemed more clinically relevant.

Stimulation intensity was compared to mean CK concentration, in attempt to help protocol development based on muscle response to NMES. Stimulation intensity was sub-divided into

categories identified in previous chapters to make analysis comparative to previous studies throughout this thesis.

7.6 Results

Forty participants completed the testing procedure, with no adverse events reported throughout. The sample size for analysis was reduced (N=32) due to unforeseen problems extracting blood in some participants, and concentrations in some samples being too low for measurement from the kinetic analysis. Raw data is presented in appendix 11. Results are displayed as Mean: Standard Deviation (SD).

Participant demographics are summarised in table 7.2. A greater number of males were recruited than females, with a wide range of ages (20-43 years). Weight also produced a high standard deviation of 11kg. Participants ranged from three different ethnicities: English, Indian and Chinese.

	Age (years)	Height (m)	Weight (kg)
Mean	26.3	1.7	72.5
SD	5.9	0.1	11.0

	Female	Male
Number	5	27

Table 7.2 Summary of participant demographics

The population recruited varied with regard to lifestyle, and level exercise performed. Information was extracted from the lifestyle questionnaire to present this. Details are offered in table 7.3.

Participant	*Num times exercise/week*	*Level of exercise*	*Type of exercise*	*Exercise last 48 hours*
1	3+	Intense	CV/ Power	RT
2	3+	Moderate	Fitness	Squats
3	No pattern	None	Fitness	None
4	1	Light	Fitness	None
5	1	Moderate	Fitness	Brisk walking, core
6	3+	Intense	CV/ Fitness	Cricket
7	3+	Moderate	Power	Core/ RT
8	3+	Moderate	CV/ Power	Aerobic/ weights
9	No pattern	None	None	Wii Fit
10	1/ month	Light	CV	None
11	3+	Moderate	CV/ Power	Tennis match
12	No pattern	None	None	Walking
13	No pattern	Light	CV/ Power	None
14	No pattern	Light	CV	None
15	1	Light	Fitness	Walking
16	No pattern	Moderate	CV	none
17	3+	Intense	CV	45 min run
18	3+	Moderate	CV	None
19	1	Moderate	CV	5 mile walk
21	3+	Moderate	CV/ Power	Half marathon
22	3+	Intense	CV/ Power	RT
24	1	Moderate	Fitness	Football match
25	1	Moderate	Fitness	Running
26	1	Moderate	Fitness	None
27	3+	Intense	CV/ Fitness	None

28	1	None	Fitness	Walking
29	3+	Intense	CV/ Power/ Fitness	None
30	No pattern	None	Fitness	Walking
31	1	Moderate	Fitness	Walking
32	No pattern	Light	Fitness	UL RT
33	3+	Intense	CV/ fitness	Martial Arts
34	3+	Intense	CV/ Power	Running

Table 7.3 Information from lifestyle questionnaire

The lifestyle questionnaire documented 'any exercise conducted in the last 48 hours', which demonstrated a range of activities ranging from no exercise to having ran a half marathon. We asked participants to quantify how intense they perceived their level of exercise, which is presented in table 7.4 (below).

Level of exercise	Number of participants	Percentage of participants (%)
None	5	16
Light	6	19
Moderate	13	41
Intense	8	25

Table 7.4 Participant demographics: perceived level of exercise

As can be seen from table 7.4, 25% of participants self-reported partaking in an intense level of exercise, and 16% reported not taking part in any exercise. The majority of participants (41%)

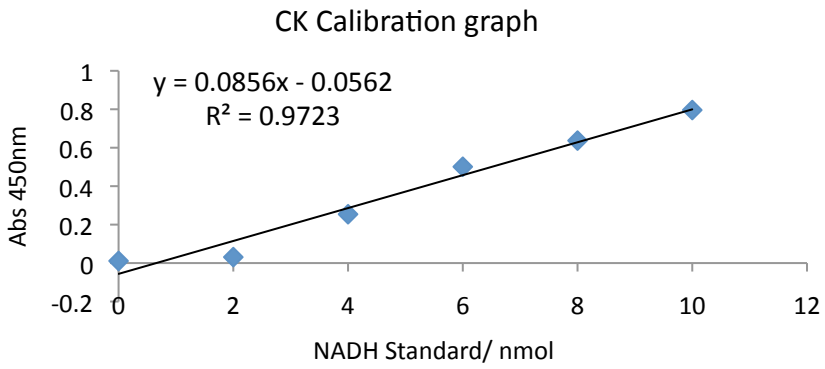
reported taking part in moderate exercise. This information will be used in conjunction with the blood biomarker analysis to investigate trends. Participants will be grouped into two groups when considering the lifestyle questionnaire in analysis; exercise or no exercise. Participants who reported 'none' or 'light' level of exercise will be considered in the no exercise group, and those who reported 'moderate' or 'intense' will be considered in the exercise group. Participants will also be considered based on their level of exercise in the previous 48 hours. This will be split into two groups based on whether they have conducted exercise in the previous 48 hours to study involvement or not: exercise, or no exercise. Groups are described in table 7.5.

	Groups	
	Exercise General	Exercise 48
Definition	Self-reported level of exercise	Exercise conducted in the previous 48 hours
Categories	Exercise (moderate, intense) No Exercise (none, light)	Exercise No exercise

Table 7.5 Exercise grouping for analysis

Creatine Kinase (CK)

Graph 7.1 presents the calibration curve constructed for CK analysis, demonstrating linearity and confidence in our assay preparation method. Analysis has been conducted looking at relationship between time points, more specifically 0-15 minutes, 15-30 minutes and finally 30-60 minutes. This allows us to establish not only whether an overall relationship exists, but also at what time point in the training period.



Graph 7.1 CK calibration graph

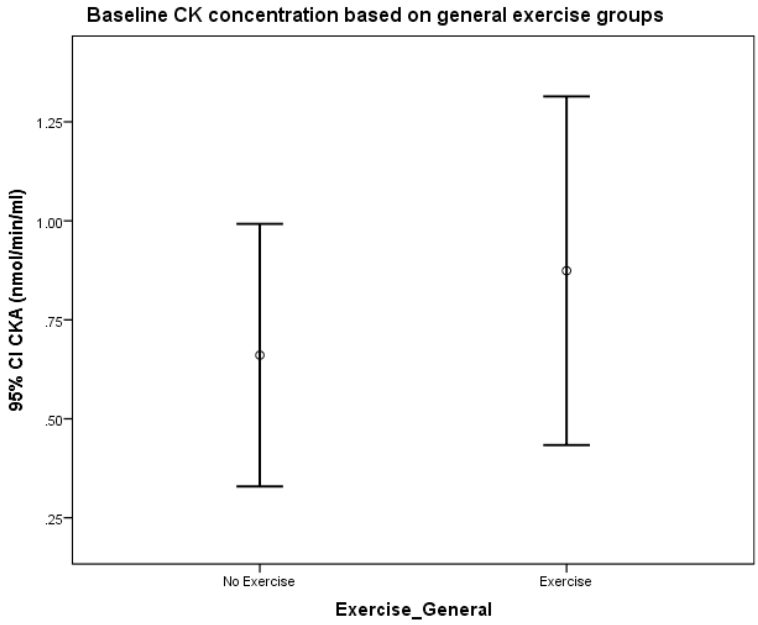
Baseline CK analysis (0 minutes)

Baseline CK will be referred to as CK-A. Analysis of baseline blood samples allowed comparison to literature in terms of concentrations extracted and to establish a baseline to reference other time points. Descriptive values are displayed in table 7.6. There is a wide range of 4.68 nmol/min/ml within the participants' baseline CK, with a mean of 0.83 nmol/min/ml, which is in the lower bracket of this range.

	Number of samples	Min	Max	Mean	SD
CK-A	27	0.33	5.01	0.83	0.86

Table 7.6 Descriptive vales for CK at baseline (nmol/min/ml)

Concentrations were considered in relation to self-reported exercise levels on the lifestyle questionnaire, as detailed in table 7.5. When looking at baseline CK levels in comparison to this response, we can see that people who exercise regularly have higher baseline CK levels (graph 7.2).



Graph 7.2 A graph to show levels of CK concentration at baseline based on self-reported exercise levels.

Descriptive statistics were conducted on participants with whom we had a full data set of blood samples for analysis (table 7.6). These indicate that a greater portion of our cohort participated in regular exercise (N=21) than those who do not (N=6). Mean values indicate that those who partake in regular exercise have a greater baseline CK value than those who do not (0.21 nmol/min/ml higher, 95% CI of the difference 1.29 to 0.46). When conducting a T-test comparing CK-A to self-reported exercise levels, there is a significant difference between values (P = 0.004, 95% CI 0.3291 to 0.9922). This indicates a relationship between those that exercise and baseline CK levels.

Exercise general	N	Minimum (nmol/min/ml)	Maximum (nmol/min/ml)	Mean	St Dev
No Exercise	6	0.33	1.24	0.66	0.32
Exercise	21	0.36	5.01	0.87	0.98

Table 7.6 Descriptive statistics for CK-A (level of CK at baseline) for people who report exercising and those that do not.

After considering people who state that they participate in exercise in general, it is important to ascertain whether people who have exercised in the previous 48 hours demonstrate similar trends in their baseline CK levels. Descriptive statistics are provided below.

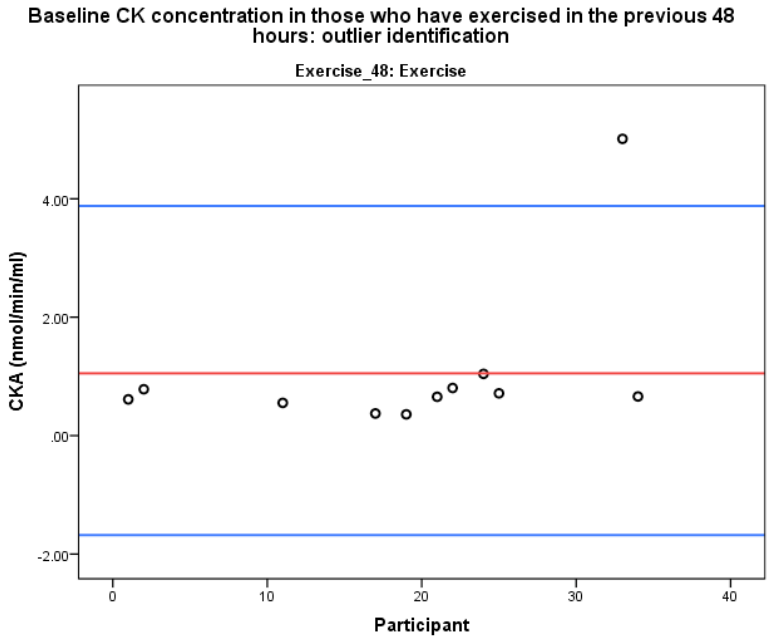
Exercise 48	N	Minimum (nmol/min/ml)	Maximum (nmol/min/ml)	Mean	St Dev
No Exercise	16	0.33	1.24	0.67	0.24
Exercise	10	0.36	5.01	1.1	1.39

Table 7.7 Descriptive statistics for CK-A (level of CK at baseline) for people who exercised in the previous 48 hours and those who did not.

Table 7.7 indicates that baseline CK levels are higher in those who exercised in the past 48 hours (0.43 nmol/min/ml higher), which is 0.22 nmol/min/ml higher than those who report taking part in general exercise. The standard deviation is higher in both exercise groups compared to the counterpart no exercise group. When conducting a paired T-test to test for differences between the two groups (general exercise and exercise in the past 48 hours), we found a significant correlation, indicating that exercise in the previous 48 hours to testing influences baseline CK values ($P = 0.006$, 95% CI 0.170 to 0.518).

The standard deviation for participants who have participated in exercise over the previous 48 hours is high (SD 1.33 nmol/min/ml) compared to that in the group who have not exercised (SD 0.24 nmol/min/ml). Confidence intervals indicate that there is variation within the dataset, specifically the exercise sub-group. Outliers have been regarding as having a CK concentration over 1.19 nmol/min/ml when utilising the median approach (Horn, Feng et al. 2001). Participant 33 has been

identified as an outlier in graph 7.3 (above median threshold and outside 2 SD parameter); this participant is a 43 year old male who partakes in intense, regular martial arts training more than 3 times a week. A training session was conducted within 24 hours prior to participation in the study. When excluding this participant from comparisons of baseline CK and exercise within the previous 48 hours, the SD is considerably reduced (0.22 nmol/min/ml).



Graph 7.3 Baseline CK levels for participants who reported exercising in the previous 48 hours. Reference lines indicate mean and +/- 2 SD.

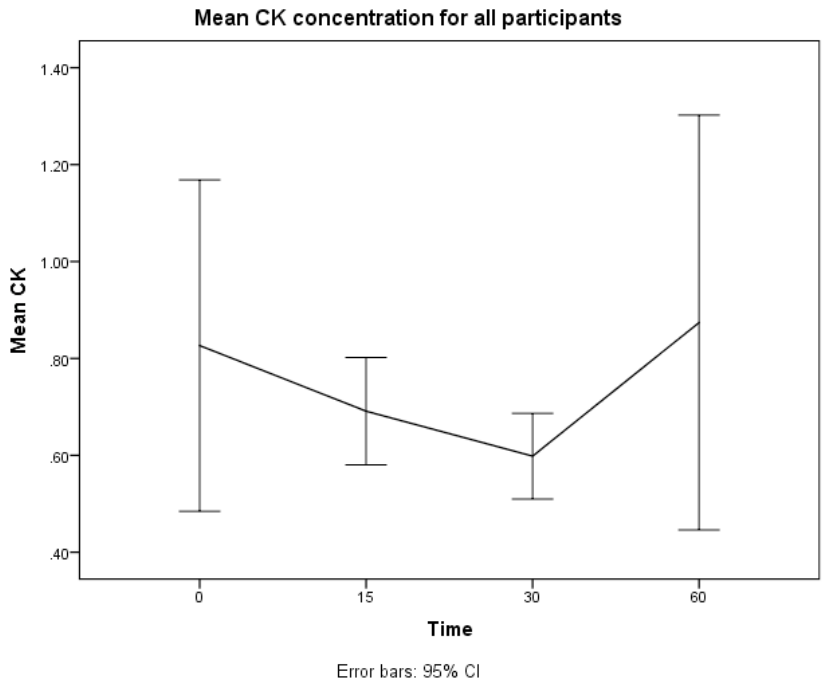
CK analysis- 0-15 minutes

Analysis of the treatment programme on CK activity during the first 15 minutes of intervention required comparison of the first two blood samples (CK-A: baseline and CK-B: after 15 minutes of treatment with NMES). Descriptive statistics show the mean of CK concentration decreased by 0.14 nmol/min/ml in the first 15 minutes of treatment (table 7.8).

	CK-A	CK-B
	(nmol/min/ml)	
Mean	0.83	0.69
SD	0.86	0.29

Table 7.8 Descriptive statistics of the first two CK blood time points. CK-A: baseline blood value (0 minutes), CK-B: Blood sample extracted at 15 minutes.

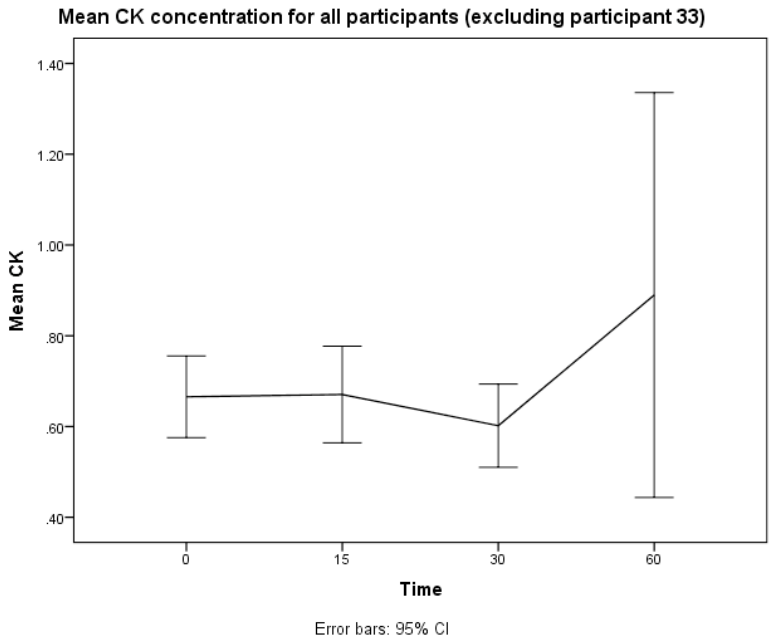
This correlates with the overall presentation of CK concentration for all participants which is shown in graph 7.4, equating to a 16.4% decrease between the first two time points.



Graph 7.4 Mean CK concentration for all participants throughout full testing protocol.

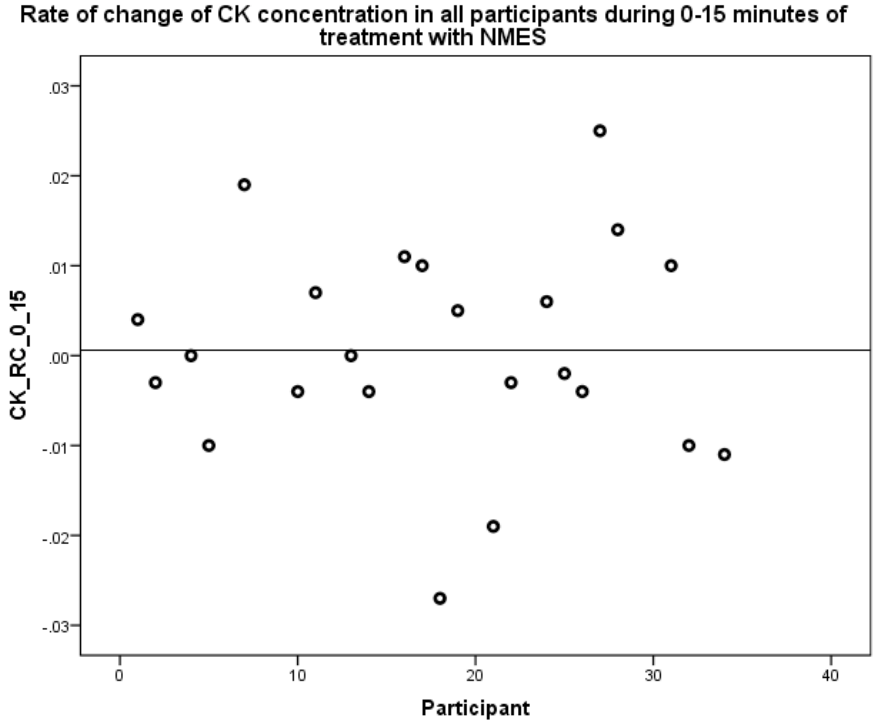
Despite clearly showing a decrease in concentration over the first 15 minutes of intervention with NMES, CK concentration displays some variability between the two time points. The SD for mean CK concentration is much larger at baseline compared to 15 minutes into treatment (0.57 nmol/min/ml larger). Previous description of participant 33 at baseline needs to be taken into account when

interpreting this high SD. Participant 33 has been excluded from the following analysis, and will be considered on an individual basis later in the chapter. Graph 7.5 excludes participant 33, demonstrating a change in the relationship of CK concentration over time. The mean concentration rises from 0.66 nmol/min/ml to 0.67 nmol/min/ml over the first two time points. The first decline of CK concentration is witnessed between 15 and 30 minutes of treatment with NMES.



Graph 7.5 Mean CK concentration for all participants, excluding participant 33 throughout full testing protocol.

Linear regression analysis indicates that within this first 15 minutes of treatment there is some variability of CK concentration (graph 7.6), indicating that some participants have increased their CK concentration, and some have decreased their CK concentration (mean b1= 0.00, SE= 0.005). When looking at specific individuals the rate of change varies between -0.027 and 0.025.



Graph 7.6 Rate of change of CK concentration based on participant during 0-15 minutes of treatment. (Positive value= increase in CK, negative value= decrease in CK). Reference line represents mean.

Given this variability in our data set previous levels of exercise may have influenced the change in concentration between the initial two time points. Self-reported exercise, or exercise in the previous 48 hours were compared to ascertain trends.

When considering self-reported exercise, CK concentration levels demonstrate an increase when people have reported exercising (b1= 0.026, SE 0.082). This difference indicates that people who exercise increase their levels of CK in the first 15 minutes of treatment with NMES (table 7.9).

	Time			
	0	0	15	15
Exercise general	Exercise	No Exercise	Exercise	No Exercise
Mean	0.67	0.66	0.68	0.64
SD	0.2	0.32	0.24	0.41

Table 7.9 Descriptive statistics of CK concentration between 0-15 of NMES, based on self-reported exercise (values shown as nmol/min/ml)

People who did not exercise demonstrated a mean CK concentration decline of 0.02 nmol/min/ml, whereas those who exercised increased 0.01 nmol/min/ml. The scale of this difference does not warrant the use of statistical testing. However it is worth highlighting from a clinical perspective, which will be reviewed in the discussion section.

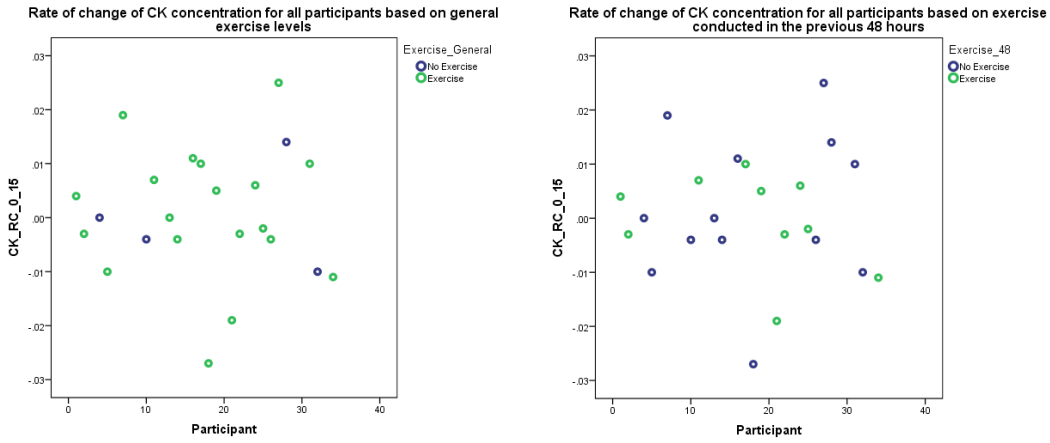
When considering exercise conducted in the previous 48 hours, the mean CK concentration declines in those who have previously exercised (table 7.10). This is supported by a reducing rate change (b1 -0.042, SE 0.069). People who report not exercising in the previous 48 hours demonstrated a mean increase in their CK concentration. The impact of exercise in the previous 48 hours is therefore greater on CK concentration than that of exercise in general.

Descriptive statistics in table 7.10 demonstrate that CK concentration shows a lower concentration at baseline in those who have exercised in the previous 48 hours compared to those who have not (-0.01 nmol/min/ml lower). At 15 minutes into treatment CK concentrations are higher in the no exercise group (0.07 nmol/min/ml difference). This indicates that those who have exercised in the previous 48 hours are demonstrating a decline in CK concentration in the initial stages of treatment

with NMES, and those who have not exercised are demonstrating a rise in CK concentration.

	Time			
	0	0	15	15
Exercise 48	Exercise	No Exercise	Exercise	No Exercise
Mean	0.66	0.67	0.63	0.7
SD	0.2	0.24	0.2	0.32

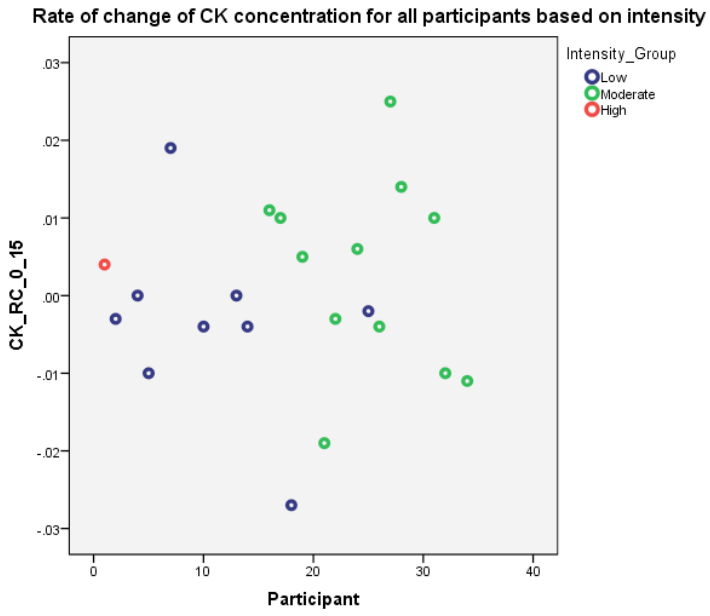
Table 7.10 Descriptive statistics of CK concentration in the first 15 minutes of treatment in those who report exercising in the previous 48 hours of study participation (values shown as nmol/min/ml)



Graph 7.7 Rate of change of CK concentration for all participants based on general exercise levels (left) and exercise conducted in the previous 48 hours (right).

Graph 7.7 indicates that there is no clear relationship between rate of change and exercise status; either general exercise or exercise conducted in the previous 48 hours. Other influential

characteristics such as stimulation intensity may have impacted the response of muscle to NMES. When looking at rate of change in the first 15 minutes of treatment based on stimulation intensity, we were able to identify some trends (graph 7.8). Those that used a low stimulation intensity (20-35ma) demonstrated a decline in CK concentration, and those who utilised a moderate stimulation intensity (36-50ma) demonstrated a rise in CK concentration. The finding was not significant on conducting an independent samples t-test (P= 0.259, CI -0.1757—0.00501). However this indicates that a relationship exists between the two factors: the higher the stimulation intensity, the greater the response from the muscle. When considering the participant who used a high stimulation intensity (n=1, participant 30, 52ma) a rise in CK concentration was observed. Participant 30 was a 20 year old male who has been considered in the 'no exercise' group in respect to both general exercise and exercised conducted in the previous 48 hours.



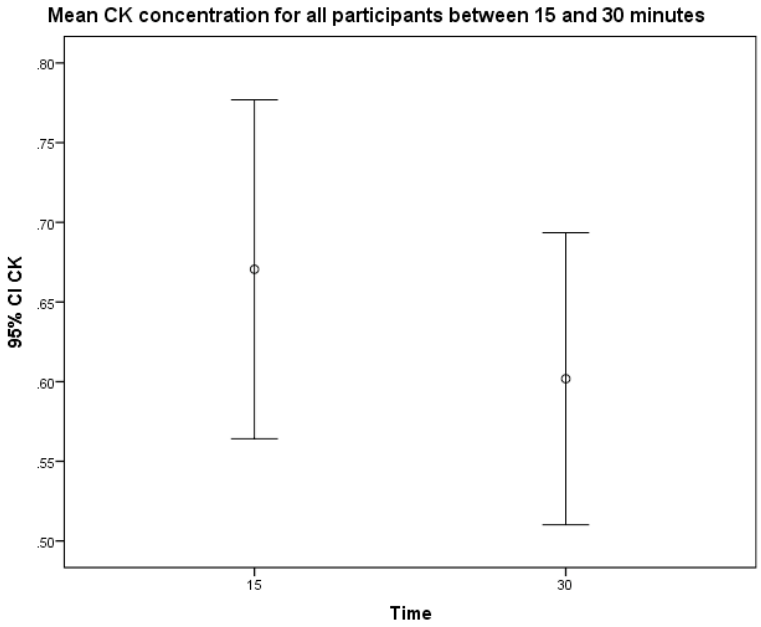
Graph 7.8 Rate of change of CK concentration for all participants based on intensity groups.

CK analysis 15-30

Investigation of CK activity during the second half of the 30 minute treatment period compared blood sample CK-B (15 minutes) and blood sample CK-C (after 30 minutes). This allowed us to establish any initial effects during or immediately after treatment with NMES. When considering descriptive statistics in table 7.11, we can see that CK concentration declines in the final 15 minutes of treatment with NMES. Concentrations reduced by a mean of 0.07 nmol/min/ml.

	CK-B	CK-C
	(nmol/min/ml)	
Mean	0.67	0.6
SD	0.27	0.23

Table 7.11 Descriptive statistics for blood sample CK-B and CK-C.

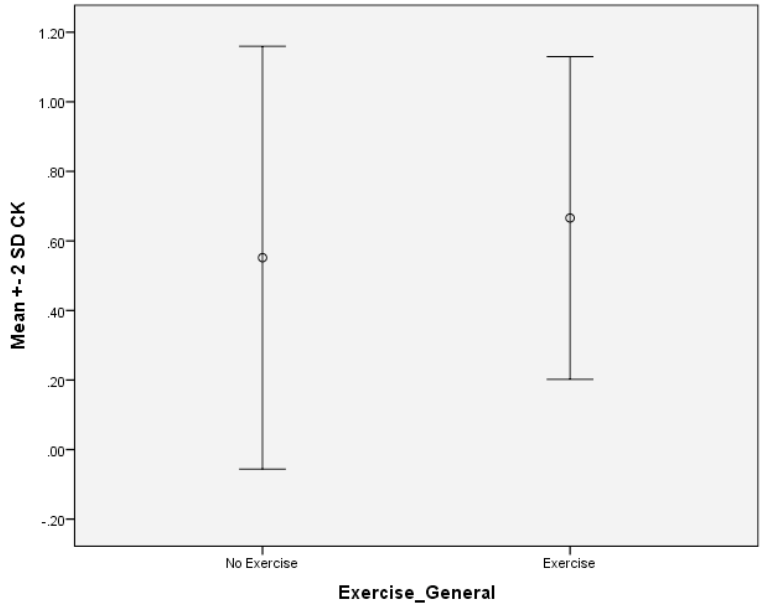


Graph 7.9 Mean CK concentrations between 15 and 30 minutes of the NMES treatment protocol.

Graph 7.9 shows the decline of CK concentration between 15 and 30 minutes of NMES treatment, and the similarity in confidence intervals. SD are also similar between both samples (table 7.11)

indicating that participants demonstrated similar responses in CK concentration at this time point of treatment. Participant 33 remains removed from analysis to enable trends to be identified.

When comparing participants who reported general exercise as a group, those who exercised demonstrated a smaller decline in CK concentration (b1= 0.114, SE 0.078). There is also a smaller SD (0.23) in the exercise group, indicating that the response to treatment is more linear in people who exercise than those who do not (graph 7.10).



Graph 7.10 Mean CK concentration during the last 15 minutes of treatment with NMES, based on perceived levels of general exercise.

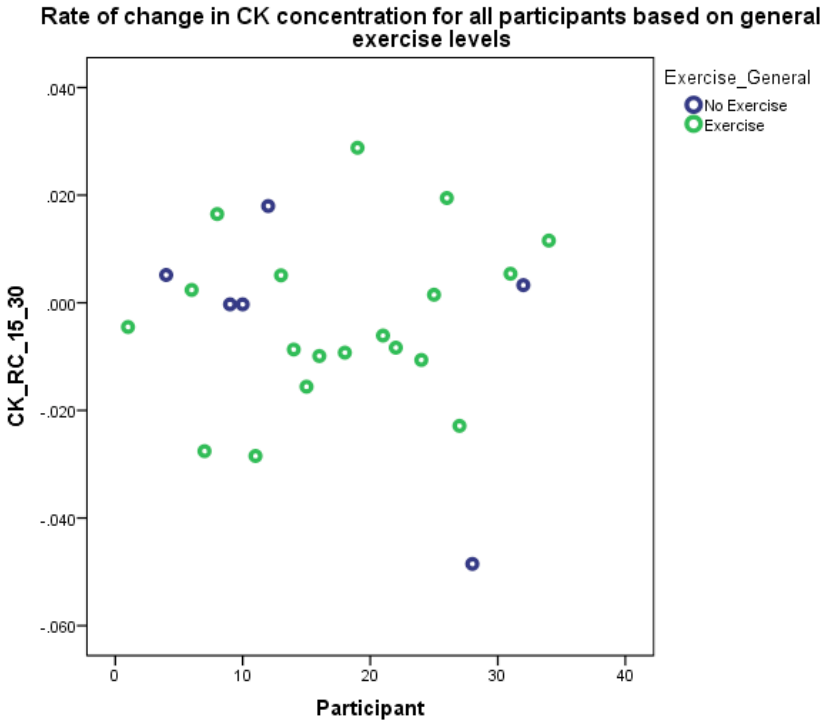
A similar finding was found with regard to exercise conducted in the previous 48 hours. The exercise group demonstrated a smaller decline in CK concentration than the no exercise group (table 7.12). CK concentration reduces by 0.13 nmol/min/ml less in people who have exercised in the previous 48 hours than those who have not. Small standard deviations indicate a linear response. Participants CK

concentration who reported taking part in exercise in the previous 48 hours decline on average 0.04 nmol/min/ml more than those in the general exercise group.

	Time			
	15	15	30	30
Exercise 48	Exercise	No Exercise	Exercise	No Exercise
Mean	0.63	0.7	0.64	0.58
SD	0.21	0.32	0.23	0.24

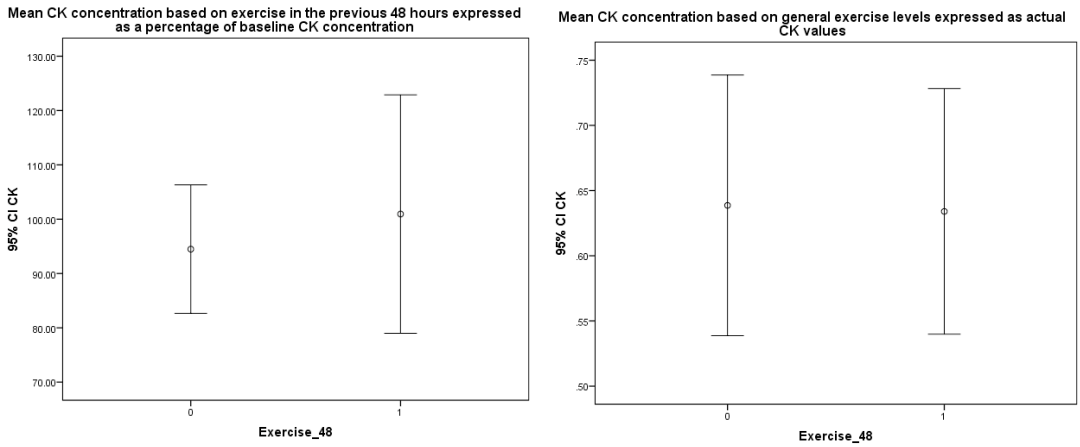
Table 7.12 Descriptive statistics for all participants during 15 to 30 minutes of treatment based on exercise conducted in the previous 48 hours.

Mean rate of change declined for all participants (b1= -0.005, SE= 0.005), however variation was witnessed within this (range= -0.051-0.029). When breaking this down into exercise groups, trends could be identified in the general exercise category (graph 7.11). Those who reported not exercising tended to display a small rise in their CK concentration, and those who exercised presented a more varied correlation, both decline and rises beyond that of the no exercise group. There is one outlier to this trend, who displayed a rate of change decline of -0.49. This data point correlates to participant 28, a 21 year old male who has been analysed in the no exercise group. This trend was not significant on t-test (P>0.005). There were no trends of note to identify when comparing rate of change based on exercise conducted in the previous 48 hours.



Graph 7.11 Rate of change in CK concentration for all participants based on general exercise levels.

When looking at CK concentration as a percentage of its baseline value (CK-A), the response differs from actual values. When comparing CK-B and CK-C (the final 15 minutes of treatment) based on exercise in the previous 48 hours, linear regression indicates a rise in CK concentration, and a b1 value of 6.457. When producing error bars to visualise the difference, as shown by graph 7.12 mean CK concentration reduces less in those who have exercised when the data is expressed as a percentage, and more in those who have exercised when expressed as actual values. Given our small sample size, all data will be interpreted as actual values, as this is deemed to have more clinical value.



Graph 7.12 Error bar to show mean CK concentration during 15-30 minutes based on exercise in the previous 48 hours expressed as a percentage (left) and actual values (right). 0= no exercise, 1= exercise.

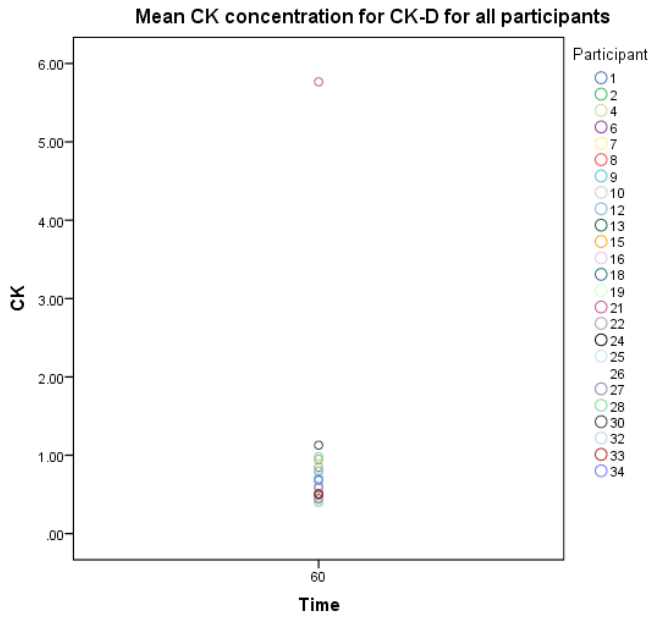
CK analysis 30-60 minutes

Analysis of the final time frame involved two blood samples; CK-C (30 minutes) and CK-D (60 minutes). This allowed us to ascertain whether there were any changes in CK concentration during the 30 minute rest period post treatment. When considering descriptive statistics the overall trend of CK concentration during these time points is a rise of 0.27 nmol/min/ml (table 7.13). This is the only rise of CK concentration noted throughout all four blood samples. The mean CK concentration of blood sample CK-A (baseline value) was 0.83, indicating that treatment with NMES raised the overall CK concentration by 0.04 nmol/min/ml.

	CK-C	CK-D
	(nmol/min/ml)	
Mean	0.6	0.87
SD	0.23	1.03

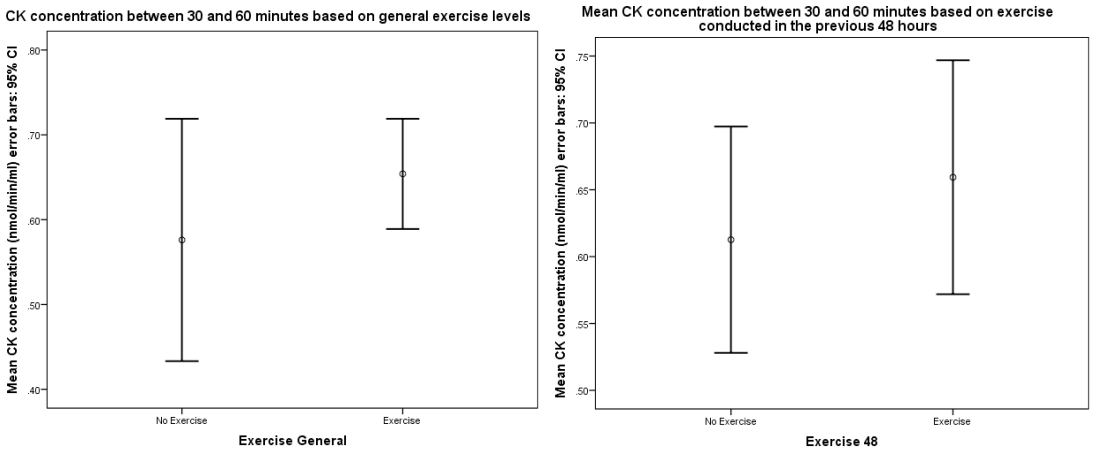
Table 7.13 Descriptive statistics of CK-C and CK-D

There is a large SD for CK-D, indicating some variation in our data set (graph 7.13). When looking more specifically for differences in CK-D participant 21 had a CK value of 5.77 nmol/min/ml. Once data for this participant was removed the SD reduced to 0.19. Participant 21 was a 28 year old British male, who ran a half marathon the previous day. He had also consumed a protein shake less than one hour prior to study participation. Participant 33 has been removed from analysis to this point, however the CK concentration for CK-D was 0.5 nmol/min/ml, which is within 1 SD of the data set. Data for this time point was therefore included in this analysis.



Graph 7.13 Mean CK concentration at CK-D for all participants.

Based on the increased concentration witnessed between time point CK-C and CK-D, we went on to further investigate whether exercise levels had an influence on initial muscle recovery post exercise with NMES. Both groups demonstrate an increase of CK concentration in the exercise group compared to the no exercise group. Confidence intervals are larger in the 48 hour exercise group (graph 7.14). Table 7.14 presents raw data.



Graph 7.14 CK concentration between 30- 60 minutes of treatment with NMES based on general exercise (left) and exercise conducted in the previous 48 hours (right)

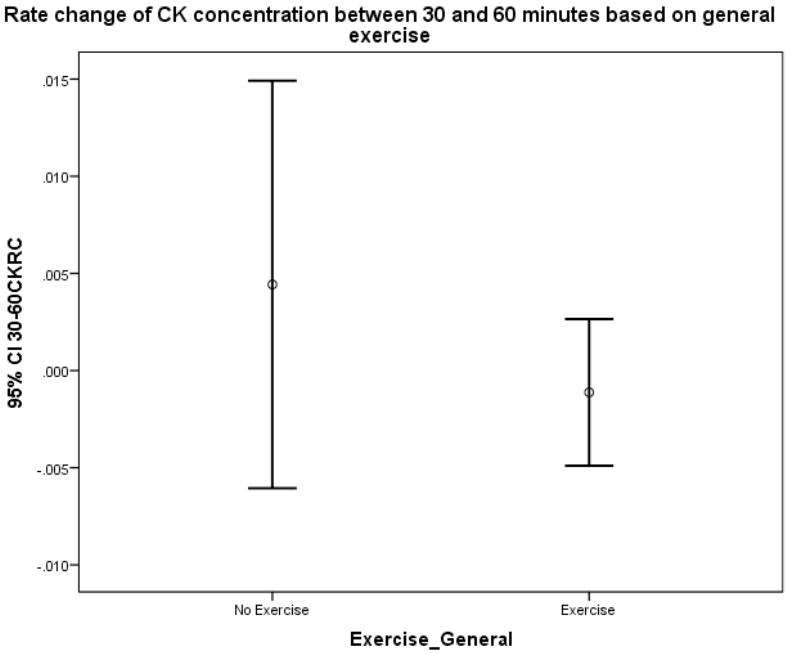
Both exercise groups demonstrate increases in mean CK concentration between 30 and 60 minutes. The greatest increase in CK concentration between time points was witnessed in the general exercise category, in the no exercise group with an increase of 0.2 nmol/min/ml. This is 0.17 nmol/min/ml larger than the increase demonstrated in the exercise sub-category of the general exercise group. When comparing changes over time, the no exercise group saw a 0.13 nmol/min/ml increase in the exercise general group, and the exercise group saw a 0.05 nmol/min/ml increase in the exercise 48 group. This indicates that exercise is more influential on CK concentration when exercise has been conducted in the previous 48 hours.

Exercise 48	Time (minutes)	
	30	60
Exercise	0.62 (0.22)	0.7 (0.15)
No Exercise	0.58 (0.24)	0.65 (0.23)

Exercise general	Time (minutes)	
	30	60
Exercise	0.64 (0.23)	0.67 (0.15)
No Exercise	0.48 (0.2)	0.68 (0.29)

Table 7.14 Descriptive statistics for CK concentration (nmol/min/ml) during 30 and 60 minutes of treatment with NMES, based on levels of exercise. Data points are expressed as mean (SD).

Linear regression analysis indicates some variability in response to CK concentration after a 30 minute rest period, with some participants increasing and some decreasing. The range was from -0.016 to 0.028 with a mean of 0.00057 (SD: 0.0087). This indicates a mean rise in CK concentration over the rest period, which is indicative of the presentation for actual values.



Graph 7.15 Rate change of CK concentration based on general exercise

Graph 7.15 demonstrates that the rate change of CK concentration during the 30 minute rest period increases more in participants who have no general exercise patterns compared to those who do. The confidence interval is wider in the no exercise sub category, indicating a level of caution should be taken when interpretation these results.

Intensity

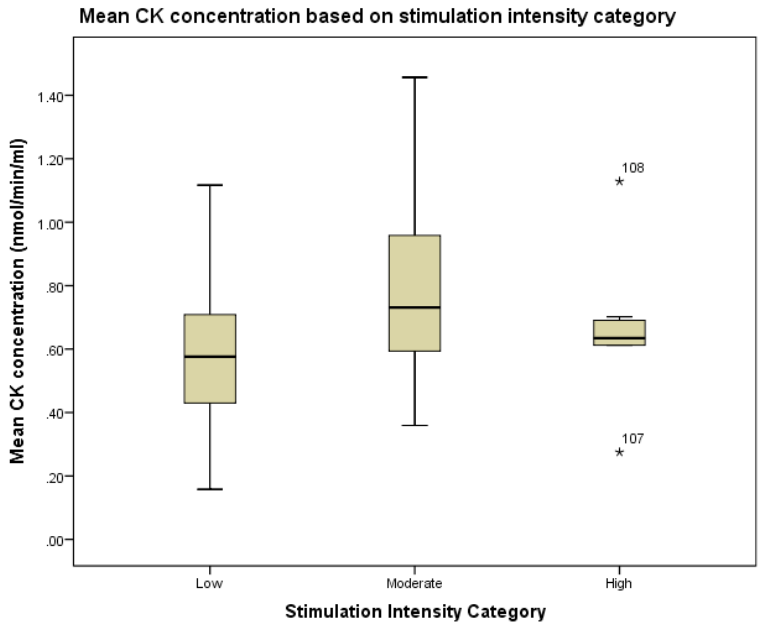
Each participant administered the treatment with NMES using maximal tolerated intensity. Intensity values ranged from 21ma-63ma. Sensation produced from NMES varies in individuals, and can be dependent on electrode adherence to skin, level of subcutaneous adipose tissue, and pain receptors (Baker, Wederich et al. 2000). This is something that cannot be accounted for when designing a study, however must be considered throughout analysis. Intensity has been considered in three sub-sections, namely low, moderate and high allowing comparison of groups. Intensity values in each sub-section are described in table 7.15.

Intensity (ma)	Group	N	Mean CK (nmol/min/ml)	SD
20-35	Low	10	0.59	0.2
36-40	Moderate	11	0.85	0.8
51+	High	2	0.66	0.3

Table 7.15 Intensity sub-groups and relation to CK concentration

From table 7.15 it is evident that participants who used a moderate intensity throughout their treatment demonstrated a higher mean CK concentration (69% increase) than those who used a low intensity. The final blood sample (60 minutes) of participant 21 was excluded from analysis in the moderate group as the value was abnormally high (5.76 nmol/min/ml) affecting interpretation. The mean CK concentration decreased in those who used a high intensity (n=2). The box plot in graph 7.16 demonstrates the mean CK concentration based on intensity. The two outliers in the high category relate to the 15 and 30 minute blood extraction time points of participant 30 (graph 7.16, points 107, 108). Participant 30 stimulated with an intensity of 52 mA, and was considered in the no exercise sub-category. Upper limb resistance training had been conducted within 48 hours prior to

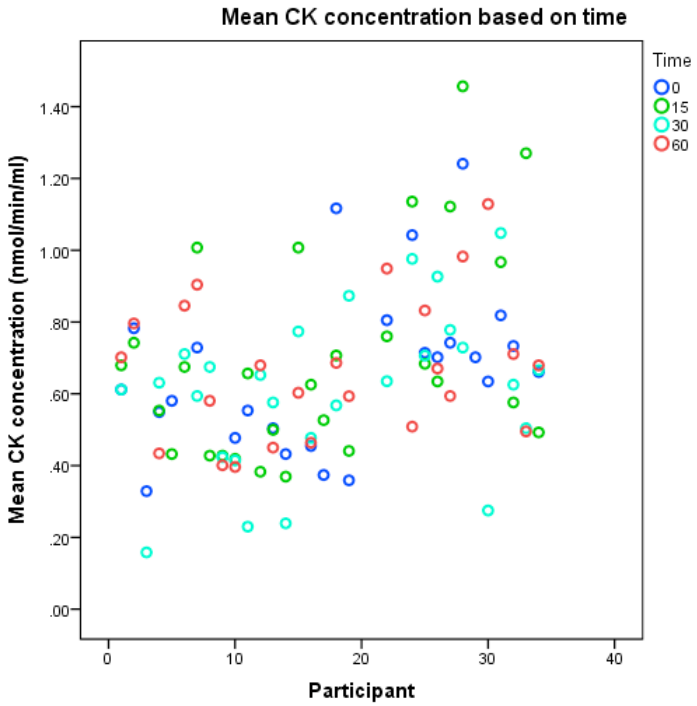
testing. This high rate of outliers (2 out of 8 inputs) in this small sub-category (n=2) indicates that response to NMES when using a high stimulation intensity is high.



Graph 7.16 mean CK concentration based on stimulation intensity sub-groups

Mean CK concentration appears to have a relationship with stimulation intensity, indicating that the higher stimulation intensity used the higher the higher the mean CK concentration. This is especially evident in the low to moderate sub-categories. Analysis of variance indicates that this relationship is significant (P= 0.003, F= 6.237 95% CI 0.6195-0.7197) despite the decline and variability witnessed in the high sub-category.

It is important to ascertain effects of mean CK concentration based on time, and whether there is a relationship over the time period that NMES was administered. Graph 7.17 demonstrates that mean CK concentration tends to rise over the four time periods, but there is no clear relationship that can be identified between them (P=0.561, F= 0.982).



Graph 7.17 mean CK concentration based on time.

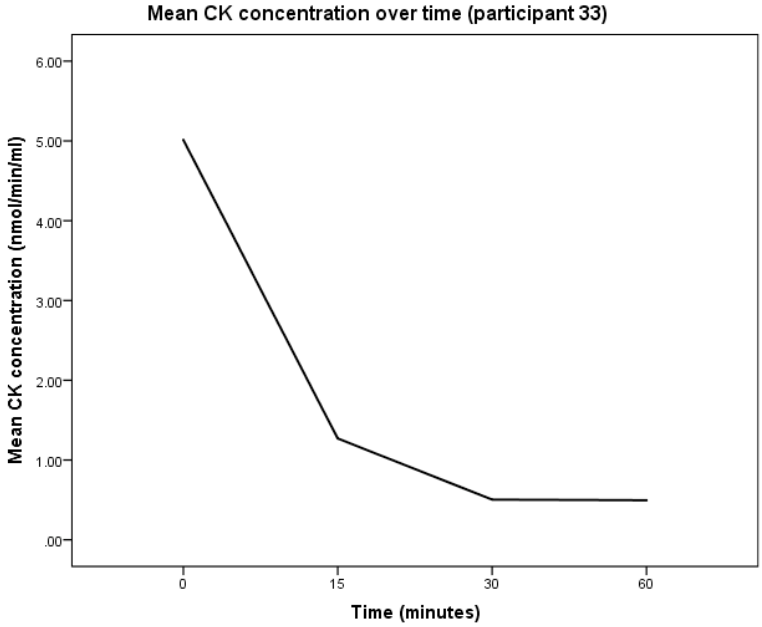
This interaction is shown in table 7.16 based on stimulation intensity category. When considering the difference between 0 and 60 minutes (the relationship of mean CK concentration over time) both the low and high stimulation intensity groups demonstrate increases: 0.01 and 0.3 nmol/min/ml respectively. The moderate stimulation intensity group demonstrates a decline of mean CK concentration over time: 0.05 nmol/min/ml. The low stimulation intensity sub-group is the only group to demonstrate a decline between 0 and 15 minutes (0.04 nmol/min/ml, SD 0.19). The moderate rises by 0.11 nmol/min/ml, however has the largest standard deviation (0.32). The high sub-group has no standard deviation value as n=1. All stimulation intensity categories demonstrated decline in the 15-30 minute time period. The moderate sub-group demonstrated a decline in the 30-60 time period of 0.1 nmol/min/ml. The highest increase in this time period was in the high sub-group of 0.48 nmol/min/ml.

Stimulation intensity category	Time (minutes)			
	0	15	30	60
Low	0.62 (0.22)	0.58 (0.19)	0.52 (0.19)	0.63 (0.2)
Moderate	0.73 (0.28)	0.84 (0.32)	0.78 (0.18)	0.68 (0.18)
High	0.62 (0.15)	0.68	0.44 (0.24)	0.92 (0.3)

Table 7.16 mean CK concentration over time, based on stimulation intensity category

Participant 33

Participant 33 was excluded from analysis, due to the high baseline CK concentration, and its influence in data interpretation. CK concentration over time is demonstrated in graph 7.18. The presentation of CK concentration over time for all participants is demonstrated in graph 7.5. The mean decline of CK concentration for all other participants is small between 0 and 15 minutes (0.01 nmol/min/ml), however participant 33 demonstrates a decline of 3.74 nmol/min/ml. Participant 33 has a baseline CK value which is 3.77 nmol/min/ml higher than the next highest value. Overall mean CK concentration demonstrates an increase of 33% between 30-60 minutes, whereas participant 33 demonstrates a decrease of 1%.



Graph 7.18 mean CK concentration over time for participant 33

Participant 33 was a 43 year old male who reported a martial arts training session less than 48 hours previous to study involvement. Training is conducted more than three times a week, and the participant reported a healthy diet and lifestyle. He is of Indian descent.

7.6.1 Overview of study five results

Analysis of CK response to treatment with NMES was conducted based on time points. Some interesting findings were witnessed with relation to information provided on the lifestyle questionnaire, primarily exercise levels. At baseline CK response was found to be elevated in those who have exercised compared to those who have not. This is the first indication that lifestyle affects response to NMES. Within the first 15 minutes of treatment with NMES CK did not respond and remained stable; however there was variability within this finding. It appeared that exercise correlated to this variability; those who had exercised tended to show decline in CK concentration, and those who did not generally increased. Some differences were noted between general self-perceived exercise and exercise conducted in the previous 48 hours, with the 48 hour group showing

a greater response to treatment. During the second half of the protocol the CK concentration was shown to decline, indicating the muscle was being stimulated to produce energy for contraction. There was however a smaller decline witnessed within the exercise groups. CK concentrations increased after the 30 minute rest period, a response which was higher in people who had reported exercising in the previous 48 hours. Finally, a proportional relationship was identified with regard to stimulation intensity; the higher the stimulation intensity used during treatment, the greater the CK response.

7.7 Discussion

The aim of this study was to ascertain how Creatine Kinase (CK) responds to a one off 30 minute treatment session with NMES. This would give us an indication as to how the muscle responds to NMES in comparison to volitional exercise. Commercially available assay preparation kits allowed extraction of enzyme concentration with confidence. Blood samples were successfully extracted during the treatment programme with NMES. CK assays were prepared and analysed with no problems, allowing concentration levels at various points through the NMES protocol to be investigated. This was able to be compared to similar durations of volitional exercise.

Forty participants were initially recruited; however eight were excluded during the treatment process and analysis. Participation required four blood samples to be extracted from each participant. This was verbalised in the initial recruitment email, and participants put forward their interest to take part with this considered. Extraction of blood was more difficult in some participants than others, due to venous presentation. This is a confounding factor associated with blood sampling that cannot be avoided. For this reason a trained phlebotomist was used to perform blood extractions, instead of training a member of the research team. This experience was deemed vital in reducing the number of participants excluded based on this factor. There were also some

participants who were lost during the analysis process. Kinetic analysis of blood was unable to be performed in some preparations. This could be attributed to the level of assay concentration not being high enough, or the equipment not reading the well in the plate. As each participant had four resulting assay readings for each blood component, if one was missing the remaining results were carried forward for analysis. This enabled as many results to be utilised in analysis as possible. The larger sample size recruited allows more reliable conclusions to be inferred from these results; however recruitment of a larger sample would have resulted in fewer encumbrances placed on assay preparation.

When recruiting a convenience sample was approached, and participants were recruited on response to an email. The email was distributed amongst staff and students from the health faculty of Keele University (Physiotherapy department). The majority of participants recruited were students (staff n=4). This study recruited five female participants. Subjective feedback from female participants was positive, with no adverse reactions, or negative comments surrounding blood extraction or treatment with NMES. Females were able to tolerate NMES protocols to a level that resulted in a strong tetanic muscle contraction. Although comparison with male response to NMES is beyond the scope of this study, the differences in muscle contraction expected based on visual feedback throughout this study are minimal. Further testing with the leg measurement device would be needed to reliably infer from gender differences.

The recruiting email addressed three main areas for the participant to consider; NMES, blood sample extraction and strength training. Literature suggests that both sexes are able to be conditioned to NMES protocols over time. Improved stimulation tolerance may influence the high failure rates of NMES currently seen in clinical settings. However males have been shown to tolerate significantly more stimulation than females, which has been linked to gender differences in the response to

noxious stimuli (Alon, Smith 2005). Females may be adjourned from participation based on the electrically induced, supra-maximal contraction used to produce strength gains. Females are accepted to hold more adipose tissue than their male counterparts (Blaak 2001), with a difference in distribution throughout the body. Males tend to store their fat in the abdominal viscera making them more at risk of cardiovascular disorders (Lemieux, Despres et al. 1994), whereas females tend to have a wider distribution, specifically around the gluteal and thigh regions. When penetrating through adipose tissue, NMES requires a higher stimulation intensity to produce a muscle contraction (Baker, Wederich et al. 2000). The comfort of stimulation could be compromised in female participants, making them less likely to tolerate stimulation compared to their male counterparts. Low levels of female participation may be related to the target audience in this study; males may be more amenable to participate in studies of this nature. There has been no link established between needle phobia and gender (Deacon, Abramowitz 2006), indicating that the inclusion of blood sampling should not have influenced our sample recruitment. The strong connotations of masculinity associated with strength training remain (Royce, Gebelt et al. 2003), despite multiple advances in female participation in sport. It must be considered that the sample approached may be predominantly males: therefore attracting a relative proportion of the population sampled. If conducting a larger study, the gender ratio may be more likely to equalise. This study may be difficult to draw conclusion based on gender, and therefore results have been considered with mixed gender. A larger study would be needed to extrapolate this information.

The Leg Measurement Device (LMD) was not utilised in outcome measure assessment as used in previous studies throughout this thesis. Although proof of concept, studies 1-4 identified the effects of NMES on strength training in human muscle. This study was conducted based on the high variability of strength increases witnessed throughout these previous studies (chapter 6 and 7). This study aimed to investigate why this high variability occurred. Blood component analysis was the only outcome measure utilised during analysis. This was appropriate to achieve the aims, and allowed a

wide range of data to be collected during analysis. This study utilised an observational design, highlighting the fact that blood assay analysis was a suitable outcome measure. Adaptations to muscle function during the NMES protocol was outside the scope of this study, and should be considered in a larger study to test the 30 minute protocol.

The lack of muscle function testing at baseline, limits the consideration for participant demographics. There has been a strong relationship identified between muscle mass and force production (Hakkinen, Kraemer et al. 2001). A decline in force production has been associated with a reduction in muscle fibre number, and a reduced firing rate of the motor neurone. There could be a wide variation of muscle demographics at baseline which would influence blood interpretation, and thus the lifestyle questionnaire becomes vital in inferring to the sample. The LMD would have limited the number of participants recruited as testing would be restricted to one participant at a time. Study participation required 1.5 hours of time per participant. If testing each participant separately data collection would have progressed beyond the time available in this PhD. Time was also a restriction for the phlebotomist extracting blood samples, who was a full time medical student. The rationale of this study predicted a varied response to NMES, therefore making a larger sample size vital in order to reliably infer from the results. Testing more than one participant at a time would also aid recruitment patterns: this enabled friendship groups to be tested together, which was exploited in a student population. With larger groups of participants being testing in one session, the phlebotomist was being maximised, allowing as many participants to be tested within the limited time he was available for testing.

Throughout analysis participants were categorised into groups based on their response to the lifestyle questionnaire. The lifestyle questionnaire allowed any observations in blood concentration to be analysed based on levels of general fitness and previously conducted exercise. Although

subjective, the answers from the questionnaire grouped participants based on self-reported exercise levels. Participants were grouped by two variables: general perceived level of exercise, and exercise conducted in the previous 48 hours. Participants were divided into 'exercise' or 'no exercise' sub-categories for each group (detailed in table 7.5). This was achieved by splitting exercise reported based on self perceived intensity. The process was conducted by two members of the research team, with a discussion of participants who sat on the border of a sub-category. Subjectivity will have influenced this process; however accuracy was maximised by discussing the content of the exercise history. Analysis of results was conducted based on mean concentrations, however also by exercise category. This was important in obtaining the most information from our results, as plasma enzyme concentrations have shown to have a high variability based on lifestyle and exercise (Brancaccio, Maffulli et al. 2007).

Creatine Kinase (CK) concentration has been shown to be a strong indicator of muscle fatigue and recovery both during and after competitive exercise (Lazarim, Antunes-Neto et al. 2009). As CK is released from the muscle membrane, growth factors are suggested to follow, resulting in adaptive changes in muscle structure with exercise. This would result in a lower CK concentration at baseline, highlighting the need to understand general levels of exercise for data interpretation. It is well researched that plasma CK levels increase during exercise, and correspond with a decline of force in the muscle (Hunkin, Fahrner et al. 2014). Increases in CK concentration can remain for up to five days post exercise, indicating that those who have conducted exercise in the previous 48 hours may have a raised concentration of CK at baseline. Without attributing variations witnessed within this chapter to lifestyle, it is difficult to make assumptions. Participants were asked if Creatine supplementation was included in their diet. This has been shown to help improve muscle performance and bone mineral density (Wallimann, Tokarska-Schlattner et al. 2011). An addition of Creatine supplementation would alter both baseline and exercise induced CK concentrations. Time

was spent expanding on information provided in the lifestyle questionnaire if required, to aid analysis of results.

This study recruited forty participants. This sample size was determined based on time available from the phlebotomist and realistic time scales for data collection. Given the wide variability of CK concentration reported in the literature with exercise (Brancaccio, Maffulli et al. 2007), a larger sample was vital to enable trends to be appropriately identified. This study recruited the largest sample size in this thesis, and although a larger sample would enable a larger amount of rigour to be postulated to the population the sample was deemed sufficient in achieving the aims of this study. A minimal sample of 120 has been recommended to identify upper and lower reference values when inferring from blood concentration results (Lazarim, Antunes-Neto et al. 2009). Although the sample used in this study is not large enough to conduct this analysis, reference values from literature can be used to ascertain whether the concentrations of biomarkers in this study are comparable to previous work. The observational design adopted in this study resulted in a control group not being required to draw conclusions from data. This maximised the use of the sample; data for each participant could be utilised in comparisons.

The role of biomarkers in volitional exercise is well established to enable a comparison of NMES to volitional exercise. The testing of a baseline blood sample from each participant allowed any outliers from normal reference values to be identified, and analysed on an individual basis (Hunkin, Fahrner et al. 2014), which can then be related to the lifestyle questionnaire. Previous studies in this thesis have adopted a stringent limb position throughout testing protocols with NMES (for description please see chapter four). The testing of muscle response to treatment required that limb position was controlled. However, this current study did not measure muscle function in response to stimulation with NMES. It was therefore concluded that participant position was not vital in

achieving the aims. The participant was positioned in a way that replicated the testing positioned used throughout this thesis. This position was modifiable given the lack of restriction to the limb, and time required to be seated. A knee brace could have been adopted to minimise movement available at the lower limb joints, however participant comfort given the extended testing time resulted in this not being carried out. In order for this study to have clinical relevance it must be comparable to previous findings relating to the effects of NMES on muscle function.

Creatine Kinase was analysed based on time points during the treatment protocol. Within this participants were compared based on their response to the lifestyle questionnaire. When considering baseline CK concentration 27 samples were used for analysis; resulting in 5 samples not having a baseline concentration available. Participants who take part in general exercise demonstrated a significantly higher baseline CK than those who did not. This indicates that levels of fitness influence the resting CK level in the body. This is widely supported in the literature (Hunkin, Fahrner et al. 2014), with CK stated to vary considerably based on pre-existing exercise levels. This provides some confidence in our data, indicating that analysis techniques have appropriate sensitivity. High intensity musculoskeletal exercise commonly results in a high degree of muscle damage (Twist, Eston 2005), which has the effect of increasing the membrane permeability of the muscle cell. Increased permeability allows the release of CK into the plasma, which can last for up to five days post exercise (Hunkin, Fahrner et al. 2014). This elevated resting concentration may give an indication to the existing force producing condition of the muscle, and how it will respond to a treatment protocol with NMES. A high CK concentration indicates that the muscle has undergone some level of adaptation to exercise, and therefore is more likely to produce a tetanic response from stimulation with NMES. Baseline CK concentration demonstrated a significantly greater increase in participants who had exercised in the previous 48 hours compared to those who take part in regular exercise. Membrane permeability or the amount of CK exiting the muscle fibre must increase in this

sample. The exact cause for this increase is unknown, and the outcome measures utilised in this study do not provide reason for how this occurred.

Type of exercise conducted in the previous 48 hours has not been linked to the baseline CK concentration. Resistance training or endurance training may result in a different amount of CK release due to the type of respiration exploited. CK has been associated with the Kreb cycle of aerobic respiration (Baird, Graham et al. 2012), which would be utilised in endurance exercise, such as long distance running. Muscle damage associated with increased cell membrane permeability often occurs of a result of eccentric muscle work (Wernbom, Paulsen et al. 2012), which would be employed during resistance or explosive anaerobic demands. Further investigation as to the type of exercise conducted would be useful to explore the effects of NMES within different exercise demands. This study did not recruit a large enough sample to perform stringent analysis based on exercise, and a larger cohort should be employed to link exercise to CK markers. The effect of baseline CK concentration in frail and pathological populations has not been investigated to date, and would be a logical next step to further the aims of this thesis.

Participant (33) had a high baseline CK concentration that was abnormally high in comparison to the remaining data set. This was highlighted through the median approach to outliers, and the value falling outside 2 standards deviations of the mean. Intense martial arts training had been conducted in the 48 hours prior to testing, which involves repeated bouts of resistance training, with eccentric input (Thibordee, Prasartwuth 2014). This type of training was conducted on a regular basis, three times a week. The muscle requirements during martial arts training indicates that a high level of muscle damage would occur during a training protocol, which would give rise to a high baseline CK concentration in this participant. The impact of exercising on previously damaged muscle tissue (progressive over-load) could result in a repetitive build-up of CK in the plasma, resulting in an

elevated baseline (Mougios 2007). Repeated high intense exercise could result in over-training syndrome (OTS), which is characterised by an acceptable level of training overload but accompanied by inadequate rest periods (Meeusen, Duclos et al. 2013). Performance decline and fatigue are the key indicators for OTS. CK concentrations are not investigated at a diagnostic stage, however it is anticipated that CK would be elevated given the repeated stress to the muscle. Clinical investigations of CK are made based on indication of pathology. Rhabdomyolysis is a condition whereby muscle tissue is damaged, leading to myoglobin being released into the blood stream often resulting in kidney damage (Anzalone, Green et al. 2010). Rhabdomyolysis is characterised by a rise in CK concentration. The sharp decline of CK in this participant once treatment had commenced indicates that this condition was not present, and another factor was influencing the value. The baseline sample was not re-tested, and an error in analysis methods cannot be ruled out in this instance. The assay preparation method was prepared once for all samples in CK analysis, however errors in the kinetic analysis may have influenced this reading. The participant was of Indian origin, and although a link between baseline CK concentration and African-Americans has been documented (Neal, Ferdinand et al. 2009) no known links with Indian descent have been established. It is anticipated that this participant is not reflective of the sample, and reflection as to the reason of variation must be considered.

The NMES protocol was commenced once the baseline sample of blood had been extracted. The following blood sample was collected after the first 15 minutes of treatment, whilst treatment continued. No issues were reported with extracting blood samples whilst the NMES protocol was in progress, as the participant was required to relax throughout the protocol. The first 15 minutes of treatment has been considered with participant 33 removed from analysis, due to the abnormally high baseline CK concentration described above. The mean CK concentration for all participants rises by 0.01 nmol/min/ml between baseline and 15 minutes of treatment. Fundamentally CK breaks down creatine using adenosine triphosphate (ATP) to create phosphocreatine (Teixeira, Borges

2012). Analysis of CK activity informs the rate of creatine breakdown to produce energy for muscle activity. Despite a small increase it can be concluded that CK concentration remained quite stable during the first 15 minutes of treatment with NMES. Stability indicates that the muscle did not require an increased amount of creatine to produce contraction within 15 minutes; resting levels were sufficient to produce the required work. Monitoring of CK in sports has been documented with the majority of samples being withdrawn at the end of a training session or day of exercise, resulting in CK being analysed approximately 24 hours post-exercise (Rodrigues, Dantas et al. 2010). As far as we are aware, literature has not established the CK response within such a short period of exercise, and the assumption is made that the muscle had enough resting creatine to produce the muscle response. The lack of desired muscle response to the NMES protocol should not be ruled out at this early time frame, however the remaining protocol should be considered before this conjecture is explored.

Linear regression analysis indicates some variability within this early time frame; some participants demonstrated an increase in CK concentration and others a decline. On consideration of general exercise, those who report exercising demonstrate an increase of CK over the first 15 minutes of treatment, and those who do not exercise exhibit a decline. Although this is not considered a significant finding, the clinical indications should be explored further. The muscle membrane may be more permeable in the exercise group, allowing a higher concentration of CK to seep through the boundary. The non-exercisers may demonstrate a similar rise in CK after treatment with NMES, however the membrane is not adapted to regular exercise, and thus the threshold for CK release into the plasma may be higher. A muscle that is accustomed to regular exercise may be more responsive to the NMES treatment protocol. Regular exercise would indicate that the function of the neuromuscular system is heightened (Byrne, Twist et al. 2004), resulting in NMES producing a larger force output from the muscle. Neuromuscular adaptations in the exercise group could result in a higher CK concentration in this sample. The level of variability of a muscle response to treatment

with NMES must be considered, although the small range in the dataset indicates a normal distribution, and testing over a larger sample would give a clearer indication of whether this is of relevance to this investigation. Participants who had exercised within 48 hours of study participation demonstrated a small decline in CK concentration in the first 15 minutes. This indicates that the muscle reacts differently compared to the general exercise group. The higher baseline CK concentration in those who have exercised in the previous 48 hours may indicate that the muscle is utilising this reserve and thus decreasing faster. Muscular adaptation to exercise could result in the muscle not demanding the same concentration of creatine to produce a muscle contraction as those who have not exercised. It must be noted that the changes described are small, and difficult to make assumptions based on a larger population. When comparing the two exercise groups no true significance is witnessed, and the sample recruited may have resulted in large individual differences making it difficult to draw conclusions.

The beginning of the treatment protocol was compared to the final 15 minutes to ascertain how the muscle responded to NMES over time. When considering all participants a mean decline in CK concentration was witnessed. This is the only decline in mean CK concentration over the entire NMES protocol. The decline in CK concentration during a training protocol with NMES indicates that artificial stimulation induces similar chemical changes to muscle function as volitional exercise. NMES produces a muscle contraction via direct stimulation of the motor neurone. Volitional exercise utilises the central nervous system to emit an efferent response to the muscle, which is bypassed in artificial contraction. NMES does however initiate anti-dromic activation patterns of the neurone (Rushton 2003), resulting in stimulation of both the efferent and afferent response. The utilisation of the CK in this final 15 minutes of the protocol indicates that creatine is being broken down to produce energy required for muscle contraction. The energy required may have been necessary to produce muscle contraction for the remaining protocol. During the treatment session, the muscle contraction visibly reduced indicating that force production was comprised; a sign of muscular

fatigue (Westerblad, Allen et al. 2002). The stimulation intensity was increased throughout the protocol to avoid unwanted fatigue, and this maintenance of contraction intensity may have been a factor that stimulated the break-down of creatine in the muscle cell. The force response of the muscle was not monitored in this study; therefore this cannot be concluded with certainty. The decline of CK witnessed after 30 minutes of NMES indicates that it can be used in a pathological population who are unable to perform volitional contraction, resulting in similar responses to exercise as a volitional programme. In terms of clinical impact this implies that NMES would be able to prevent unwanted muscle atrophy in patients who are unable to produce a volitional contraction, or begin to stimulate hypertrophy in a population.

When looking at the final 15 minutes of treatment of NMES with reported exercise it can be concluded that there was a smaller decline in CK concentration in those who reported exercising. This indicates that muscle previously adapted to exercise does not require as much breakdown of creatine, potentially related to the increased baseline value documented. Previous demands of exercise placed on the muscle may have induced a learning effect on the muscle resulting in a lower threshold of demand to produce similar work outputs. When conducting linear regression analysis, variability was witnessed. Participants who reported not exercising demonstrated a rise in CK concentration, and those who exercised produced a more varied response. Non-exercisers required more CK to break down creatine than exercisers. This may be influenced by muscle structure based on existing demand, or a methodological issue such as variation of stimulation intensity. It is anticipated that people who report exercising would use a stimulation intensity that is higher than those who do not. Regular exercise to induce muscle hypertrophy requires a motivational input to drive the need for improvement (Spiegel, Grant-Pillow et al. 2004). This would indicate that those who exercise have a higher drive to produce the greatest force output from the muscle. It would be interesting to investigate whether a positive relationship exists between force output and stimulation intensity, however further measurement of muscle output would be required for this.

The final blood sample investigated the response of CK to the 30 minute rest period after the NMES protocol. The mean CK concentration increased after a 30 minute rest post-treatment. The level of CK concentration may have increased due to the predicted demand to carry out further exercise. Research indicates that the muscle membrane will have increased its permeability (Hunkin, Fahrner et al. 2014), allowing an influx of CK into the plasma as a response to exercise. CK concentrations are being investigated as a marker of muscle damage, and injury prevention in elite sport (Lazarim, Antunes-Neto et al. 2009). This elevated state of CK post exercise, indicates that the muscle may be in a state of fatigue, and thus continued exercise may induce injury. Participant 21 had an elevated CK concentration that was substantially greater than other participants. This participant had completed a half marathon the day previous to study testing, which resulted in a high resting CK concentration, but also suggested that his muscle was accustomed to aerobic exercise. The elevated final blood sample may be reflective of his resting level; this participant completed the highest level of aerobic exercise of all participants. The relationship between aerobic training and risk of injury would require a threshold of CK concentration to be set, however the sample size in this study is insufficient for this calculation. It is important to ascertain how exercise impacts treatment with NMES, as clinical applications may be considered for patients who would normally conduct exercise on a regular basis. The convenience sample recruited may not be reflective of a wider population, and thus outliers to the trends identified should be considered in the wider context. A larger sample would enable further conclusions from this to be drawn. This study aimed to ascertain whether the response to training with NMES is similar to that of volitional exercise. This raise in CK concentration indicates that the aim was accepted, the response of CK to exercise with NMES has produced biomarker changes that indicate the muscle has responded to exercise.

Stimulation intensity (SI) was governed by participant comfort during the treatment protocol, and ranged from 21-63 ma. All participants were accepting of the sensation produced from NMES, and no problems were encountered when modifying intensity based on strength of muscle output. The SI was documented and recorded for all participants, based on the highest tolerated intensity during the 30 minute protocol. Analysis was conducted by grouping the SI into three categories, which allowed any trends between SI and mean CK concentration to be identified. The majority of participants were stimulated in the low or moderate categories, with 10 and 11 participants respectively. Although this indicates that a tetanic muscle contraction was produced with a relatively low SI, the participants in this study were reluctant to increase SI to high intensities. This may be reflective of the sample; students studying physiotherapy may be more likely to participate in sport. Weight was documented in the lifestyle questionnaire, however skin fold thickness and anthropometric measurements of the muscles being tested may give more of an indication of how subcutaneous fat influenced impedance to stimulation. A low subcutaneous adipose layer would allow better passage of the electrical current to the muscle, and therefore stimulation with a lower intensity would be sufficient to produce a similar muscle contraction (Baker, Wederich et al. 2000). This may also be reflective of the method. Participants may be more receptive to increasing SI if muscle force production were being tested as an outcome measure. The element of self-competition to increase force production could have profound implications (Zilioli, Watson 2012) with respect to motivation to increase SI to produce a strong muscle contraction. Some participants appeared anxious about increasing SI as the effects repeated stimulation over 30 minutes were unknown to them.

When comparing the three categories, a positive relationship existed between the category and mean CK concentration; the higher the SI the higher the mean CK concentration. The rise in mean CK concentration indicates that the muscle has required more energy to produce the muscle contraction. By encouraging participants to increase the SI, the protocol is maximising the potential

from the muscle. When applying NMES for strength training, this implies that participants should use a SI that is on the higher end of their tolerance level, and should be increased based on becoming accustomed to the sensation. When increasing the intensity in the high category a large amount of variation was witnessed. The CK response was inconsistent, indicating that a clear relationship cannot be identified. Application of NMES at a high intensity produced both a high and a low mean CK, indicating that more testing needs to be conducted to ascertain the effects throughout a treatment protocol. It may be that the linear response continues, and that the level of CK increases in a linear fashion, implying a larger force out from the muscle. The low CK response to the high SI may indicate that the participant was accustomed to high intensity exercise. A high load of resistance training has been shown to increase muscle soreness, however maintain a low CK concentration in trained individuals (Vincent, Vincent 1997). Conversely, aerobic fitness has been correlated with elevated pre-match CK concentration in athletes (Hunkin, Fahrner et al. 2014). This data point relates to a 30 year old male, who was analysed in the exercise group. Resistance training was conducted in the 48 hour prior to testing, with an exercise history that suggests a high load of resistance training. It must be considered that the sample who used a high SI was low (n=2), and thus conclusions are difficult to extrapolate without testing a larger sample.

Result	*Link to NMES*	*Link to clinical relevance*
Those who exercise have a higher baseline CK concentration	Those who exercise regularly may be more responsive to treatment with NMES. High CK may be indicative of fatigue: may not tolerate NMES due to synchronicity of firing	Baseline CK can be used as a measure of muscle fatigue and predictor of injury Future work: establishing a link between type of exercise and CK concentration may highlight exercise history that may respond better to NMES
CK concentration remained stable in the first 15 minutes of treatment with NMES	The muscle did not require additional energy to produce muscle contraction as a result of NMES Did the protocol work the muscle sufficiently?	Little literature to relate to this short time period NMES protocols must be of sufficient time to induce changes in CK concentration
CK concentration decreased in the final 15 minutes of treatment	NMES has demanded that the muscle produce more energy to maintain contraction Stimulation intensity must be progressively increased to maintain contraction	Change in CK are of a similar pattern to volitional exercise NMES may be able to induce adaptations in muscle in a clinical population to maintain muscle structure during periods of forced rest
Variability was witnessed throughout the full treatment protocol; some participants increased, some decreased	The variation in CK response is similar to variation found during NMES in previous studies	Larger studies are required to ascertain effects on different ages, pathologies and exercise levels to identify trends within variation
CK concentration increased after 30 minutes rest	The NMES protocol worked the muscle enough to maintain an increased permeability post treatment Response to NMES is similar to volitional exercise	Investigation into treatment time is warranted to ensure the muscle is not over-trained
The higher stimulation intensity used during treatment resulted in a higher CK concentration (some variability in higher intensities)	Time should be spent accommodating to stimulation intensity to maximise output of treatment Participant comfort is paramount to successful treatment	Encouragement to increase stimulation intensity would maximise treatment effects Larger study needed to ascertain effects of high stimulation intensities

Table 7.17 Summary of study five results, with links to how the results affect application of an NMES protocol and clinical relevance

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7.8 Conclusion

The aim of this study was to ascertain whether there was a physiological reason behind the variability in response to NMES that has been witnessed in previous trials within this thesis. This study administered a one off 30 minute treatment session with NMES, and monitored CK activity throughout the protocol, and for 30 minutes after completion. Table 7.17 (above) summarises the main findings and clinical relevance of this study. **The overall pattern of CK activity during the treatment session correlated with CK monitoring in the sporting literature; indicating that treatment with NMES requires the muscle to produce energy, which is likely to cause muscular adaptation**. The fact that changes in muscle response have been noted in previous trials reinforces the fact that NMES has the ability to produce adaptation. Further investigation is required to ascertain how the muscle responds to a full treatment protocol (as would be administered if prescribing NMES to produce strength gains). This would allow study into how often sessions should be conducted per week. The synchronicity of firing which is well understood during NMES suggests that fatigue of the muscle may be a problem in administering multiple sessions per week. Further development in understanding of this process would aid protocol development in different clinical populations. The variability witnessed throughout previous trials in this thesis has been reinforced in this study. Although the variability demonstrated a similar overall pattern, some participants responded better than others. **It appears pre-existing muscular condition (level of exercise) may influence how a muscle responds to NMES**, however a larger sample with stratification based on lifestyle is warranted to fully understand this. Factors such as nutritional status, weight, and age may also influence how a muscle responds to NMES. Previous thoughts have anticipated that artificial stimulation will produce a standardised response in every muscle applied; however it appears factors that influence performance in exercise may also influence application of NMES.

Chapter 8

Clinical discussion

The primary aim of this thesis was to establish the role of neuromuscular electrical stimulation (NMES) in reversing age-related and pathological muscle atrophy. Five clinical studies were completed to fulfil this objective. The studies have been detailed in chapter's four to eight and document the progression of this thesis in aiming to achieve these aims. Firstly the identification of an optimal frequency was investigated and tested in a healthy population. This gave justification for the protocol to be used throughout remaining studies. The protocol was tested on varying ages and in a sample of acute stroke patients. Testing healthy participants enabled effects of the treatment protocol to be established before testing on a pathological population. Results indicate that NMES would be an effective adjunct to a physiotherapy programme; however some variability was witnessed that required investigation. The final clinical study aimed to ascertain reason for this variability. Blood samples were extracted during a NMES treatment session to determine physiological muscle response to treatment. Various findings were highlighted throughout the journey and will be discussed in relation to embedding NMES as an adjunct to a physiotherapy programme in a clinical setting. A summary of the main clinical findings has been provided in table 8.1.

Study	Main findings
Study one (Chapter 4): Establishing treatment parameters and dose	1. An optimal frequency of 50Hz was decided upon 2. This was based on greatest rate of change over varying frequencies 3. Early analysis may have limited conclusions from this study: 60

	Hz may be more representative of the findings 4. 50Hz produced a fatiguing response from the muscle that would be desired from a strength training protocol 5. Some fatigue throughout a strength training protocol would be considered an asset for hypertrophy development
Study two (Chapter 5): Testing in old and young muscle	1. NMES is able to increase volitional force output of young and old muscle 2. The response time of muscle to NMES improves over time 3. A muscle will not receive the full 'on' time which is programmed into a NMES protocol 4. Young and old muscle require similar intensities to produce strength increases 5. Variability witnessed- some participants responded better to treatment than others
Study three (Chapter 6): Testing in a pathological population (stroke)	1. NMES is practical to administer in an acute stroke setting 2. NMES has the potential to improve the probability of walking post stroke. Quicker restoration of walking ability would reduce the time period for muscle deterioration post stroke 3. NMES appears to improve strength gains post stroke 4. Identification of a strength threshold required for completion of functional activities would maximise potential of rehabilitation 5. NMES appears to improve pennation angle despite the muscle not being able to produce a volitional output 6. NMES appears to influence cutaneous sensation post stroke
Study four (Chapter 7): Testing variability of response	1. People who exercise have a higher baseline creatine kinase concentration and thus may be more responsive to treatment with NMES 2. The muscle does not show a response to exercise after 15 minutes of treatment with NMES: NMES protocols should be greater than 15 minutes in length 3. Stimulation intensity should be progressed to maximise the output of muscle 4. The response of a muscle to NMES is similar to that of volitional exercise

	5. Variation in (Creatine Kinase) CK response to stimulation with NMES is similar to that of the response noted throughout this thesis

Table 8.1 summary of findings found in clinical trials

The Leg Measurement Device (LMD)

Consideration should first be given to the leg measurement device (LMD), which was designed and developed to monitor the muscle response to NMES throughout this thesis. The LMD was able to produce accurate measurement of knee extensor and ankle plantarflexor moment; key muscles targeted in a rehabilitation setting for functional activity. The portability of the device enabled ease of use both in an outpatient or a hospital ward environment. The device was utilised both resting on a chair and positioned in a hospital bed throughout this thesis, highlighting the adaptability of its use. A measurement tool of this nature to be used in clinical practice is not currently available and allows measurements to be conducted for both research and clinical use. The LMD is able to be modified in accordance with patient limb size, which is simple to conduct whilst the limb is in position to be tested. This is something that clinical staff could perform with training and no problems in this aspect of measurement would be anticipated. Accurate measurement of muscle strength is something that is regularly performed in a physiotherapy department, however manual muscle testing is often employed for discharge criteria. It is anticipated that a device of this nature could be used to assess differences in muscle strength before and after surgery, to ascertain effects of rehabilitation or muscle atrophy.

The high sensitivity of force transducers could also be used to identify small changes in strength or onset of initial muscle activation post intervention. This would allow rapid feedback to be given to the patient enabling higher motivation to improve strength during rehabilitation. The device was

also used to monitor muscular response during NMES treatment protocols. This allows another biofeedback application during a treatment programme and a way of a clinician being confident in the appropriateness of treatment for the specific individual. The high sensitivity of muscle force change monitored by the LMD would allow application in neurological rehabilitation; a form of physiotherapy that uses small increases in muscle strength to transfer to large increases in muscle function (Umphred, Lazaro et al. 2013). Further development is warranted to ascertain the onset of muscle contraction in relation to force output on the foot plate. Integrated application of electromyography would be an effective tool to enable this and would be able to monitor compensations to allow strength to be achieved. Compensations are strategies often employed by patients and although allowing functional movement patterns to be produced, they highlight a weakness that may hinder progression of rehabilitation. Further development and testing is required but at this initial stage the LMD appears to be a measurement tool that would be welcomed for multiple applications in a clinical setting.

Study One: Optimal Frequency

Study one aimed to identify an optimal frequency to be used during strength training with NMES. An optimal frequency was identified (50 Hz) which was shown to produce the greatest change in force of the muscle over the programme. Linear regression analysis allowed this change of force to be identified which was considered appropriate based on the variable response to stimulation on assessment of maximal moment. Based on subsequent findings within this thesis, there appears to be many factors affecting the response of muscle to NMES. This includes application variations (electrode position, impedance) as well as lifestyle factors, such as pre-existing condition of muscle, nutritional status and level of fitness. It may be that further investigation into lifestyle groups is required and a protocol devised for clinicians to apply NMES based on placing the individual to be treated into one of these groups. NMES initiates muscle response by artificially inducing axonal

propagation. The resultant muscle contraction is produced via identical means as volitional muscle contraction (Lieber 2009). Debate exists as to the mechanism of motor unit recruitment; whether large diameter motor units are recruited before small diameter, or whether a random pattern of recruitment is produced (Kim, Bangsbo et al. 1995). Larger axons are associated with type two fatigable muscle fibres and are considered to have a lower threshold for stimulation therefore highlighting a mechanism of muscle fatigability (Bickel, Gregory et al. 2011). The supra-maximal contraction of muscle achieved throughout this thesis indicates that a non-selective recruitment pattern is associated with NMES. The response over a training period could however be associated with current muscle condition. This response has been proposed as the applied stimulus with NMES is constant, however the muscle output varies. A muscle that has been extensively pre-conditioned (one that is part of a regular resistance training programme) will be able to withstand constant firing at maximal intensity without overloaded fatigue. This is in response to training as would be seen in a voluntary resistance training programme. This muscle will demonstrate a response from NMES that is indicative of strength training: a small rate of force decline indicating the muscle has been worked. The muscle that has undergone little pre-conditioning will suffer substantial effects of fatigue and effectively produce an automatic protective decline in force output in response. The levels of energy source associated with muscle contraction (adenosine-triphosphate) have been depleted and not restored to allow further contraction. It is not proposed that the under conditioned muscle should not be included in a NMES treatment session, but instead that stimulation intensity is gradually increased during the programme to enable a continuous muscle response that is reflective of strength training properties.

Study two: Protocol Development

The stimulation parameters set in study one were tested on a protocol consisting of 45 muscle contractions. This was based on three sets of fifteen repetitions, which were conducted three times

a week over 6-weeks for remaining studies. NMES literature is ambiguous with regard to the duration and intensity of muscle contraction required to induce hypertrophy (Maffiuletti, Pensini et al. 2002). Recommendation for the duration of treatment in this protocol was obtained from volitional strength training literature. Outcome measures were utilised to establish any changes in muscle architecture; giving a true indication of muscle response to treatment. This thesis proposes the use of NMES as adjunct to a physiotherapy programme, as opposed to a stand-alone treatment. However, application in solitude was necessary to establish that NMES was producing desired muscle effects. In reality NMES may only be utilised until the muscle is able to achieve sufficient contraction intensities to induce hypertrophy; as a means of preventing muscle atrophy associated with weakness. The muscle displayed a small average decline of force over the 45 muscle contractions. This response is suggested to be a beneficial stimulus for hypertrophic process to occur (Stevens-Lapsley, Balter et al. 2012) and thus the 45 muscle contractions applied with tested parameters appear sufficient to fulfil strength training aims.

Study three: Investigation of the protocol on varying ages

When testing the 6-week stimulation protocol on healthy participants of differing ages the muscle was able to produce a greater moment after treatment; indicating that strength had been improved. The LMD was utilised to assess muscle response to treatment. Interestingly the time the muscle was in a contracted state in response to treatment was shorter than the 'on' time set in the stimulation protocol. The time of muscle contraction increased during the 6-week period indicating that the muscle adapted its response time as a reaction to stimulation. Firstly, when administering NMES in a clinical environment, it is suggested that the protocol is applied with a greater 'on' time than what is required. This thesis stimulated for 3 seconds a time frame that was chosen to mimic a burst of muscle activity that would be utilised to maximise muscle strength through a voluntary resistance training programme. However, it is recommended that this time frame is doubled to 6 seconds;

which would result in 2.7 seconds of actual muscle work. This figure would be anticipated to increase as the muscle became more responsive to treatment. The 'on' time inputted into the stimulator does not consider the time frame of the neuromuscular system to detect a stimulus and initiate its response. This time appears to reduce with a strength training protocol with NMES. A quicker neuromuscular response indicates that the muscle will be able to produce more muscle power; the amount of strength produced in a unit of time (Lieber 2009). This becomes important when treating both an athletic population and the frail elderly. An athletic population often suffer musculoskeletal injuries such as ruptured anterior cruciate ligament, which often results in surgical procedures. NMES allows training of the muscle without undue compression (through resistance or weight bearing) on the joint which is often contraindicated early in rehabilitation. This indicates that NMES could be used acutely after surgery with the aim of restoring the ability of the quadriceps to control the knee when the patient initially starts to weight bear to prevented unwanted stresses on the newly reconstructed ligament. Treatment in this regard would not be required in all patients but allows a safe and effective treatment to maintain muscle function if forced rest is implemented. The same principles could apply to an elderly population; the ability to produce enough power to control knee flexion and prevent falling would enable the individual to continue mobilisation with increased confidence and help to prevent the progression of sarcopenia. In terms of the elderly, maintaining physical activity would help to defer pathology associated with a decline of health.

Results indicate that both young and older muscle require similar intensities to produce strength improvements with NMES. The older muscle demonstrated a rapid initial adaptation and then demonstrated steady improvements over the remaining training period. This suggests that the neuromuscular system in older individuals is not working to its full potential and has the ability to develop within a short time scale. This maybe through repetition of neural stimulation resulting in decreased response time of the stimulus between the nodes of Ranvier which heighten the speed of conduction. The heightened activity of ion channels may be produced as a result of stimulation with

NMES (Poliak, Peles 2003). Development of the neuromuscular system enables the muscle to receive more frequent and potentially stronger impulses, increasing the likelihood of adaptation in structural properties. It appears the internal muscle structure is slower to respond to treatment with NMES than its younger counterpart; however changes have been reported in thesis. It was originally thought that older muscle would respond at a quicker rate as a result of the new stimulus imposed on an unconditioned muscle. It is likely that the unconditioned state of older muscle along with the effects of general deterioration with age results in slower response of the satellite cells and thus a slower rate of hypertrophy. The ability of older muscle to respond to treatment with NMES indicates that this may be an effective treatment to use in a clinical setting. There was however variability witnessed throughout the strength advances in both ages, a factor that consideration of lifestyle may be able to justify.

Ultrasound as a hypertrophy outcome measure

The mechanism associated with strength training with NMES is not fully understood (Gondin, Guette et al. 2005). Initial advances in force production are attributed to an increased neural drive to the muscle, both in young and old muscle. Ultrasonography was utilised as an outcome measure to ascertain differences in pennation angle of the muscle. The pennation angle is the angle at which muscle fibres attach into the aponeurosis of muscle and are thought to have a proportional relationship with strength gains (Aagaard, Andersen et al. 2001). However some authors dispute this, indicating that the two processes have little relationship (O'Sullivan, Sainsbury et al. 2009). Pennation angle was seen to increase in both the young, old and pathological muscle within this thesis. An increase in pennation angle indicates that more muscle fibres are being stored in parallel, effectively increasing the physiological cross sectional area of the muscle. Muscle fibres arranged in this fashion are indicative of the ability to produce more force (Lieber 2009) highlighting that the 6-week NMES protocol produced hypertrophy on top of neural adaptation. Clinically this implies that

NMES is able to prevent deterioration, or even advance internal muscle structure in a period of rest. The carry over effect of this finding was not tested in this thesis, but should be monitored over an extended period of time, especially in the elderly. This would indicate whether training with NMES as an adjunct to physiotherapy was able to over-come the onset of muscle atrophy associated with sarcopenia to improve long term mobility.

Study four: Investigation of the protocol on a pathological population

Testing of the 6-week NMES training protocol on an acute stroke ward proved feasible and effective in achieving adaptation of muscle. Results have to be considered on an individual basis as a consequence of the small sample in this study. Small advances in strength were gained after the 6-week NMES intervention, which were higher than the control counterparts. This indicates that NMES was able to induce hypertrophic muscle adaptation, which was confirmed by ultrasonography measurements. Ultrasonography highlighted some interesting changes in internal muscle structure in this population. Pennation angle demonstrated improvements in participants who did not restore volitional control of muscles post stroke. This indicates that NMES is able to advance internal muscle structure ensuring the muscle has the capacity to contract if volitional control is restored. Atrophy associated with conditions such as stroke is rapid, especially considering the advanced age of sufferers. Although neuroplastic changes are thought to occur in the acute phase post stroke, the ability to use NMES between insult and motor restoration provides a treatment that will prevent the effects of disuse atrophy. The muscle would be likely to reduce in size; however the structure of the muscle fibres present would be maintained. This would be advantageous in rehabilitation as it would enable functional mobility (such as transition from sitting to standing) to be conducted with a quicker onset. This would also be beneficial in orthopaedic rehabilitation allowing muscle structure to be maintained during a period of illness or forced non-weight bearing as a result of surgical intervention.

During rehabilitation of a patient after a stroke accurate measurement of strength is rarely conducted. Muscle strength is monitored through manual assessment, however is often thought to be subjective and difficult to compare between examiners (Pradon, Roche et al. 2013). The use of a device such as that designed in this thesis would enable accurate measurement to be performed within all severities of stroke. The level of strength required to conduct functional activities after neurological insult has not been investigated to date. The introduction highlights the concept of an independence threshold; the level of muscle contraction required for independent function. Although not directly measured in this thesis, progress has been made in identifying this threshold. Measurement of quadriceps moment during sit to stand would indicate the level of quadriceps moment required to achieve the movement; and thus produce an aim of a strength training protocol. However ability to measure precise moments in a functional pattern is challenging. Use of the LMD to measure moment on a session by session basis would allow measurement of the lower limb muscles once independent standing was achieved. The results from the NMES protocol appear to transfer to walking ability in this study which has also been indicated in the literature (Yan, Hui-Chan et al. 2005). This indicates that isometric application of NMES has the ability to transfer to functional ability and thus assumption can be proposed that isometric strength can transfer to functional strength. Knowledge of the strength threshold for independent function would allow a goal of the NMES strength training protocol in initial rehabilitation stages post stroke.

Another interesting finding when NMES was applied to participants with acute stroke was the restoration of cutaneous sensation as a result of NMES. It is difficult not to rule out the role of natural recovery in this; however sensation progressed during application of the protocol and it was therefore assumed that it played a role in restoration. Electrodes applied onto the skin are non-selective in the structures that they innervate. Cutaneous receptors such as Meissner's and Pacinian

corpuscles detect changes in vibration and produce responses in the somatosensory system. These mechanoreceptors are located superficially within the skins epidermis and thus stimulation that penetrates through to innervate motor axons will also initiate a response in anything that travels within its path (Baker, Wederich et al. 2000). Stimulation of the mechanoreceptors will have resulted in a repetitive afferent response from them which may have developed restoration of sensation. Cutaneous sensation becomes vitally important for self-management of a neurological condition. Ability to monitor changes in pressure can prevent pressure sores which can often result in infection and a prolonged period of bed-rest as a treatment method. Stroke patients are often required to wear orthotic devices as a means of safety to mobilise (Carse, Bowers et al. 2014). The ability to sense the orthosis and notice areas of high pressure again becomes vital in maintaining healthy tissue. The overall impact on proprioception will be increased by normalised sensation of lower limb movements and thus assist in effective rehabilitation. The ability of the NMES protocol proposed in this thesis to influence strength and sensation highlights a dual role in rehabilitation.

Study five: Investigation of blood biomarkers

A high level of variability was obtained throughout the two studies which tested the 6-week strength training protocol with NMES. This indicates that NMES had influence on muscle structure; however the response produced was not consistent. The studies in this thesis did not allow comparison within gender or fitness level as both were proof of concept and thus recruited a small sample size. Work of this nature should be conducted to highlight any trends within participant groups. Creatine Kinase (CK) was monitored through a one off treatment session with NMES. The NMES protocol was altered slightly to maximise the activity of the muscle in a single application. Baseline CK was elevated in participants who reported regular exercise. This indicates that the cell membrane was more permeable as a result of exercise (Wernbom, Paulsen et al. 2012). Increased permeability at baseline indicates that the muscle may be more responsive to treatment with NMES. This is due to activity of

CK having a greater opportunity to produce energy by the threshold of permeability already being low. This suggests that a physiotherapy programme conducted alongside a NMES protocol may be an adjunct to participant responsiveness. Although NMES as applied in this thesis does not require active muscle involvement in contraction, consideration should be given to spending some time replicating the movement volitionally. It would be interesting to see whether NMES triggered by electromyography would enhance this effect, potentially increasing response of the muscle to treatment even further. This response is easier to manipulate in a musculoskeletal population as they have the ability to generate if not attempt muscle contraction to assist the NMES programme.

Creatine kinase (CK) concentration has been shown to elevate during high intensity exercise that induces muscular hypertrophy (Brancaccio, Maffulli et al. 2007). This is produced in response to high intensity exercise damaging the membrane permeability of the muscle cell. CK is stored within the M-line of the sarcolemma and thus released in response to permeability changes. The lack of CK response to treatment after 15 minutes indicates that the muscle had enough resting energy to produce contraction or the muscle was not working to a sufficient level or long enough duration to induce membrane damage. This highlights the importance of NMES being applied at a maximal tolerated intensity and increasing the stimulation intensity as the participant begins to show a reduced force output. The requirement of a treatment protocol to last more than 15 minutes in order to work the muscle over a sufficient time period also appears vital.

As the treatment with NMES progressed the response of CK was similar to that of volitional resistance training; a depletion following by a corresponding increased concentration. This indicates that NMES is producing similar effects to volitional exercise despite the variability witnessed within results of this thesis. Although application of the protocol was slightly different, this suggests that the stimulation parameters proposed are indicative of true strength response of a muscle. CK

depletion is thought to be a process of energy utilisation to produce contraction and the subsequent increase a preparation for further exercise. CK response was not measured over a longer period of time and while voluntary resistance training induces elevation for up to 5 days, the effects of CK activity over a comparable period are unknown in relation to NMES strength training. CK has been proposed as a marker of injury susceptibility in sports medicine; an exceptionally elevated CK level at baseline indicates over-load of exercise and fatigue. Understanding the carry over effect of CK after NMES would allow the monitoring of muscle fatigue throughout an NMES protocol, a process which is highly relevant given the non-selective recruitment of fatigable muscle fibres in artificial contraction. CK concentration increased after 30 minutes rest with NMES, however this increase may have continued if tested over a longer period of time. The intensive nature of the strength protocol may have caused undue fatigue, thus limiting the strengthening effects witnessed in this application. Parameter setting has been established and tested within this thesis; however the protocol design was influenced through volitional literature for easy comparison. Further testing of the training regimen would enable the protocol to be administered with more certainty. The effects of CK elevation may be present after the initial few applications of the protocol, hence the full 6-weeks of training may not be required to inducing fatiguing effects in the muscle. This would become problematic in a population who use NMES for strength training as an intensive means of training before voluntary training commences. However, the results from clinical studies imply that muscle response did not induce excess fatigue and strength responses noted. Further research is warranted however the clinical findings produced from this research are still recommended.

It appears that fitness or exercise level is a key determinant in how somebody responds to treatment with NMES. The variability witnessed in CK concentration demonstrated trends in relation to previous levels of exercise, which has previously been discussed in individual response to contractions inducing via NMES. It may be that protocols are devised for applications in participants with differing fitness levels, for example; sedentary, mild, moderate and intense. This would enable

the stimulation to be administered based on an understanding of neuromuscular response to treatment with NMES. However, the results of this thesis indicate that the muscle is able to respond to treatment to NMES despite variation of muscle condition and thus gives justification for use in a clinical environment.

The following parameters and key determinants have been recommended for application of NMES for strength training as a result of this thesis:

Stimulation parameters	***Key determinants***
• 50 Hz frequency • 450 µs pulse width • 6 seconds on • 10 seconds off • 0.5 second ramp times • Maximal tolerated intensity	• Apply with maximal stimulation intensity • Gradually increase stimulation intensity in line with a visual decline in muscle force • Combine NMES with volitional muscle contraction (use as a physiotherapy adjunct)

Table 8.2 Key features of a NMES protocol for strength training

Chapter 9

Conclusion and Recommendations

9.1 Conclusions

Frailty is a growing concern in an ageing population. As frailty advances and co-morbidities develop, the level of physical activity concomitantly reduces. This poses two problems; the individual loses confidence in their ability to mobilise (which deepens the problem), and their muscles will experience significant disuse atrophy. This is heightened by age, with age related muscle disorders such as sarcopenia adding to the effect of disuse. Frailty renders an individual more prone to pathology such as stroke. This process can be exacerbated in someone who has a stroke, with forced rest advancing muscle atrophy, and neurological insult having a detrimental effect on balance, proprioception and mobility. NMES has been explored as an intervention to prevent muscle atrophy in these two conditions, whilst physiotherapy commences.

The Leg Measurement Device (LMD) was designed and developed in this thesis to accurately measure moments about the knee and ankle joints. The device was successful in measuring moments, with high sensitivity to detect small changes in force. This allowed monitoring of muscle response during the treatment protocol to ascertain deeper understanding of the treatment effect. The LMD was utilised both in an outpatient and inpatient setting. The portability and hygienic wipe clean use were key attributes to its successful integration in testing a clinical population. A small modification was made in the early stages to maximise ease of participant position, which proved successful when testing on an acute clinical population who were unable to mobilise.

Controversy exists as to the optimal parameters to stimulate muscle with, and thus work to further explore parameter setting has been conducted. Frequency was investigated as the dependant variable, as the inversely related pulse duration and stimulation intensity are better understood. Stimulating at a frequency of 50 Hz has been shown to produce the greatest rate of change in force in this research, whilst not producing detrimental muscle fatigue. In addition, these parameters appear successful in producing a muscle response indicative of a strength response from the muscle. This appeared to identify a protocol that required testing over a full training protocol. The strength training protocol was utilised in healthy individuals of different ages. NMES literature suggests that the training effects are better understood on young muscle (Maffiuletti, Minetto et al. 2011) and this study set to understand whether the muscle response was similar in aged muscle. The effects appear similar, with both age groups shown to increase their muscle strength, which occurred to a greater extent in young muscle. The response rate of the neuromuscular system to stimulation with NMES reduced with training, and the muscle did not receive the full stimulation time set. It is therefore suggested that the stimulation time is increased beyond what is considered a desired length of contraction. A proportional relationship exists between muscle force output and stimulation intensity; indicating that the intensity should be increased to maximal tolerated to achieve optimal treatment effects.

The treatment programme was tested in a population who had suffered a stroke, and results indicate that strength can be advanced in this population. Strength increases were small however were able to transfer to functional ability alongside traditional rehabilitation. It must be taken into consideration that the sample size was small, and the results were forced to be considered on an individual basis which limits the interpretations that can be made. Pennation angle was seen to increase despite any restoration of volitional muscle control. The ability of NMES to maintain internal muscle structure in the initial stages of a physiotherapy programme presents an application of NMES that would advance rehabilitation time scales post stroke. NMES has also been shown to

increase cutaneous sensation as applied in this thesis, highlighting dual application of the protocol to enhance rehabilitation. Cortical control of sensation would enable key determinants in a physiotherapy programme, such as balance to be restored in a shorter time scale. Despite these findings, a high level of variability was presented in the findings, with the small sample sizes (indicative of proof of concept) making the variability difficult to account for.

Creatine Kinase is a biomarker which was monitored through a treatment session with NMES. Creatine kinase displayed similar responses in concentration as volitional strength training, highlighting that the protocol is effective despite the variability witnessed. It also gives an indication that the responsiveness to NMES could be linked to exercise history; those that exercise respond better to the treatment protocol. Creatine kinase has been shown to remain elevated post exercise with NMES, which could indicate fatigue as a result of the intensive nature of the strength protocol (Lazarim, Antunes-Neto et al. 2009).

9.2 Recommendations

It is from these results that we are able to recommend avenues for future development of work. The results of this thesis provide an insight into the muscle response to treatment with NMES, but do not provide firm conclusions. It is noted that the studies described in this thesis recruited a small convenience sample, which was imperative for establishing trends to justify further investigation. It can be concluded that the results provide scientific justification for larger randomised controlled trials, with a higher powered methodology to be conducted. Although control groups were utilised where appropriate, blinding was not performed throughout this thesis mainly as a consequence of the author being a single tester throughout. Further development of methodology is required to confidently present the mechanism of muscle response into the literature as solid findings.

Four areas of future development have been highlighted as a result of this thesis, and are outlined below:

1. Development of the Leg Measurement Device (LMD). Although the LMD was successful in achieving the aims of this thesis, further development is required to enable amalgamation into a clinical environment. It is anticipated that surrounding muscles were activated in compensation for lack of control to produce strength, a finding that was especially prudent in a neurological population such as stroke. Integration of electromyography into the device would allow feedback of which specific muscle activated to produce the force, and also the interplay between muscles; does one muscle activate before another. A means of displaying real time moment values during testing would allow instant feedback to participants using the device; something that would be important to include if utilised on patients for discharge criteria. At present the LMD is indicative of a research tool; something that was vital for initial testing, but required uploading of results, and conversion of moments on a laptop. This process was not a problem for testing within this thesis; however it is required when thinking about clinical usage.

2. Establishing stimulation parameters for secondary aims of NMES. The stimulation parameters in this thesis have been specific for the use in strength training. NMES has been postulated to have other uses, such as improving voluntary muscle activation, assisting recovery after injury and improving the endurance properties of muscle (Deley, Babault 2014). All aims have been identified to use differing stimulation parameters, and research now needs to address this issue. It is the hope that a stimulator with programmed parameters for each aim of treatment be designed, to enable ease of use in a clinical environment.

3. Identifying the level of strength required for functional independence in stroke rehabilitation. Monitoring of the knee extensor moment required for functional independence in stroke would enable a strength training programme with NMES to be administered with a primary goal; to reach the strength threshold. Use of the LMD to monitor strength during rehabilitation would enable this level of functional strength to be identified.

4. Investigating concentration of Creatine Kinase to assist with development of training intensity. Creatine Kinase has been shown to elevate for up to five days after volitional strength training. Monitoring of concentration ceased after 30 minutes in this thesis. Monitoring over a longer period of time (5 days) may demonstrate further increases in concentration; something that may be indicative of the synchronous firing pattern of NMES during application. Elevated Creatine Kinase in the muscle is suggested to be a marker of muscle fatigue, and risk of injury in sports medicine (Lazarim, Antunes-Neto et al. 2009). Elevated Creatine Kinase may be able to guide development of the training intensity used to administer the stimulation protocol with greater accuracy, to avoid detrimental fatigue. Fatigue associated with NMES has been highlighted as a barrier to clinicians incorporating it into clinical treatments, and work of this nature may help NMES integrate into clinical practice.

5. The ability to increase strength in an elderly population has been indicated. It would be interesting to ascertain whether a ceiling effect exists as to the level of strength that can be restored in this population. This is outside the scope of this thesis, but would be recommended for future research.

The primary aim of this thesis was to ascertain the role of NMES in preventing age-related and pathological muscle atrophy. It is postulated that NMES is successful in achieving this aim; NMES would be a suitable treatment modality to use in the initial stages of a physiotherapy programme to prevent unwanted disuse atrophy of skeletal muscles to maximise rehabilitation potential.

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12. Appendix

Appendix One Ethical approval studies 1-3 (Chapter 4-6)

NHS

National Research Ethics Service

South Staffordshire Research Ethics Committee

Medical Institute
Hartshill Road
Hartshill
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18 January 2010

Miss L Claydon
Research Physiotherapist
NHS
ORLAU
Robert Jones & Agnes Hunt Hospital
Gobowen, Shropshire
SY10 7AG

Dear Miss Claydon

Study Title: **Are changes in muscle structure and function, following treatment with Neuromuscular Electrical Stimulation similar in younger and older adults?**
REC reference number: 09/H1203/92
Protocol number: 1.0

Thank you for your letter of 22 December 2009, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of

the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		10 November 2009
REC application		
Protocol	1.0	09 November 2009
Investigator CV		10 November 2009
GP/Consultant Information Sheets	1.0	09 November 2009
Letter from Sponsor		27 February 2009
Student CV		10 November 2009
GP Information Study 1	1.0	09 November 2009
GP Information Study 2	1.0	09 November 2009
GP Information Study 3	1.0	09 November 2009
Funder Letter		01 July 2009
Flow diagram	1.0	09 November 2009
Recruitment e-mail	1.0	26 October 2009
Participant Information Sheet: Study One	3.0	22 December 2009
Participant Information Sheet: Study Two	2.0	22 December 2009
Participant Information Sheet: Study Three	2.0	22 December 2009
Participant Consent Form: Study One	3.0	22 December 2009
Participant Consent Form	1.0	22 December 2009
Recruitment Poster	2.0	22 December 2009
Response to Request for Further Information		22 December 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1203/92	**Please quote this number on all correspondence**

Yours sincerely

Professor Tim Reynolds
Chair

Email: barbara.cannings@uhns.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Caroline Stewart ORLAU Robert Jones & Agnes Hunt Hospital Gobowen, Shropshire SY10 7AG*
Ms T Jones, Robert Jones and Agnes Hunt Orthopaedic and District Hospital NHS Trust, Oswestry, Shropshire, SY10 7AG

Appendix 2 Raw Data Study One, Chapter Four

Freq (Hz)	20_1	20_2	30_1	30_2	40_1	40_2	50_1	50_2	60_1	60_2	70_1	70_2	80_1	80_2	100_1	100_2
1	13.67	15.23	16.05	19.27	16.98	19.65	19.23	21.67	25.50	23.56	21.85	32.73	23.63	22.65	24.54	31.73
2	17.73	15.11	23.59	28.76	23.87	22.98	77.10	81.37	24.16	31.67	26.75	27.16	32.61	30.74	35.58	29.82
3	6.69	9.67	6.77	9.76	7.36	9.65	8.11	9.26	34.96	36.98	28.34	30.19	16.06	20.34	42.07	39.67
5	9.54	9.74	14.35	15.21	15.70	21.56	13.56	11.24	10.86	11.59	11.31	10.23	13.46	12.63	15.10	13.63
6	5.34	10.13	5.47	7.34	7.20	11.87	8.65	8.43	10.59	12.45	11.46	12.84	12.00	12.98	11.75	15.65
8	7.84	10.21	7.95	8.76	5.91	6.21	7.54	9.23	7.50	9.78	8.05	10.36	7.85	8.92	8.90	10.87
9	7.93	9.65	12.31	14.32	10.76	13.54	4.24	5.78	15.56	19.99	9.29	12.62	10.63	14.64	10.78	19.87
11	15.51	13.22	9.07	10.87	15.55	15.76	12.38	14.64	16.78	24.76	15.88	15.76	16.92	24.76	17.98	22.64
12	1.84	6.54	2.06	6.76	3.19	6.87	2.87	3.98	5.28	9.37	7.15	9.27	6.42	5.49	6.48	11.67
14	13.12	15.48	10.98	12.98	9.64	11.22	11.00	12.11	8.93	14.74	7.24	8.21	9.59	7.92	10.19	8.45
15	4.16	8.64	4.32	5.67	4.77	10.08	4.68	5.23	3.77	6.21	5.89	7.26	3.17	9.47	2.46	9.34
16	23.83	19.69	28.89	26.72	35.64	30.37	33.10	29.82	25.90	24.99	28.51	30.64	32.05	26.96	24.86	27.89
17	5.09	8.45	2.99	7.32	7.54	9.54	7.54	8.67	6.46	8.64	11.15	8.76	13.16	15.73	19.38	15.32
18	10.28	13.43	10.49	9.76	8.30	10.25	11.82	13.34	12.14	14.27	11.24	10.29	8.27	11.38	9.94	11.26
19	8.14	11.12	8.89	9.21	9.64	9.60	10.24	9.24	8.84	8.66	9.30	11.67	9.30	11.97	4.49	5.04
Mean	10.05	11.75	10.94	12.85	12.14	13.94	15.47	16.27	14.48	17.18	14.23	15.87	14.34	15.77	16.30	18.19
SD	5.82	3.50	7.43	7.06	8.49	6.83	18.56	19.19	9.18	9.33	8.07	9.23	8.78	7.58	11.34	10.08
SE	1.56	0.93	1.98	1.89	2.27	1.82	4.96	5.13	2.45	2.49	2.16	2.47	2.35	2.03	3.03	2.69
Mean day 1 and 2	10.90		11.90		13.04		15.87		15.83		15.05		15.06		17.24	

Study one raw data: Maximal Moment for each frequency. Data presented in Nm. 1: day one of testing, 2: day two of testing, SD: Standard Deviation, SE: Standard Error.

Appendix 3 Raw Data Study Two, Chapter 5

	Participant number										
Attempt	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
1	11.3	16.9	53.0	9.6	10.2	17.1	39.3	5.9	34.4	10.6	13.2
2	11.8	17.1	56.4	11.0	11.4	22.8	42.0	8.2	33.4	8.5	13.1
3	11.0	17.3	59.7	11.6	15.2	26.0	41.1	8.1	34.7	12.4	11.6
4	10.8	18.0	66.4	11.6	15.6	10.3	43.2	7.3	33.2	13.5	11.6
5	12.1	19.5	67.6	12.0	14.7	15.7	44.4	6.9	34.8	8.5	11.6
6	10.8	18.4	67.1	12.1	15.3	12.7	42.4	6.1	34.5	7.7	12.0
7	11.0	16.4	68.5	11.0	15.3	10.9	43.8	4.6	35.1	8.7	12.2
8	11.1	18.3	67.9	12.2	16.0	10.7	43.5	3.9	36.6	5.7	11.8
9	10.4	19.1	62.9	10.7	16.3	11.3	42.9	4.4	35.4	8.4	12.0
10	12.9	17.9	61.6	11.4	16.0	12.6	42.6	5.4	35.4	6.9	15.5
11	13.6	16.3	63.2	11.3	16.4	7.5	44.5	7.0	35.7	8.8	16.0
12	13.3	18.0	61.1	10.0	15.7	2.6	44.7	6.0	36.3	7.5	15.8
13	12.4	16.0	60.5	13.1	16.7	3.3	44.1	5.7	36.8	9.3	16.2
14	12.7	20.4	60.1	10.9	16.3	3.1	44.3	6.6	37.2	8.9	14.8
15	12.6	16.6	61.5	9.7	15.8	4.7	44.6	6.7	38.3	10.7	14.4
16	10.7	15.2	56.0	10.1	16.0	12.3	48.3	6.6	37.9	5.4	11.8
17	17.5	16.0	56.3	10.9	16.9	11.2	44.6	8.7	41.4	4.4	11.9
18	13.2	17.9	63.3	11.1	17.5	9.9	44.8	10.0	40.8	4.0	10.9
19	12.8	20.3	61.1	10.9	17.4	16.2	45.0	7.2	40.0	4.0	11.3
20	11.5	16.9	57.8	12.5	18.3	9.7	46.7	3.0	41.3	5.2	12.4
21	12.3	18.3	58.4	9.4	17.1	11.6	46.7	2.9	40.3	3.4	11.9
22	11.9	17.0	56.1	11.0	19.4	11.3	47.3	3.5	40.2	1.8	11.5
23	11.8	16.9	55.0	13.2	17.1	6.3	46.2	4.0	40.8	3.9	12.2
24	12.0	19.4	55.4	14.2	20.3	11.8	47.5	3.8	41.1	3.4	12.3
25	11.2	15.8	62.4	14.9	17.7	10.3	47.9	11.3	41.0	4.4	12.5
26	11.9	17.0	63.0	13.7	16.7	7.7	48.5	9.4	42.0	3.8	11.0
27	12.3	15.7	62.1	12.9	18.4	7.0	47.1	11.7	41.4	2.8	10.7
28	13.2	20.5	65.9	13.2	16.4	3.7	48.8	5.7	40.8	3.2	10.7
29	14.7	16.3	64.9	13.2	17.9	5.1	47.8	10.3	42.0	2.3	10.0
30	12.6	16.3	65.2	11.1	17.4	0.4	49.5	5.6	42.1	1.8	10.3
31	8.7	17.8	51.2	9.9	14.2	8.4	49.0	7.5	39.9	5.9	10.7
32	8.5	18.9	51.2	9.7	16.4	8.2	47.5	3.5	38.3	5.0	9.7
33	9.1	18.3	53.4	10.3	16.0	8.7	48.5	9.6	38.5	5.8	12.1
34	10.6	23.9	53.0	12.0	13.9	7.8	47.4	11.6	34.6	6.0	11.6
35	9.8	21.2	43.3	9.6	13.7	8.4	48.2	12.6	36.5	5.3	12.0
36	9.2	20.3	42.4	13.1	14.9	7.1	50.0	12.0	34.9	6.1	10.6
37	8.3	20.2	42.9	10.7	16.1	6.2	48.6	6.8	35.3	4.7	12.2
38	8.1	18.9	42.2	11.3	16.0	6.3	49.4	10.9	34.8	5.2	10.7
39	8.1	20.1	42.5	10.6	15.0	10.1	51.1	14.3	34.4	4.0	12.3
40	7.3	20.4	41.9	10.5	15.7	8.4	48.3	13.0	32.6	6.1	11.4
41	8.6	19.0	43.1	10.5	16.1	5.7	48.3	9.5	35.5	3.5	10.2
42	8.4	21.0	44.1	11.6	14.4	5.8	48.6	13.1	34.7	3.2	10.3
43	9.0	19.7	40.7	12.9	16.1	5.2	49.2	8.1	37.0	3.6	10.7

44	8.0	23.3	41.2	12.3	15.1	3.4	50.5	8.6	35.8	2.5	10.9
45	7.6	19.8	40.7	11.9	16.3	6.8	51.6	6.3	35.4	3.5	10.8
Average	11.0	18.4	55.9	11.5	16.0	9.2	46.4	7.6	37.4	5.8	12.0
SD	2.13	1.98	8.97	1.34	1.76	4.95	2.84	3.02	2.87	2.81	1.59

Raw Data Study two: Maximal Isometric Force Production (MIFP), measured in Nm

Appendix 4a Raw data Study Three, Chapter 6

		Pre-Treatment						Post-treatment						
Subject	**Group**	**Gastroc 1**	**Gastroc 2**	**Gastroc 3**	**Mean MIFP**	**Maximal MIFP**	**SD**	**Gastroc 1**	**Gastroc 2**	**Gastroc 3**	**Mean MIFP**	**Maximal MIFP**	**SD**	**Increase**
S2P1	T60	11.7	19.4	21.6	**17.57**	**21.60**	**5.20**	21.3	20.3	26.4	**22.67**	**26.40**	**3.27**	4.80
S2P2	C60	45.2	58.6	67.9	**57.23**	**67.90**	**11.41**	73.5	87.5	86.4	**82.47**	**87.50**	**7.78**	19.60
S2P3	T60	22.3	28.2	41.6	**30.70**	**41.60**	**9.89**	72.5	78.7	70.8	**74.00**	**78.70**	**4.16**	37.10
S2P4	T30	na	na	na	**na**		**na**	na	na	na	**na**		**na**	0.00
S2P5	C30	31.3	34.5	32.6	**32.80**	**34.50**	**1.61**	37.3	32.1	29.4	**32.93**	**37.30**	**4.02**	2.80
S2P6	T60	60.1	70.7	67.5	**66.10**	**70.70**	**5.44**	72.6	62.7	67.2	**67.50**	**72.60**	**4.96**	1.90
S2P7	C60	0	0	0	**0.00**	**0.00**	**0.00**	0	0	0	**0.00**	**0.00**	**0.00**	0.00
S2P8	C30	34.4	62.8	68.8	**55.33**	**68.80**	**18.38**	23.6	54.2	67.4	**48.40**	**67.40**	**22.47**	-1.40
S2P9	C30	39.3	55.1	61	**51.80**	**61.00**	**11.22**	63.2	54.1	36.3	**51.20**	**63.20**	**13.68**	2.20
S2P10	C60	53.7	71.3	70.4	**65.13**	**71.30**	**9.91**	38.8	54.5	66.1	**53.13**	**66.10**	**13.70**	-5.20
S2P11	T30	58.5	79.8	88.7	**75.67**	**88.70**	**15.52**	120.8	133.2	144.3	**132.77**	**144.30**	**11.76**	55.60
S2P12	T30	89.9	96	97.8	**94.57**	**97.80**	**4.14**	141.2	149.2	164	**151.47**	**164.00**	**11.57**	66.20
S2P13	T60	59.8	61.3	52.9	**58.00**	**61.30**	**4.48**	68.1	69.8	73.5	**70.47**	**73.50**	**2.76**	12.20
S2P14	T60	24.6	31.8	26.4	**27.60**	**31.80**	**3.75**	66.9	69.5	73.4	**69.93**	**73.40**	**3.27**	41.60
S2P15	C30	46.7	71.9	49.6	**56.07**	**71.90**	**13.79**	72.5	75.1	62.7	**70.10**	**75.10**	**6.54**	3.20
S2P16	T30	67.5	59.5	50.7	**59.23**	**67.50**	**8.40**	42.8	57.4	66.2	**55.47**	**66.20**	**11.82**	-1.30
S2P17	C60	36.4	38.7	44.2	**39.77**	**44.20**	**4.01**	33.4	38.3	40.7	**37.47**	**40.70**	**3.72**	-3.50
S2P18	T30	52.1	43.4	48.3	**47.93**	**52.10**	**4.36**	58.9	64.2	54.3	**59.13**	**64.20**	**4.95**	12.10
S2P19	C60	34.2	43.1	40.8	**39.37**	**43.10**	**4.62**	42.4	52.3	31.7	**42.13**	**52.30**	**10.30**	9.20
S2P20	C30	78.4	79.2	68.9	**75.50**	**79.20**	**5.73**	77.1	74.3	71.2	**74.20**	**77.10**	**2.95**	-2.10

Raw data Study three: Maximal Isometric Force Production (MIFP) Pre and Post treatment, measured in Nm. Gastroc: Gastrocnemius, 1,2,3: attempt, SD: Standard Deviation

Appendix 4b Raw data Study Three, Chapter 6

Participant	Group	Pennation angle Pre Treatment	Pennation angle Post Treatment	Difference
1	T60	11	15	-0.6
1	T60	11	13	0.5
1	T60	10	15	0.2
2	T60	15	13	0.4
2	T60	10	13	-0.2
2	T60	11	11	0.5
3	T60	13	15	1.3
3	T60	11	13	-0.3
3	T60	11	15	-0.1
4	T60	10	13	0.7
4	T60	10	16	-1.2
4	T60	10	13	1.3
5	T60	12	13	0.1
5	T60	13	13	0.4
5	T60	11	12	0.5
6	T30	10	12	1
6	T30	10	13	-0.4
6	T30	13	13	0.3
7	T30	15	17	2.6
7	T30	16	17	2.5
7	T30	15	18	4
8	T30	15	20	-1.6
8	T30	16	16	3.4
8	T30	16	17	-0.3
9	T30	11	14	1.1
9	T30	11	15	0.5
9	T30	11	15	0.8
11	C30	18	22	4.4
11	C30	20	19	1.7
11	C30	22	20	4.3

Participant	Group	Pennation angle Pre Treatment	Pennation angle Post Treatment	Difference
12	C60	12	13	1.7
12	C60	13	14	3.1
12	C60	15	14	0.1
13	C30	16	17	0.9
13	C30	14	17	3.3
13	C30	16	16	0.4
14	C30	11	11	3.8
14	C30	10	10	-1.7
14	C30	12	13	-1.4
15	C60	12	12	4.8
15	C60	12	12	-0.1
15	C60	12	13	1.2
16	C30	15	16	0.2
16	C30	16	16	-0.2
16	C30	16	16	1.1
17	C30	11	12	0.6
17	C30	12	11	0.7
17	C30	11	11	-1.7
18	C60	8	10	3.5
18	C60	9	9	5.5
18	C60	9	9	2.1
19	C60	10	10	2
19	C60	9	10	1.1
19	C60	9	10	3.4
20	C60	13	13	3.2
20	C60	12	10	4.5
20	C60	11	12	3.6

Raw Data study three: Pennation angle (measured in degrees) pre and post treatment. Difference: post treatment measurement - pre-treatment measurement

Appendix 4c Raw data Study Three, Chapter 6

Participant	Week	Attempt	Day	MFP-ES	DUR
1	1	1	1	18.859	0.8
1	1	2	1	19.114	0.75
1	1	3	1	19.114	0.75
1	1	4	1	19.519	0.85
1	1	5	1	20.324	0.75
1	1	6	1	19.267	0.75
1	1	7	1	19.165	0.75
1	1	8	1	20.159	0.85
1	1	9	1	21.004	0.8
1	1	10	1	20.447	0.75
1	1	11	1	20.283	0.75
1	1	12	1	18.393	0.55
1	1	13	1	18.247	0.8
1	1	14	1	18.062	0.8
1	1	15	1	17.708	0.75
1	1	16	1	16.94	0.85
1	1	17	1	16.135	0.85
1	1	18	1	16.736	0.75
1	1	19	1	15.436	0.8
1	1	20	1	16.802	0.75
1	1	21	1	17.108	0.8
1	1	22	1	16.703	0.85
1	1	23	1	15.538	0.8
1	1	24	1	16.39	0.85
1	1	25	1	16.189	0.8
1	1	26	1	15.53	0.75
1	1	27	1	15.181	1.2
1	1	28	1	15.137	0.85
1	1	29	1	15.042	0.85
1	1	30	1	14.944	0.8
1	1	31	1	16.94	0.85
1	1	32	1	16.135	0.85
1	1	33	1	16.736	0.75
1	1	34	1	15.436	0.8
1	1	35	1	16.802	0.75
1	1	36	1	17.108	0.8
1	1	37	1	16.703	0.85
1	1	38	1	15.538	0.8
1	1	39	1	16.39	0.85
1	1	40	1	16.189	0.8
1	1	41	1	15.53	0.75
1	1	42	1	15.181	1.2
1	1	43	1	15.137	0.85
1	1	44	1	15.042	0.85
1	1	45	1	14.944	0.8
1	1	1	2	13.429	1.9
1	1	2	2	12.424	0.65
1	1	3	2	10.409	0.6

Participant	Week	Attempt	Day	MFP-ES	DUR
1	1	15	2	13.76	0.65
1	1	16	2	7.492	0.7
1	1	17	2	8.697	0.75
1	1	18	2	9.935	1.6
1	1	19	2	10.956	0.6
1	1	20	2	8.046	0.6
1	1	21	2	9.106	0.65
1	1	22	2	10.154	0.6
1	1	23	2	9.098	0.6
1	1	24	2	9.098	1.45
1	1	25	2	8.992	1.85
1	1	26	2	6.632	0.55
1	1	27	2	5.737	0.65
1	1	28	2	6.141	0.6
1	1	29	2	7.091	0.7
1	1	30	2	4.571	0.55
1	1	1	3	19.056	0.8
1	1	2	3	20.505	0.85
1	1	3	3	20.462	0.8
1	1	4	3	20.859	0.7
1	1	5	3	21.157	0.75
1	1	6	3	21.008	0.75
1	1	7	3	20.859	0.7
1	1	8	3	21.255	0.75
1	1	9	3	20.29	0.75
1	1	10	3	20.541	0.75
1	1	11	3	19.984	0.8
1	1	12	3	19.937	0.7
1	1	13	3	20.286	0.75
1	1	14	3	20.232	0.75
1	1	15	3	20.931	0.75
1	1	16	3	16.095	0.75
1	1	17	3	15.789	0.75
1	1	18	3	15.636	0.8
1	1	19	3	15.691	0.75
1	1	20	3	15.49	0.75
1	1	21	3	16.834	0.85
1	1	22	3	16.535	0.8
1	1	23	3	15.534	0.75
1	1	24	3	16.084	0.75
1	1	25	3	15.235	0.75
1	1	26	3	15.33	0.75
1	1	27	3	14.427	0.75
1	1	28	3	15.086	0.8
1	1	29	3	14.838	0.8
1	1	30	3	15.385	0.75
1	1	31	3	13.276	0.75
1	1	32	3	13.531	0.75

1	1	4	2	13.429	0.6
1	1	5	2	14.78	0.8
1	1	6	2	15.118	0.8
1	1	7	2	11.502	0.6
1	1	8	2	14.361	0.6
1	1	9	2	13.454	0.55
1	1	10	2	13.163	0.6
1	1	11	2	14.721	0.6
1	1	12	2	16.12	0.55
1	1	13	2	15.37	0.55
1	1	14	2	13.665	0.55

1	1	33	3	12.795	0.7
1	1	34	3	13.09	0.7
1	1	35	3	10.254	0.7
1	1	36	3	9.445	0.75
1	1	37	3	10.381	0.75
1	1	38	3	9.98	0.75
1	1	39	3	13.189	0.75
1	1	40	3	12.69	0.7
1	1	41	3	12.18	0.75
1	1	42	3	13.829	0.75
1	1	43	3	10.894	0.75
1	1	44	3	10.84	0.75
1	1	45	3	12.446	0.75

Raw data study three: A sample of the data extracted from the LMD. Data relates to participant one. MFP-ES: Maximal electrically stimulated force production (Nm), DUR: Duration of contraction (Seconds)

KEELE UNIVERSITY

NHS Aintree Hospitals
NHS Foundation Trust

University Hospital Aintree
Lower Lane

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STROKE TEAM FOR AUDIT AND RESEARCH

DEPARTMENT OF MEDICINE FOR THE ELDERLY

Tel: 0151 529 3978

Information Sheet for:

Strength training with electrical stimulation – Is this is a viable method of facilitating independent mobility and improving quality of life after a moderate to severe stroke?

We would like to invite you to take part in a study of a treatment which may be used to improve your leg strength and therefore help you walk better. If you are interested in supporting this research project, then it is important that you understand why the research is being done and what it will involve. Please read the following information carefully, and discuss it with others, if you wish, prior to confirming your willingness to participate in this study. If any information is unclear, or if you would like more information, please contact **Dr Ramesh Durairaj**. Do take time to decide whether you wish to take part. If you do not want to take part in this study, we would like to assure you that your current medical care will not be affected or compromised in anyway and we would like to thank you for giving us a bit of your time.

The purpose for this study

Loss of strength in the leg muscle can compromise the ability to independently walk after a stroke. Treatment with electrical stimulation can be used to strengthen muscles that cannot be exercised actively and as a result the ability to walk may improve. The aim of this study is to find out whether treatment with electrical stimulation can be used to help with the recovery of walking function after a stroke. This study forms part of an educational program undertaken by Ms Leanne Claydon, registered physiotherapist.

What is Electrical Stimulation (ES)?

ES is a way of using small electrical impulses to the cause paralysed muscles to contract. Two self-adhesive pads (electrodes) are placed on the skin close to the nerve that activates the muscle or over the muscle itself. The electrodes are connected to a small battery powered stimulator, which supplies the electrical impulses that cause the muscle to contract.

Who is it suitable for?

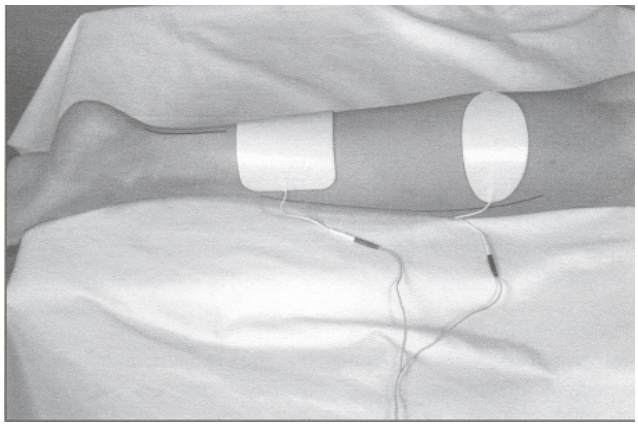
It is most commonly used for people who have suffered a stroke, but it is also used for people with multiple sclerosis, cerebral palsy, incomplete spinal cord injury and with some people who have had head injuries.

How is it used?

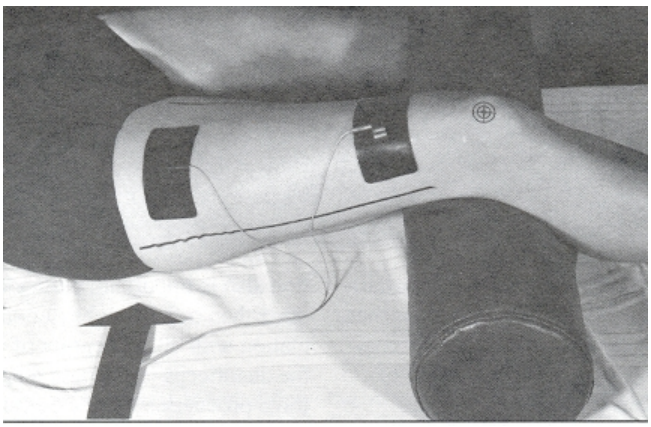
To exercise and strengthen muscles. ES is used to exercise muscles in the legs and also in the arm and hand. The stimulation can also help to relax muscles that are tight. This may be done as part of a physiotherapy treatment programme. Patients typically use electrical stimulation for 30 – 60 minutes a day and continue to use the stimulator until the goals of the treatment are reached.

What are the risks?

ES causes a 'tingling' sensation (a bit like pins & needles). Most people get used to this quite quickly and do not find it uncomfortable. Some people do not like the sensation and may decide not to continue with ES. Occasionally there may be some slight skin irritation from the electrodes. If this problem persists then hypoallergenic electrodes can be used. ES is not suitable for use by people who have poorly controlled epilepsy, people with implanted electronic devices (e.g. cardiac pacemakers). ES should not be used during pregnancy.



Stimulation of calf muscles



Stimulation of quadriceps muscles

Who is eligible to participate in this study

All adult patients admitted to the Aintree Combined Stroke Unit who have lost the ability to walk after their stroke but who are able to sit will be eligible to participate. Not all people can be treated with electrical stimulation and

you will be required to undergo a screening process to check you have no contraindications to treatment once consent has been given for study participation.

Do I have to take part?

Participation in this clinical trial is entirely voluntary. You are not obliged to support this clinical trial and the standard of care you receive will not be affected in any way.

However, if you do decide to take part you will be given this information sheet to keep and be asked to sign a Consent Form. Also, even if you decide to take part you are still free to withdraw at any time without giving reason. A decision to withdraw at any time, will not affect the standard of care you receive.

What will happen, if I decide to take part in this trial and what will I have to do?

If you are interested in supporting this trial and have read the information leaflet you will be asked to sign the consent form. We will then ask you questions about your health and wellbeing, consult your medical notes for information relating to your stroke, perform a medical examination to see how the stroke has affected you and take some measurements of your muscle structure using an ultrasound scanner and an MRI scanner. We will also carry out tests to see that it is safe to treat you with electrical stimulation. The time taken for this initial assessment will, normally, not exceed two hours in total.

- On completion of these initial assessments you will be randomly allocated into one of two groups (T-group and C-group). Neither you nor the researcher will have any influence on which treatment group you are allocated to. This will be done independently by a computer. Randomization means that you are put into a group by chance. It is like flipping a coin. Which group you are put in is done by a computer. Neither you nor the researcher will choose what group you will be in. This is known as 'blinding'. You will have a 1 in 2 chance of being placed in any group.

- People in the **T-group** will be given additional treatment with electrical stimulation once a day each (from Monday to Friday) over a period of 6 weeks. Stretching will be carried out daily and strengthening treatment will be done on alternate days.

- People in **C-group** will receive no additional treatment.

Some important points related that to the study treatment are described below.

- Everyone recruited to this trial will still get NHS based rehabilitation therapy as normal.
- We will keep a diary to record the sessions of all treatment that you receive.
- All treatment will be provided by a state registered and trained physiotherapist.

Both groups will be treated the same in every way apart from the fact that one group receives the electrical stimulation and the other one does not. This will enable us to make comparisons and to find out whether the electrical stimulation helps with the recovery of arm function or not.

To identify any benefits associated with treatment we will assess your recovery six-week treatment period. This will involve a visit to the hospital to see a member of our team who will ask you questions about your recovery and repeat some of the assessments taken at the start of the trial. The time required for these should not normally exceed two hours. If you have already been discharged from hospital by the time of these assessments

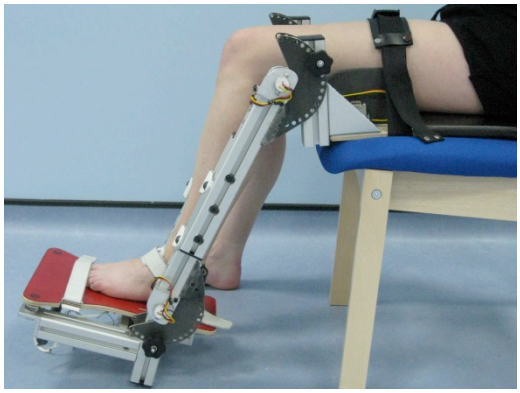
we will arrange free transport for you to come to the clinic, if you wish. You will have to travel to the hospital for 18 visits in total.

Details of treatment

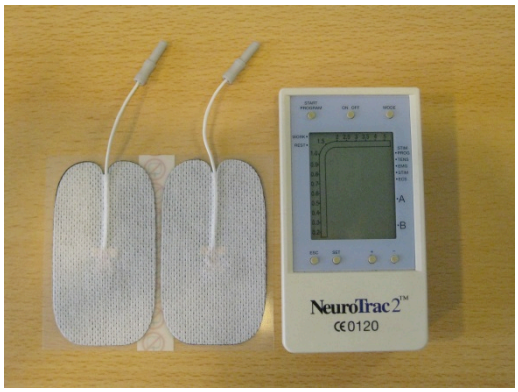
The equipment that is used for electrical stimulation is shown in the picture (this machine is very similar to a TENS machine which is used to treat pain). The electrodes that deliver treatment are placed on the surface of the skin as shown on the picture on the left. You will be seated comfortably on a chair or a plinth (a specialised bed used for physiotherapy treatment). Your leg will be placed on a support or left free (depending on whether you have a scheduled exercise or stretching session respectively). Electrodes will be placed over your muscle. Once you are ready treatment will commence. A small electrical current will then be passed via the electrodes to your muscles. This will result in

- a muscle contraction and a movement of the limb (the same as you would when you move your limb actively by yourself) accompanied by a mild to moderate tingling sensation if you are participating in the stretching protocol or
- a muscle contraction (a tightening of the muscle without movement) accompanied by a strong tingling sensation (this may be uncomfortable but should not cause pain) if you are participating in the strengthening protocol.

We have experience of using this equipment and do not expect it to cause any significant side effects. However, some people may find the initial sensation of their limbs moving automatically disturbing.



Device to rest your leg, and measure your strength



Equipment used to deliver Electrical Stimulation

During each treatment session (a maximum of one hour in any session) you will be required to sit and relax as much as possible, however, if you feel any discomfort or have any concerns you must inform the therapist immediately. When treatment is completed the equipment will be taken off and you can then move about and carry on with other things. [NB: A therapist will supervise all treatment sessions for this study.]

Are there any alternatives to the treatment that is being used?

There is no known alternative to treatment with surface electrical stimulation that is currently being used.

What are the possible disadvantages and advantages of taking part in the research?

To the best of our current knowledge, there is no known disadvantage of taking part in this research study, as we would have ensured that you have no contraindications to treatment (and informed you of this) prior to the commencement of this study. It is possible that you may see faint redness of the skin under or surrounding the electrode pad following treatment and this is nothing to be concerned about. However, if the redness persists you will need to inform the treatment therapist or your doctor/nurse. As stated before, some people may find the initial sensation of their muscles moving automatically during treatment with electrical stimulation disturbing.

What if new information becomes available?

If this happens we will give this information to you and then discuss with you whether you want to continue in the study. If you decide to withdraw, your medical treatment and therapy will not be affected in any way. However, if you decide to continue in the study you will be asked to sign an updated consent form.

What happens when the research study stops?

The treatment that we are testing is currently not available via the NHS. However, if you wish to continue treatment after the study period is completed then the NHS consultant will review your case and prescribe the appropriate treatment required for you.

What if something goes wrong during this study?

Although the risks associated with treatment involving electrical stimulation are low it is important to point out that if taking part in this research project harms you there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

Will my taking part in this study be kept confidential?

If you consent to take part in the research we will need to look at your medical records and notes. However, your name and contact details will not be divulged to any third party outside the research team. Also it will not be possible to identify you from the data collected. Any data we have of you will be stored on a secure computer. Any information, about you, which leaves the hospital, will have your name and address removed so that you cannot be recognised from it. We will inform your GP about your participation in this study if you provide us with permission to do the same.

What will happen to the results of the research study?

On completing this trial we will need to analyse the data to identify whether early treatment with electrical stimulation can help with strengthening and/or walking ability. The results of this study will be published in

Conferences and Medical Journals. Copies of any such publications can be obtained by contacting **Dr. Ramesh Durairaj**. Once again we would like to stress that you will not be identified from the published data.

It is also possible that we will want to use the data collected in this study to support our other research projects. If you have no objections for us to use the data collected from you during the course of this study, you will need to indicate this on the appropriate section in the consent form. If the data is used to support future research it will not be possible to specifically identify you from the data.

We would now like to thank you for having taken the time to enquire about our study. If you have any further questions please contact us and we will be happy to answer all your questions.

If we have answered all your questions and you want to support this study you will now need to sign the consent form. If however, you have decided not to participate, we would like to thank you for taking the time to find out about this study.

Thank you.

Dr Ramesh Durairaj
Consultant Physician for Stroke and General Medicine
Dept of Medicine for the Elderly
Aintree University Hospitals NHS Foundation Trust
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A study funded by

action medical research
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Registered Charity No. 208701

Appendix 6a The Barthel Index Study Four, Chapter 7

Provided by the Internet Stroke Center — www.strokecenter.org

THE Patient Name: _____
BARTHEL Rater Name: _____
INDEX Date: _____

Activity Score

FEEDING
0 = unable
5 = needs help cutting, spreading butter, etc., or requires modified diet
10 = independent _____

BATHING
0 = dependent
5 = independent (or in shower) _____

GROOMING
0 = needs to help with personal care
5 = independent face/hair/teeth/shaving (implements provided) _____

DRESSING
0 = dependent
5 = needs help but can do about half unaided
10 = independent (including buttons, zips, laces, etc.) _____

BOWELS
0 = incontinent (or needs to be given enemas)
5 = occasional accident
10 = continent _____

BLADDER
0 = incontinent, or catheterized and unable to manage alone
5 = occasional accident
10 = continent _____

TOILET USE
0 = dependent
5 = needs some help, but can do something alone
10 = independent (on and off, dressing, wiping) _____

TRANSFERS (BED TO CHAIR AND BACK)
0 = unable, no sitting balance
5 = major help (one or two people, physical), can sit
10 = minor help (verbal or physical)
15 = independent _____

MOBILITY (ON LEVEL SURFACES)

0 = immobile or < 50 yards
5 = wheelchair independent, including corners, > 50 yards
10 = walks with help of one person (verbal or physical) > 50 yards
15 = independent (but may use any aid; for example, stick) > 50 yards _____

STAIRS
0 = unable
5 = needs help (verbal, physical, carrying aid)
10 = independent _____

TOTAL (0–100): _____
Provided by the Internet Stroke Center — www.strokecenter.org

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor
and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient,
friends/relatives and nurses are the usual sources, but direct observation and common sense are also
important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer
periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

References
Mahoney FI, Barthel D. “Functional evaluation: the Barthel Index.”
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***Arch Phys Med Rehabil.* 1980;61:355-358.**
Collin C, Wade DT, Davies S, Horne V. “The Barthel ADL Index: a reliability study.”
***Int Disability Study.*1988;10:61-63.**
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purposes with the following citation:
Mahoney FI, Barthel D. “Functional evaluation: the Barthel Index.”
Maryland State Med Journal 1965;14:56-61. Used with permission.

Permission is required to modify the Barthel Index or to use it for commercial purposes.

Appendix 6b The Nottingham Extended Activities of Daily Living Questionnaire Study Four, Chapter 7

Nottingham Extended ADL Scale
The following questions are about everyday activities. Please answer by ticking ONE box for each question. Please record what you have ACTUALLY done in the last few weeks.

DID YOU…….	**Not at all**	**with help**	**on your own**	**on your own with difficulty**
1. Walk around outside?				
2. Climb stairs?				
3. Get in and out of a car?				
4. Walk over uneven ground?				
5. Cross roads?				
6. Travel on public transport?				
7. Manage to feed yourself?				
8. Manage to make yourself a hot drink?				
9. Take hot drinks from one room to another?				
10. Do the washing up?				
11. Make yourself a hot snack?				
12. Manage your own money when out?				
13. Wash small items of clothing?				
14. Do your own housework?				
15. Do your own shopping?				
16. Do a full clothes wash?				
17. Read newspapers or books?				
18. Use the telephone?				
19. Write letters?				
20. Go out socially?				
21. Manage your own garden?				
22. Drive a car?				

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Appendix 7 Raw data Study Four, Chapter 7

Participant Num	Group	Quads Pre	Quads 3-wk	Quads Post	Difference pre-post	Gastroc Pre	Gastroc 3-wk	Gastroc Post	Difference Pre-Post
1	Treatment	0	0	0	0	0	0	0	0
2	Control	0	13	19	19	0	1.1	2.6	2.6
3	Treatment	0	1.5	4.2	4.2	0	14	26	26
4	Treatment	0.4	3.5	7.5	7.1	1.3	6	21	19.7
7	Treatment	0	1.8	1.5	1.5	0	5	8.2	8.2
8	Control	0	0	0	0	0	0	0	0

Raw data study 4: Maximal Isometric Force Production (MIFP) Measured in Nm

Participant	Timed up and go test (seconds)	
	Baseline	6-weeks
1	0	0
2	0	16.4
3	0	16.6
4	0	14.4
5	0	NA
6	0	NA
7	0	13.8
8	0	0
9	0	NA

Raw data study 4: Timed up and go test. Measured in seconds

Participant	Group	Week able to walk
2	C	6
3	T	5
4	T	6
7	T	2

Raw data study 4: Functional ability

Appendix 8 Ethical approval Study Five, Chapter 8

The Robert Jones and Agnes Hunt NHS
Orthopaedic and District Hospital NHS Trust

Oswestry
Shropshire
SY10 7AG

Telephone 01691 404000
Minicom/text 01691 404558

Direct Line: 01691 404193
Email: mccall@rjah.nhs.uk

Ms Leanne Claydon
ORLAU
Robert Jones & Agnes Hunt Orthopaedic & District Hospital Trust

Date: 27 February 2009
Project Ref: RL1 401

Dear Ms Claydon

Re: Are changes in muscle structure and function, following treatment with neuromuscular electrical stimulation similar in younger and older adults?

I am pleased to confirm that **The Robert Jones & Agnes Hunt Orthopaedic & District Hospital NHS Trust** has reviewed your research study entitled '**Are changes in muscle structure and function, following treatment with neuromuscular electrical stimulation similar in younger and older adults?'** and gives approval for you to conduct this research within the Trust. Your research has been entered into the Trust's Research database.

Please reply to this letter confirming the expected start date and duration of the study. As part of the Research Governance Framework it is important that the Trust is notified as to the outcome of your research and as such we will request feedback once the research has finished along with details of dissemination of your findings. We may also request brief updates of your progress from time to time, dependent on duration of the study. Similarly, if at any time details relating to the research project or researcher change, the R&D department must be informed.

Our understanding is that LREC approval has been applied for. Please note that Trust does not give approval for you to commence research until LREC approval is received. Please advise the R&D office once you have made your ethical approval submission.

If you have any further questions regarding this or other research you may wish to undertake in the Trust please feel free to contact me again. The Trust wishes you success with your research.

Yours sincerely

Professor I McCall
Medical Director

INVESTOR IN PEOPLE

Patron: H.R.H. The Duchess of Kent

Theatre Recovery Unit
Awarded for excellence

Appendix 9 Participant Information Sheet Study Five, Chapter 8

Keele University

Title: Investigation of Biomarker(s) Resulting from Ex Vivo Electrical Stimulation of Muscle
(Version 2.0 Date 11/06/2012)

You are being invited to take part in a research study. Before you decide whether to take part or not, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Our contact details are on the back page, and we welcome you to get in touch with us if any of the information is unclear. Please take time to decide whether or not you wish to participate.

What is the purpose of this study?
Muscle weakness is a significant problem for elderly people, for those with long term neurological conditions and for those recovering after injury. People with weak muscles struggle to maintain their independence. The literature suggests that strength training is the best route to recovery and this can be provided by electrical stimulation, even in people who are unable to work their muscles themselves. Our research shows that some people respond very well, but others much less so. In order to understand individual responses the underlying mechanisms need to be identified. During this trial, we will do two things (a) measure the water content in your body non-invasively and (b) collect four small samples of blood from volunteers undergoing electrical stimulation. The blood will be analysed to look for key biological markers so see if they explain the response of the muscle. We will also study the relationship between the changes in the water content and the blood results. This project is especially timely as the population is ageing. If we can better understand the processes of muscle weakness we may be able to develop new therapies or drug treatments, targeted at the right people.

What is Electrical Stimulation (ES)?
Electrical Stimulation is a way of using small electrical impulses to cause muscles to contract. Two self-adhesive pads (electrodes) are placed on the skin close to the nerve that activates the muscle or over the muscle itself. The electrodes are connected to a small battery powered stimulator, which supplies the electrical impulses that cause the muscle to contract. Similar electrodes will be used to measure the water content in the body.

What are the risks?
Electrical Stimulation causes a 'tingling' sensation (a bit like pins & needles). Most people get used to this quite quickly and do not find it uncomfortable. However, some people do not like the sensation and may decide not to continue with Electrical Stimulation. Occasionally there may be some slight skin irritation from the electrodes. If this problem persists then hypoallergenic electrodes can be used. Electrical Stimulation is not suitable for use by people who have poorly controlled epilepsy, people with implanted electronic devices (e.g. cardiac pacemakers). Electrical Stimulation should not be used during pregnancy.

Why do we want to take blood samples and measure water content, and who will take these?

We want to take samples of blood in order to understand how the muscle responds to electrical stimulation. Our previous work in this area shows that people respond differently to electrical stimulation, and looking at markers in your blood will help us understand this. Blood will be taken by a trained health care worker. The changes in the water content of the treated area may also be a surrogate indicator for the changes in the composition of blood. If we can confirm that this is the case it will be easier to translate the findings from this study to the hospital setting. The researcher who will provide electrical stimulation will also take the bio-impedance measurement.

Who is eligible to take part in the study?
Adults aged between 18 years and older are eligible to take part in this study. Not all people can be treated with Electrical Stimulation and you will be required to undergo a screening process to check you have no contraindications to electrical stimulation once consent has been given for study participation. This information will be gathered in the lifestyle questionnaire.

Do I have to take part?
Participation in this clinical trial is entirely voluntary, if you decide not to participate we will thank you for your time, and take no further action. However, if you do decide to take part you will be given this information sheet to keep and be asked to sign a Consent Form. You will have the opportunity to ask the research team any questions that may arise about the study before signing the consent form. Also, even if you decide to take part you are still free to withdraw at any time without giving reason.

All information collected will remain confidential, and stored on a secure computer. Your name and contact details will be replaced by a subject number and you will therefore not be recognisable from anything collected.

What will happen during the trial and what is expected of me?
The trial requires that you attend for a one off session with electrical stimulation.
Firstly we will attach the electrodes to the skin over your quadriceps muscle (muscle on the front of your thigh), and let you feel how the stimulation feels. We will then disconnect the electrical stimulator and connect the electrodes to a machine that will non-invasively measure the water content of your body (using a machine called a bio-impedance monitor). This is a non-invasive measurement and will cause you no discomfort as the currents used to measure your water content will be much smaller than that used to stimulate your muscle. Once this is done we will reconnect the electrodes to the stimulator and we will slowly turn up the intensity, to a maximal level that you are comfortable with. The feeling should be intense, however not painful; everything we do will be within your pain limits. The stimulation will cause your quadriceps muscle to contract. We will discuss this more on the day, and will go through this part of the process slowly. Once you are happy with the intensity, we will start the stimulation program. This will last for 30 minutes. Your quadriceps muscle will contract for 15 seconds, and then will have a 5 second rest; this sequence will be repeated for the 30 minute period.
We also want to look at how your muscle responds to the electrical stimulation, which means we have to take small samples of blood. We will take four samples altogether. The first will be before we have conducted any stimulation, and then after 15, 30 and 60 minutes after that. We will re-measure the water content after 30 minutes and 60 minutes along with taking blood.
We will also ask you to fill out a short questionnaire about your lifestyle, including things you like to eat and any exercise that you conduct.
We anticipate that you will be with us for about one and a half hours in total.

What if something goes wrong during the study?
The person to contact for complaints is Ms N Leighton, Research Governance Officer.

Tel: 01782 733306; Email: n.leighton@keele.ac.uk

Every precaution will be used in this study to ensure your safety. Although the risks associated with electrical stimulation are low it is important to point out that if taking part in this research project harms you there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal university complaints mechanisms will be available to you.

What will happen to the results of this study?
The results of this study will be published in Conferences and Medical Journals. Copies of any such publications can be obtained by contacting any member of the research team. Once again we would like to stress that you will not be identified from the published data.
This study is also contributing to an educational program being conducted at Keele University, by Leanne Claydon, registered Physiotherapist.

Thank-you for your time in reading this information sheet.

Contact information

Dr Paul Roach: Institute for Science and Technology in Medicine, Keele University, Hartshill Campus, Stoke-on-Trent, Staffordshire, ST4 7QB. Tel 01782 555226, Email P.Roach@istm.keele.ac.uk

Dr Anand Pandyan: School of Health and Rehabilitation, McKay Building, Keele University, Staffordshire, ST5 5BG, Tel 01782 734252, Email a.d.pandyan@istm.keele.ac.uk

Dr Caroline Stewart: ORLAU, RJAH Orthopaedic Hospital, Oswestry, Shropshire, SY10 7AG, Tel 01691 404666, Email Caroline.Stewart@rjah.nhs.uk

Miss Leanne Claydon: St Mary's University College, Waldegrave Road, Strawberry Hill, Twickenham, TW1 4SX Tel 020 8240 4000, Email leanne.claydon@smuc.ac.uk

KEELE UNIVERSITY

Investigation of Biomarker(s) Resulting from Ex Vivo Electrical Stimulation of Muscle

Thank you for agreeing to take part in this study. In order to understand how your muscle responds to electrical stimulation, it is important to know a little bit about your lifestyle. Both your current exercise levels, and the type of food you eat will both impact on how your muscle responds to electrical stimulation, and knowing this information will allow us to take these factors into consideration. We will also need to ensure it is safe to use electrical stimulation on your leg.

Below are a few questions about your general lifestyle, thank-you for taking the time to fill them out.

Please indicate your

Age:

Height:

Weight:

How often do you participate in exercise? (please put a X in the appropriate box)

3 or more times a week ☐

1-2 times a week ☐

Once a week ☐

Once a month ☐

With no regular pattern ☐

Comments:……
…….

At what level would you say you exercise, if at all? (please put a X in the appropriate box)

Intense exercise ☐

Moderate exercise ☐

Light exercise ☐

I don't regularly exercise ☐

Comments:……
…….

What type of exercise do you conduct? (please put a X in all the boxes that apply)

Aerobic/ Cardiovascular ☐

Power/ strength ☐

To maintain general fitness ☐

Comments:……
…….

What exercise, if any have you conducted in the last four days?

……
…….

How would you consider your diet? (please put a X in all the boxes that apply)

I eat a combination of all food stuffs, and consider my diet healthy ☐

I take supplements to enhance my healthy diet ☐

I generally eat healthily, however like to indulge now and again ☐

I generally don't have time to prepare healthy meals ☐

I eat a mix of 'heat up' meals and take-aways ☐

I don't consider my diet, I eat what I fancy ☐

Comments:

What did you eat for your evening meal last night?

……
…….

Thank you for taking time to fill out this questionnaire. We will be happy to answer any questions should they arise.

Participant has no contraindications to electrical stimulation (i.e. active implantable devices, cancer, scar tissue at stimulation site, uncontrolled epilepsy, DVT, metallic implants at site of stimulation)

Signature of person screening:

Name: Ms Leanne Claydon Date:

Appendix 11 Raw Data Study Five, Chapter 8

Participant	Stim Intensity	Exercise_ 48	Exercise_ General	CKA	CKB	CKC	CKD	%CKA	%CKB	%CKC	%CKD
1	63	1	1	0.612	0.679	0.612	0.702	100	111.0	100.0	114.7
2	21.5	1	1	0.783	0.742		0.796	100	94.8		101.7
3	22	0	0	0.329		0.158		100		48.1	
4	21	0	0	0.549	0.554	0.631	0.434	100	100.8	114.9	79.1
5	25	0	1	0.581	0.432			100	74.5		
6	21	1	1		0.675	0.711	0.846				
7	35	0	1	0.729	1.007	0.594	0.904	100	138.2	81.5	124.0
8	31	1	1		0.428	0.675	0.581				
9	31	0	0		0.428	0.423	0.401				
10	33	0	0	0.477	0.419	0.414	0.396	100	87.8	86.8	83.1
11		1	1	0.554	0.657	0.230		100	118.7	41.6	
12		0	0		0.383	0.652	0.679				
13	33	0	1	0.504	0.500	0.576	0.450	100	99.1	114.3	89.3
14	28	0	1	0.432	0.369	0.239		100	85.4	55.3	
15	36	0	1		1.007	0.774	0.603				
16	44	0	1	0.455	0.625	0.477	0.464	100	137.5	104.9	102.0
17	42	1	1	0.374	0.527			100	140.9		
18	35	0	1	1.117	0.707	0.568	0.686	100	63.3	50.8	61.4
19	41	1	1	0.359	0.441	0.873	0.593	100	122.8	243.2	165.2
21	46	1	1	0.655	0.367	0.276	5.765	100	56.1	42.1	879.7
22	50	1	1	0.805	0.760	0.635	0.949	100	94.4	78.9	117.9
24	37	1	1	1.042	1.136	0.976	0.509	100	109.0	93.7	48.8
25	34	1	1	0.714	0.684	0.706	0.832	100	95.7	98.9	116.5
26	40	0	1	0.702	0.634	0.926	0.670	100	90.4	132.0	95.5
27	48	0	1	0.742	1.121	0.778	0.594	100	151.1	104.8	80.0
28	39	0	0	1.241	1.457	0.729	0.982	100	117.4	58.7	79.2
29		0	1	0.702				100			
30	52	0	0	0.634		0.275	1.129	100		43.3	177.9
31	37	0	1	0.819	0.967	1.048		100	118.1	128.0	
32	47	0	0	0.733	0.576	0.625	0.711	100	78.6	85.3	96.9
33		1	1	5.012	1.270	0.504	0.495	100	25.3	10.1	9.9
34		1	1	0.661	0.493	0.666	0.679	100	74.5	100.8	102.8

Raw data study five: Creatine Kinease analysis (CK) measured in nmol/ml/min. A/B/C/D refer to time points in analysis. 0: no exercise, 1: exercise